CLINAM

European Foundation for Clinical Nanomedicine

12/2020

Basel, October 26–28

SUMMIT PROCEEDINGS

CLINICAL NANOMEDICINE AND THE IMPACT OF DIGITALIZATION AND ARTIFICIAL INTELLIGENCE FOR PRECISION MEDICINE

THE TECHNOLOGIES FOR DIAGNOSIS & THERAPY IN PATIENT-CENTRIC MEDICINE

Summit under the Auspices of the Swiss Confederation and with 36 NPO Programme Supporters

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12TH EUROPEAN AND GLOBAL SUMMIT FOR CLINICAL NANOMEDICINE, TARGETED DELIVERY AND PRECISION MEDICINE

THE BUILDING BLOCKS TO PERSONALIZED MEDICINE

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EDITORIAL

For the twelfth time the CLINAM Summit is held - this year it is a live stream Summit.

As an international platform serving the community since many years, we felt that stopping the Summit because of the COVID-19 is not a good idea. In difficult times, an excellent exchange of scientific knowledge and strong personal networking between all stakeholders is particularly important and should be made possible.

While the Summit was planned for May in Basel, it became evident in March that postponing is unavoidable, leading to the October 25 – 28 as meeting date in Basel, full of hope that by then infection rates would have come down drastically. We were wrong. All over Europe the numbers of infected people are rising.

Our original plan to have 150 members in Basel physically and from all over the world in live stream connection seemed feasible. Several practical questions were difficult to solve in a good way: for a conference that emphasizes personal interaction, how will catering over lunch work out, how to organize evening events with ballet and an orchestra? Many people would say: Skip this. We are here for excellent science and not for eating. However, CLINAM is more than just science. It also lives from meeting others and jointly build the field. CLINAM is an enabler for new creativity, for cooperation and for new projects. Therefore the four dinners and all lunches are a vital element in the philosophy of this event. The recent rise in infection rates restricting travelling even within Europe made the decision to turn to virtual unavoidable.

Feedback from participants over many years reveal an enormous benefit of the CLINAM network. Meeting new people and making friends is often a key to success in working together. CLINAM as scientific Summit strives for the ambiance where you feel great, can open all your senses and release your energy towards novel findings, novel research, novel plans and audacious endeavors.

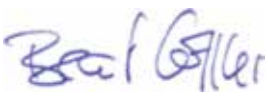
The fully virtual Summit this year has now 36 supporting collaborating organizations, facilitating the exchange of knowledge and research results in Nanomedicine and related fields. CLINAM has grown continuously. Nanomedicine as cross technology touches most fields that participate in recent medical innovations. Can we virtually create trust and creative mutual discussions about ideas and projects between the participants? Almost 500 participants and the exhibitors in this summit dare to take the challenge. Having a Summit virtually certainly will also confer opportunities, and although we may not yet fully grasp all of them, let us learn together how we can make the best of it and create excellence in the constrained setting forced upon us by the pandemic.

As always, CLINAM aims to be at the forefront of the evolution of medicine. “Clinical Nanomedicine and the Impact of Digitalization and Artificial Intelligence for Precision Medicine” is this year’s focus, and it turns out that this is a timely topic since we start by having the summit digitally.

Together with all speakers, poster presenters and participants we shall be prepared to follow the challenging schedule and to achieve a live streaming event bringing the maximum possible benefit to participants globally. It is evident that times in which life on Mars is debated, the technology in live streaming will advance rapidly. Also due to COVID-19 we get more and more familiar with this technology for conferences.

We thank you for taking the effort to be with us to meet on screen. And hopefully in 2021 in real life!

Basel, October 2020



Dr. med. h.c. Beat Löffler, MA
CEO of the CLINAM-Foundation

Prof. Dr. med. Patrick Hunziker
CSO of the CLINAM-Foundation

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CURRICULA VITAE SPEAKERS

Ibane Abasolo

Dr. Abasolo is coordinator of the Functional Validation & Preclinical Research (FVPR) area of the CIBBIM-Nanomedicine, at the Vall d'Hebron Research Institute (VHIR, Barcelona). As head of FVPR, Dr. Abasolo is responsible for standardizing trials with which to test therapeutic agents, nanosystems or candidate genes, offering industry and other research groups a technological platform with which to advance the preclinical development of their compounds. In fact, FVPR is part of unit 20 (Experimentation *in vivo*) of the platforms of the CIBER-BBN (Spanish Network in Bio-enginery, Biomaterials and Nanomedicine) and of the Singular Technical Scientific Infrastructure (ICTS) Nanbiosis.

Ibane Abasolo obtained the Bachelor's degrees in Biochemistry and Biology from the University of Navarra in 1997 and 1998, respectively. During her doctorate in the laboratories of Dr. Alfonso Calvo (CIMA, Pamplona) and Prof. Zhou Wang (Northwestern University, Chicago, USA), he studied the role of a peptide hormone, adrenomedullin, in prostate cancer. Later, Dr. Abasolo continued her post-doctoral training in the group of Prof. F.X. Real (IMIM, Barcelona), where he focused on the study of key molecules in the progression of pancreatic cancer and cerebellar development. During this time, Dr. Abasolo gained experience in experimental mouse models, from the generation of transgenic models to the molecular and cellular characterization of pre-existing models. In 2016, Dr. Abasolo moved to Insitut d'Alta Tecnologia (PRBB, Barcelona), where she trained in molecular imaging techniques such as microPET, SPECT and CT. In 2017, she joined the lab of Dr. Simó Schwartz at the CIBBIM-Nanomedicine center at VHIR and since then she has been working on the preclinical validation (*in vitro* and *in vivo*) of different type of nanomaterials. She has been focusing in two main indications, drug-resistant and difficult-to-treat cancers and of lysosomal storage disorders, currently lack a definitive curative treatment.

Currently Dr. Abasolo leads 2 European research projects (DiamESTar and Smart4Fabry) and participates directly in the European projects NoCanTher, Evo-Nano and Safe-Med-Tech led by Dr. Schwartz (CIBBIM-Nanomedicine, VHIR). Importantly, Dr. Abasolo is the PI of a national grant (PI18/00871, by ISCiii) focused on improving the treatment of lysosomal storage disorders using different nanometric systems, extracellular vesicles, among them. Research career of Dr. Abasolo is supported by her publications in the field of cancer, lysosomal diseases and nanotechnology, as well as her extensive network of collaborators nationally and internationally.

RECENT PUBLICATIONS

- Pesarrodona M, Jauset T, Díaz-Riascos ZV, Sánchez-Chardi A, Beaulieu ME, Seras-Franzoso J, Sánchez-García L, Baltà-Foix R, Mancilla S, Fernández Y, Rinas U, Schwartz S Jr, Soucek L, Villaverde A, Abasolo I, Vázquez E. Targeting Antitumoral Proteins to Breast Cancer by Local Administration of Functional Inclusion Bodies. Adv Sci (Weinh). 2019 Jul 24;6(18):1900849. doi: 10.1002/advs.201900849. eCollection 2019 Sep 18. PubMed PMID: 31559131; PubMed Central PMCID: PMC6755514.
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- Cacicedo ML, Islan GA, León IE, Álvarez VA, Chourpa I, Allard-Vannier E, García-Aranda N, Díaz-Riascos ZV, Fernández Y, Schwartz S Jr, Abasolo I, Castro GR. Bacterial cellulose hydrogel loaded with lipid nanoparticles for localized cancer treatment. Colloids Surf B Biointerfaces. 2018 Oct 1;170:596-608. doi: 10.1016/j.colsurfb.2018.06.056. Epub 2018 Jun 26. PubMed PMID: 29975908.
- Rocas P, Fernández Y, García-Aranda N, Foradada L, Calvo P, Avilés P, Guillén MJ, Schwartz S Jr, Rocas J, Albericio F, Abasolo I. Improved pharmacokinetic profile of lipophilic anti-cancer drugs using αvβ3-targeted polyurethane-polyurea nanoparticles. Nanomedicine. 2018 Feb;14(2):257-267. doi: 10.1016/j.nano.2017.10.009. Epub 2017 Nov 7. PubMed PMID: 29127040.
- Giannotti MI, Abasolo I, Oliva M, Andrade F, García-Aranda N, Melgarejo M, Pulido D, Corchero JL, Fernández Y, Villaverde A, Royo M, García-Parajo MF, Sanz F, Schwartz S Jr. Highly Versatile Polyelectrolyte Complexes for Improving the Enzyme Replacement Therapy of Lysosomal Storage Disorders. ACS Appl Mater Interfaces. 2016 Oct 5;8(39):25741-25752. Epub 2016 Sep 22. PubMed PMID: 27610822.

Karin Abitorabi

Karin Abitorabi obtained her Diplom in Immunology and Microbiology from the University of Konstanz, Germany.

Karin has had a very distinguished and successful career with over 20 years of experience from discovery research to development of therapeutic drugs and is an internationally recognized expert in cell therapy development.

Prior to joining Novartis, she worked independently as a consultant at Cell Therapy and Flow Cytometry and worked on various clinical studies, at PCT (Progenitor Cell Therapy) leading Process Development in Mountain View, California, in the departments of Hematopoietic Transplantation at SyStemix, Inc., Preclinical Oncology and Immunology at Cell Genesys Inc., and Research at Schering-Plough BioPharma (formerly DNAX, now Merck Research Labs). She has worked as a key team member on separate therapeutic programs leading to IND filings.

In 2013 Karin joined the Cell and Gene Therapy department at Novartis in Basel and significantly contributed to the success of a variety of pipeline programs including the Kymriah program. Karin collaborated with a network of international CMOs and effectively facilitated the transfer of Novartis process technology. She was also instrumental in process improvements and for the progression of several hematopoietic stem cell program efforts into the clinic.

Gabriel Aeppli

Gabriel Aeppli is professor of physics at ETH Zürich and EPF Lausanne, and head of the Synchrotron and Nanotechnology division of the Paul Scherrer Institute. All of his degrees are from MIT and include a BSc in Mathematics and Electrical Engineering, and MSc and PhD in Electrical Engineering. A large fraction of his career was in industry, where, starting as a work-study student at IBM and after his PhD moving to Bell Laboratories and then NEC, he worked on problems ranging from liquid crystals to magnetic data storage. He was subsequently co-founder and director of the London Centre for Nanotechnology and Quain Professor at University College London. Aeppli also cofounded the Bio-Nano Consulting Company, of which he remains a non-executive director. He is a frequent advisor to numerous private and public entities worldwide (including China, Australia, Europe and the US) engaged in the funding, evaluation and management of science and technology. Honors include the Mott Prize of the Institute of Physics (London), the Oliver Buckley prize of the American Physical Society, the Néel Medal/International Magnetism Prize of the International

Union of Pure and Applied Physics, and election to the American Academy of Arts and Sciences and the Royal Society (London).
Aeppli's scientific research is currently focused on the applications of nanotechnology and photon science to biomedicine and quantum information processing. Projects include the development of optical and microwave tools for medical diagnostics and pharmacology, where we are interested in new drug-target and antibody-antigen binding assays. We pay particular attention to obtaining specific, quantitative results, as well as to ease of use and (eventual) low cost for our engineered systems. Photons are also at the heart of efforts to control and read out quantum states in solids, including especially silicon, for which we exploit coherent, tunable pulses of THz radiation from a free electron laser. Our most recent work (2015) describes the electrical detection of coherent orbital superpositions in a commercial silicon wafer. A related topic is adiabatic quantum computing, where calculations are performed by mapping problems onto networks of bits, and then relaxing the networks via quantum mechanics. This procedure is most easily modeled in the limits of very large networks using magnets (although programmable medium size networks containing of order 1000 bits are now being implemented using superconducting junctions), where the bits correspond to Ising spins with either up or down magnetization, and quantum mechanics is introduced via a "transverse" field, which allows tunneling between the up and down states.

Kirill Afonin

Associate Professor

Dr. Afonin graduated from Saint Petersburg State University with a M.S. in Chemistry, followed by a Ph.D. in Photochemistry earned from Bowling Green State University, Ohio. In addition, he also obtained a Graduate Certificate in Bioinformatics, Proteomics/Genomics. In the following three years, Dr. Afonin completed a Postdoctoral Fellowship in Chemistry and Biochemistry at the University of California Santa Barbara. In 2011, he was invited as a Research Fellow to the National Cancer Institute, NIH where he established and managed an experimental branch within the Computational RNA Structure Group. He started his tenure-track appointment at UNC Charlotte in 2015 and in 2019, was promoted with permanent tenure to the rank of Associate Professor. Dr. Afonin currently serves as a founding council member and vice-president of International Society of RNA Nanotechnology and Nanomedicine.

RECENT PUBLICATIONS

- Chandler M, Johnson B, Panigaj M, Afonin KA. Innate immune responses triggered by nucleic acids inspire the design of immunomodulatory nucleic acid nanoparticles (NANPs). Current Opinion in Biotechnology, 63: 8-15, 2020. PMID: 31778882
- Ke W, Hong E, Saito RF, Rangel MC, Wang J, Viard M, Richardson M, Khisamutdinov EF, Panigaj M, Dokholyan NV, Chammas R, Dobrovolskaia MA, Afonin KA. RNA-DNA fibers and polygons with controlled immunorecognition activate RNAi, FRET, and transcriptional regulation of NF-kB in human cells. Nucleic Acids Research, 47(3): 1350-1361, 2019. PMID: 30517685
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- Johnson MB, Halman JR, Satterwhite E, Zakharov AV, Bui MN, Benkato K, Goldsworthy V, Kim TJ, Hong E, Dobrovolskaia MA, Khisamutdinov E, Marriott I, Afonin KA. Programmable nucleic acid-based polygons with controlled neuroimmunomodulatory properties for predictive QSAR modeling. Small, 13(42), 2017. PMID: 28922553

Maria Pilar Aguar-Fernandez

Head of Unit Health innovations
DG RTD

Ms. Aguar joined the EC working for the DG for Health & Consumers on evaluation of the performance of national authorities and food-safety control systems in 2001.
From 2005 to 2012, she was involved in development and management of European Research Programmes in DG Research & Innovation in the area of Industrial Technologies.
From 2012 to 2017, she was Head of Unit at the Joint Research Centre. Her portfolio included developing testing methodologies for policymakers in the framework of EU legislation on chemicals and consumer products and working on safety and risk-benefit assessments of nanomaterials, nanomedicines and health technologies.
Since 2017, she has returned to DG Research & Innovation, managing European research programmes in health and environment and in regenerative medicine. From June 2019, she is in charge of the Unit "Health Innovations".
Pilar holds degrees in Pharmacy and in Food Science and Technology with additional studies in Toxicology. Before joining the European Commission, she worked for the Spanish Ministry of Health in different positions.

Zahraa Al-Ahmady

Dr Zahraa Al-Ahmady is senior lecturer at the Pharmacology Department, Nottingham Trent University, UK. She also holds a honorary research fellow position at the University of Manchester, UK. She obtained her BSc Degree in Pharmacy with a distinction from the College of Pharmacy, University of Baghdad in 2004. After training as a clinical pharmacist, she was awarded a scholarship to study the Masters in Drug Delivery at the UCL School of Pharmacy, where she won the AstraZeneca Prize for the best overall performance. Zahraa completed her PhD studies with the Nanomedicine Lab at UCL School of Pharmacy on the design, characterization and biological performance of temperature-sensitive vesicles for cancer therapy in 2012. She then joined the NANOSOLUTIONS (FP7-NMP) European project as a postdoctoral research associate at the University of Manchester. Her work was mainly focused on the structure – biological function relationship that determines the safety of engineered nanomaterials. Following that she worked as a research fellow with the North West Centre of Advanced Drug Delivery (NoWCADD), a join post between the division of pharmacy at the University of Manchester, the Nanomedicine Lab and AstraZeneca, working on the development of innovative therapeutic and *in vivo* imaging approaches. Dr Al-Ahmady lab explores effective and efficient delivery approaches for cancer and neurodegenerative diseases.

Khuloud T. Al-Jamal

Chair of Drug Delivery & Nanomedicine, King's College London

Professor Khuloud T. Al-Jamal is a Chair of Drug Delivery & Nanomedicine, King's College London. She is also a registered pharmacist at the General Pharmaceutical Council. She started her academic career as a lecturer at King's College London in 2011. She has completed her pre-registration pharmacy training at The University College London Hospital and was awarded the Overseas Research Award Scheme (ORSA) Scholarship from The University of London (2000-2004) to complete her PhD in Drug Delivery from The School of Pharmacy, University of London (currently known as UCL-School of Pharmacy).

She was awarded the prestigious CW Maplethorpe Research and Teaching Postdoctoral Fellowship from The University of London (2005-2007) to explore the use of cationic dendrimers as anti-angiogenic agents for growth inhibition of solid and metastatic tumours. She has developed an extensive experience in designing and developing novel nanoscale delivery systems including dendrimers, liposomes, quantum Dots (QDs), polymers, viral vectors, chemically functionalised carbon nanotubes and graphene oxide. Her current work involves pre-clinical translation of novel nanomaterials designed specifically for drug, protein, nucleic acids and radionuclide delivery for therapeutic or diagnostic applications.

She was awarded and is managing a number of research projects funded by The Royal Society, Worldwide Cancer Research, EPSRC, BBSRC, FP6, FP7 and ITN Marie Curie research programmes. In February 2012, she was awarded the BBSRC New Investigator award exploring the use of chemically functionalised carbon nano-needles as vectors for delivering therapeutics across the BBB. In 2012, she was awarded the prestigious Royal Pharmaceutical Society Science Award in recognition for her outstanding scientific achievements in the field of Nanomedicine. In 2019, she received the CRS Nanomedicine and Nanoscale Delivery Award. She is a three-time winner of the Wellcome Trust Image Award (2014-2016). She is a management board member and is on the steering committee of the London Centre of Nanotechnology and the Children Brain Tumour Drug Delivery Consortium. She is on the Editorial Board of Biomaterials Science (RSC), Scientific Reports (Nature Publisher) and Journal of Drug Targeting.

RECENT PUBLICATIONS

- Wang JT-W, Klippstein R, Martincic M, Pach E, Feldman R, Sefl M, Michel Y, Sosabowski JK, Kalbac M, Da Ros T, Ménard-Moyon C, Bianco A, Kyriakou I, Emfietzoglou D, Saccavini J-C, Ballesteros B*, Al-Jamal KT* and Tobias G*. (2019) Neutron activated samarium-153 encapsulated single- and multi-walled carbon nanotubes for *in vivo* imaging and tumour radiotherapy. ACS Nano. (In press; 10.1021/acsnano.9b04898)
- Sunoqrot S*, Al-Debsi T, Al-Shalabi E, Ibrahim LH, Faruqu FN, Walters A, Palgrave R, Al-Jamal KT. (2019) Bioinspired Polymerization of Quercetin to Produce a Curcumin-Loaded Nanomedicine with Potent Cytotoxicity and Cancer-Targeting Potential in Vivo. ACS Biomaterials Science and Engineering. 5(11): 6036-6045
- Llenas M, Sandoval S, Costa PM, Oró-Solé J, Lope-Piedrafita S, Ballesteros B, Al-Jamal KT*, Tobias G*. (2019) Microwave-Assisted Synthesis of SPION-Reduced Graphene Oxide Hybrids for Magnetic Resonance Imaging (MRI). Nanomaterials (Basel). 9(10)
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Wafa Al-Jamal

Reader in Nanomedicine and Drug Delivery/ Queen's University Belfast

Dr Wafa Al-Jamal completed her PhD in Drug Delivery and Nanomedicine in 2008 from the School of Pharmacy, University of London (now known as UCL-School of Pharmacy). She is currently a Reader in Nanomedicine and Drug Delivery at School of Pharmacy, Queen's University Belfast. She is also a Prostate Cancer Fellow (2014-2019). Dr Al-Jamal joined School of Pharmacy, University of East Anglia, Norwich, as a Lecturer in Drug Delivery (2013-2017), after working as a Senior Research Fellow at University College London, and King's College London (2008-2013).

Dr Al-Jamal's main research interest focuses on engineering novel nanomaterials for biomedical applications. Her current research aims to design smart vectors to deliver a wide range of therapeutic agents and targeting moieties, and to fabricate multifunctional nanoparticles for combinatory therapy and theranostic applications. Her long-term research career is to facilitate the translation of nanoparticle-based therapeutics from the lab to the clinic.

Dr Al-Jamal is the GSK Emerging Scientist Award winner for 2015, and Gro Brundtland Award winner for 2017. Her multidisciplinary research has been funded by the Royal Society, Prostate Cancer UK, The Engineering and Physical Sciences Research Council (EPSRC), and Rosetrees Trust. She has published over 50 papers in high impact factor journals. Currently, she is a member the Prostate Cancer UK (PCUK) Research Advisory Committee, and a Visiting Professor at Guizhou Medical School, China.

RECENT PUBLICATIONS

- Cheung CCL, Ma G, Karatasos K, Seitsonen J, Ruokolainen J, Koffi CR, Hassan HAFM, and Al-Jamal WT. 2019. Liposome-Templated Indocyanine Green J- Aggregates for In Vivo Near Infrared Imaging and Stable Photothermal Heating. Nanotheranostics. Accepted
- Cheung CCL, Ma G, Ruiz A, and Al-Jamal WT. 2019. Microfluidics production of lysolipid-containing temperature-sensitive liposomes. JOVE. In press.
- Cheung CCL and Al-Jamal WT. 2019. Sterically stabilized liposomes production using staggered herringbone micromixer: Effect of lipid composition and PEG-lipid content. International Journal of Pharmaceutics, 566:687-696.
- Pereira SGT, Hudoklin S, Kreft ME, Kostevsek N, Stuart MCA, Al-Jamal WT. 2019. Intracellular activation of a prostate specific antigen-cleavable doxorubicin prodrug: a key feature toward prodrug-nanomedicine design. Mol Pharmaceutics, 16(4):1573-1585.
- Silva V, Al-Jamal WT. 2017. Exploiting the Cancer Niche: Tumour-Associated Macrophages and Hypoxia as Promising Synergistic Targets for Cancer Therapy. J Control Release, 253: 82-96

Cameron Alexander

Professor of Polymer Therapeutics

Cameron Alexander is Professor of Polymer Therapeutics and a Royal Society Wolfson Research Merit Fellow at the School of Pharmacy, University of Nottingham, UK.

Professor Alexander received degrees (BSc and PhD) in Chemistry from the University of Durham, UK and carried out post-doctoral research at the Melville Laboratory for Polymer Synthesis, University of Cambridge. He is a Fellow of the Royal Society of Chemistry, the Institute of Materials, Minerals and Mining, and the Higher Education Academy. He

is a member of the Science Engineering and Technology Board of the Engineering nd Physical Sciences Research Council (EPSRC) and held EPSRC Leadership and Impact Fellowships from 2009-2019. From 2006-2016, Professor Alexander led the EPSRC Centre for Doctoral Training in Advanced Therapeutics and Nanomedicines at Nottingham and University College London with leading pharmaceutical industry partners. He has published > 200 papers and received the UK Macro Group Medal in 2014 for contributions to polymer science. Professor Alexander has been fortunate to collaborate with fellow Europeans in 'NanoFar', the first joint doctoral programme in Nanomedicine in Europe, and to work with scientists from more than 20 countries in his research group

RECENT PUBLICATIONS

- Al-Natour, M. A.; Yousif, M. D; Cavanagh, R. J.; Selo, A. A.; Apebende, E.; Ghaemmaghami, A.; Kim, D-H.; Aylott, J.; Taresco, V.; Chauhan, V. and Alexander, C. Facile Dye-Initiated—Polymerization of Lactide-Glycolide Generates Highly Fluorescent—Poly(lactic-co-glycolic Acid) for Enhanced—Characterization of Cellular Delivery. ACS Macro Letters 2020, https://doi.org/10.1021/acsmacrolett.9b01014
- Giannakopoulou, N.; Williams, J.; Moody, P. R.; Sayers, E.J.; Magnusson, J. P.; Pope, I.; Payne, L.; Alexander C.; Jones, A. T.; Langbein, W.; Watson, P. and Borri, P. Four-wave-mixing microscopy reveals non-colocalisation between gold nanoparticles and fluorophore conjugates inside cells. Nanoscale 2020, https://doi.org/10.1039/C9NR08512B.
- Vasey, C. E.; Pearce, A. K.; Sodano, F.; Cavanagh, R. J.; Abelha, T.; Cuzzucoli Crucitti, V.; Anane-Adjei, A. B.; Ashford, M. B.; Gellert, P. R.; Taresco, V. and Alexander, C. Amphiphilic tri- and tetra-block co-polymers combining versatile functionality with facile assembly into cytocompatible nanoparticles. Biomaterials Science 2019, 7, 3832-3845.
- Francini, N.; Cochrane, D.; Illingworth, S.; Purdie, L.; Mantovani, G.; Fisher, K.; Seymour, L. W.; Spain, S. G. and Alexander, C. Polyvalent diazonium polymers provide efficient protection of oncolytic adenovirus Enadenotucirev from neutralising antibodies while maintaining biological activity *in vitro* and *in vivo*. Bioconjugate Chemistry 2019 30 (4), 1244–1257
- Alazzo, A.; Al-Natour, M.; Spriggs, K. A.; Stolnik, S.; Ghaemmaghami, A.; Kim, D. H. and Alexander, C. Investigating the molecular effects of hyperbranched polycation-DNA complexes on lung cancer cells using LCMS-based metabolite profiling. Molecular Omics 2019 15, 77—87.

Christoph Alexiou

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Prof. Dr. Christoph Alexiou, received his Ph.D. in 1995 from the Technical University of Munich, Medical school. After finishing his internship in the Gastroenterology Department at the Universityhospital of the Technical University he started as a physician and researcher at the Department of oto-rhino-laryngology, head and neck surgery and founded a research group working on the field of local chemotherapy with magnetic nanoparticles (Magnetic Drug Targeting). In the year 2000 he received his degree as an ENT-Physician and 2002 he changed to the ENT-Department in Erlangen, Germany, where he performed his postdoctoral lecture qualification (Habilitation). He is working there as an assistant medical director in the clinic and leads the Section for Experimental Oncology and Nanomedicine (SEON). Since 2009 he owns the Else Kröner-Fresenius-Foundation-Professorship for Nanomedicine at the Universityhospital Erlangen. He receives grants from the European Union, German Research Community (DFG), Ministry of Education and Science (BMBF) and Bavarian State Ministry of the Enviroment and Consumer Protection and is a member of the Executive Board of the European Technology Platform for Nanomedicine (ETPN). His research is addressing the emerging fields of Diagnosis, Treatment and Regenerative Medicine using magnetic nanoparticles and the translation from basic research into clinical trials. He received for his research several national and international renowned awards.

RECENT PUBLICATIONS

- Cicha I, Scheffler L, Ebenau A, Lyer S, Alexiou C, Goppelt-Struebe M: Mitoxantrone-loaded superparamagnetic iron oxide nanoparticles as drug carriers for cancer therapy: Uptake and toxicity in primary human tubular epithelial cells. Nanotoxicology 10: 557-566, 2015

To analyze the cellular responses to mitoxantrone-carrying SPIONs (SPION-MTO), and to the drug released from SPIONs, we used an *in vitro* system that allows comparison of primary human cells with different endocytotic capacities, namely, epithelial cells from proximal and distal parts of the nephron. Uptake did not affect cell viability or morphology and we show that whereas the uptake of SPIONs does not affect cellular functions or viability, the toxicity of drugloaded SPIONs depends essentially on the type of drug bound to nanoparticles.

- Friedrich RP, Zaloga J, Schreiber E, Toth IY, Tombacz E, Lyer S, Alexiou C: Tissue plasminogen activator binding to superparamagnetic iron oxide nanoparticle-covalent versus adsorptive approach. Nanoscale Res Lett, 11: 297, 2016

In this study, we used tangential flow filtration (TFF) method to purify the drugs before the reaction and used the frequently applied and clinically available recombinant tissue plasminogen activator (tPA; Actilyse®) as a proof of concept. We then coupled the tPA preparation to polyacrylic acid-co-maleic acid (PAM)-coated superparamagnetic iron oxide nanoparticles (SPIONs) using an amino-reactive activated ester reaction and compared these particles to PAM-coated SPIONs with electrostatically adsorbed tPA. Covalent linkage significantly improves the reactivity and long-term stability of the conjugated SPION-tPA system compared to simple adsorption.

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The design and use of materials in the nanoscale size range for addressing medical and health-related issues continues to receive increasing interest. Research in nanomedicine spans a multitude of areas, including drug delivery, vaccine development, antibacterial, diagnosis and imaging tools, wearable devices, implants, high-throughput screening platforms, etc. using biological, nonbiological, biomimetic, or hybrid materials. Many of these developments are starting to be translated into viable clinical products. Here, we provide an overview of recent developments in nanomedicine and highlight the current challenges and upcoming opportunities for the field and translation to the clinic.

- Pöttler M, Fliedner A, Bergmannn J, Bui LK, Mühlberger M, Braun C, Graw M, MD, Janko C, Friedrich O, Alexiou C, Lyer S: Magnetic Tissue Engineering of the Vocal Fold Using Superparamagnetic

Iron Oxide N anoparticles. Tissue Engt Part A 25:1470-1477, 2019
This study aims at nanotechnology for regenerative medicine by magnetic tissue engineering (MTE). New approaches for vocal fold (VF) reconstruction are desperately needed. Superparamagnetic iron oxide nanoparticles offer innovative, scaffold-free potentials for tissue engineering: MTE. By using MTE we could generate functional multilayered human VF cell constructs, which can consequently be used to regenerate the voice in patients with VF injuries.

- Mühlberger M, Janko C, Unterweger H, Friedrich R,Friedrich B, Band, Cebulla N, Alexiou C, Dudziak D, Lee G ,Tietze R: Functionalization Of T Lymphocytes With Citrate-Coated Superparamagnetic Iron Oxide Nanoparticles For Magnetically Controlled Immune Therapy. Int J Nanomedicine. 14: 8421–8432, 2019

T cells can be "magnetized" by incorporation of SPIONCitrate for magnetic targeting. The production of the particle-cell hybrid system is straightforward, as the loading process only requires basic laboratory devices and the loading efficiency is sufficient for cells being magnetically controllable. For these reasons, SPIONCitrate are potential suitable candidates for magnetic T cell targeting.

Lajos (Lou) P. Balogh

Editor-in-Chief, Precision Nanomedicine

Dr. Lajos (Lou) P. Balogh, Ph.D. is the Publisher and Editor-in-Chief of Precision Nanomedicine (PRNANO) designated as the official journal of the International Society for Nanomedicine and CLINAM, the European Society for Clinical Nanomedicine. Dr. Balogh has thirty-five years of experience in teaching, scientific research, and academic publishing with a strong interdisciplinary background. Lou is considered to be an international expert in Nanomedicine and scholarly publications. He he has published 228 scientific papers, gave >230 invited lectures, and has been awarded 12 patents in chemistry, physics, nanotechnology, and nanomedicine. His publications have been cited over 8000 times (19 papers with more than 100 citations, 8 with more than 200 citations, and one cited over 1000 times; h-index=40).
Dr. Balogh is one of the five Founders of the American Society for Nanomedicine. He serves on the US Technical Advisory Group to ISO TC 229 Nanotechnology and on the Board of a number of international European and US organizations. Some recent awards include the Fulbright Scholarship, Visiting Professorship for Senior International Scientists (Chinese Academy of Sciences, Beijing), and the KOFST Fellowship, Seoul National University, Seoul, Korea. Prof. Balogh has held faculty positions at several universities and research institutions: The Kossuth Lajos University of Sciences and the Art, (Debrecen, Hungary), at the University of Massachusetts Lowell, MA, at the Michigan Molecular Institute, Midland, MI, The University of Michigan, Ann Arbor, MI, at the Roswell Park Cancer Institute, Buffalo, NY, and at the University at Buffalo, Buffalo, NY. Between 2008-2016 Lou, as Editor-in-Chief, took Nanomedicine: Nanotechnology, Biology and Medicine (Elsevier) from 5 editors and no impact factor in 2008, to 20 editors and IF =6.9, (5-year IF=7.5) in 2016. He increased readership from 96,000 to over 480,000 downloads/year.
Today, he is the President of Andover House, Inc. a registered not-for-profit online publishing company (www.Andoverhouse.org), the owner and Chief Scientific Advisor of AA Nanomedicine & Nanotechnology (AANMNT), a science consulting firm (baloghl@aananomedicine.com). He also serves as the Executive Editor of Manuscript Clinic (www.manuscriptclinic.us), which helps investigators to successfully publish their research results in the fields of Nanomedicine and Nanotechnology. He gave courses, seminars, and semesters to many scientists while teaching at the Chinese Academy of Sciences (2014 and 2016), at the Academia Sinica, Taiwan, at the Seoul National University Hospital, Seoul, Korea (2017), at The Hong Kong University of Science and Technology (2018), and – as a Fulbright scholar - at the Semmelweis University, Budapest Hungary (2019).

RECENT PUBLICATION

- Balogh, LP, Balancing Interests of Science, Scientists, and the Publishing Business. Prec. Nanomed. 2018 Apr; 1(1):5-14. DOI:10.29016/180418.1
- Parak W, Pelaz B, Alexiou C, Alvarez-Puebla RA, Alves F, Andrews AM, Ashraf, S, Balogh LP, et al, Diverse Applications of Nanomedicine, ACS Nano. ACS Nano, 2017, 11 (3), pp 2313–2381. DOI: 10.1021/acsnano.6b0604

Yechezkel Barenholz

Professor Emeritus Yechezkel (Chezy) Barenholz is head of the Liposome and Membrane Research Lab at the Hebrew University-Hadassah Medical School, Jerusalem, Israel and is also the Daniel G. Miller Professor in Cancer Research at Hebrew University of Jerusalem. He has been on the faculty at Hebrew University since 1968 and has been a visiting Professor at leading Universities around the world. His current research focuses on the development of drugs and nano-drugs based on drug delivery systems (DDS) best exemplified by the anticancer Doxil®, the first liposomal drug as well as the first nano-drug approved by the FDA (1995) with over 700,000 cancer-patients treated so-far world-wide. Few of the liposomal drugs he and his team developed are now at different stages of pre-clinical and clinical stages. Professor Barenholz is an author of >420 scientific publications, cited >35,500 times, with h-index 93 Barenholz is a co-inventor in > 55 approved patent families >50% of them licensed. He is a founder of four 6 start-ups, NasVax (now Therapix) Moebious, PolyPid, LipoCureRX, RebioticsRX, and Ayana Pharma. He received many National and International awards including Honorary Doctorate degree from the Technical University of Denmark (2012) and the 2012 founders award of the International Controlled Release Society.

Matthias Barz

Professsor for Biotherapeutic
Delivery, Leiden Academic Center for Drug Research (LACDR)

Matthias Barz was born in 1981 in Frankfurt Main (Germany). After finishing high school he studied chemistry at the Johannes Gutenberg-University Mainz (Germany) and Seoul National University (South Korea), received a diploma degree in chemistry in 2006 and a PhD in polymer chemistry from the Johannes Gutenberg-University Mainz (Germany) working under the supervision of Prof. R. Zentel in 2009. After finishing his PhD he did postdoctoral stays in the labs of Maria J. Vicent (CIPF, Valencia, Spain) and Tom Kirchhausen (Boston Children's Hospital, Harvard Medical School, Boston, USA). In 2013 he became independent junior research group leader and started his habilitation at the Institute of Organic Chemistry at the Johannes Gutenberg-University Mainz (Germany), which he finished in 2016. In 2020 he was appointed to full professor for biotherapeutic delivery at the Leiden Academic Center for Drug Research (LACDR) (Leiden, Netherlands).

He received numerous awards for his independent research including the Dozentenpreis des Fonds der Chemischen Industrie (FCI, Most prestigious junior faculty award in Chemistry in Germany), Scholarship of the Dr. Hermann-Schnell-Stiftung, German Chemical Society (GDCh), Young Investigator, Polymer Science and Engineering (PMSE), American Chemical Society (ACS) and the Roche pRED Award for Excellence in Applied Cellular and Molecular Biology in Drug Delivery.
His main research interest is the development of nanomedicines based on polypept(o)ides.

RECENT PUBLICATION

- E. J. L. Steen, J. T. Jørgensen, K. Johann, K. Nørregaard, B. Sohr, D. Svatunek, A. Birke, V. Shalgunov, P. E. Edem, R. Rossin, C. Seidl, F. Schmid, M. S. Robillard, J. L. Kristensen, H. Mikula, M. Barz, A. Kjær, M. M. Herth. "Trans-Cyclooctene-Functionalized PeptoBrushes with Improved Reaction Kinetics of the Tetrazine Ligation for Pretargeted Nuclear Imaging." ACS Nano 2019, DOI:10.1021/acsnano.9b06905.
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Patrick Baumhof

Vice President Formulation & Delivery
trained in Chemistry, at the University of Leipzig. His scientific expertise includes chemistry, pharmaceutical sciences and immunology. He joined CureVac in July 2007 when he was responsible for the development and preclinical testing of new formulations for mRNA vaccines and therapeutics. He is inventor of several patents and he co-authored several publications on mRNA technology. Currently he is heading the department for Product design an formulations and is Program manager for the RNAoptimizer Program.

RECENT PUBLICATION

- Proximity Ligation Assays for In Situ Detection of Innate Immune Activation: Focus on In Vitro-Transcribed mRNA Emmeline L. Blanchard, Kristin H. Loomis, Sushma M. Bhosle, Daryll Vanover, Patrick Baumhof, Bruno Pitard, Chiara Zurla, Philip J. Santangelo Mol Ther Nucleic Acids. 2019 Mar 1; 14: 52–66. PMID
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Yaelle Bavli

Yaelle is a researcher at the Laboratory of Membrane and Liposome Research at the Hebrew University of Jerusalem. After completing her M.Sc. degree in Pharmacology with a specialization in pre-clinical models from the Louis Pasteur University of Strasbourg (France), she joined the laboratory of Prof. Yechezkel Barenholz for her PhD. Her Ph.D. thesis was focused on the adverse effects of nano-liposomal drugs upon local and systemic administration. Her research interests include new procedures to evaluate in animal models the adverse effects of local and systemic injection of nanoliposomal formulations, with a special focus the immunological response (complement activation and anti-PEG antibody synthesis).

Beatrice Beck Schimmer

Professor of Anesthesiology

Beatrice Beck Schimmer is full professor of anesthesiology at the University of Zürich (UZH). She studied medicine at the University of Bern. From 2005 to 2018, in parallel to her research and teaching activities, she was Chief of Service at the Institute of Anesthesiology at the University Hospital Zurich. From 2012 to 2018 she was a member of the Research Council of the Swiss National Science Foundation, where she was not only involved in evaluating research, but also as president of the specialized committee 'Careers' and member of the Presiding Board.
Her research focuses on volatile anesthetics-induced organ protection as well as on nanomedicine, covering basic research, translational as well as clinical studies. With the help of magnetic nanoparticles proteins, metals, but also cells are extracted from blood as a complex biological fluid. This is a promising technology to be translated into clinical scenarios. Together with partners from the ETH Zurich she holds a patent within this field. Since mid 2018 she is Vice President Medicine at the UZH and in charge of the leadership and management of the Faculty of Medicine. She is responsible for the strategic development of academic medicine at UZH in cooperation with the ETH Zurich and the four University hospitals.

François Berger

University of Grenoble, School of Medicine

François Berger, MD, PhD had a dual scientific and clinical education in the field of neurology, oncology and molecular and cell biology. He continues to have a dual clinical and research activity has professor of cell biology and neuro-oncology. He develops a translational research activity, trying to validate innovative technologies at the preclinical/clinical level in close collaboration with micro-nanotechnology groups. Exploring the best modalities to accelerate translation of technology innovation at the bedside is the main focus of his research. As Clinatec director he explored the feasibility of clinical research delocalization inside Minatec technology campus as an experimentation to accelerate translation of technology Innovation. Difficulties to export an academic research mode, cost to manage safely patients outside the hospital, the progressive migration to a techno-centric position and at the end the ethical questioning of this position were the main bottlenecks. The development of an innovative translational strategy to catch disruptive innovation outside the health sector was the main success in the context of CEA excellence in the field of technology. After two years, he came back inside the hospital as director of a new research unit INSERM U1205 associating INSERM-Grenoble University and Grenoble university hospital. He is now director of a new research unit INSERM U1205-BrainTech Lab. The objectives of this group are to develop innovative technologies for a better understanding, prevention and therapy of Brain diseases and cancer. It is also to accelerate the transfer of technology innovation at the bedside implementing innovating translational methodologies from cellular, preclinical investigations to human proof of concept trials.

RECENT PUBLICATION

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- Zaccaria A, Bouamrani A, Chabardès S, El Atifi M, Seigneuret E, Lobrinus JA,Dubois-Dauphin M, Berger F, Burkhard PR (co-last authors) . Deep brain stimulation-associated brain tissue imprints: a new *in vivo* approach to biological research in human Parkinson's disease. Mol Neurodegener. 2016 Jan 28;11:12.
- Zaccaria A, Roux-Dalvai F, Bouamrani A, Mombrun A, Mossuz P, Monsarrat B, Berger F. Accessing to the minor proteome of red blood cells through the influence of the nanoparticle surface properties on the corona composition. International Journal of Nanomedicine 2015, 10:1869-1883.
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Pol Besenius

Pol Besenius was born and raised in Luxemburg, studied Chemistry at the Vienna University of Technology in Austria, and at the University of Strathclyde in Glasgow, Scotland. He received his PhD degree from the same institution in 2008, under the supervision of Prof. Peter Cormack and Prof. David C. Sherrington FRS, in collaboration with Prof. Sijbren Otto and Prof. Jeremy K.M. Sanders FRS at the University of Cambridge. As a Marie-Curie Fellow Pol undertook postdoctoral studies at the Eindhoven University of Technology with Prof. Anja Palmans and Prof. E. W. "Bert" Meijer. In 2011, Pol moved to the University of Münster to set up an independent research group at the Organic Chemistry Institute, supported by a Liebig Fellowship. He was also elected as young fellow to the North Rhine-Westphalian Academy of Sciences and Arts. In 2015, he took up a Professorship at the Institute of Organic Chemistry at the University of Mainz. Since 2019 he is the editor of a new journal Organic Materials, and recently he was awarded an ERC Consolidator Grant. His research interests include macromolecular chemistry, self-assembly in water and at interfaces, responsive supramolecular polymers, viromimetic particles and synthetic vaccines.

Tobias Bopp

I am a basic researcher and immunologist who specializes in the field of immunological tolerance to foster our knowledge about context- and tissue-specific regulation of immune responses in health and disease. After completing my training in molecular biology at Johannes-Gutenberg-University Mainz in 2003 I joined the Institute for Immunology (IFI) at University Medical Center (UMC) Mainz to conduct my doctoral thesis in 2006. As a faculty member and later on as a W2 Professor for Molecular Immunology in the IFI at UMC Mainz, I focus on a better understanding of the molecular and transcriptional regulation of T cell- and macrophage-regulated immune responses in situ. Since 2019 I am continuing my research at UMC as chair and director of the IFI.

AREA OF EXCELLENCE

My scientific interests and expertise revolve around the regulatory T cell-, T Helper cell- and macrophage-mediated immune regulatory mechanisms. Since my postdoc and subsequent appointment as professor for molecular Immunology at the IFI of UMC Mainz I have endeavored to provide excellent research in order to enhance our knowledge on the molecular mechanisms underlying the transcriptional regulation of T cells and macrophages in the regulation of context- and tissue-specific immune responses, resulting in app. 100 publications in internationally renowned immunological journals (e.g. Nature Immunology, Cell, Immunity, Cell Metabolism, Journal of Clinical Investigations and the Journal of Experimental Medicine). Besides the honors of being a co-speaker of the DFG-funded comprehensive research Center (CRC) 1292, a project leader in the CRCs 1066, 1292, TR52, TR128, a member of the steering committee of the University Cancer Center Mainz, a faculty member of the German cancer Consortium, expert reviewer for national and international research agencies and renowned journals, I was elected in 2013 to become the head and coordinator of the largest research focus group within the German Society for Immunology (DGfI), T cells: Subsets and functions.

SUPPORTING ACTIVITIES

In addition to providing excellent teaching and basic research, I am also involved in the development and pursuit of superior scientific

and university's administrative structures. Here, I am not only a founding member of the "Research Center for Immunotherapy" (FZI) of UMC Mainz, but also the chairman and coordinator of the FZI's leadership council, a member of the educational and the scientific committee of UMC and a board member of the Mainz Research School of Translational Biomedicine (TransMed).

HONORS AND AWARDS

On the basis of my work I had the honor to receive funding for excellent junior scientists by the Carl Zeiss Foundation from 2007-2009, the Fritz-and-Ursula-Melchers award in 2010 and the Boehringer Ingelheim award in 2008.

Gerrit Borchard

Gerrit Borchard is a licensed pharmacist and obtained his Ph.D. in pharmaceutical technology from the University of Frankfurt (Germany). After holding several academic positions at Saarland University (Germany) and at Leiden University (The Netherlands), he joined Enzon Pharmaceuticals, Inc. (USA) as Vice President Research. In 2005, he was appointed Full Professor of Biopharmaceutics at the University of Geneva (Switzerland), and in 2015, he was an invited professor at Graz University (Austria).

Prof. Borchard has published more than 140 scientific papers (9534 citations, h-factor 51) and 23 book chapters, he edited two books, and is named as inventor on 10 patents. Since 2014, he is president of the Swiss Academy of Pharmaceutical Sciences (SAPhS). In 2012 he joined the Non Biological Complex Drugs (NBCD) working group hosted at Lygature (Utrecht, The Netherlands), joining its steering committee in 2015. He was nominated Chair of the NBCD working party at the European Directorate for the Quality of Medicines & Health Care (EDQM) by Swissmedic, and joined the External Advisory board of the EU-Nanotechnology Characterization Laboratory (EU-NCL) in 2016. He was appointed to the steering board of contactpointnano.ch, a platform fostering SMEs in the field of nanomedicine, in January 2019.

Due to his working in both academia and industry, and living in four countries, Prof. Borchard has acquired extensive experience in diverse working and cultural environments, and is fluent in Dutch, English, French and German (native). Being an enthusiastic long-distance runner and triathlete, he loves to roam the trails and by-roads of the Jura mountains on foot and bike.

Sven Even Borgos

Invited speaker

Sven Even Borgos (born 1976) earned both his undergraduate and PhD degrees at the Norwegian University of Science and Technology in Trondheim, the main technical university of Norway. His undergraduate was in Biophysics and Medical Technology. His PhD, however, was in molecular biology, concerned with genetic engineering of the antibiotic-producing soil bacterium Streptomyces noursei in order to develop mutants producing derivatives of the clinically important antifungal antibiotic nystatin and related compounds, with improved pharmacological properties. He then did a post doc in systems biology, developing and validating a genome-scale metabolic model of the alginate-producing bacterium Pseudomonas fluorescens. Since 2006, he has been working in SINTEF (Norway), which is one of the largest independent research institutes in Europe with more than 2000 employees. Here, he has been working with advanced analytical chemistry, mainly based on mass spectrometry coupled to chromatography. The last years, he has been specializing in physicochemical characterisation of nanomaterials, with an emphasis on nanomedicines. He has been assay group leader for chemical characterization in the European Nanomedicine Characterisation Laboratory (EUNCL) H2020 project. Following on to this, he is WP leader in the REFINE H2020 project supporting the relevant authorities in ongoing developement and optimisation of regulatory framworks for nanomedicines. This also includes ongoing collaborations with e.g. Joint Research Centre (JRC) and National Institute of Standards and Technology (NIST) on standardization of nanomedicines analytical methods. Since 2017, the SINTEF Mass Spectrometry group is also the main analytical partner in the B-SMART H2020 project developing RNA-based nanomedicines against neurodegenerative disorders, and since late 2019 Borgos is SINTEF's PI in the new EXPERT project which seeks to build a platform for nanomedicine-based delivery of therapeutic mRNA, with a lead indication against triple-negative breast cancer. He has a keen interest in all aspects of RNA as therapeutics, and lately also as synthetic mRNA vaccines. This goes in particlar for development and characterization of novel mRNA delivery systems.

RECENT PUBLICATIONS

- Sulheim, E., Mørch, Y., Snipstad, S., Borgos, S.E., Miletic, H., Bjerkvig, R., Davies, C.d.L. and Åslund, A.K.O. (2019) Therapeutic Effect of Cabazitaxel and Blood-Brain Barrier opening in a Patient-Derived Glioblastoma Model. Nanotheranostics, 3, 103-112.
- Ritsema, J., Herschberg, E., Borgos, S., Løvmo, C., Schmid, R., Te Welscher, Y., Storm, G. and van Nostrum, C.F. (2018) Relationship between polarities of antibiotic and polymer matrix on nanoparticle formulations based on aliphatic polyesters. International journal of pharmaceutics, 548, 730-739.
- Fusser, M., Overbye, A., Pandya, A.D., Morch, Y., Borgos, S.E., Kildal, W., Snipstad, S., Sulheim, E., Fleten, K.G., Askautrud, H.A. et al. (2018) Cabazitaxel-loaded Poly(2-ethylbutyl cyanoacrylate) nanoparticles improve treatment efficacy in a patient derived breast cancer xenograft. J Control Release, 293, 183-192.
- Bremer-Hoffmann, S., Halamoda-Kenzaoui, B. and Borgos, S.E. (2018) Identification of regulatory needs for nanomedicines. Journal of Interdisciplinary Nanomedicine, 3, 4-15.
- Borgos, S.E.F. (2016) In Cornier, J., Owen, A., Kwade, A. and Van de Voorde, M. (eds.), Pharmaceutical Nanotechnology: Innovation and Production, 2 Volumes. John Wiley and Sons, Vol. 1, pp. 135-156.

Susanne Bremer-Hoffmann

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Susanne Bremer-Hoffmann is responsible for a laboratory assessing the interaction of nanomaterials with biological systems which is part of the JRC's open Nanobiosciences Laboratory. She is representing the Joint Research Centre in the Horizon 2020 project "REFINE" where she focuses on the identification of regulatory needs of nanotechnology-enabled health products. Susanne Bremer-Hoffmann is a biologist by education with a specialisation in cell biology, molecular biology and in vitro toxicology. After having obtained her Ph.D. from the Free University Berlin in Germany, she worked at the Federal Institute for Risk Assessment in Germany before joining the European Commission's Joint Research Centre in 1995. Susanne Bremer-Hoffmann was involved in the establishment of European Reference Laboratory for Alternatives to Animal Testing (EURL-ECVAM) and contributed to a number of validation studies related to endocrine disruption and developmental toxicity. She participated in several European and

international research projects, published more than 90 scientific manuscripts and contributed to various European and international expert groups relevant for the implementation of European legislations in the field of chemicals and cosmetics.

Donald Bruce

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Dr Donald Bruce holds doctorates in chemistry and in theology. He is managing director of the independent consultancy Edinethics Ltd., working on the ethics and public engagement of emerging technologies. After working 15 years as a chemist in nuclear energy research, risk regulation, and energy policy, he became Director of the Church of Scotland's Society, Religion and Technology Project (SRT) from 1992-2007. In this role he did pioneering ethical assessment of many emerging technologies including GM crops and animals, cloning and stem cells. He has worked extensively on nanomedicine and related technologies from 2003 to the present, in a series of EC projects Nano2Life, NanoBio-Raise, NanoMedRound, Ethentech (on human enhancement), and NanoAthero. An integral part of this work has been in developing and writing public engagement tools with Perry Walker formerly of the New Economics Foundation. He helped develop the Democs/Decide card games and Open-up argument map concepts, on such issues as GM crops, synthetic biology, human enhancement, and stem cells for therapy and for toxicity testing. He created a Democs game on nanomedicine for the NanoAthero EC FP7 project on nanodevices to detect and treat atherosclerosis. He has a diverse range of ethical research interests, including the ethics of genome editing in food animals and in humans, ethics and livestock breeding in the context of global warming and changing food habits (EC H2020 BovReg project), the role of PGD in addressing genetic conditions, end of life issues, and ethical investment. He is a member of the UK Animals in Science Committee, which advises the UK government on animal research. He was a former member of the Scottish Science Advisory Committee, the Societal Issues Panel of Engineering and Physical Sciences Research Council, the Public Affairs advisory group of Biotechnology Research Council, and of the Advisory Board of the Institute of Nanotechnology.

Horacio Cabral

Horacio Cabral is an Associate Professor in the Department of Bioengineering, Graduate School of Engineering, The University of Tokyo. He received his Ph.D. in Materials Engineering from The University of Tokyo in 2007 under the supervision of Prof. Kazunori Kataoka. Dr. Cabral was an Assistant Professor at the Center for Disease Biology and Integrative Medicine, Graduate School of Medicine, The University of Tokyo, until 2010, when he joined the Department of Bioengineering of The University of Tokyo as a Lecturer. In 2014, he was promoted to his current position. Dr. Cabral currently serves on the editorial board of Science and Technology of Advanced Materials, Nanomaterials and the advisory board of Macromolecular Bioscience. Dr. Cabral's major research interests relate to the development of nanomedicines for diagnosis and therapy, particularly systems directed to intractable cancers. His work has provided a basis for several nanomedicines aimed for tumor-targeted therapy and imaging, showing that the design parameters of nanomedicines greatly influences their activity, and some of these formulations have proceeded into clinical studies, offering improved survival and reduced side effects.

RECENT PUBLICATIONS

- J. D. Martin, H. Cabral, T. Stylianopoulos, R. K. Jain, Improving cancer immunotherapy using nanomedicines: Progress, opportunities and challenges. Nat. Rev. Clin. Oncol. (2020) (DOI: 10.1038/s41571-019-0308).
- P. Mi, H. Cabral, K. Kataoka, Ligand-installed nanocarriers towards precision therapy. Adv. Mater. e1902604 (2020) (DOI: 10.1002/adma.201902604).
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- H. Cabral, K. Miyata, K. Osada, K. Kataoka, Block copolymer micelles in nanomedicine applications. Chem. Rev. 118 (14) 6844-6892 (2018) (DOI: 10.1021/acs.chemrev.8b00199).

Luigi Calzolai

Project Leader
European Commission, Joint Research Centre

Luigi joined the Joint Research Center of the European Commission in 2009 where his work focuses on the development of methods for the characterization of nanomedicine and the detection and identification of nanoparticles in complex matrices more in general. He is a member of the Core Expert Team of the European Union Nanomedicine Characterization Laboratory.
Luigi obtained his M.S. and Ph.D. in chemistry from the University of Florence and the University of Siena, respectively.
After a Postgraduate Research at the University of California, Davis, he joined, in 1998, the Swiss Institute of Technology in Zurich, in the laboratory of the then Nobel Laureate Kurth Wuthrich, where he determined (in solution, by NMR) the three dimensional structure of prion proteins responsible of neurological disorders, such as Mad Cow Disease and Creutzfeld-Jacob disease.
In 2007 he moved to the School of Pharmacy of the University of Kent (UK) as Senior Lecturer in biochemistry.

RECENT PUBLICATIONS:

- Physical characterization of liposomal drug formulations using multi-detector asymmetrical-flow field flow fractionation. Jeremie Parot; Fanny Caputo; Dora Mehn; Vincent Hackley; Luigi Calzolai. Journal of Controlled Release. In press.
- Measuring particle size distribution of nanoparticle enabled medicinal products, the joint view of EUNCL and NCI-NCL. A step by step approach combining orthogonal measurements with increasing complexity. Caputo F, Clogston J, Calzolai L, Rösslein M, Prina-Mello A. Journal of Controlled Release. 2019
- Are existing standard methods suitable for the evaluation of nanomedicines: some case studies. Gioria S, Caputo F, Urbán

P, Maguire CM, Bremer-Hoffmann S, Prina-Mello A, Calzolai L, Mehn D. Nanomedicine. 2018, 13:539-54.
- Analytical ultracentrifugation for analysis of doxorubicin loaded liposomes. Mehn D, Iavicoli P, Cabaleiro N, Borgos SE, Caputo F, Geiss O, Calzolai L, Rossi F, Gilliland D. International journal of pharmaceutics. 2017. 523:320-6.

José M Carballido

José M. Carballido is an Executive Director at the Novartis Institutes for Biomedical Research (NIBR) in Basel (CH). José obtained his M. Sc. degree in Biology at the University of Barcelona (ES) in 1987 and, after a short training in Immunology at the Pharmaceuticals Research Division of Ciba-Geigy, Basel, he entered the Swiss Institutes for Allergy and Asthma Research (SIAF) in Davos (CH), to work towards his Ph. D. (Dr. sc. Nat. for the University of Zurich, 1992). José spent one additional year at SIAF, as leader of the Allergy group and then he reached to Palo Alto, CA (US), to perform a Postdoctoral training at the DNAX Research Institute of Molecular and Cellular Biology. In 1997, José joined the Novartis Research Institute in Vienna (AT), where he held positions of increasing importance until he became the Head of the Fully Integrated Program of Psoriasis. Following the closure of the Vienna site (2008), he was appointed Executive Director at the Autoimmunity, Transplantation and Inflammation Disease Area of NIBR, in Basel (CH). Since October 2019, he is Executive Director at the Translational Medicine / Preclinical Safety department of NIBR Basel.
José has a strong background in basic and clinical immunology, particularly in the areas of Allergy, Dermatology, Transplantation and Autoimmunity. José serves as ad-hoc reviewer for various scientific journals and private or governmental funds and he is an active member of several academic societies. He is author of over 60 scientific publications, has published 11 international patents and holds more than 20 inventions. José is currently focused on bringing to the clinic immune tolerance approaches, particularly using nanomedicines to ameliorate and cure type 1 diabetes. In his current role he is also responsible for the evaluation of immunosafety across the Novartis portfolio.

Myriam Cevallos

Myriam Cevallos has been scientific advisor to the State Secretariat for Education, Research and Innovation since 2015. She represents Switzerland in the Health Programme under Horizon 2020, is a member of the State Representative Group of the Innovative Medicines Initiative (IMI) and represents Swiss interests in the Governing Board of the European Open Science Cloud. She is a former member of the Swiss National Covid-19 Science Task Force, which advises the Swiss Federal Council.
Myriam studied Biology at the University of Basle and holds a master's degree in epidemiology of the London School of Hygiene and Tropical Medicine She started her career at the SwissTPH in Basel, where she conducted an NIH research project in Latin America in collaboration with the University of California Berkeley. She then worked for seven years at the Institute for Social and Preventive Medicine at the Clinical Trials Unit of the Bern University Hospital. She was responsible for the management of international projects, including the STROBE Collaboration. Before joining the SERI, she supported the Federal Office of Public Health in the evidence-based development and piloting of concepts for the regulation of clinical research in the Human Research Act.

Chunying Chen

Ph.D. and Prof.
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Dr. Chen received her Bachelor's degree in chemistry (1991) and obtained her PhD degree in Biomedical engineering from Huazhong University of Science and Technology of China in 1996. She worked as a postdoctoral research fellow at the Key Laboratory of Nuclear Analytical Techniques, Institute of High Energy Physics of Chinese Academy of Sciences (1996-1998) and at the Medical Nobel Institute for Biochemistry of Karolinska Institute, Sweden (2001-2002). From 2002 onwards, she is working as a group and project leader at the China Nanosafety lab. She is one of the earliest researchers in this new field in China. Dr. Chen currently is a principal investigator at Key Laboratory for Biomedical Effects of Nanomaterials and Nanosafety in National Center for Nanoscience and Technology of China. She has authored/co-authored over 150 peer-reviewed papers/book chapters and 3 books. She has been authorized 13 granted patents and one international standard. She has served as editorial board members of peer-reviewed journals. She is the principle investigator of several domestic and international projects, such as China MOST 973 Program and projects from Natural Science Foundation of China, the EU-FP6 and EU-FP7, IAEA Coordinated Research Project (2009-2012), Danish Council for Strategic Research (2013-2015), Germany BMBF Cooperation Project (2011-2014), and Japan photon factory cooperation projects (2006-2007, 2008-2009). She has been awarded the National Award for Innovation and Outstanding Service to the Standard authorized by Standardization Administration of the People's Republic of China in 2011, the Second Prize of Beijing Science and Technology (ranked second) in 2008, the Second Prize of the National Natural Science Award (ranked second) in 2012. She has been selected as one of Highly Cited Researchers in Pharmacology & Toxicology field during 2002-2012 by Thomson Reuters in 2014.Her research interests include the potential toxicity of nanoparticles, therapies for malignant tumors using theranostic nanomedicine systems and vaccine nano-adjuvants using nanomaterials. She has authored/co-authored over 280 peer-reviewed papers. She has been awarded the second prize of the National Natural Science Award (2018, 2012), Outstanding Female Awards of the Chinese Academy of Sciences, National Science Fund for Distinguished Young Scholars, Chinese Outstanding Young Female Scientists Awards. She serves as Editor-in-Chief for NanoImpact and an Associate Editor for Science Bulletin, Nanoscale, Nanoscale Advances, etc.

RESEARCH INTERESTS:

- Development of novel nanomedicine with high efficiency and low toxicity for tumor theranostics.
- Investigation on the interaction of engineered nanomaterials with biological systems.
- Integrating advanced nuclear techniques and biotechnologies for nanomaterials exposure and molecular mechanisms.
- Exposure scenarios and the occupational exposure to nanomaterials.

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Mark Chiu

Mark received his training from: BA Biophysics at UC Berkeley, PhD Biochemistry at University of Illinois Urbana-Champaign, and conducting postdoctoral work at ETH-Zürich and Biozentrum of University of Basel. His work experience has spanned from being an Organic Chemist at Microgenics developing chemical conjugation of enzymes; Chemistry Professor at Seton Hall University getting grants on prokaryotic membrane protein biochemistry; Research Investigator at Abbott Labs on Mammalian Membrane Protein Drug Discovery; Associate Director at Janssen Research and Development leading the Process Analytical Sciences Team responsible for clinical development of biotherapeutics, cell, and gene therapies. He is now CSO for Tavotek Biotherapeutics working on differentiated products for auto-immune and oncology diseases.

Insung S. Choi

Insung S. Choi is Professor of Chemistry and of Bio and Brain Engineering at KAIST, Korea, and the Director of the Center for Cell-Encapsulation Research (Creative Research Initiative; 2012-). He obtained his BS and MS degrees in Chemistry at Seoul National University in 1991 and 1993, and did his PhD degree in Chemistry at Harvard University in 2000 under the supervision of George M. Whitesides. After postdoctoral work with Robert Langer at the Department of Chemical Engineering of MIT, he joined the faculty at KAIST in 2002. He was awarded KCS-Wily Young Chemist Award (2003), Thieme Journal Award (2003), Presidential Young Scientist Award (2004; KAST), and JANG SEHEE Research Achievement Award (2013; KCS). His research interests include biomimetic chemistry and machine learning. He has published over 250 peer-reviewed papers (>10000 citations, h-index > 55). He is the editorial board member of Chemistry-An Asian Journal (Wiley-VCH), ChemNanoMat (Wiley-VCH), Material Today BIO (Elsevier), Scientific Reports (NPG), and Polymers (MDPI), and the editorial advisory board member of Advanced Healthcare Materials (Wiley-VCH).

Daan J.A. Crommelin

em-professor Dept Pharamceutics,
Utrecht University

Prof. Daan Crommelin is professor emeritus from the Department of Pharmaceutics at Utrecht University. Until December 2011 he was scientific director of the Dutch Top Institute Pharma – a public private partnership - in Leiden. He is adjunct professor at the Department of Pharmaceutics and Pharmaceutical Chemistry at the University of Utah. Crommelin is co-founder of OctoPlus, a Leiden based company specialized in the development of pharmaceutical (mainly protein based) product formulations and advanced drug delivery systems. He published over 350 scientific articles, many book chapters and edited a number of books. He was Editor-in-Chief of the AAPS book series 'Advances in the Pharmaceutical Sciences'. He advises venture capital groups and acts as a consultant/expert witness for several big pharma companies and SME's. He chaired the Board of Pharmaceutical Sciences of the International Pharmaceutical Federation (F.I.P.). He is past president of the European Federation of Pharmaceutical Sciences (EUFEPS) and past vice-chair of the scientific advisory board of the European Innovative Medicines Initiative (IMI).

Jon de Vlieger

Lygature - Non Biological Complex Drugs Working Group

Jon de Vlieger obtained his doctoral degree in bio analytical chemistry from the VU University in Amsterdam. In 2011 he joined Lygature (former Top Institute Pharma), an independent not-for-profit organization based in the Netherlands that catalyzes the development of new medical solutions by driving public-private collaboration between academia, industry, and society. Dr. de Vlieger coordinates several international public private partnerships, such as the European Lead Factory and the Non Biological Complex Drugs Working Group. He is a co-editor of the book on NBCDs in the AAPS Advances in the Pharmaceutical Sciences Series and a co-author on a series of key-papers related to regulatory challenges for NBCDs.

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Joke Den Haan

Associate Professor
My current research is focused on the development of vaccines that target tumor antigens to CD169+ macrophages in the spleen for the induction of anti-tumor immune responses. We have developed techniques to study CD169+ macrophages and have mouse models that are deficient in these cells (CD169-DTR) or express mutant CD169/Siglec-1 molecules (collaboration Prof.dr. P.R. Crocker). Previously, we were the first to discover that CD169+ macrophages transfer antigens to dendritic cells and thereby stimulate T cell and anti-tumor immune responses and recently identified CD169 as an adhesion molecule for dendritic cells. These studies indicate that CD169+ macrophages are important in the activation of adaptive immune responses and that vaccinations strategies that target antigens to these macrophages could be utilized for the stimulation of anti-cancer immune responses. Currently we investigate antibody-antigen complexes and liposomes as vaccine modalities that target CD169+ macrophages. For the liposomal targeting I have an ongoing collaboration with Prof.dr. G. Storm (University of Utrecht). My research is embedded in the Cancer Center Amsterdam research institute and is well connected with clinicians.

RECENT PUBLICATIONS:

- van Dinther, D., M. Lopez Venegas, H. Veninga, K. Olesek, L. Hoogterp, M. Revet, M. Ambrosini, H. Kalay, J. Stockl, Y. van Kooyk, and J. M. M. den Haan. 2019. Activation of CD8(+) T Cell Responses after Melanoma Antigen Targeting to CD169(+) Antigen Presenting Cells in Mice and Humans. Cancers (Basel) 11.
- Grabowska, J., M. A. Lopez-Venegas, A. J. Affandi, and J. M. M. den Haan. 2018. CD169(+) Macrophages Capture and Dendritic Cells Instruct: The Interplay of the Gatekeeper and the General of the Immune System. Front Immunol 9: 2472.
- van Dinther, D., H. Veninga, M. Revet, L. Hoogterp, K. Olesek, J. Grabowska, E. G. F. Borg, H. Kalay, Y. van Kooyk, and J. M. M. den Haan. 2018. Comparison of Protein and Peptide Targeting for

the Development of a CD169-Based Vaccination Strategy Against Melanoma. Front Immunol 9: 1997.
- van Dinther, D., H. Veninga, S. Iborra, E.G.F. Borg, L. Hoogterp, K. Olesek, M.R. Beijer, S.T.T. Schetters, H.Kalay, J. Garcia-Vallejo, K.L. Franken, L.B. Cham, K.S. Lang, Y. van Kooyk, D.Sancho, P.R. Crocker, Joke M.M. den Haan. 2018. Functional CD169 on macrophages mediates interaction with dendritic cells for CD8+ T cell cross-priming. Cell reports 22: 1484-1495.
- van Dinther, D., D. A. Stolk, R. van de Ven, Y. van Kooyk, T. D. de Gruijl, and J. M. M. den Haan. 2017. Targeting C-type lectin receptors: a high-carbohydrate diet for dendritic cells to improve cancer vaccines. J Leukoc Biol 102: 1017-1034.

Neil P. Desai

Neil Desai is Founder/CEO of Aadi Bioscience Inc, a clinical stage start-up developing targeted mTOR therapeutics for oncology/cardiovascular applications and Founder/CEO of Aadigen, LLC, a company focused on delivery of nucleotide therapeutics. He was formerly VP of Strategic Platforms at Celgene Corp and also SVP of Global R&D at Abraxis Bioscience (Los Angeles, California, USA, acquired by Celgene in 2010 for approximately $3B) where he led the development of Abraxane®, the company's flagship nanotechnology product. Dr. Desai is an inventor of the nanoparticle-albumin bound (nab®) drug-delivery platform and Abraxane, which is approved for metastatic breast cancer, non-small cell lung cancer and pancreatic cancer. Prior to Abraxis, Dr. Desai held positons of increasing seniority at American Bioscience, Inc., VivoRx, Inc. and VivoRx Pharmaceuticals, Inc. (predecessor companies of Abraxis), where he worked on the early discovery and development of Abraxane, developed novel encapsulation systems for living cells and was part of the team that performed the world's first successful encapsulated islet cell transplant in a diabetic patient. Dr. Desai has over 25 years of experience in novel therapeutic delivery systems with over 100 issued patents, over 40 peer-reviewed publications and book chapters, and over 200 presentations at scientific meetings. He is reviewer for several scientific journals, an active participant in FDA and EU Nanotechnology initiatives and a member of the Steering Committee for the National Cancer Institute (NCI) Alliance for Nanotechnology in Cancer. He holds board and advisory positions in various start-ups. Dr. Desai recieved a M.S and Ph.D. in Chemical Engineering from the University of Texas at Austin, USA, and a B.S. in Chemical Engineering from the University Institute of Chemical Technology in Mumbai, India.

László Dézsi

PhD, DrHabil, Senior Research Associate, Nanomedicine Research and Education Center, Institute of Translational Medicine, Semmelweis University, Budapest, Hungary (*in vivo* animal laboratory).

He obtained his MSc degree in biology at Eötvös Loránd University and his PhD in physiology at Semmelweis University Medical School, Budapest, Hungary. He conducted teaching and research activities at Semmelweis University (1981-1999), and meanwhile he received fellowships at Albert Ludwigs Universität, Freiburg, Germany working in the field of local regulation of blood flow in skeletal and cardiac muscle studying nitric oxide; and at the University of Pennsylvania, Philadelphia, USA, Cerebrovascular Research Center working in the field of cerebral blood flow and metabolism as well as cerebral ischemia and reperfusion in animal stroke models. He had been head of laboratory, CRO monitor and research project manager in vascular and safety pharmacology at Gedeon Richter (GR) Pharmaceutical Plc. (1999-2012). He was manager of Analgesic Research Laboratory (2006-2012), a joint venture of GR and University of Pécs, Department of Pharmacology. He was involved in curriculum development and had been Secretary of Biomedical Engineering (BE) Course Committee (1994-2000), currently member of the MSc BE Committee at Technical University, Budapest. He made his habilitation at Semmelweis University in 2005 and became Adjunct Professor (PD) of physiology in 2006. He established his own teaching course in 2008 entitled "Cardiorespiratoric and neurophysiological measuring techniques" at the Department of Translational Medicine. He participates in postgradual education in nanomedicine. He is a physiology and pathophysiology teacher at Semmelweis University Medical and Health Faculties. Currently he is working at the Nanomedicine Research and Education Center (2012-) in the field of nanomedicine investigating cardiopulmonary and immunological effects of nanoparticles in various *in vivo* models of complement activation related pseudoallergy (CARPA) and participates in the development of new models. He was a member of the EU FP7 "NanoAthero" Consortium (2013-2018), now he participates in "EXPERT" project EU's Horizon 2020 research and innovation programme (2019-).

RECENT PUBLICATIONS

- Unterweger H , Dézsi L , Matuszak J , Janko C , Poettler M , Jordan J , Bäuerle T , Szebeni J , Fey T , Boccaccini A R , Alexiou C , Cicha I. Dextran-coated superparamagnetic iron oxide nanoparticles for magnetic resonance imaging: Evaluation of size-dependent imaging properties, storage stability and safety. INTERNATIONAL JOURNAL OF NANOMEDICINE 13: pp. 1899-1915. (2018)
- Cicha I, Chauvierre C, Texier I, Cabella C, Metselaar JM, Szebeni J, Dézsi L, Alexiou C, Rouzet F, Storm G, Stroes E, Bruce D, MacRitchie N, Maffia P, Letourneur D. From design to the clinic: practical guidelines for translating cardiovascular nanomedicine. CARDIOVASCULAR RESEARCH 114: pp. 1714-1727 (2018)
- Dézsi L, Mészáros T, Őrfi E, Fülöp TG, Hennies M, Rosivall L, Hamar P, Szebeni J, Szénási G. Complement Activation-Related Pathophysiological Changes in Anesthetized Rats: Activator-Dependent Variations of Symptoms and Mediators of Pseudoallergy. MOLECULES 24: pp. 3283-3295 (2019)
- Őrfi E, Mészáros T, Hennies M, Fülöp T, Dézsi L, Nardocci A, Rosivall L, Hamar P, Neun BW, Dobrovolskaia MA, Szebeni J, Szénási G. Acute physiological changes caused by complement activators and amphotericin B-containing liposomes in mice. INTERNATIONAL JOURNAL OF NANOMEDICINE 14: pp. 1563-1573. (2019)

Gilles Divita

Dr. Gilles DIVITA is founder and CEO of DivInCell and Chief Scientist at Aadigen LLC, California (USA). a BioPharmaceutical NanoMedicine Start-up pioneering a novel drug delivery technology for the treatment of cancer and genetic diseases. Dr. DIVITA has over 25 years of experience in drug delivery systems, peptide-drugs and oligonucleotide therapeutics. Dr Divita's work focuses on strategies to probe and perturb the behaviour of biomolecules in physiological and pathological settings. He is the pioneer of the "non covalent cell penetrating peptide-based strategy" for therapeutic delivery. Dr. DIVITA is author of over 180 articles in peer reviewed scientific journals and of 15 patents. Dr. DIVITA holds a Ph.D. in Biochemistry/Biophysic from the University in Lyon, France. From 1992-1994, he worked as an Associate Scientist at the Max Planck Institute for Medical Research in Heidelberg-Germany. Dr. DIVITA joined the National Center for Scientific Research (CNRS) in 1996 and was from 1999 to 2016, Research Director, head of Chemical

Biology and Nanotechnology for Therapeutics Team at the CNRS in Montpellier-France.

Marina Dobrovolskaia

Director of Operations, Nanotechnogy Characterization Lab

Dr. Dobrovolskaia is the Director of Operations and the Head of Immunology Section at the Nanotechnology Characterization Laboratory (NCL). In her role as the Director of Operations, Dr. Dobrovolskaia leads the NCL operations to provide preclinical nanoparticle characterization services to the nanotechnology research community, advance the translation of promising nanotechnology concepts from bench to the clinic, and contribute to the education of the next generation of scientists in the field of preclinical development of nanotechnology-based products, the activities emphasized in the NCL mission. She also directs the performance of Immunology, Client Relations and Administrative sections of the NCL. Closely integrated functioning of these sections plays a critical role in advancing the NCL's key strategic goals, and in supporting the missions of the Frederick National Laboratory for Cancer Research. In her role as the Head of the Immunology Section, Dr. Dobrovolskaia leads a team conducting preclinical studies to monitor nanoparticles' toxicity to the immune system both *in vitro* and *in vivo* using variety of immune function animal models. Prior to joining the NCL, Dr. Dobrovolskaia worked as a Research Scientist in a GLP laboratory at PPD Development, Inc. in Richmond, VA, where she was responsible for the design, development and validation of bioanalytical ligand-binding assays to support pharmacokinetic and toxicity studies in a variety of drug development projects. She received her M.S. degree from the Kazan State University in Russia; Ph.D. from the N.N. Blokhin Cancer Research Center of the Russian Academy of Medical Sciences in Moscow, Russia; and MBA from the Hood College in Frederick, MD. Since 2016, she is also a member of Project Management Institute and a certified Project Management Professional.

RECENT PUBLICATIONS

- Szebeni J, Simberg D, González-Fernández Á, Barenholz Y, Dobrovolskaia MA. Roadmap and strategy for overcoming infusion reactions to nanomedicines. Nat Nanotechnol. 2018 Dec;13(12):1100-1108.
- Leong HS, Butler KS, Brinker CJ, Azzawi M, Conlan S, Dufés C, Owen A, Rannard S, Scott C, Chen C, Dobrovolskaia MA, Kozlov SV, et al. On the issue of transparency and reproducibility in nanomedicine. Nat Nanotechnol. 2019 Jul;14(7):629-635.
- Hong E, Halman JR, Shah A, Cedrone E, Truong N, Afonin KA, Dobrovolskaia MA. Toll-Like Receptor-Mediated Recognition of Nucleic Acid Nanoparticles (NANPs) in Human Primary Blood Cells. Molecules. 2019 Mar 20;24(6).
- Ilinskaya AN, Shah A, Enciso AE, Chan KC, Kaczmarczyk JA, Blonder J, Simanek EE, Dobrovolskaia MA. Nanoparticle physicochemical properties determine the activation of intracellular complement. Nanomedicine. 2019 Apr;17:266-275.
- Schubert MS, Cedrone E, Neun B, Behlke MA, Dobrovolskaia MA. Chemical Modification of CRISPR gRNAs Eliminate type I Interferon Responses in Human Peripheral Blood Mononuclear Cells. J Cytokine Biol. 2018;3(1).

Ryan Donnelly

Professor of Pharmaceutical Technology

Professor Ryan Donnelly holds the Chair in Pharmaceutical Technology at Queen's University Belfast and is Director of the university's new interdisciplinary research programme Materials & Advanced Technologies for Healthcare, comprising 58 academics from Pharmacy, Chemistry & Chemical Engineering, Mechanical & Aerospace Engineering, Biological Sciences, Nursing and Medicine. His personal research is centred on design and physicochemical characterisation of advanced polymeric drug delivery systems for transdermal and intradermal drug delivery, with a strong emphasis on improving patient outcomes. He is currently developing a range of novel microneedle technologies through independent research, but also in collaboration with several major pharma partners. He has obtained substantial research funding and authored over 500 peer-reviewed publications, including 4 patent applications, 6 textbooks, 23 book chapters and approximately 170 full papers. He has been an invited speaker at numerous national and international conferences. Professor Donnelly is Editor-in-Chief of Recent Patents on Drug Delivery & Formulation and Associate Editor of Drug Delivery & Translational Research. He has won Evonik's Resomer Award (2018), the Controlled Release Society's Young Investigator Award (2016), BBSRC Innovator of the Year and the American Association of Pharmaceutical Scientists Pharmaceutical Research Meritorious Manuscript Award (2013), the GSK Emerging Scientist Award (2012) and the Royal Pharmaceutical Society's Science Award (2011).

RECENT PUBLICATIONS

- McCrudden, M.TC., Larrañeta, E., Clark, A., Jarrahian, C., Rein-Weston, A., Creelman, B., Moyo, Y., Lachau-Durand, S., Niemeijer, N., Williams, P., McCarthy, H.O., Zehrung, D., Donnelly, R.F. (2019). Design, formulation and evaluation of novel dissolving microarray patches containing rilpivirine for intravaginal delivery. Advanced Healthcare Materials. In Press.
- Khan, S., Minhas, M.U., Tekko, I.A., Donnelly, R.F., Thakur, R.R.S. (2019). Evaluation of microneedles-assisted in situ depot forming poloxamer gels for sustained transdermal drug delivery. Drug Delivery and Translational Research. In Press.
- McCrudden, M.TC., Larrañeta, E., Clark, A., Jarrahian, C., Rein-Weston, A., Lachau-Durand, S., Niemeijer, N., Williams, P., Haeck, C., McCarthy, H.O., Zehrung, D., Donnelly, R.F. (2018). Design, formulation and evaluation of novel dissolving microarray patches containing a long-acting rilpivirine nanosuspension. Journal of Controlled Release. 292,119-129.
- Courtenay, A.J., McCrudden, M.T.C, McAvoy, K.J., McCarthy, H.O., Donnelly, R.F. (2018). Microneedle-mediated transdermal delivery of bevacizumab. Molecular Pharmaceutics. 15, 3545-3556.
- Vora, L.K., Vavia, P.R., Larrañeta, E., Bell, S.E.J., Donnelly, R.F. (2018). Novel nanosuspension-based dissolving microneedle arrays for transdermal delivery of a hydrophobic drug. Journal of Interdisciplinary Nanomedicine. 3, 89-101.

Falk Ehmann

Falk Ehmann is currently working at the European Medicines Agency (EMA) in the Division Science and Innovation Support Office. His main responsibilities include managing the Innovation Task Force promoting Innovation and novel methodologies in drug development with focus in the areas of Pharmacogenomics (Clinical Pharmacology), Nanomedicines, Borderline and Combined Medicinal

Products, and other -omics especially in connection with Personalized Medicine. Further areas of expertise include policy development of Similar Biological Medicinal Products (Biosimilars) with focus on monoclonal antibodies and Vaccines. He held various positions and responsibilities at the EMA since 2004, including Scientific Advice during product development and working as Product Team Leader in the Oncology and Anti-Invectives therapeutic areas of the EMA Unit for Human Medicines Development and Evaluation. As part of engagement in Pharmacoeconomics, Health Care Market Place and Early Drug Development with King's College Falk created a cost-benefit decision model, designed protocols for First in Man clinical studies and proposed a definition for Innovation in Health Care in order to value it, which has been shared with the National Institute for Health and Care Excellence (NICE) and the UK ministry of Health.Prior to joining the EMA Dr Ehmann studied European and International law at the University Berlin, worked as Public Health Researcher at the Robert Koch Institute, at the Representation of the European Commission in Berlin andas Medical Intern at different University Hospitals. He attended his military service in the German Air Force. Falk Ehmann wrote his PhD thesis on Molecular Intra Cellular Cell Signallingat the Institute of Biochemistry and Molecular Biology at the University Hospital Hamburg-Eppendorf. His Master Thesis discusses coping mechanisms and responses of European Health Care Systems to the 2009 H1N1v Influenza Pandemi.

Rosy Favicchio

Senior Editor

Rosy developed approaches for cancer diagnostics and therapeutics using metabolic imaging at Imperial College London, and most recently she branched out into proteomics and cell signalling. Her expertise lies primarily in quantitative data analysis, which earlier in her career she applied to the characterization of DNA–protein interactions. Working with fluorescent-based assays sparked her interest in imaging, which led Rosy to complete a PhD in molecular imaging in 2010 from the Foundation for Research & Technology in Crete (Greece) and the University of Portsmouth in the UK, after developing a fluorescence molecular-tomography system and its *in vivo* applications. Rosy joined Nature Biomedical Engineering in June 2016.

Lukas Engelberger

Since 2014, Dr. iur., LLM, Lukas Engelberger (CVP) is a member of the Government of the Canton Basel-Stadt and Head of the Public Health Department. After his studies and doctorate in law at the universities of Basel, Fribourg and Bern, he was practising as an atorney at Bär & Karrer in Zürich (2003-2005) and as a legal counsel at Hoffmann-La Roche Ltd. in Basel (2005-2014). He is member of the executive committee of the Swiss Conference of the State Ministers of Public Health (GDK). He is married and father of three children.

Xavier Fernàndez-Busquets

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CURRENT RESEARCH: NANOBIOMEDICINE

- Single-molecule studies of proteoglycan and glycosaminoglycan interactions.
- Application of nanotechnology to the study of functional amyloids.
- Development of nanovectors for the targeted delivery of antimalarial drugs.

ACADEMIC BACKGROUND

1986: Graduate in Biological Sciences, area of Biochemistry. Universitat Autònoma de Barcelona, Spain.
1988: Dissertation for University degree, area of Enzymology/Organic Chemistry. CIBA-GEIGY AG, Basel, Switzerland / Universitat Autònoma de Barcelona.
1988: Master in Biochemistry and Molecular Biology. Universitat Autònoma de Barcelona.
1992: PhD Thesis in Biological Sciences. Universitat Autònoma de Barcelona.
2004: Diploma in University Teaching, Institut de Ciències de l'Educació, Universitat de Barcelona.

POSITIONS HELD

November 2001 - November 2006: 5-year tenure track Ramón y Cajal Position. Research Center for Bioelectronics and Nanobioscience. Barcelona Science Park, Universitat de Barcelona, Spain.
May 1999 - November 2001: Postdoctoral position. Plant Biotechnology Group. Department of Biochemistry and Molecular Biology, School of Pharmacy, Universitat de Barcelona, Spain.
April 1993 - April 1999: Postdoctoral position. Novartis AG-Friedrich Miescher Institut, Basel, Switzerland, and Marine Biological Laboratory, Woods Hole, USA.
October 1992 - March 1993: Postdoctoral position. Institute of Agroalimentary Research and Technology (IRTA), Cabrils, Spain.
February 1987 - September 1992: PhD Thesis. Department of Biochemistry and Molecular Biology, Universitat Autònoma de Barcelona, Spain.
July - October 1985 and July - December 1986: Trainee student. Zentrale Forschungslaboratorien, CIBA-GEIGY AG, Basel, Switzerland.

RECENT PUBLICATIONS

- Moles, E., Kavallaris, M., and Fernàndez-Busquets, X. (2019) Modeling the distribution of diprotic basic drugs in liposomal systems: perspectives on malaria nanotherapy. Front. Pharmacol. 10, 1064.
- Biosca, A., Dirscherl, L., Moles, E., Imperial, S., and Fernàndez-Busquets, X. (2019) An immunoPEGliposome for targeted antimalarial combination therapy at the nanoscale. Pharmaceutics 11, E341.
- Martí Coma-Cros, E., Biosca, A., Marques, J., Carol, L., Urbán, P., Berenguer, D., Riera, M.C., Delves, M., Sinden, R.E., Valle-Delgado, J.J., Spanos, L., Siden-Kiamos, I., Pérez, P., Paaijmans, K., Rott-

mann, M., Manfredi, A., Ferruti, P., Ranucci, E., and Fernàndez-Busquets, X. (2018) Polyamidoamine nanoparticles for the oral administration of antimalarial drugs. Pharmaceutics 10, E225.
- Moles, E., Galiano, S., Gomes, A., Quiliano, M., Teixeira, C., Aldana, I., Gomes, P., and Fernàndez-Busquets, X. (2017) ImmunoPEGliposomes for the targeted delivery of novel lipophilic drugs to red blood cells in a falciparum malaria murine model. Biomaterials 145, 178-191.
- Aláez-Versón, C.R., Lantero, E., and Fernàndez-Busquets, X. (2017) Heparin: new life for an old drug. Nanomedicine 12, 1727-1744.

Mauro Ferrari

Mauro Ferrari, Ph.D., is the President and CEO of DXT, Inc., the Silicon Valley-based subsidiary of Dompe' Farmaceutici, dedicated to the creation of a new generation of high-tech pharmaceutical products.
He also serves as Affiliate Professor of Pharmaceutics at the University of Washington, in Seattle, WA., and on the Board of Directors of Arrowhead Pharmaceuticals (NASDAQ: ARWR), BrYet, Inc., and the University of Saint Thomas.
His prior positions include: President of the European Research Council (ERC), and President and CEO of Houston Methodist Research Institute (USA), where he directed more than 2,300 employees and credentialed clinicians engaged in basic science and over 1,000 clinical research protocols in cancer, cardiovascular diseases, neurology, and many other domains of medicine. He also served as Executive Vice President of the Houston Methodist Hospital System, recognized by U.S. News & World Report as one of the top twenty hospitals in the USA.
In his prior university life, he served as Senior Associate Dean and Professor of Medicine at Weill Cornell Medical School in Manhattan, New York, and as tenured professor of engineering and/or medicine at several institutions including the University of California, Berkeley, the University of Texas Medical School and M.D. Anderson Cancer Center, and the Ohio State University. He directed the launch of the national program in cancer nanotechnology of the USA, while serving as Special Expert and Eminent Scholar at the National Cancer Institute.
His laboratory focuses on research and development of new cancer drugs, using novel methods derived from mathematics, computer science, and advanced technologies. Some of his experiments were flown on the International Space Station. He is recognized as the pioneer of nanomedicine and transport oncophysics. He has published over 500 scientific articles and 7 books, and is the inventor of over 50 patents issued in the USA and worldwide. He has won the Blaise Pascal Medal of the European Academy of Sciences, and numerous other scientific awards and recognitions, in the USA, Italy, and worldwide.
Mauro Ferrari is a Foreign Member of the Italian National Academy of Sciences (Accademia dei Quaranta), a Corresponding Member of the Pontifical Academy for Life, by appointment of Pope Francis, a Member of European Academy of Sciences, the National Academy of Inventors of the USA, the American Association for the Advancement of Science.
Born in Italy, he holds a degree in Mathematics from the University of Padua, a Master and Ph.D. in Mechanical Engineering from the University of California, Berkeley, and attended medical school at The Ohio State University. He received his executive leadership education at the Harvard Business School, and the CEO Academy of the Wharton School of Business of the University of Pennsylvania.
As a singer, as well as baryton and tenor saxophonist, he performs with blues legends Milton Hopkins and Texas Johnny Boy in the United States, while his European musical collaborators include the Rhythm and Blues Band, Barbara Errico, Mauro Costantini and Daniele Dagaro.

Anne Field

Dr. Field obtained her PhD in Pharmacology from University College, London, before moving to Australia to take up a Postdoctoral Research Fellowship in Neuroscience at the Australian National University in Canberra. She started working the Therapeutic Goods Administration (TGA) in 1995, and has worked in a number of regulatory areas since then. Her current role is Senior Toxicologist in the Scientific Evaluation Branch, Medicines Authorisation Division. She has extensive experience in the evaluation of nonclinical data submitted to support marketing applications across a wide range of therapies. Dr Field represents the TGA on the IPRP nanomedicines working group, and was recently appointed as its co-chair. She is a member of the Australasian Society of Clinical and Experimental Pharmacologists and Toxicologists (ASCEPT) and the Australasian College of Regulatory Toxicology and Risk Assessment, and is a panel member of the Australian Cardiovascular Alliance's Drug Discovery Flagship.

Beat Flühmann

Pharmacist, MBA

Dr. Flühmann is Pharmacist by training and holds a PhD in molecular biology. He was working in various position in the field of pharmaceuticals and functional nutrition. He was at Roche Deputy Department Head of a R&D department for 8 years. Dr. Flühmann was leading a global multidisciplinary research and development team at Roche/DSM nutritional products developing novel compounds for the prevention and treatment of diabetes. In his current position Dr. Flühmann is Global Lead Nanomedicines at Vifor Pharma Ltd Switzerland, with a main interest in regulatory aspects of nanomedicines. He is Steering Committee Member of the Non-Biological Complex Drugs Working Group hosted at Lygature, a non for profit organization. The Non-Biological Complex Drugs Working Group has been set up to discuss appropriate and harmonized science-based approval and post-approval standards to ensure patient safety and benefit with Non Biological Complex Drugs. The working group engages in activities to publish and discus the corresponding scientific evidence with authorities, experts, health care providers. Moreover the group is involved in scientific education and training on the above mentioned topics to relevant stakeholders.

Cristina Fornaguera i Puigvert

My scientific career begins in 2011, when after graduating in Biotechnology at the Autonomous University of Barcelona, and finishing the MSc in Respiratory Medicine, I started my doctoral thesis at the Colloidal and Interfacial Chemistry Group, of the Institute of Advanced Chemistry of Catalonia, of the CSIC. The objective of my thesis, framed within a Pharmaceutical program of the University of Barcelona, was the development of polymeric nanoparticles from nano-emulsions, designing nanoparticles with the right properties to cross the blood-brain barrier and become promising nanosystems for the treatment of diseases at the cen-

tral nervous system level. During the thesis, I was able to perform techniques from colloidal chemistry (RSC Adv, 2016 (1)), to the manipulation of laboratory animal models (J Control Release, 2015). All this without forgetting all the intermediate steps, (e.g. organic chemistry, analytical chemistry and cellular and molecular biology) (Eur J Pharm Biopharm, 2015; Nanoscale, 2015 (1)). In addition, the versatile nature of nanoparticles enabled their use for multiple biomedical applications, for the encapsulation of reporter molecules (Colloids Surf B, 2017; Pharm Res, 2017; Colloids Surf B, 2016; Eur Pol J, 2018), becoming diagnostic elements, as well as active compounds, becoming therapeutic nanosystems for drug (J Control Release, 2015; CSR Adv, 2016 (2), Colloids Surf B, 2015 and Nanoscience, 2015 (2)) and genetic therapies (Int J Pharm, 2015).
For this reason, when finishing the thesis, evaluated with excellent cum laude and awarded with the Extraordinary Prize of 2015, I joined as a postdoctoral researcher at Sagetis Biotech, a spin off of the Sarrià Chemical Institute (IQS) of the Ramon Llull University (URL), where I have developed my degree until last month. In Sagetis, thanks to the close collaboration with the Materials Engineering Group (GEMAT) of IQS, I have been able to maintain my link with the academic world, tutoring students and directing final degree and master's work. It should also be noted that during my postdoctoral stage I had the opportunity to teach classes of drug release systems to the degree of pharmacy of the URL, thus beginning my teaching career. My activity in Sagetis consisted of the design of new advanced nanosystems for the treatment of illnesses currently without cure, in the field of drug therapy (Drug Delivery, 2017) and gene (Adv Healthcare Mat, 2018; Int J Pharm, 2019; Adv Healthcare Mat, 2019). Thus, with polymers previously designed in the group, we managed to encapsulate different types of genetic material, in order to obtain non-viral genetic therapy vectors. In addition, with these polymers, we also managed to cover different types of viruses, to change tropism and, at the same time, to re-administer encapsulated viruses, which is impossible without the polymer coating, due to problems of activation of the immune system.
As is clear from this brief summary of my career, I have had cross-cutting and multidisciplinary experience in the field of nanomedicine, so that I have acquired knowledge and skills in the development of nano-systems since the engineering of biomaterial with the necessary properties for the desired therapeutic objective, going through the physical-chemical characterization of the nanoparticles, as well as studies of safety and efficacy in cell cultures, even reaching animal models. It is also worth noting that I have not only acquired experience, but the studies I have been involved in can be considered successful since, on the one hand, they have resulted in the publication of several scientific articles and even and all reviews of the topics I have worked on (Curr Pathobiol Rep, 2016; J Pers Med, 2017 (1); J Pers Med, 2017 (2); Pharm Nanotechnol, 2018; Int J Pol Sci, 2018) and book chapters. Of my publications, I would like to remark the ones related to the work presented here:

RECENT PUBLICATIONS

- mRNA Delivery System for Targeting Antigen-Presenting Cells In Vivo, Fornaguera, C; Guerra-Rebollo, M; Lazaro, Ml; Castells-Sala, C; Meca-Cortes, O; Ramos-Perez, V; Cascante, A; Rubio, N; Blanco, J; Borros, S, Adv Healthcare Mat (2018).
- SPIONs' Enhancer Effect on Cell Transfection: AnUnexpected Advantage for an Improved Gene Delivery System, Balcells, L; Fornaguera, C; Brugada, P; Guerra-Rebollo, M; Meca-Cortes, O; Martinez, G; Rubio, N; Blanco, J; Santamaria, J; Cascante, A; Borrós Sr, ACS Omega (2019).
- Tracking the DNA complexation state of pBAE polyplexes in cells with super resolution microscopy, Riera, R; Feiner-Gracia, N; Fornaguera, C; Cascante, A; Borros, S; Albertazzi, L, Nanoscale (2019).
- In Vivo Retargeting of Poly(beta aminoester) (OM-PBAE) Nanoparticles is Influenced by Protein Corona, Fornaguera, C; Guerra-Rebollo, M; Lazaro, M; Cascante, A; Rubio, N; Blanco, J; Borros, S, AdvHealthcare Mat (2019).
- Development of an optimized freeze-drying protocol for OM-PBAE nucleic acid polyplexes, Fornaguera, C.; Castells-Sala, C.; Lazaro, M. A.; Cascante, A.; Borros, S., Int J Pharm (2019).

Sara Fortuna

Sara Fortuna is the coordinator of the Self-Assembly, Recognition, and Applications group at the Department of Chemical and Pharmaceutical Sciences, University of Trieste, Italy.

Sara Fortuna graduated in Chemistry in 2007 from the University of Trieste (Italy), has been awarded her PhD in Chemistry from Warwick University (UK) in 2010, and carried out postdoctoral activity in SISSA, Trieste (Italy). In 2013 she started her present research activity under the mentorship of Prof.G.Scoles at the University of Udine (Italy), then Nova Gorica (Slovenia), and SISSA. In 2017 she settled in the University of Trieste (Italy) where she is now Assistant Professor of Medicinal Chemistry and she coordinates a multidisciplinary collaboration aiming to provide novel theoretical solutions to problem of medical and biological interest, with particular interest on the development of novel binders for medical, pharmaceutical, and biological applications
Her area of expertise is in the multiscale modelling of non-covalently bound molecular systems. She looks at surface adsorbed molecular layers, peptides and nanobody design for protein recognition, including new molecular architectures for biosensing. In her career to date she has gained experience in applying both classical molecular simulation (lattice and off-lattice models, Monte Carlo and molecular dynamics simulations) and quantum chemical (DFT) calculations to these systems. This combination of techniques gives her the ability to meet the needs of her collaborators both by suggesting new (macro)moleculas designs and by interpreting their results.

She also supports medical doctors in modeling protein mutants, and synthetic medicinal chemists in modeling potential drugs for systems of medical interest.

RECENT PUBLICATIONS

- M.A. Soler, B. Medagli, M.S. Semrau, P. Storici, G. Bajc, A. de Marco, A. Laio, and S. Fortuna, A consensus protocol for the in silico maturation of antibody fragments, Chem. Comm., 2019, in press
- D. Zampieri, S. Fortuna, A. Calabretti, M. Romano, R. Menegazzi, D. Schepmann, B. Wuensch, S. Collina, D. Zanon, and M.G. Mamolo, Discovery of new potent dual sigma receptor/GluN2b ligands with antioxidant property as neuroprotective agents, Eur. J. Med. Chem., 2019, 180, 268-282
- M.A. Soler, S. Fortuna, A. de Marco, and A. Laio, Binding affinity prediction of nanobody-protein complexes by scoring of molecular dynamics trajectories, Phys. Chem. Chem. Phys., 2018, 20 , 3438-3444 (2018 PCCP HOT Article!)
- V. Salpietro, C. L. Dixon, H. Guo, O. D. Bello, G. H. Heimer, L. Burglen, S. Valence, E. Torti, M. Cho, M. Hacke, J. Rankin, H. Tariq, S. Efthymiou, E. Colin, V. Procaccio, P. Striano, K. Mankad, A. Lieb, S. Chen, L. Pisani, C. Bettencourt, R. Manniko, A. Manole, A. Brusco, G. B. Ferrero, J. Armstrong Moron, A. Van Haeringen, C. Ruivenkamp, C. Nava, D. Heron, F. Zara, C. Minetti, A Skabar, A. Fabretto, 100,000 Genome Project, Deciphering Developmental Disorders Study, SYNAPS Study Group, M. Raspall-Chaure, M. Chez, A. Tsai, E. Fassi, M. Shinawi, J. Costantino, R. De Zorzi, S. Fortuna, B. Keren, D. Bonneau, M. Choi, B. Benzeev, J. Clayton-Smith, A. Macaya, J. E. Rothman, E. Eichler, D. M. Kullmann, and H. Houlden, AMPA receptor GluA2 subunit defects are a cause of neurodevelopmental disorders, Nat. Commun., 2019, 10, 3094
- M.A. Soler, A. Rodriguez, A. Russo, A. Feyisara Adedeji, C.J. Dongmo Foumthuim, C. Cantarutti, E. Ambrosetti, L. Casalis, A. Corazza, G. Scoles, D. Marasco, A. Laio, and S. Fortuna, Computational design of cyclic peptides for the customized oriented immobilization of globular proteins, Phys. Chem. Chem. Phys., 2017, 19, 2740-2748
- M.A. Soler, A. de Marco, and S. Fortuna, Molecular dynamics

simulations and docking enable to explore the biophysical factors controlling the yields of engineered nanobodies, Sci. Rep., 2016, 6, 34869

For further information visit www.sarafortuna.eu and http://monalisa.uniud.it

Alberto A. Gabizon

Alberto Gabizon received his M.D. degree from the School of Medicine, University of Granada, Spain, and his Ph.D. in Cancer Immunology from the Weizmann Institute of Science, Rehovot, Israel (1979). He completed his training and certification in Radiation and Medical Oncology at Hadassah-Hebrew University Medical Center, Jerusalem, Israel (1985), followed by a Research Fellowship at the Cancer Research Institute of UCSF Medical Center, San Francisco, CA (1985-88).

Dr. Gabizon pioneered the development of a new generation of long-circulating liposomes known as Stealth liposomes, and played a key inventorship and research contribution in the development of Doxil® (pegylated liposomal doxorubicin), a unique anticancer formulation extensively used in the clinic with important pharmacologic and safety advantages over conventional chemotherapy. His most recent invention, currently in clinical studies, is Promitil® (pegylated liposomal mitomycin-C prodrug), a formulation with improved safety over the parent drug mitomycin C, that may be particularly useful in chemo-radiotherapy.

Dr. Gabizon has received the Spain National Prize of Medicine to university graduates, the Research Career and Professorship Awards of the Israel Cancer Research Fund, the Hebrew University Kaye Innovation Award, the Tel Aviv University Sarnat Lectureship, and the Alec Bangham Life Time Achievement Award of the International Liposome Research Society.

Dr. Gabizon is active in the medical oncology practice, as well as in pharmacology research with special emphasis on applications of liposomes in drug delivery, targeting of drugs, and experimental cancer therapy. He has published over 150 articles and specialized book chapters, and is an inventor of 14 USPTO-approved patents.

He is currently Director of the Nano-oncology Center at Shaare Zedek Medical Center, and Professor of Oncology at the Hebrew University-Faculty of Medicine in Jerusalem. In 2011, he founded Lipomedix Pharmaceuticals Inc., a start-up company aimed at developing Promitil® and other inventions in the field of cancer nanomedicine.

Google Scholar (Jan 2020): Citations=24,178; h-Index=72; i10-index=136.

Ruth Gabizon

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After getting my PhD from the Hebrew University Medical School I went to Dr. Prusiner's lab in UCSF for my postdoctoral studies on prion diseases. In 1988, I established my own prion lab in the Neurology department of the Hadassah University Hospital. Since then, I investigate features of neurodegenerative diseases and in particular those of genetic prion diseases, since the E200K PrP mutation is common among Jews of Libyan origin living in my country. In the last years, we are generating nanotechnology based formulations of natural antioxidants that can prevent/delay the outbreak of neurodegenerative diseases in at risk individuals. A few years ago, I founded Granalix Biotechnologies, together with Prof Magdassi, and we have established our first product, GranaGard, a nanotechnology based formulation of Pomegranate seed oil.

Jérôme Galon

Dr. Jérôme Galon is Director of Research at INSERM (French NIH), and Head of the laboratory of Integrative Cancer Immunology, in Paris, France. Dr. Galon was trained as an immunologist at the Pasteur Institute and at the Curie Institute (Paris, France). He holds a Ph.D. degree in Immunology (Jussieu University, Paris, France, 1996). Between 1997 and 2001 he worked at the NIH (National Institute of Health, Bethesda, USA). Since his full-tenured position at INSERM in 2001, he directs interdisciplinary research programs on tumor-Immunology. He is associate Director and co-founder of European Academy of Tumor Immunology (EATI), board Director of the Society for Immunotherapy of Cancer (SITC). His work on the comprehensive analysis of the tumor-microenvironment and the role of T-cells in human cancer led to the demonstration of the importance of adaptive pre-existing immunity in human cancer, and the concept of cancer immune-contexture. He pioneered the Immunoscore. He is the co-founder of HalioDx company and the Chairman of its scientific council. His contributions have been recognized with numerous awards, including the William B. Coley Award, an international prize which honors the best scientists in the fundamental and cancer immunology, the Award from the National Academy of Science and from the National Academy of Medicine. In 2019, he was the winner of the prestigious European Inventor Award from the European Patent Office.

Robert Geertsma

Senior Scientist, Centre for Health Protection, National Institute for Public Health and the Environment (RIVM),
Robert.Geertsma@rivm.nl

Robert Geertsma has worked at the Dutch National Institute for Public Health and the Environment (RIVM) for more than twentysix years. As a senior scientist and project leader he is responsible for the provision of scientific advice to regulators on quality and safety of medical technology and nanomedicine. He works on multiple research projects on opportunities as well as risks of nanotechnologies and nanomaterials in medical applications, performing both desk research and experimental research. He participated in FP7-projects ObservatoryNano and NanoMedRoundTable, and is currently one of the partners in the H2020 project REFINE (Regulatory Science Framework for Nano(bio)material-based Medicinal Products and Medical Devices). He is also one of the experts of the Risks of Nanotechnology Knowledge and Information Centre (KIR nano), a Dutch government-supported observation organisation based at RIVM. His areas of expertise include risk management, biological safety, nanotechnology and emerging medical technologies. He participates actively in international ISO/CEN Standards Committees on these subjects and he is chairman of the joint CEN/CENELEC/TC3 responsible for horizontal standards on topics like

quality and risk management systems. He was a member of the SCENIHR WG that wrote the Scientific Opinion "Guidance on the Determination of Potential Health Effects of Nanomaterials Used in Medical Devices". He is chairing the ISO/TC194/WG17 on Biological Evaluation of Medical Devices – Nanomaterials, and he is a member of the Nanomedicines WG of the International Pharmaceutical Regulators Programme. Furthermore, he frequently represents the Dutch competent authority in European Commission's working groups such as the New Technologies WG, of which he was appointed co-Chair in 2009. He is a member of the European Society for Nanomedicine and the European Technology Platform Nanomedicine.

PUBLICATION LIST

- Halamoda-Kenzaoui B, Box H, Elk M van, Gaitan S, Geertsma RE, Gainza Lafuente E, Owen A, Del Pozo A, Roesslein M, Bremer-Hoffmann S. Launching stakeholder discussions on identified regulatory needs for nanotechnology-enabled health products. Prec. Nanomed. 2020 July;3(3):608-621.
- Giannakou C, Park MVDZ, Bosselaers IEM, Jong WH de, Laan JW van der, Loveren H van, Vandebriel RJ, Geertsma RE. Nonclinical regulatory immunotoxicity testing of nanomedicinal products: Proposed strategy and possible pitfalls. WIREs Nanomed Nanobiotechnol. 2020;e1633.
- Giannakou C, Aimonen K, Bloois L van, Catalán J, Geertsma RE, Gremmer E, Jong WH de, Keizers PHJ, Schwillens PLWJ, Vandebriel RJ, Park MVDZ. Sensitive method for endotoxin determination in nanomedicinal product samples. Nanomedicine 2019; 14:1231-1246.
- Brand W, Noorlander CW, Giannakou C, Kooi MW, Jong WH de, Vandebriel RJ, Park MVDZ, Bosselaers IEM, Scholl JHG, Geertsma RE. Nanomedicinal products - Survey on specific toxicity and side effects. Int J Nanomedicine 2017; 12:6107-29.
- Giannakou C, Park MVDZ, Jong WH de, Loveren H van, Vandebriel RJ, Geertsma RE. A comparison of immunotoxic effects of nanomedicinal products with regulatory immunotoxicity testing requirements. International Journal of Nanomedicine 2016; 11: 2935–2952.

Matthieu Germain

CEO, Curadigm

Matthieu holds a doctorate in biotechnology. He has joined Nanobiotix in 2004. With over 15 years in nanomedicine, he is the author of more than 15 publications and scientific communications and is the inventor of 15 patents. He has held multiple positions in Nanobiotix before taking the lead at Curadigm and managed both domestic and international projects

Stephan Grabbe

Stephan Grabbe, MD (born 1961), is a Dermatologist and currently holds the position as Director and Chairman of the Department of Dermatology, University of Mainz Medical Center (UMMC), Germany. He received his medical and scientific education at the University of Münster, Germany (Department of Dermatology (1987-2003), as well as at Harvard Medical School (Research fellowships at the Department of Dermatology, Massachusetts General Hospital, Harvard Medical School, Boston, USA (1989-1992) and at the Department of Dermatology, Brigham and Womens' Hospital, Harvard Medical School, Boston, USA (1998)). Before being appointed to his current position, Stephan Grabbe was Director and Chairman, Dept. of Dermatology, University of Essen Medical center (2003-2007). His clinical focus is on skin oncology and immune-mediated skin dieseases. Currently, he is also Head of the UMMC Skin Cancer Center, Member of the board of the University of Mainz Cancer Center (UCT) , Head of the Biomedical Research Center of the Johannes Gutenberg University, and co-speaker of the Research Center Immunotherapy (FZI) of the University of Mainz.
Stephan Grabbes scientific focus is in the field of cellular immunology and immunotherapy, dendritic cells, as well as nanoparticle-mediated immunomodulation. In this respect, he is deputy speaker of the collaborative research center SFB 1066 of the German Research Council ("Nanoparticle-mediated immunotherapy"), and deputy speaker of the collaborative research center SFB TR156 ("Skin imunology"). Stephan Grabbe has published more than 150 original papers in peer-reviewed journals, has been cited more than 10.000 times and has an h-index of 50.

Piotr Grodzinski

Dr. Piotr Grodzinski is a Chief of the Nanodelivery Systems and Devices Branch (NSDB) within Cancer Imaging Program, Division of Cancer Treatment and Diagnosis, National Cancer Institute. He directs the NCI Alliance for Nanotechnology in Cancer program dedicated to the development of nanotechnology-based cancer interventions.
Dr. Grodzinski graduated from the University of Science and Technology (AGH) in Krakow, Poland and continued his studies at the University of
Southern California in Los Angeles, where he researched novel semiconductor materials used in low threshold lasers. In mid-nineties, Dr. Grodzinski left the world of semiconductor research and got interested in biotechnology. He built a large microfluidics program at Motorola Corporate R&D in Arizona. The group made important contributions to the development of integrated microfluidics for genetic sample preparation with its work being featured in Highlights of Chemical Engineering News and Nature reviews. After his tenure at Motorola, Dr. Grodzinski was with Bioscience Division of Los Alamos National Laboratory where he served as a Group Leader and an interim Chief Scientist for DOE Center for Integrated Nanotechnologies (CINT). At the National Institutes of Health (NIH), in addition to his programmatic responsibilities, he co-chaired Trans-NIH Nanotechnology Task Force, which is coordinating the nanotechnology efforts across 27 institutes of the agency.
Dr. Grodzinski received Ph.D. in Materials Science from the University of Southern California, Los Angeles in 1992. He is an inventor on 17 patents and published over 70 peer-reviewed papers and 10 book chapters. Dr. Grodzinski was inducted into the College of Fellows of the American Institute for Medical and Biological Engineering (AIMBE) and the American Association for the Advancement of Science (AAAS).

Rainer Haag

Univ.-Prof. Macromolecular Chemistry
Rainer Haag, is Full Professor in Organic and Macromolecular Chemistry at the Freie Universität Berlin. Since 2008 he is the head of a collaborative research center SFB 765 "Multivalency a chemical organization and action principle". His research interests are dendritic polymers as highly functional polymeric supports for catalysis, macromolecular nanotransporters for DNA- and drug-delivery and protein resistant material surfaces. In 2004 his group was awarded the young investigator NanoFutur award from the German Ministry of Science (BMBF) and in 2010 the Athur K. Doolittle Award of the American Chemical Society (ACS) for their work on "multifunctional nanotransport systems". He is also advisor for two start-up companies (nanopartica and Dendropharm) that license the group's technology. With Dendropharm he received the Innovation award Berlin-Brandenburg 2016. In 2019 he became an elected member of acatech the German Academy of Technical Sciences.
Rainer Haag completed his PhD thesis in 1995 with Prof. A. de Meijere, University of Göttingen, Germany. In 1996-1997 he worked as a Postdoc with Prof. S. V. Ley, University of Cambridge (England) and as a research fellow (1997-1999) with Prof. G. M. Whitesides, Harvard University, Cambridge (USA). From 1999 until 2002 he completed his habilitation and was research group leader at the Materials Research Center (FMF) of the University of Freiburg (Mentor: Prof. Rolf Mülhaupt). In 2003 he became Associate Professor in Organic Polymer Chemistry at the University of Dortmund.

RECENT PUBLICATIONS

- N. Rades, K. Achazi, M. Qiu, C. Deng, R. Haag, Z. Zhong, and K. Licha, J. Control. Release, 2019, 300, 13-21. Reductively cleavable polymer-drug conjugates based on dendritic polyglycerol sulfate and monomethyl auristatin E as anticancer drugs.
- M. Ferraro, K. Silberreis, E. Mohammadifar, F. Neumann, J. Dernedde, and R. Haag, Biomacromolecules, 2018, 19(12), 4524–4533. Biodegradable Polyglycerol Sulfates Exhibit Promising Features for Anti-inflammatory Applications
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Heinrich Haas

Vice President RNA Formulation & Dug Delivery
BioNTech RNA Pharmaceuticals GmbH,
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Heinrich has more than 20 years of experience in academic research and industrial pharmaceutical development. His focus is on development for advanced drug delivery systems. After he received his Ph.D. in physical chemistry, he researched lipid membranes and organized biomolecular systems. He was responsible for projects in different biopharmaceutical companies for research and development of diagnostic and therapeutic carrier systems. Joining BioNTech in 2010, he helped build the formulation development and analytics unit, which develops formulations for delivery of RNA and small molecules.

Stefan Halbherr

Ph.D.
Manager Research and Development
InnoMedica

Studied Biochemistry at the University of Bern/Switzerland. At the University Institute for Immunology in the Insel hospital in Bern he investigated disease-specific antibody signatures in Hemophilia patients using Designed Ankyrin Repeat Protein (DARPin) technology. During his PhD, he developed genetically engineered self-amplifying RNA vaccines against influenza A viruses. During his doctoral studies already, he played a crucial role in the founding team of InnoMedica, and restructured the company into a cutting-edge nanodrug company. Since then he led the R&D department and brought the research concepts to a marketable product, introducing many innovations in the processes of industry-scale liposome assembly, drug loading, and especially in the design of biologically functional liposome surfaces. At the same time, he was tightly involved in the vertical integration of all business-relevant value chains into the firm, ultimately leading to the creation of the GMP-approved large-scale liposome manufacturing facility of InnoMedica in Marly/Switzerland. InnoMedica today manufactures its innovative medicines 100% in its own facilities up until the final vial with label and card box, being innovative at all steps of creation. In 2019 Stefan Halbherr then took the role as Country Manager and President of the Board of InnoMedica Switzerland AG, while also leading the R&D team to the creation of a well-diversified product pipeline with breakthrough potential. Disruptive products are strategically positioned in the fields of oncology (Talidox, TaliTrace, TaliTaxel) and neurology (Talineuren), ready to make patients to people again.

Adelina Haller

PhD student

Adelina completed her study of molecular medicine at the Friedrich-Alexander University of Erlangen-Nuremberg (Germany) in 2017. After having completed her thesis in the field of molecular pathology, for her PhD she changed to the subject of nanomedicine.
Building up a tight junction together with chemists, who synthesize novel types of nano-capsules, she is working at the Department of Dermatology, University Medical Center of the Johannes Gutenberg-University Mainz (Germany) and the Max-Planck-Institute for Polymer Research (Mainz). In vivo she investigates the behavior of novel nano-capsuels under the supervision of Prof. Volker Mailänder. Her project is supported by the Collaborative Research Center 1066 which focuses on nanodimensional polymer therapeutics for tumor therapy.

Inge Herrmann

Department of Mechanical and Process Engineering, ETH Zurich and Empa

Inge Herrmann is a chemical engineer (MSc ETH Chem Bio Eng, 2007) with additional training in (pre)clinical research (incl CAS Clinical Trial Management). After graduating with a PhD from ETH Zur-ich in 2010 (Stark Lab), she underwent further training at the University Hospital Zurich (USZ, Beck-Schimmer Lab), the University of Illinois in Chicago (US, Minshall Lab) and the Imperial College Lon-don (UK, Stevens Lab). She is an expert in nanoparticle synthesis and characterization, microscopy and spectroscopy and translational nanomedicine. She has spearheaded several translational nanomedicine projects, and serves as a scientific advisor of the spin-off company hemotune. Since 2015, she is heading a research group at Empa specialized in the design and development of particle-enabled approaches for di-agnostics and drug delivery, jointly with academic and clinical partners around the world. Inge Herrmann has won various awards, including the Bayer Healthcare Award and the Johnson & Johnson Award. She is principle investigator (PI) of several national and international projects supported by the Swiss National Science Foundation (Project grant and Eccellenza grant), the Personalized Health and Related Technolo-gies Initiative (PHRT), the Novartis FreeNovation program and several medical foundations (incl. the Swiss Heart Foundation, the Bangerter Rhyner Foundation, the Horten Foundation, the Mayenfisch Foundation, etc). In 2019, Inge Herrmann joined the Department of Mechanical and Process Engineering (D-MAVT) of ETH Zurich as an Assistant Professor where she is heading the Nanoparticle Systems Engi-neering Lab.

Eggehard Holler

Following an education in Chemistry, Physical Chemistry, Enzymology, Mineralogy (Frankfurt/Germany 1960-1967) received PhD in 1966 on “Trypsin Enzyme Mechanism” (Professor Hermann Hartmann, Theoretical Chemistry) with Summa Cum Laude. After Postdoctoral Fellowships with Professor George P. Hess (Ithaca, Fast enzyme kinetics, 1968-70) and Nobel Laureate Professor Melvin Calvin (Berkeley, Enzymology of the translation genetic code, 1970-72), Venia Legendi and Professorship in Biochemistry at University Regensburg/Germany.

The summary of the publication record for different periods of scientific career: Genetic Code-correct activation of amino acids; DNA replicating enzyme complexes, enzyme folding, protein-nucleic acids complexes, pleiotypic AppppA and similar effectors, 1972-1988, 57 peer reviewed publications; Various platinum drugs and estrogen receptor affinity drugs in anti-cancer mechanisms, DNA-polymerases in various prokaryotes and Physarum polycephalum, cell cycle regulation, 1987-1992, 17 publications; Discovery of polymalic acid as regulator of DNA synthesis in Physarum (1989). Phylogeny of polymalate, mechanisms for synchrony of DNA replication and nuclear division, enzymology of polymalic acid biosynthesis and its degradation. Coupling of polymalic acid production and the life-cycle of Physarum. Gene of polymalate hydrolase and its multistage expression. Interaction profile with cell cycle-expressed molecules in the plasmodium, activation of sporulation, chemical/physical characterization, 1989 -2006, 32 peer reviewed publications; Polymalic acid in drug delivery, historically first report of nanoconjugate as novel prototype for tumor treatment with targeted delivery across BBB, endosomal escape and drug activation, antisense *in vivo* inhibition of tumor marker synthesis in 2006, multiple drugs and targeting (e.g. antibody), brain tumor treatment, delivery of synthetic pharmaceuticals, breast cancer treatment, mechanism of drug delivery, seeding work on Her2+ breast cancer treatment by immune stimulation and complex chemo therapeutics, post synthetic analysis of Polycefin nanodrugs, MRI- and NIR-imaging, multi-blockage of tumor pathways, 2006-2015, 39 peer reviewed publications; Mini nano drugs, tumor microenvironment, air-pollution and cancer/Alzheimer’s disease, multi-delivery into healthy and diseased brain, local disease immune systems, 2016-2019, 9 peer reviewed publications; Book chapters: 13 and Editorials and Reviews: 14.

I had the fortune to join the Nanomedicine Research Center at the Department of Neurosurgery, Cedars-Sinai Medical Center (2009) and since then collaboration with Professor Dr. Julia Y. Ljubimova where I was in charge of designing a novel class of tumor multifunctional drug treatment to regulate the tumor environment and signaling based on the tumor markers. These projects were supported by numerous NIH grants favoring polymalic acid as a unique biodegradable, non-toxic, non-immunogenic and highly versatile macromolecular platform for nanomedicine translated into drug candidates for targeted imaging and treatment of breast- and brain-tumors, brain metastases and recently in the forefront of local tumor immune responses and delivery to treat neurodegenerative diseases.

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Byung Hee Hong

Byung Hee Hong (b. 1971) received the BS (1998), MS (2000) and PhD (2002) degrees in chemistry from POSTECH in Korea. After spending 3.5 years as a postdoctoral researcher at the Columbia University (Advisor: Philip Kim), he joined the Department of Chemistry, Sungkyunkwan University (SKKU) as an Assistant Professor, in 2007. He moved to Seoul National Univ. in 2011 as an Associate Professor, and now he is a Full Professor and Director of Graphene Research Center at Seoul National Univ. since March, 2017.

Byung Hee Hong pioneered the large-scale synthesis of graphene by CVD, which triggered chemical research studies toward the practical applications of graphene. His first report on the CVD synthesis of graphene (Nature 457, 706 (2009)) has recorded the world highest citations in chemistry among the papers published since 2009. A year after, Byung Hee Hong developed the synthesis of ultra-large graphene based on roll-to-roll methods and applied the material to flexible touch screens (Nature Nanotech. 5, 574-578 (2010)), which is believed to be the first demonstration of the utilization of graphene materials in practical electronic devices. He is a Founding/Regional Editor for 2D Materials journal. He spun-off a

company, Graphene Square Inc. in 2012, specialized in high-quality graphene and 2D materials synthesis equipment, and Biographene Inc. in 2017 for therapeutic applications of graphene-based materials.

Delyan Hristov

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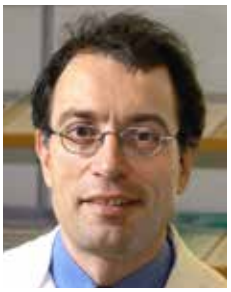
Delyan R. Hristov earned his B.Sc. in chemistry at the Faculty of Chemistry, Sofia University in 2011 and went on to a PhD program under Prof. Kenneth A. Dawson at University College Dublin. His viva panel consisted of Prof. Susan Quinn, Prof. Gareth Redmond and Prof Michael Grätzel. After graduating Dr. Hristov worked with Prof. Dawson as a postdoc for a year where his work focused on developing techniques for nanoparticle characterization, developing and characterizing nanoparticles for drug delivery with an emphasis on material surface properties. Following this Dr. Hristov worked with Prof. David Brayden for a further year where he helped develop and test a new type of encapsulated insulin nanoparticle which had between 40 and 60% loading capacity for oral drug delivery. In 2018 Dr. Hristov took an appointment with Prof. Kimberly Hamad-Schifferli where he develops immunoprobes for use in paper based immunoassays.
His current research is focused on understanding how immunoprobe design impacts nanoparticle functionality in paper based assays. Specifically how surface properties affect particle affinity for the antigen in a variety of conditions. Lessons learned are applied to diagnosis of disease including development of a Coronavirus assay.
Dr. Hristov is a co-author on fifteen papers published in a range of peer reviewed journals including Nature Nanotechnology, JACS and ACS Nano.

Alexander Huber

Dr. Alexander Huber started his career in the pharmaceutical industry at the research department of F. Hoffmann- La Roche Ltd in Basel, Switzerland in 2002. He held several positions with increasing responsibility. 2009 he joined Novartis Pharma technical research and development (TRD) as director of the global centre of excellence for parenteral clinical supply. In 2013 he changed to the Cell & Gene-Therapy group as global CMC head. He is responsible of managing a global team for clinical and commercial manufacturing of CTL019 / Kymriah in Japan and Luxturna in Europe. He also manages several CMOs to ensure supply of critical materials for this therapy.
Alexander received his PhD from the Federal Institute of Technology (ETH) Zürich in the field of molecular neuropharmacology, developing a first ex-vivo gene therapy approach against focal epilepsy in an animal model. He also holds a MBA from the same university.



Patrick Hunziker

Patrick Hunziker has studied Medicine the University of Zurich, Switzerland. He received a doctoral decree based on thesis work in experimental immunology from the University of Zurich and did further research in experimental haematology at University Hospital in Zurich, Switzerland. He earned specialist degrees in Internal Medicine, Cardiology and Intensive Care Medicine. As a fellow the Massachusetts General Hospital, Harvard Medical School, worked on cardiac imaging in a joint project with the Massachusetts Institute of Technology, Cambridge. 2008 Patrick Hunziker became professor for Cardiology and Intensive Care Medicine at the University of Basel. His professional activities in Europe, the U.S., Africa and China gave him a broad insight into the needs for the medicine of the future in a variety of settings. Hunziker became involved in medical applications of Nanoscience in the late nineties and has been the pioneer physician in Nanomedicine in Switzerland since then. With improved prevention, diagnosis and cure of cardiovascular disease as his main research topic, he worked in the nanoscience fields of atomic force microscopy, nanoptics, micro/nanofluidics, nanomechanical sensors and polymer nanocarriers for targeting. He is the co-founder of the European Society of Nanomedicine, cofounder of the European Foundation for Clinical Nanomedicine and co-initiator of the European Conference for Clinical Nanomedicine and is clinically active as deputy head of the Clinic for Intensive Care Medicine at the University Hospital Basel, Switzerland. After being founding president of the European Society for six years he was elected as President of the International Society for Nanomedicine, which is uniting members from all continents in the world and realizes regular Summer schools on Nanomedicine.

David Jacob

Technical Director

David Jacob (53) holds a PhD in lasers physics from the University of Rennes (1995) and a diploma in aeronautics (ENSAE 1991). David Jacob is Technical Director of Cordouan Technologies (Pessac, 33). He has over 20 years of industry experience in the design and industrialization of optoelectronic systems and R&D management; Co founder and technical director of Cordouan technologies since 2007, he is in charge of collaborative R&D project management and of the development and industrialization of innovative instruments dedicated to the physico-chemical characterization (size, charge, shape) of nanoparticles. David Jacob represents Cordouan Technologies on two AFNOR standardization committees at the national level (AFNOR / X457 Nanotechnologies commission) and internationally (ISO / TC 24 / SC 4 & ISO / TC 229). He is also a member of the French Nanometrology association

RECENT PUBLICATIONS

- Thermosensitive polymer-grafted iron oxide nanoparticles studied by in situ dynamic light backscattering under magnetic hyperthermia ; Gauvin Hemery · Elisabeth Garanger · Sébastien Lecommandoux · [...] · Olivier Sandre ; Dec 2015 · Journal of Physics D Applied Physics
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berger · B. De Roo · D. Jacob · [...] · J.P. Locquet ; Jan 2015 · Nuclear Instruments and Methods in Physics Research Section B Beam Interactions with Materials and Atoms

Nikhil Jain

Dr. Nikhil Jain is currently a Junior Group Leader and Lecturer at the Institute of Translational Medicine, ETH Zurich. He carried out his doctoral studies at the National University of Singapore under the supervision of Prof. Paul Matsudaira and G.V. Shivashankar (2008-2014). As a Ph.D. student, by combining microfabrication techniques, high-resolution-imaging and "omics" toolkit, he elucidated functional and regulatory coupling between cellular architecture and the gene expression program. Using genome-wide transcriptome analysis and by generating multi-dimensional plots, he linked specific gene clusters with distinct cell-geometries in fibroblast and stem cells. In 2014, he joined the laboratory of Prof. Viola Vogel as a post-doctoral fellow, where he contributed to the formulation of a new paradigm of homeostasis and inflammatory gene expression regulation of immune-cells by forces, which exist in healthy and diseased tissues. According to this, macrophage activation is directly regulated by spatial confinement. During his postdoctoral tenure, he was awarded an SNF fellowship followed by an EMBO fellowship to carry out a project to study epigenomic changes during macrophage inflammation in collaboration with Prof. Yuval Ebenstein at the Tel-Aviv University and Prof. Chuan He at the University of Chicago along with spending time at the Cornell University in Jan Lammerding's lab to work on nuclear mechanics. His current research interest is towards understanding how physical forces, which exist in tissues, shape the behaviour of immune cells during different pathological conditions mainly aging and neuro-degenerative diseases.

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Lloyd Jeeffs

Director of Clinical Manufacturing Solutions
Precision NanoSystems, Inc.

Dr. Jeffs joined Precision NanoSystems in June 2018 as Director of Clinical Manufacturing Solutions, based at the Vancouver, Canada Headquarters. He is responsible for developing and executing custom programs to meet the clinical manufacturing needs of PNI's clients.
He is an expert in developing Lipid-based nanotherapeutics and has over 20 years of experience in this field, including: formulation and process development, scale-up and technology transfer.
Dr. Jeffs has played key roles in manufacturing GMP RNA-LNP drugs for more than 10 Clinical Stage programs. He has previously worked at Arbutus Biopharma Corporation (and its predecessor companies Tekmira Pharmaceuticals and Protiva Biotherapeutics) where he was Director of Manufacturing & Supply Chain Management, and at Northern Lipids Inc. as a Formulation Scientist.
Dr. Jeffs received his PhD. in Applied Microbiology from the University of Saskatchewan and has B.Sc. and M.Sc. degrees from the University of British Columbia. He is a co-author of numerous peer-reviewed publications dealing with the development of Lipid Nanoparticle therapeutics and is a co-inventor of key patents in this field.

Gerard M. Jensen

Gerard M. Jensen earned a B.S. in Biochemistry from U.C.L.A. and a Ph.D. in Physical Chemistry from the University of Southern California, with Professor Philip Stephens FRS as advisor. Dr. Jensen then completed a post-doctoral fellowship in Molecular Biology at The Scripps Research Institute. His academic research has involved chiroptical and electron paramagnetic resonance spectroscopies, theoretical modeling of metalloproteins, site-directed mutagenesis, protein purification, protein X-ray crystallography, experimental and theoretical study of small molecule binding to natural and engineered protein cavities, and ab initio calculation of fundamental properties of small molecules.
Since 1995, Dr. Jensen has worked in the biotechnology industry. At NeXstar Pharmaceuticals and later Gilead Sciences, he has led efforts in formulation development, scale-up and manufacturing of liposomal and oligonucleotide based therapeutics, analytical method development and product and process characterization. He is presently the Executive Director of Technical Operations at the Gilead commercial manufacturing facility in San Dimas California and a new facility in La Verne California. The group provides primary process and analytical chemistry development and support, validation services, and project management and engineering services for the Gilead parenteral manufacturing sites. Commercial products developed, approved, manufactured and commercialized from this site include AmBisome® (liposomal amphotericin B injection), a product that treats life threatening fungal infections, DaunoXome® (liposomal daunorubicin citrate for injection), a product that treats Kaposi's sarcoma, Macugen® (pegaptanib sodium injection) an oligonucleotide aptamer treatment for age-related macular degeneration, and Veklury® (Remdesivir) for the treatment of COVID-19.
Dr. Jensen has been an Adjunct Professor at the Keck Graduate Institute in Claremont, at the California State University at Fullerton, the California Polytechnic State University at Pomona, and at Azusa Pacific University. He has taught graduate courses in Pharmaceutical Development and Regulatory Affairs. Dr. Jensen is an Associate Editor of the Journal of Liposome Research and has authored or co-authored over 40 publications or patent applications.

Wenlei Jiang

Dr. Wenlei Jiang currently serves as a Senior Science Advisor in the Office of Research and Standards (ORS)/Office of Generic Drugs (OGD)/Center for Drug Evaluation and Research (CDER). She has been championing regulatory research in the areas of generic nanomaterials, narrow therapeutic index drugs, and modified release products to support review standards development and ensure post-market safety and efficacy of these drug products. Currently she is leading complex drug product classification and research, as well as promoting global bioequivalence harmonization. She also serves as Vice Chair at Product Quality Research Institute (PQRI) Steering Committee and Chair at Biopharmaceutical Technical Committee. Prior to joining FDA, she was at Novartis Pharmaceutical Corporation where her responsibilities included formulation development of conventional liquid and solid dosage forms, and advanced parenteral drug delivery systems. She received her PhD in Pharmaceutics and Pharmaceutical Chemistry from The Ohio State University.

Michael Johnston

Dr. Michael Johnston completed his PhD in the Department of Biochemistry and Molecular Biology at the University of British Columbia in 2006, where his research focused on regulated drug release from liposomal delivery systems. Dr. Johnston subsequently joined Health Canada as a post-doctoral fellow and was then hired as a research scientist in 2007 to establish a nanomedicines research program. Since then Dr. Johnston has further developed his research program to focus on understanding parameters affecting critical quality attributes of nanoscale drug delivery systems with a particular interest in nanoparticles generated with recombinant protein expressed in plants. Dr. Johnston is also concerned with nanoscale vaccine adjuvants. He also currently chairs the International Pharmaceutical Regulators Forum (IPRF) working group for nanomedicines.

Olivier Jordan

Olivier Jordan is Senior Lecturer at the School of Pharmacy, University of Geneva. He graduated in engineering and received his PhD from EPFL working on the microencapsulation of insulin-secreting cells for the treatment of diabetes. In the team of Prof Aebischer at the Lausanne University Hospital, he focused on biomaterials engineering for cartilage and nerve prosthesis. Moving at School of Pharmacy, he developed projects in the field of novel delivery carriers for drugs, protein and therapeutic heat, based on in situ forming implants, nano- or microparticles. He is the (co-)author of 70 peer-reviewed publications, 9 patents, and founded two startups in the biomedical field.

RELEVANT PUBLICATIONS:

- P. Maudens, C.A Seemayer, C. Thauvin, C. Gabay, O. Jordan, E. Allémann. Nanocrystal-Polymer Particles: extended delivery carriers for osteoarthritis treatment. Small 14(8):1703108 (2018).
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Steve Kanner

Steve is the Chief Scientific Officer of Caribou Biosciences responsible for R&D activities related to therapeutic discovery and development. Before joining Caribou, Steve served in positons of increasing responsibility in both oncology and immunology/inflammation drug discovery at Bristol-Myers Squibb, Agensys, and Astex Pharmaceuticals, and was most recently VP, Head of Biology at Arrowhead Pharmaceuticals leading a department in discovery of RNAi therapeutics for oncology, genetic diseases and other indications. Steve has authored numerous publications in both peer-reviewed journals and books, and is an inventor on multiple patents and patent applications. Steve earned his undergraduate degree in Genetics from the University of California, Berkeley, his Ph.D. in Immunology and Microbiology from the University of Miami's Miller School of Medicine, and did post-doctoral work in oncology at the University of Virginia.

Michael Keller

Michael Keller studied Chemistry & Biochemistry at the ETH Zürich from 1989-1994. The award of the ETHZ-Imperial College London exchange scholarship 1994 enabled him to pursue a MSC/DIC in Chemical Research at Imperial College London, before joining the Research group of Professor Manfred Mutter at the University of Lausanne where he carried out a PhD in Bioorganic Chemistry. After a year as lecturer at the same Institute, he joined Imperial College London Genetic Therapies Centre as Academic Visitor specializing in nonviral delivery systems for nucleic acids. He co-founded the Anglo/Japanese Biotech company IC-Vec Ltd. in 2002 developing novel cationic lipids and nanomedicines for siRNA delivery, before joining Novartis Pharma AG Basel to build up siRNA formulation in Technical Research & Development. He was awarded the Novartis Leading Scientist Award in 2009 for his work on siRNA delivery. In late 2017 he joined the preclinical CMC unit at Hoffmann-La Roche Ltd. Basel to work on locked nucleic acids (LNAs), with a particular focus on disruptive concepts to enable enhanced delivery of LNA

Lora Kelly

Lora has lectured on her cancer journey to motivate scientists in their research for CRS (New York 2018), the World Molecular Imaging Congress, (Philadelphia, 2017), John's Hopkins (2017), Let's Win Pancreatic Cancer (2016), and has been awarded NPF's Courageous Patient Award recommended by her Hopkins' physicians (2015). Lora shared her journey at ICONAN 2019 in Munich to help scientists understand what patients endure and to honor the journeys' of those patients who are no longer here to advocate for themselves. Lora has endured many rigorous trial based treatments and continues to fight to win. Sharing from her heart and life- Lora puts clearly into perspective the importance of research and the sacrifice of cancer patients and their caregivers.

Ajay Khopade

Dr. Ajay Khopade is a Vice President R&D (Formulation Development) and Heading non-oral division at Sun Pharma Advanced Research Co. Ltd. (SPARCL), a pharma research and drug discovery company separated out from a leading speciality pharma Sun Pharmaceutical Industries Limited.
With over 18 years of experience in pharmaceutical product development, in his current role as VP-R&D, is responsible for development of SPARC's innovative and differentiating drug product portfolio and product life-cycle management through strategic innovation planning & road mapping. Currently he is leading a team of scientists supporting development of niche products and platform technologies. Dr. Khopade has extensive end-to-end (ideation-technology development-preclinical POC-clinical-commercial) development experience across multiple therapeutic areas in novel parenteral dosage forms. He has participated as CMC expert in the submission of number of INDs, NDAs and technology evaluation for in-licensing opportunities. He is an inventor of a platform nanotechnology in the field of oncology (Nanotecton®), ophthalmics (GFR®, SMM®and TearAct®) and depot injections protected by various IPs globally with over a dozen patents. Most of these technologies have endured clinical tests to reach into the market. Dr. Khopade has been a Humboldt post-doctoral fellow at Max Plank institute of Colloids and Interfaces, Germany. He holds a Ph.D. degree in Pharmaceutical Sciences from the University of Sagar, MP, India. His areas of interest are understanding physical chemistry of drug delivery system design.

Sarah Kraus

Sarah Kraus graduated at Tel Aviv University, Tel Aviv, Israel and completed her Ph.D. studies in Immunology and Cell Biology on the involvement of second messengers and intracellular signaling in the development of tumor cell resistance to the complement system. She continued her research as a postdoctoral fellow at the Weizmann Institute of Science, Rehovot, Israel in the field of signal transduction in cancer and investigated the activation of mitogen-activated protein kinase (MAPK) pathways and signals induced by G-protein coupled receptors, using the GNRH receptor as a prototype, in prostate and breast cancer models. She then continued her studies as a Research Associate at the Department of Microbiology and Cancer Center, University of Virginia Health System, Charlottesville, Virginia, USA where she investigated signaling pathways and scaffold proteins involved in the development of prostate cancer. After returning to Israel in 2006, Dr. Kraus joined the Integrated Cancer Prevention Center at the Tel Aviv Sourasky Medical Center, Israel as a Senior Scientist and Head of the Molecular Biology research laboratory. She also joined the teaching staff at the Faculty of Medicine, Tel Aviv University as an Assistant Prof. and Lecturer. On 2016, and after completing an M.B.A. degree in Biomedical Management, she joined New Phase Ltd., a company engaged in the development of a novel therapeutic strategy for cancer treatment, based on Nanotechnology.

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Silke Krol

Laboratory for personalized medicine,
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Since 2018 Silke Krol is with IRCCS Ospedaliero Specializzato in Gastroenterologia "Saverio de Bellis" developing novel nanoparticle-based therapeutic and diagnostic approaches for inflammatory bowel disease. From 2010 till 2018 Silke Krol was with Fondazione IRCCS Istituto Neurologico "Carlo Besta" in Milan, Italy heading the laboratory for Nanomedicine. There she studied the transport mechanisms for differently functionalized gold nanoparticles across the blood brain barrier and how this is influenced by blood-derived proteins. Moreover, different novel metallic and non-metallic delivery systems for various other diseases (cardiovascular, prion disease, epilepsy, glioma, lymphomas, viral diseases) were designed for projects funded by Italian and European foundations. In collaboration with Prof. Stellacci in Lausanne, Switzerland, they discovered the role of nanoparticles in vaccine stabilization, antiviral action and enhancer for viral infectivity for gene delivery. From 2016-2018 she worked in parallel for the IRCCS Istituto tumori "Giovanni Paolo II" in Bari, Italy leading the laboratory for translational Nanotechnology with focus on early diagnosis and advanced therapy of cancer.
She studies the application of multifunctional polymer/nanogold based drug or drug delivery systems as well as diagnostic tool for medical applications such as a hand-held point-of-care device for measuring chemotherapeutic drugs in real-time at the bedside of

the patient. Moreover, the multilayer-nanocoating was used for encapsulation and immune protection of living cells like e.g. pancreatic islets. She has several pending patents for possible future drugs for prion disease and cancer treatment, viral diseases, and cancer diagnostics.
In 2009 she worked as an expert consultant for the United Nations and serves as external expert reviewer for National projects in France, Italy, Georgia and Greece. She worked as project technical advisor in 3 EU-FP7 projects and was external expert for the evaluation of EU project. She is member of the international advisory committee of the International scientific spring conference in Islamabad, Pakistan. She is member of the editorial board of the journal "Precision Medicine", and associate editor of "Frontiers in Nanobiotechnology". Since 2013 she is adjunct faculty member at the Pakistan Institute of engineering and applied science (PIEAS). Recently she founded a start-up, EnCytos with a team from the University of Twente.

Twan Lammers

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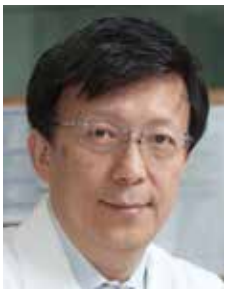
Twan Lammers obtained a DSc degree in Radiation Oncology from Heidelberg University in 2008 and a PhD degree in Pharmaceutics from Utrecht University in 2009. In the same year, he started the Nanomedicine and Theranostics group at the Institute for Experimental Molecular Imaging at RWTH Aachen University. In 2014, he was promoted to full professor at the faculty of medicine at RWTH Aachen. He published over 200 research articles and reviews, and he received multiple scholarships and awards, including a starting and consolidator grant from the European Research Council, and the young investigator award of the Controlled Release Society. He is associate editor for Europe for the Journal of Controlled Release and he serves on the editorial board of several additional journals. His primary research interests include drug targeting to tumors and to the brain, image-guided drug delivery, and functional and molecular imaging of liver and kidney fibrosis.

Raphaël Lévy

Raphaël Lévy obtained a PhD in Physics (Strasbourg, France, 2002) studying the conformation of biopolymers adsorbed on surfaces using the atomic force microscope then moved to Liverpool to work as a postdoctoral researcher on the applications of nanoparticles in biology. After publishing articles on the functionalisation of gold nanoparticles with peptides, he obtained in 2006 a 5 year Fellowship (BBSRC David Phillips) to establish his independent research group in this field. In the following years, in collaboration with biologists and chemists, he made contributions to the design, optimization and characterization of nanoparticles,[1, 2, 3] the understanding of the interactions of nanoparticles with cells,[4,5] and the development of nanoprobes for the evaluation of cell therapy safety.[6,7]
In parallel, from 2009, he started to challenge a series of reports published in high profile journals (e.g. Nature Materials, JACS, PNAS, Science) which claimed that nanoparticles with a particular structure (itself an imaging artefact) had a number of extraordinary properties; this led to the publication of Stripy Nanoparticles Revisited [8] after more than three years of peer review, marking the beginning of a public controversy that lasted several years. The near impossibility of timely correction of the scientific record via traditional peer review led him to experiment alternative practices to generate a much-needed space for scientific discussion of disputable findings. This includes his blog (https://raphazlab.wordpress.com, over 300,000 views and over 100,000 visitors since launching in 2008), many contributions to www.PubPeer.com, Twitter and preprints,[9,10, 11]. He is using this combination of scientific communication tools in a second, still unfolding, controversy related to the SmartFlares (or Spherical Nucleic Acids). Those are nanoparticles that according to many high profile articles were supposed to detect mRNAs inside cells, but that have now been withdrawn from the market after our critiques and their commercial failure (see RNA Detection Tool Debate Flares Up at ACS Meeting, The Scientist, September 2018).
DOIs: [1.] 0.1021/nn204214x; [2.] 10.1021/nn204214x; [3.] 10.1039/B910657J; [4.] 10.1021/nn9006994; [5.] 10.1371/journal.pone.0121683; [6.] 10.1021/acsnano.6b03246; [7.] 10.7554/eLife.33140; [8.] 10.1002/smll.201001465; [9.] 10.1038/s41551-018-0218-x; [10.] 10.1101/029447; [11.] 10.5281/zenodo.3900212



Dong Soo Lee

As nuclear medicine physician since 1990, and nanomedicine pursuer since 2009, I endeavored to establish radionanomedicine (combined nuclear and radionanomedicine) and looked for the application of radionuclide-labeled nanomedicines to preclinical and possibly in human (clinical situration) in the near future. Using radiolabled nanomaterials, their *in vivo* distribution was easily traced with tracer kinetic. When therapeutic radionuclide was labeled to (for example) iron oxide, we could do the dosimetry in animals. Therapy and simultaneous diadnostic imaging with radionuclide is called theranostics and we use radionuclide for imaging and this is nuclear theranostics. Among many nanomaterials, we are recently interested in graphenes and exosomes. I (We) believe that *in vivo* distribution and characteraization of physiologic determinants thereof is the gate for preclinical/clinical usablity of any nanomaterials (labeled with radionuclides) at least at first in the efforts of translation to clinics.

RECENT PUBLICATIONS

- Lee DS, Suh M, Kang SY, Hwang DW. Physiologic constraints of using exosomes *in vivo* as systemic delivery vehicles. Precision Nanomedicine. 2019 Jul 30;2(3):344-69.
- JB Park, D Sung, S Park, KA Min, KW Kim, Y Choi, HY Kim, E Lee, HS Kim, BH Hong, DS Lee. 3D graphene-cellulose nanofiber hybrid scaffolds for cortical reconstruction in brain injuries 2D Materials 6 (4), 045043
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- Suh M, Lee DS. Brain Theranostics and Radiotheranostics: Exosomes and Graphenes In Vivo as Novel Brain Theranostics. Nucl Med Mol Imaging. 2018 Dec;52(6):407-419.
- Oh HJ, Kim J, Park H, Chung S, Hwang DW, Lee DS. Graphene-oxide quenching-based molecular beacon imaging of exosome-mediated transfer of neurogenic miR-193a on microfluidic platform. Biosens Bioelectron. 2019 Feb 1;126:647-656.

Hans Lehrach

Max Planck Institute for Molecular Genetics, Ihnestr. 63-73, 14195 Berlin, Germany
Founder and Scientific Director, Dahlem Centre for Genome Research and Medical Systems Biology Founder and Chairman of the Board, Alacris Theranostics GmbH, Max-Planck-Str. 3, 12489 Berlin

Hans Lehrach obtained his Ph.D. at the Max Planck Institute for Experimental Medicine and the Max Planck Institute for Biophysical Chemistry in 1974. Next he moved on to Harvard University, Boston (1974-1978) for a postdoc and then became group leader at EMBL, Heidelberg (1978-1987). He then moved to the Imperial Cancer Research Fund, London (1987-1994) to become head of the Genome Analysis Department. In 1994 he returned to Germany to become Director at the Max Planck Institute for Molecular Genetics (since 1994, em. 2014). He holds a position as a Visiting Professor at the Berlin Institute of Health (BIH), Berlin, Germany as well as at the Southern University of Science and Technology (SUSTech) ind Shenzhen, China.
His expertise lies in genetics, genomics, systems biology and personalized medicine. Highlights include his key involvement in several large-scale genome sequencing projects, such as the human, rat, and Schizosaccharomyces. His group was part of the team which identified the Huntington's disease gene. Dr. Lehrach also performed key work on technologies such as protein microarrays, protein interactome analysis, yeast artificial chromosomes and RNAseq. He has been pioneer in the application of next generation sequencing techniques and systems medicine for the development of personalized therapies in cancer (Virtual Patient Model). He was partner in two German ICGC projects and partner in the European IHEC project BLUEPRINT, a steering committee member of the 1000 Genomes project, leader of the managing entity of the IMI OncoTrack project and co-ordinated the FET Flagship pilot initiative IT Future of Medicine (ITFoM, www.itfom.eu), a finalist of the FET Flagship Call, which has established a strong technological roadmap and network of partners from 33 countries. In this current initiative, Hans Lehrach represents a growing network of research institutions, industry representatives, scientists and patient groups that share a long-term vision for sustainable health care (www.healthcarecompactforeurope.eu) and the personalization of medicine in his new FET Flagship initiative Digital Twins for Better Health (DigiTwins).
Dr. Lehrach has founded several biotechnology companies such as Sequana Therapeutics, GPC Biotech, Scienion, Prot@gen, PSF Biotech, Atlas Biolabs. Dr. Lehrach is founder of the Berlin-based company Alacris Theranostics GmbH, specialising in the development of new approaches for personalised medicine for cancer patient diagnosis, treatment and drug stratification. He is chairman of the Supervisory Board and scientific advisor of the company since 2008. In 2010 he founded the non-for-profit research institute The Dahlem Centre for Genome Research and Medical Systems Biology (DCGMS).

Beat Löffler

Dr. med. h.c. Beat Löffler, MA studied, Philosophy, Communication Sciences and Politics at the Free University in Berlin, graduating with an MA From the University of Basel he received an MD h.c. .1984 he co-founded an Agency for New Media. 1994 he founded his company L&A Concept Engineering for translation of science-based visions in the application and establishment of worldwide networks (mission and strategy for realizing projects out of visions of clients). He was for 6 years was secretary general and coach of the trinational BioValley Promotion Team, with the mission of establishing the trinational Upper-Rhine Biotechnology network. From 2003 to 2006, he worked for NEC Hightech Performance Computing as mandated Leader Life Sciences Business Development in Biology and Medicine. In 2007 he founded the European Foundation for Clinical Nanomedicine with Patrick Hunziker. The aim of the foundation is the research and development of nanomedicine with regard to its use as an innovative technology, better medical care in the future and the establishing an international network in nanomedicine and related fields. Today is his twelfth written programme and organization of the scientific summit on clinical nanomedicine under the name CLINAM 12 /2020 (Clinical Nanomedicine). CLINAM shaped a neutral high-level debate platform organized by the nonprofit foundation, which serves also as meeting place for the international regulatory authorities in the field of nanotechnologies in health. (IPRP). The foundation launched the European Journal of Nanomedicine of which today PRNANO as official nonprofit Gold Open Access Journal of CLINAM, ISNM and ESNAM. Beat co-founded the European Society for Nanomedicine (ESNAM) and the International Society for Nanomedicine, (ISNM) which realizes every year a Nanomedicine Summer school.

Roderick Lim

Argovia Professor for Nanobiology, Biozentrum and the Swiss Nanoscience Institute, University of Basel

Roderick Lim studied applied physics at the University of North Carolina at Chapel Hill. He received his PhD in 2003 from the National University of Singapore for his research work on the nanotribological properties of confined liquids using the atomic force microscope (AFM). In 2004, he moved to the Biozentrum, University of Basel for postdoctoral research with Prof. Ueli Aebi. During this time, he struck on the idea of applying biomimetic approaches to study how nuclear pore complexes (NPCs) regulate nucleocytoplasmic transport (NCT) and subsequently received the 2008 P-G. de Gennes Prize "From Solid State to Biophysics". In 2009, he was appointed Argovia Professor for Nanobiology at the Biozentrum and the Swiss Nanoscience Institute, University of Basel, where he received tenure in 2014.
Over the past decade, his main focus has been to study the fundamental control mechanisms of NCT. His lab investigates NCT across biophysical, nanoscale and cellular levels to shed light on the basic underlying principles that govern such biological function. This includes (i) uncovering the dynamic barrier-forming characteristics of intrinsically disordered proteins in the NPC known as phenylalanine-glycine nucleoporins (FG Nups) by high-speed AFM (https://youtu.be/o9sDGBYRF_M) [1], (ii) elucidating the multivalent interactions that facilitate NPC transport selectivity and speed [2], and (iii) discovering that soluble transport receptors known as karyopherins maintain NPC barrier and transport function [3]. Most recently, Lim and colleagues have leveraged on their understanding of NCT to target polymer vesicles known as polymersomes into the cell nucleus with potential applications in drug delivery and gene therapy [4]. He is also a co-inventor of ARTIDIS® "Automated and Reliable Tissue Diagnostics", an AFM-based innovation for cancer diagnosis [5].

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4. Zelmer et al. (2020) Organelle-specific targeting of polymersomes into the cell nucleus. Proceedings of the National Academy of Sciences USA, 117(6) 2770-2778.
5. Plodinec et al. (2012). The nanomechanical signature of breast cancer. Nature Nanotechnology 7(11), 757-65.

Neill Liptrott

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Dr Liptrott has a background in pharmacolDr Liptrott has a background in pharmacology, immunology and molecular cell biology. His research is focused on investigating biocompatibility and immunological safety of conventional and nanotechnology-enabled medicines. Dr Liptrott's research to date has helped underpin the successful translation of solid drug nanoparticle formulations through GMP manufacture towards healthy volunteer bioequivalence studies. Dr Liptrott heads the nanotechnology and advanced materials biocompatibility research programmes at the University of Liverpool. Additionally Dr Liptrott is a member of the Executive Board and core expert team (CET) of the recently established European Nanomedicine Characterisation Laboratory (EU-NCL). He leads the University of Liverpool work packages on nanoparticle biocompatibility and structure-activity relationships.

SELECTED PUBLICATIONS

- David, C. A. W., M. Barrow, P. Murray, M. J. Rosseinsky, A. Owen and N. J. Liptrott (2020). "In Vitro Determination of the Immunogenic Impact of Nanomaterials on Primary Peripheral Blood Mononuclear Cells." International journal of molecular sciences 21(16).
- Hobson, J. J., S. P. Rannard, A. Owen and N. J. Liptrott (2020). "Safety assessment of a new nanoemulsion-based drug-delivery system reveals unexpected drug-free anticoagulant activity." Nanomedicine (London, England) 15(14): 1361-1373.
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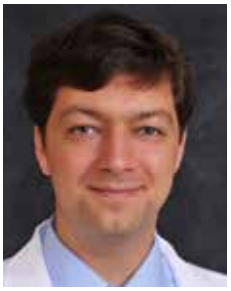
- David, C. A., A. Owen and N. J. Liptrott (2016). "Determining the relationship between nanoparticle characteristics and immunotoxicity: key challenges and approaches." Nanomedicine (London, England) 11(11): 1447-1464.



Vladimir Ljubimov

Dr. Vladimir Ljubimov, MD, is a 4th year Neurosurgery resident at Cedars-Sinai Medical center. He graduated from the University of Southern California's Keck School of Medicine.

Dr. Ljubimov's interests are neurosurgical oncology and engineering new technologies for the treatment of tumors of the central nervous system. He has worked on many projects in the past including early ALS diagnosis and immunotherapies for Alzheimer's disease, mechanisms of pituitary tumor senescence, how inflammation stops neurogenesis through a p21-regulated mechanism and how to reverse it using antidepressants. He co-founded a start-up company designing a novel device for bone marrow harvesting for stem cell regenerative medicine. He is currently working with the Cedars-Sinai Nanomedicine center on projects involving targeted drug delivery to brain tumors and novel imaging technology for brain tumor diagnosis.
Dr. Ljubimov is a co-author on multiple manuscripts including ones published in Nature Communications, PNAS, Cancer Research and on nanotechnological platforms for drug delivery to the CNS and virtual biopsy (ACS Nano) using the technology developed at Cedars-Sinai Medical Center.

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- Sun, T., et al. Blockade of a laminin-411-Notch axis with CRISPR/Cas9 or a nanobioconjugate inhibits glioblastoma growth through tumor-microenvironment crosstalk. Cancer Research 79, 1239-1251 (2019).
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- Patil, R., et al. MRI virtual biopsy and treatment of brain metastatic tumors with targeted nanobioconjugates: Nanoclinic in the brain. ACS Nano 9, 5594-5608 (2015).

Julia Ljubimova

Dr. Julia Ljubimova, MD, PhD, is professor of Neurosurgery and Biomedical Sciences and Director of the Nanomedicine Research Center in the Department of Neurosurgery at Cedars-Sinai Medical Center, Los Angeles USA.
Dr. Ljubimova's main interests are drug delivery through multiple biological barriers and in particular, across blood brain barrier (BBB). She is studying differential cancer genomic/proteomic signatures as tools for finding novel/early markers of cancer growth and is involved in development and design of new nanomedicine drugs against these and other tumor targets. Her group has developed biodegradable and customizable nano bioconjugates that selectively target brain and breast cancers and that could have less dose-limiting toxicity than current therapies. Recently, radically new nano immunomedicines were developed for cancer treatment, with the ability to stimulate both general and local anti-tumor immune responses.
Dr. Ljubimova's studies have been published in numerous high-impact peer-reviewed journals, including Cell, Nature Communications, Proc Natl Acad Sci USA, Cancer Research, Nanomedicine,

Journal of Controlled Release, American Journal of Pathology, Angiogenesis, Biomaterials, Angewante Chemie, and ACS Nano. She is an inventor on 22 USA and foreign patents. She has trained over 70 undergraduate and graduate students, postdocs, and clinical fellows.

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- Israel, L.L., et al. A combination of tri-Leucine and Angiopep-2 drives a poly-anionic polymalic acid nanodrug platform across the blood-brain barrier. ACS Nano 13, 1253-1271 (2019).
- Ljubimova, J.Y., et al. Covalent nano delivery systems for selective imaging and treatment of brain tumors. Advanced Drug Delivery Reviews 113, 177-200 (2017).
- Sun, T., et al. Blockade of a laminin-411-Notch axis with CRISPR/Cas9 or a nanobioconjugate inhibits glioblastoma growth through tumor-microenvironment crosstalk. Cancer Research 79, 1239-1251 (2019).
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François Lux

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90 publications, 15 patents on nanoparticles and their use for medicine, 4 book chapter, h-factor of 15. Co-fundation of NH TherAguix (2015) company specialized in nanomedicine and MexBrain, company specialized in *in vivo* metallic extraction.

EDUCATION

2019-2024: Institut universitaire de France. Member.
2009-: University Lyon 1. Associate Professor in the team of Pr Olivier Tillement. Specialization in nanomedicine.
2007-2009: Ecole Normale Supérieure of Lyon. Lecturer with work on theoretical surface chemistry.
2004-2007: Ecole Normale Supérieure of Lyon. PhD in chemistry on the synthesis of new ligands and complexes for luminescence studies.
2000-2004: Ecole Normale Supérieure of Lyon. Studentship as a state employee for the ministry of education.

RECENT PUBLICATIONS

- V. –L. Tran, V. Thakare, F. Rossetti, A. Baudouin, G. Ramniceanu, B. –T. Doan, N. Mignet, C. Comby-Zerbino, R. Antoine, P. Dugourd, F. Boschetti, F. Denat, C. Louis, S. Roux, T. Doussineau, O. Tillement, F. Lux, « One-pot direct synthesis for multifunctional hybrid silica nanoparticles », J. Mat. Chem. B, 2018, 6, 4821-4834.
- A. Detappe, E. Thomas, M. W. Tibbit, S. Kunjachan, O. Zavidij, N. Parnandi, E. Reznichenko, F. Lux, O. Tillement, R. Berbeco, « Ultrasmall silica-based bismuth gadolinium nanoparticle for dual MR-CT guided radiation therapy », Nano Lett., 2017, 17, 1733-1740.
- S. Kotb, A. Detappe, F. Lux, F. Appaix, E. L. Barbier, V. –L. Tran, M. Plissoneau, H. Gehan, F. Lefranc, C. Rodriguez-Lafrasse, C. Verry, R. Berbeco, O. Tillement, L. Sancey, « Gadolinium-based nanoparticles and radiation therapy for multiple brain melanoma metastases : Proof of concept before phase I trial », Theranostics, 2016, 6, 418-427.
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Volker Mailänder

Univ.-Prof. Dr. med.

Volker Mailänder studied medicine at the University of Ulm supported by a stipend from the Studienstiftung des Deutschen Volkes and was in the graduate program “Molecular Biology”. He worked in the Blume/Negrin lab in Stanford, California, on natural killer cells and was involved in patient care in the bone marrow transplantation unit. Afterwards he received training in internal medicine (haematology/oncology) in the Charité hospital in Berlin. After relocating to the Institute for Clinical Transfusion Medicine, University Clinic of Ulm, he worked on stem cell manipulation, the interaction of nanoparticles with cells and especially uptake mechanisms and the intracellular pathway. He was board certified in transfusion medicine. Further work focused on using polymeric nanoparticles for labelling or manipulation of stem cells and other cell types. Since 2008 he is leading a joint research group between the University Medical Clinic and the MPI for Polymer Science in Mainz. He has been appointed a professorship dealing with the translation of nanocarriers into medical applications. He is proficient in the procedures of manipulating, freezing and storing stem and immune cells for patients care as the head of production and qualified person. He is active in several cooperative projects (SFB1066 “Nanodimensional polymeric therapeutics for tumor therapy”, BMBF projects) and is vice speaker of the center BiomaTiCS (Biomaterials, Tissues and Cells in Science) of the University Medical Center. Since 1.1.2016 he is W2 professor at the University Medicine Mainz and associated to the Dermatology department and heads the Center for Translational Nanomedicine – CTN. He is especially interested in understanding and overcoming the hurdles of applying nanocarriers for use in clinical applications. Therefore, protein corona, targeting and GMP-conform production of nanocarriers are the main focus of his research.

PUBLICATIONS

- Prozeller, D., J.Pereira, J. Simon, V. Mailänder, S. Morsbach, K. Landfester (2019). “Prevention of Dominant IgG Adsorption on Nanocarriers in IgG-Enriched Blood Plasma by Clusterin Precoating.” Adv Sci 6(10):1802199. doi: 10.1002/advs.201802199.
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Harald Mangge

Deputy Head of the Clinical Institute for Medical and Chemical Laboratory Diagnostics (CIMCL), Medical University of Graz, Austria

Harald Mangge is a Medical Doctor and Professor at the Department of Laboratory Medicine of the Medical University of Graz, Austria. His research focuses on cardiovascular-, metabolic-, and oncologic diseases with emphasis on Immunology and Nanomedicine. In the framework of the STYJOBS/EDECTA cohort project, Harald Mangge conducts a large prospective, observational study to improve the understanding of metabolic and cardiovascular risk in obesity (http://clinicaltrials.gov/ct2/show/NCT00482924). Further, he holds the position of a Deputy Head of the Clinical Institute of Medical and Chemical Laboratory Diagnosis (CIMCL) and is vice speaker of the Cardiovascular Research Field of the Medical University of Graz.

RECENT PUBLICATION

- Immune-Mediated Inflammation in Vulnerable Atherosclerotic Plaques. Mangge H, Almer G. Molecules 2019 PMID: 31450823
- Subcutaneous adipose tissue distribution and telomere length. Mangge H, Renner W, Almer G, Gruber HJ, Zelzer S, Moeller R, Horejsi R, Herrmann M. Clin Chem Lab Med. 2019 PMID: 30913032
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Marianne Marchioni

PharmD, PhD

Marianne is the Application specialist of Izon science.
After a first education as a Pharmacist, she has specialized in nano-formulation through an internship in Catalent Pharma solution (France). Pursuing her studies in the nano field, she completed a PhD at the interface of Biology and Chemistry focusing on silver nanoparticles at the CEA-Grenoble. During these three years, she has mastered and/or used several Nano-characterization technics from the simplest (used for routine characterization), to the most complicated ones.
Izon's expertise is in Tunable Resistive Pulse Sensing (TRPS) for highly accurate particle measurement, in extracellular vesicle (EV) separation with the automated AFC and qEV column platform, and more recently in RNA extraction from EVs (in partnership with Norgen Biotek).

Mira Marcus-Kalish

Dr. Mira Marcus-Kalish is the Director of International Research Collaborations at Tel Aviv University. Her main areas of research are mathematical modelling, converging technologies and data mining.
Dr. Kalish holds a Ph.D. in Operations Research from the Technion, Israel Institute of Technology, where she developed one of the first computerized systems for electrocardiogram (ECG) diagnosis. Her postdoctoral training was at Harvard University, the MBCRR (Molecular Biology Computer Research and Resource) laboratory and at the Dana Farber Cancer Institute. She was awarded her B.Sc. in Statistics and Biology from the Hebrew University of Jerusalem.
Upon her return to Israel, she joined the Tel Aviv University Business School, establishing the pioneering Medical Management program focusing on Medical Informatics. Dr. Kalish later joined the Weizmann Institute of Science, working with Prof. Ephraim Katzir on protein interactions, specificity and sensitivity. She moved back to Tel Aviv University to the Biotechnology Department, taking active part in cross disciplinary research, Converging Technologies and contributing to the EU-US Wtec-NBIC2 activities and publication.
In the private sector, Dr. Kalish served as the scientific advisor and then the head of the Enterprise Marketing Department of IBM Israel. She played an active role in many EU framework projects such as: the Nano2Life Network of Excellence, SkinTreat, ReNaChip, EpoCan, NanoAthero, GLAM, ENATRANS etc. Currently she is the Vice Chair of the Medical Informatics Subproject – the EU Human Brain Flagship Project. Her focus is on disease signature identification, based on targeted analysis of the micro & macro environmental clinical features, as well as on newly-developed approach and analytical tools to meet the challenges of big versus small data analysis towards reliable, personalized and precise medicine for the benefit of society.
Other areas of research include rehabilitation of the discrete sensory motor, learning function, drug toxicity, data mining, and, most recently, a broad band interdisciplinary initiative on "Healthy Aging".
Dr. Kalish was one of the founders of the "Dead Sea Research Institute" in Masada. The goal was "Life in Extreme Conditions – A lesson from nature". To study the broad band of areas at the lowest place of earth, the adaption and survival of human life and culture, especially

Scott E. McNeil

Prof Dr Scott McNeil
Department of Pharmaceutical Sciences
Faculty of Science, University of Basel
Klingelbergstrasse 50, 4056 Basel,
Switzerland

Dr. McNeil is a research group leader in the Dept. of Pharmaceutical Sciences at the University of Basel. His research deals with nanomedicine-based drug delivery and the characterization of nanoformulations. He is the former Director of the Nanotechnology Characterization Laboratory at NCI / NIH.

Clive Meanwell

Clive is Executive Chair of Population Health Partners, a global investment firm based in New York, San Francisco, and London, focused on technologies which arrest the drivers of common and burdensome health conditions such as heart disease, diabetes, anxiety, and depression.
Clive was the founder, Chief Executive and Chief Innovation Officer of The Medicines Company, acquired by Novartis for $9.7 billion in early 2020. Prior to that, he was a Partner at MPM Capital, and before then, he held executive positions with Roche in Switzerland and the United States.
Clive trained in medicine, with post-graduate specialization in cancer research, clinical trials, statistics, and epidemiology. He holds MB ChB and MD cum laude degrees from the University of Birmingham, UK.

Florian Meier

Florian Meier holds a PhD in Analytical Chemistry earned from University of Ulm, Germany in 2013 and joined Postnova Analytics in 2014 as Group Leader Research.
In this position, he gained vast experience in the application of various Field-Flow Fractionation (FFF) techniques and related detection systems such as for example Multi-Angle Light Scattering, Dynamic Light Scattering or Inductively-Coupled Plasma Mass Spectrometry.
As a passionate researcher in an industrial environment, his research focuses on the characterization of samples in the nano- and micrometer size range (e.g. engineered nanomaterials, micro- and nanoplastics, environmental colloids, proteins, polymers, viruses, nano-enabled pharmaceuticals and many more), thereby exploiting and continuously pushing the limits of multi-detector FFF. In this respect, he was and is involved in several collaborative national and international research projects
Being a designated member of the "Arbeitsausschuss Nanotechnologien" of the German Institute for Standardization (DIN), he enjoys bringing in his FFF-expertise as an appointed expert for the ISO/TC 229 "Nanotechnologies".

LIST OF NATIONAL AND INTERNATIONAL COLLABORATION PROJECTS (EXCERPT)

- NanoCELL, German BMBF, ongoing (project coordinator): https://www.nanopartikel.info/en/projects/current-projects/nanocell
- SubµTrack, German BMBF, ongoing: https://bmbf-plastik.de/en/joint-project/submtrack
- ACEnano, EU Horizon 2020 Programme, ongoing: http://www.acenano-project.eu/
- NanoUmwelt, German BMBF, 2014-2017 (project coordinator): https://www.nanopartikel.info/en/projects/completed-projects/nanoumwelt
- SamrtNano, EU Framework 7 Programme, 2012-2016: https://www.linkedin.com/in/smartnano-project-47496763/

LIST OF PEER-REVIEWED PUBLICATIONS (EXCERPT)

- C. Schwaferts, V. Sogne, R. Welz, F. Meier, T. Klein, R. Niessner, M. Elsner, N.P. Ivleva, „Nanoplastic Analysis by On-line Coupling of Raman Microscopy and Field-Flow Fractionation Enabled by Optical Tweezers", Analytical Chemistry, 2020, 92(8), 5813-5820.
- M. Hesler, L. Aengenheister, B. Ellinger, R. Drexel, S. Straskraba, C. Jost, S. Wagner, F. Meier, H. von Briesen, C. Büchel, P. Wick, T. Buerki-Turnherr, Y. Kohl, "Multi-endpoint toxicological assessment of polystyrene nano- and microparticles in different biological models *in vitro*", Toxicology in Vitro, 2019, 61, 104610.
- D. Müller, M. Nogueira, S. Cattaneo, F. Meier, R. Drexel, C. Contado, A. Pagnoni, T. de Vries, D. Cohen, M. Portugal-Cohen, A.J. deMello, "Integration of inverse Supercritical Fluid Extraction and miniaturized Asymmetrical Flow Field-Flow Fractionation for the rapid analysis of nanoparticles in sunscreens", Analytical Chemistry, 2018, 90(5), 3189-3195.
- V. Sogne, F. Meier, T. Klein, C. Contado, "Investigation of Zinc Oxide particles in cosmetic products by means of Centrifugal and Asymmetrical Flow Field-Flow Fractionation", Journal of Chromatography A, 2017, 1515, 196-208.
- K. Eskelin, M. Lampi, F. Meier, E. Moldenhauer, D.H. Bamford, H.M. Oksanen, "Asymmetric flow field flow fractionation methods for virus purification", Journal of Chromatography A, 2016, 1469, 108-119.

Josbert Metselaar

Josbert M. "Bart" Metselaar (Rotterdam, July 6th 1971) obtained a MSc degree in Pharmaceutical Sciences in 1995 and a PharmD degree in 1998, both at Utrecht University. During his study he completed a research internship in pharmacology and PK/PD at the Dept of Pharmaceutics, University of Florida, US. In 1999 he started a PhD at the Dept of Pharmaceutics and the Dept of Immunology in Utrecht where he studied novel targeted formulations of anti-inflammatory medicines.
After completing his PhD and a Post Doc fellowship, he decided to translate part of his accomplishments into investigational medicinal products by starting his company Enceladus in 2005, with which he raised more than 6 million Euros funding over the years. With these investments and additional non-equity funding he performed a series of preclinical and clinical development projects on three liposomal products, which were scaled up to full industrial production under his supervision. Together with Sun Pharma as a large industrial partner he successfully completed a pivotal phase III trial on one liposomal product.
In 2012 he took a part-time academic position in the group of Targeted Therapeutics at the University of Twente and in 2015 he transferred his activities to the Dept. of Experimental Molecular Imaging at the RWTH Aachen University Clinic in Germany, where he works on drug carrier design and formulation development in the field of advanced drug delivery for inflammation, atherosclerosis, and cancer. In 2018 he co-founded Liposoma Health BV, a company that focuses on design, development and manufacturing of advanced nutraceutical products.

Nathalie Mignet

Dr Nathalie MIGNET is Research Director at the National Center for Scientific Research (CNRS) in France. She is the head of the Laboratory Chemical and Biological Technologies for Health located at Université de Paris, also supported by CNRS and INSERM.
After a PhD in France in organic chemistry, Dr Mignet was hired by the company Lynx Therapeutics in San Francisco. She then joined the University of Sheffield in UK. In 1998, she was hired by the French biotech company Capsulis to work on onion-based nanoparticles called spherulites. She joined the CNRS as a research Scientist in 2000 to work on non-viral gene delivery. Since then, she expanded her domain of interest from drug delivery sys-

tems to nanomedicine designed for triggered delivery or imaging. With her team, she is interested in nanomedicine for delivery or imaging going from fundamental to preclinical studies.
She is also the founder and the president of the French Society for Nanomedecine, SFNano.

RECENT ARTICLES

1. K. Lemdani, N. Mignet, V. Boudy, J. Seguin, E. Oujagir, O. Bawa, F. Peschaud, J-F. Emile, C. Capron, R. Malafosse Local immunomodulation combined to radiofrequency ablation results in a complete cure of local and distant colorectal carcinoma, Oncoimmunology. 2019;8(3):1550342.
2. H. Salmon, R. Gahoual, P. Houzé, T. Ibrahim, M. Bessodes, D. Scherman, J. Seguin, N. Mignet Europium labelled lactosylated albumin as a model workflow for the development of biotherapeutics Nanomedicine, 2019, 18, 21-30.
3. W. Khaled, J. Piraquive, B. Leporq, J. H. Wan, S. Lambert, N. Mignet, B-T. Doan, S. Lotersztajn, P. Garteiser, B. Van Beers, In Vitro Distinction Between Proinflammatory and Antiinflammatory Macrophages With Gadolinium-Liposomes and Ultrasmall Superparamagnetic Iron Oxide Particles at 3.0T, J. Magn. Res. Imaging 2019, 49(4):1166-1173.
4. Manta S, Renault G, Delalande A, Couture O, Lagoutte I, Seguin J, Lager F, Houzé P, Midoux P, Bessodes M, Scherman D, Bureau MF, Marie C, Pichon C, Mignet, N. Cationic Microbubbles and Antibiotic-Free Plasmid for sustained Ultrasound-mediated Transgene Expression in liver. J. Controlled Rel. 2017, 262:170-181.
5. Ramniceanu G., Doan B-T., Vezignol C., Graillot A., Loubat C., Mignet N., Berret J-F. Delayed hepatic uptake of multi-phosphonic acid poly(ethylene glycol) coated iron oxide measured by real-time magnetic resonance imaging RSC Advances 2016, 6 (68), 63788-63800

Moein Moghimi

Moein Moghimi is a Professor of Pharmaceutics and Nanomedicine (School of Pharmacy) and Research Professor (Translational and Clinical Research Institute) at Newcastle University (UK); Adjoint Professor at the Department of Pharmaceutical Sciences, Skaggs School of Pharmacy and Pharmaceutical Sciences, University of Colorado-Denver Medical Center (USA); and Deputy Editor of Molecular Therapy (Cell Press). Professor Moghimi is co-founder of S. M. Discovery Group Inc. (Colorado, USA) and S. M. Discovery Group Ltd. (UK). His earlier appointments have included Professor and Chair in Pharmaceutics at the School of Medicine, Pharmacy and Health, Durham University, UK (2016-2017); Full Affiliate Member/ Professor at the Methodist Research Institute, Houston Methodist Hospital Systems, Houston, Texas, USA (2013-2017); Visiting Professor at Università Degli Studi Di Padova, Padova, Italy (2015); Professor of Nanomedicine (at the Department of Pharmacy), Professor of Pharmaceutical Nanotechology (at the NanoScience Center), and Founder/Director of the multi-million Dollar Center for Pharmaceutical Nanotechnology and Nanotoxiocology, University of Copenhagen, Denmark (2008-2016); and Honorary Professor of Nanomedicine at the Multidisciplinary Research Center, Shantou University, China (2008-2010).
Prof. Moghimi's research is centred on fundamental and translational aspects of nanomedicine engineering and performance, with the overall goal of advancing fundamental understanding of biological barriers, and particularly the role of innate immune system, in relation to nanoparticle performance and safety, and within the context of precision medicine applicable to cancer, cardiovascular diseases, immune disorders, and disease of the central nervous system. As of Jan 2020, Prof. Moghimi has over 280 peer-reviewed publications/patents in the field and has given over 400 invited talks, keynotes and plenaries.

EDUCATION:

Alma Mater (Biochemistry): University of Manchester, UK
PhD (Biochemistry): Charing Cross and Westminster Medical School, University of London (Imperial College), UK

SELECTED RECENT REPRESENTATIVE PUBLICATIONS:

2019 Nature Communications 11: 4635 | 2019 Nature Nanotechnology 14: 629–635 | 2019 Nature Nanotechnology 14: 260–268 | 2018 Molecular Therapy 26: 933–934 | 2018 ACS Nano 12: 5834–5847| 2017 ACS Nano 11: 11584–11593 | 2017 Nature Nanotechnology 12: 589–594 | 2017 Nature Nanotechnology 12: 387–393 | ACS Nano 11: 12–18 | Biomaterials 112: 141–152.

Brett P. Monia

Dr. Monia is the chief executive officer and a founding member of Ionis Pharmaceuticals. His contributions at Ionis include research into the medicinal chemistry and mechanisms of action of RNA-targeting modalities to treat human diseases, most notably antisense-based therapeutic strategies. Dr. Monia has extensive experience across a range of therapeutic areas, including oncology, metabolic disease, inflammation, neurological disease and cardiovascular disease, which have resulted in a broad range of successful clinical achievements and in marketing approvals for new medicines.
Dr. Monia has published more than 200 primary research manuscripts, reviews and book chapters, and is an inventor on more than 100 issued patents. He serves as a senior editor for the journal Nucleic Acid Therapeutics, is on the board of directors for Dynacure, and has served as president of the Oligonucleotide Therapeutics Society (OTS). Dr. Monia is also an adjunct professor of biology at San Diego State University where he lectures at the graduate level on pharmacology.
Dr. Monia received his Ph.D. in Pharmacology from the University of Pennsylvania and B.S. degrees in Molecular Biology and Analytical Chemistry from Stockton University in Pomona, New Jersey.

Carlos Mota

Assistant Professor, Maastricht University

Dr. Carlos Mota is an Assistant Professor in the Department of Complex Tissue Regeneration, MERLN Institute for Technology-inspired Regenerative Medicine, Maastricht University. In 2013, he was a postdoc at the department of Tissue Regeneration, University of Twente, the Netherlands where he developed, in partnership with Screvo B.V., a multiwell array platform for high content screening, targeting the effect of small molecules and biopharmaceutical in cancer therapeutics in vitro and in vivo.
Dr. Mota received his PhD in Biomaterials from the BIOS research doctorate school in Biomolecular Sciences at the University of Pisa, Italy, in March 2012. His doctoral studies were focused on the development of new approaches for the fabrication of polymeric scaffolds for Tissue Engineering applications. Furthermore, he was a researcher at the department of Neurosciences, University of Pisa, where he developed scaffolds for otology surgery applications.
Currently, his main research interests are focused on biofabrication, bioprinting and additive manufacturing techniques for the development of tissue engineered constructs.

Debabrata Mukhopadhyay

Debabrata (Dev) Mukhopadhyay,
Ph.D.Professor, Departments of
Biochemistry and Molecular Biology and
Physiology and Biomedical Engineering;
Florida DOH Cancer Research Chair
Mayo Clinic College of Medicine and
Science
Jacksonville, Florida, USA

Debabrata (Dev) Mukhopadhyay: Professor of Biochemistry and Molecular Biology, Mayo Clinic College of Medicine and Science, Jacksonville, Florida, has a joint appointment with the Department of Physiology and Biomedical Engineering. He has specific expertise in key research areas including Cancer, Cardiovascular Diseases, Neurodegenerative diseases and Diabetes. As a postdoctoral fellow, later as an Associate Professor at Harvard Medical School, Boston, he carried out angiogenesis and tumor microenvironment related research. After moving to Mayo Clinic as a Professor and also as leaders of both Tumor Microenvironment Program and Translational Nanomedicine Program, he has been supervising additional research areas including targeted drug delivery systems, novel drug development, nano-mechanics in biological systems. His laboratory has been funded mostly by the government agencies including NIH, Department of Defense and also by other private agencies for more than two decades. He also trained more than 60 investigators and several of them are now renowned Professors and faculties in different institutions throughout the world. He has also been serving as reviewer for several study sections in NIH, Department of Defense and also international funding agencies and participating as editorial board members for several distinguish journals. Recently, he has been appointed as the Florida Department of Health Cancer Research Chair to develop a new Mayo Clinic Translational Nanomedicine Center. He has published more than 216 peer-reviewed manuscripts in different journals including Nature, Nature Medicine, Caner Cell, Cancer Research, ACS Nano, Nano Letters, Gastroenterology and other highly rated journals. He has several patents and has been involving to develop different start-up companies in the area of therapy and diagnosis of different diseases including cancer.

Willem Mulder

Biomedical Engineering and Imaging Institute, Department of Radiology, Icahn School of Medicine at Mount Sinai, New York, NY, USA.
Department of Oncological Sciences, Icahn School of Medicine at Mount Sinai, New York, NY, USA.
Laboratory of Chemical Biology, Department of Biomedical Engineering and Institute for Complex Molecular Systems, Eindhoven University of Technology, Eindhoven, Netherlands.

Willem Mulder, Ph.D., a biomedical engineer with a background in nanochemistry, is Professor of Radiology and Professor of Oncological Sciences at Icahn School of Medicine at Mount Sinai (ISMMS) in New York, as well as Professor of Precision Medicine at the Eindhoven University of Technology (TU/e), The Netherlands. Mulder obtained an M.Sc. in Chemistry from the Utrecht University (2001) and a Ph.D. in Biomedical Engineering from TU/e (2006).
Mulder pioneered the exploitation of nanomaterials as highly tunable immunotherapeutics and imaging agents. His research focuses on (nano-)immunotherapy and precision imaging in cardiovascular diseases, cancer and transplantation. Mulder established library technology, encompassing nanomaterials derived from natural apolipoproteins (nanobiologics), that allows meticulously designing targeted immunotherapies. When appropriately designed, such nanobiologics can be applied to empower the immune system's ability to fight disease, by promoting or inhibiting an immune response, by polarizing macrophage function, or by targeting myeloid cell dynamics.
Mulder has published more than 150 scientific publications in top scientific journals, including Nature Nanotechnology, Nature Reviews Drug Discovery, Nature Communications, Science Translational Medicine, Immunity, etc. His H-index is 67 and his work has been cited more than 13.000 times. Mulder is the principal investigator of multiple National Institutes of Health grants. In 2013, Mulder received a Vidi grant and in 2018 he was awarded a Vici grant, both from the Dutch Science Foundation (NWO).

Bert Müller

Director

Bert Müller holds the Thomas Straumann-Chair for Materials Science in Medicine at the University of Basel, Switzerland. He is the founding director of the Biomaterials Science Center (BMC). In 1989, he received his MS degree in physics from the Dresden University of Technology, Germany. His PhD degree in experimental physics was awarded from the University of Hannover, Germany in 1994. With the related oral and poster presentations, Bert Müller won the Morton M. Traum Award of the Surface Science Division of the American Vacuum Society in 1994. Since 2001, he has been "Privat-Dozent" in experimental physics and, therefore, has regularly taught Physics in Medical Research at the ETH Zurich, Switzerland. Prof. Müller is the author of more than 300 journal papers and many book chapters. His current research interests include high-resolution hard X-ray imaging, nanocontainer for targeted drug delivery, dielectric elastomer transducers for medical applications, and physics-based approaches in dentistry. Bert Müller is Fellow of SPIE and active Member of the European Academy of Sciences. Recently, he has become a Research Councilor of the Swiss National Science Foundation. In 2019, Bert Müller became co-founder of two nanotechnology-based start-up companies from BMC, i.e. Bottmedical AG and Acthera Therapeutics AG, both with domicile in Basel, Switzerland.
The major scientific achievements of Bert Müller during the last ten years are:
1. NANOTECHNOLOGY-BASED MUSCLES TO TREAT INCONTINENCE
The aging of our society has led to the increasing prevalence of social and economic burdening by age-related diseases including urinary and fecal incontinence. In severe cases, artificial sphincter systems are applied, which currently rely on fluid-filled cuffs. One of the main drawbacks is the constant pressure acting on the hollow organ. The natural counterpart, however, adapts to external factors such as climbing stairs or resting in bed, so that the function is guaranteed and the tissue can regenerate. Hence, sensor-controlled devices with the necessary time response have to be developed. As dielectric elastomer actuators (DEA) not only provide the necessary forces, strains, and response time but can simultaneously be operated as sensors, I suggested that these artificial muscles should become the basis of future active implants [Swiss Medical Weekly 139 (2009) 591; Annals Biomed. Eng. 44 (2016) 1355]. Within a nano-tera.ch funded initiative, my team used molecular beam deposition to prepare single-layer actuators that provide the necessary strain at a voltage as low as 12 V [Sens. Actuators A 233 (2015) 32]. Also within nano-tera.ch, we invented compliant Au electrodes [Adv. Mater. Technol. 2 (2017) 1700105]. We are going to found the first spin-off company in this field. Here, we can rely on the key players in the field and started a collaboration with Medtronics.
2. SHEAR-SENSITIVE LIPOSOMES FOR TARGETED DRUG DELIVERY
Till Saxer, a vital medical doctor with specialization in internal and

intensive care approached me to revolutionize the treatment of cardiovascular diseases – the leading cause of death in our society. During our discussions, I developed the idea to take advantage from the increased blood flow within the constrictions to be widened [Cardiovasc. Res. 99 (2013) 328]. Together with the organic chemist Andreas Zumbühl, we invented metastable liposomes of nanometer size that can release their cargo upon increased shear stress [Nature Nanotech. 7 (2012) 536]. Together with further partners I established a protocol to determine the lumen of healthy and constricted human arteries [Nature Protocols 9 (2014) 1401]. These anatomical data formed the basis of state-of-the-art flow simulations necessary for improving the liposomes toward clinical trials. Prerequisites for such trials are successful *in vitro* and animal studies. In close collaboration with the world-leading Hungarian specialists my team found a surprising lack of complement activation for our artificial liposomes corroborated with a series of pig experiments [Nanomedicine: NBM 12 (2016) 845]. Finally, our development resulted in nano-containers even stable at elevated body temperature [Prec. Nanomed. 1 (2018) 42, incl. cover page].

3. IMAGING HUMAN BRAIN TISSUES DOWN TO ATOMIC LEVEL

Since 1999 I have been dealing with hard X-ray tomography. To the best of my knowledge, my team was the first one able to three-dimensionally visualize hundreds of individual ganglion cells after osmium staining [J. Microsc. 234 (2009) 95 incl. cover page]. Later, by means of grating interferometry we visualized Purkinje cells without staining [J. Roy. Soc. Interface 7 (2010) 1665 and image of Research SNSF 10/2011]. Very recently, we have visualized paraffin-embedded, non-stained Purkinje cells using a lab source [NeuroImage 139 (2016) 26]. Even higher spatial resolution with sub-cellular features including dendrites, we have reached this year as well [Sci. Rep. 6 (2016) 32156]. Very recently, we published pioneering results on nano-holotomography and reached a spatial resolution beyond the optical limit, i.e. below 100 nm [Adv. Sci. (2018) 1700694, incl. frontispiece], which is at least a factor of three better in each of the three orthogonal directions than histology using optical micrographs. These three-dimensional methods that we developed will have a huge impact on the pathological analysis of human tissues. Related measurements by means of spatially resolved small-angle X-ray scattering using synchrotron radiation at the cSAXS beamline of the Swiss Light Source, Villigen, Switzerland demonstrate true molecular resolution of oriented periodic structures such as the myelin sheaths. These high-resolution brain imaging data quantitatively decode the neurodegenerative diseases.

RECENT PUBLICATIONS

- G. Rodgers, G. Schulz, H. Deyhle, W. Kuo, C. Rau, T. Weitkamp, B. Müller; Optimizing contrast and spatial resolution in hard X-ray tomography of medically relevant tissues; Applied Physics Letters 116 (2020) 023702
- M. Buscema, S. Hieber, G. Schulz, H. Deyhle, A. Hipp, F. Beckmann, J. A. Lobrinus, T. Saxer, B. Müller ; Ex vivo evaluation of an atherosclerotic human coronary artery via histology and high-resolution hard X-ray tomography; Scientific Reports 9 (2019) 14348;
- S. Matviykiv, H. Deyhle, J. Kohlbrecher, F. Neuhaus, A. Zumbuehl, B. Müller; Small-angle neutron scattering study of temperature-induced structural changes in liposomes; Langmuir 35 (2019) 11210-11216
- M. Buscema, H. Deyhle, T. Pfohl, A. Zumbuehl, B. Müller; Spatially resolved small-angle X-ray scattering for characterising mechano-responsive liposomes using microfluidics; Materials Today BIO 1 (2019) 100003
- C. Bikis, G. Rodgers, H. Deyhle, P. Thalmann, A. Hipp, F. Beckmann, T. Weitkamp, S. Theocharis, C. Rau, G. Schulz, B. Müller ; Sensitivity comparison of absorption and grating-based phase tomography of paraffin-embedded human brain tissue ; Applied Physics Letters 114 (2019) 083702

André Nel

Distinguished Professor of Medicine
Chief, Division of NanoMedicine
Department of Medicine UCLA
Research Director California NanoSystems Institute

Dr. Nel is a Distinguished Professor of Medicine at UCLA, where he has successfully established a large federally-funded nanotechnology research program. The team science efforts he has put together as Research Director of the California Nanosystems Institute is spearheading nanomedicine translation on the UCLA campus. Professor Nel is a recipient of the Harry Truman Award and received the 2013 California Governor's Environmental Economic Leadership Award. He plays national leadership roles in science, biomedical research, nanotechnology and policy. He was a member of the US Bilateral Presidential Commission for technology cooperation with Russia, and served on Pres. Obama's PCAST panel for planning NNI technological innovation and commercialization. Dr Nel represented the NIH in a cooperative research agreement with the Chinese Academy of Sciences, in which he was elected as Honorary Foreign Professor. Dr. Nel is peer selected as one of the Best Doctors in America and received the John Salvaggio Award for outstanding service to the American Academy of Allergy and Immunology. He has been included as a Highly Cited Scientist (top 1% in chemistry) by Clarivate Analytics. He is a productive inventor, with numerous patents, some of which have contributed to the launching of two startup companies

RECENT PUBLICATION

- Liu X, Jiang J, Chan R, et al. Improved Efficacy and Reduced Toxicity Using a Custom-Designed Irinotecan-Delivering Silicasome for Orthotopic Colon Cancer. ACS Nano. 2019;13(1):38–53. doi:10.1021/acsnano.8b06164
- Khademhosseini A, Nel AE, Bunje H, et al. Nanoscience and Nanotechnology at UCLA. ACS Nano. 2019;13(6):6127–6129. doi:10.1021/acsnano.9b04680
- Poole JA, Barnes CS, Demain JG, et al. Impact of weather and climate change with indoor and outdoor air quality in asthma: A Work Group Report of the AAAAI Environmental Exposure and Respiratory Health Committee. J Allergy Clin Immunol. 2019;143(5):1702–1710. doi:10.1016/j.jaci.2019.02.018
- Zhao Y, Bai C, Brinker CJ, et al. Nano as a Rosetta Stone: The Global Roles and Opportunities for Nanoscience and Nanotechnology. ACS Nano. 2019;13(10):10853–10855. doi:10.1021/acsnano.9b08042
- Liu Q, Wang X, Liu X, et al. Use of Polymeric Nanoparticle Platform Targeting the Liver To Induce Treg-Mediated Antigen-Specific Immune Tolerance in a Pulmonary Allergen Sensitization Model. ACS Nano. 2019;13(4):4778–4794. doi:10.1021/acsnano.9b01444

Julien Nicolas

CNRS Director of Research (equiv. full Professor) at Univ. Paris-Sud
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CNRS Director of Research (equiv. full Professor) **since 2016**
Institut Galien Paris-Sud, UMR CNRS 8612, Univ. Paris-Sud.

HABILITATION

2013

"From Macromolecular Synthesis of Advanced Nanoparticulate

Systems for Drug Delivery". Committee: Profs. R. S. Langer, K. Matyjaszewski, J. M. J. Fréchet, P. Couvreur, E. Fattal, B. Charleux, S. Lecommandoux, J.-C. Leroux

CNRS researcher 2007

Institut Galien Paris-Sud, UMR 8612, Faculté de Pharmacie de Châtenay-Malabry, Univ. Paris-Sud. CNRS researcher 1st class since 2011

Postdoctoral fellowship 2006

Department of Chemistry, Univ. of Warwick, UK (w/ Pr. D. M. Haddleton). Marie-Curie IEF grant

PhD in Chemistry and Physical Chemistry of Polymers 2005

Laboratory of Polymer Chemistry, Univ. Pierre and Marie Curie, Paris (w/ Pr. B. Charleux). "Nitroxide-Mediated Polymerization in Aqueous Dispersed Media: Use of SG1-based, Water-Soluble Alkoxyamines". Industrial funding (Arkema)

Master 2 in Chemistry and Physical Chemistry of Polymers. 2001–2002

Laboratory of Polymer Chemistry, Univ. Pierre and Marie Curie, Paris

Master's degree in Chemistry and Chemical Engineering ("Ingénieur") 1998–2001

Ecole Supérieure de Chimie Organique et Minérale (ESCOM), Cergy-Pontoise

RESEARCH TOPICS

1. Orthogonal functionalization of biodegradable nanoparticles for drug delivery

- Development of 3rd generation nanocarriers (i.e., biodegradable, stealth and targeted) made of poly(alkyl cyanoacrylate) against cancer and Alzheimer's disease
- Synthesis of functionalized poly(lactic acid) and polypeptide nanoparticles by means of 'click chemistry' for application in oncology and coagulation disorders

2. Polymer prodrugs for cancer therapy

- Development of multifunctional squalene-based prodrug nanoparticles
- Design of polymer prodrug nanoparticles obtained by the 'drug-initiated method'

3. Reversible deactivation radical polymerization (RDRP): mechanism and synthesis of innovative biomaterials

- Design of well-defined degradable vinyl polymers by radical ring-opening polymerization (rROP)
- Study of nitroxide-mediated polymerization (NMP) of methacrylic esters
- Development of PEG-based (co)polymers, nanoparticles and bioconjugates obtained by NMP

Jaroslav Nikolaev

Principal Scientist in Computational Biology, InterAx Biotech
Dual education – M.Sc. degrees in experimental biology and computer science. A PhD in Nuclear Magnetic Resonance spectroscopy.

After Ph.D. co-founded a company focusing on AI-driven information management in academic research.
After exiting the company worked as a Scientist at ETH Zurich – developed new approaches for Magnetic Resonance spectroscopy in Systems Biology applications.
Now is a Principal Scientist at InterAx Biotech – guiding Computational / Mathematical Biology to speed up drug discovery.

RECENT PUBLICATION

(as corresponding author):

- Systems NMR: single-sample quantification of RNA, proteins and metabolites for biomolecular network analysis. Yaroslav Nikolaev, Nina Ripin, Martin Soste, Paola Picotti, Dagmar Iber, Frédéric H-T Allain; Nat Methods 2019
- Systematic mapping of protein-metabolite interactions in central metabolism of Escherichia coli. Maren Diether †, Yaroslav Nikolaev †, Frédéric Allain, Uwe Sauer; († - equal contribution); Mol Syst Biol. 2019
- Systematic identification of protein-metabolite interactions in complex metabolite mixtures by ligand-detected Nuclear Magnetic Resonance spectroscopy. Yaroslav Nikolaev, Karl Kochanowski, Hannes Link, Uwe Sauer, Frédéric Allain; Biochemistry 2016

Lutz Nuhn

Dr. Lutz Nuhn is leading the Emmy Noether research group "Macromolecular Therapeutics" at the Max-Planck-Institute for Polymer Research (MPIP) in Mainz (Germany).
He studied biomedical chemistry at the Johannes Gutenberg-University Mainz (Germany) and received his diploma degree in 2010. In 2008/09, he practiced first research experience in the laboratories of Prof. Robert Langer (MIT, USA). For his doctoral degree he studied in the group of Prof. Rudolf Zentel, and during summer 2013 also in the group of Prof. Kazunori Kataoka (University of Tokyo, Japan). In 2014, he was awarded a PhD with distinction from Johannes Gutenberg-University Mainz. For his postdoctoral research, he moved to Belgium and worked together with Prof. Bruno De Geest and Prof. Richard Hoogenboom at Ghent University as a Feodor-Lynen fellow of the Alexander-von-Humboldt Foundation. Since summer 2017, Lutz Nuhn returned to Germany and joined the group of Tanja Weil at the MPIP as a Liebig fellow of the Fonds der Chemischen Industrie (FCI). Since 2019, he has been appointed as Emmy Noether group leader supported by the German Research Foundation (DFG).
Lutz Nuhn was a member of the Max Planck Graduate School with the Johannes Gutenberg-University Mainz (MPGC) and received scholarships from the German National Academic Foundation, the Alexander-von-Humboldt-Foundation, the Research Foundation Flanders ("Fonds Wetenschappelijk Onderzoek Vlaanderen, FWO") and the "Fonds der Chemischen Industrie" (FCI). He is currently also a project leader the Interdisciplinary Research Center "Nano-Sized Polymer Therapeuctis for Tumor Immunotherapy" (SFB 1066) in Mainz.
His is research focuses on multi-responsive and degradable polymeric nanocarriers, especially for advanced immunotherapies.

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- Lien Lybaert, Karim Vermaelen, Bruno G. De Geest, Lutz Nuhn – "Immunoengineering through Cancer Vaccines – a Personalized and Multi-step Vaccine Approach towards Precise Cancer Immunity", Journal of Controlled Release 2018, 289, 125-145.
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- Lutz Nuhn, Simon Van Herck, Andreas Best, Kim Deswarte, Maria Kokkinopoulou, Ingo Lieberwirth, Kaloian Koynov, Bart N. Lambrecht, Bruno G. De Geest – "FRET Monitoring of Intracellular Ketal Hydrolysis in Synthetic Nanoparticles", Angewandte Chemie International Edition 2018, 57, 10760-10764.
- Lutz Nuhn, Nane Vanparijs, Ans De Beuckelaer, Lien Lybaert, Glenn Verstraete, Kim Deswarte, Stephan Lienenklaus, Nikunj M.

Shukla, Alex C. D. Salyer, Bart N. Lambrecht, Johan Grooten, Sunil A. David, Stefaan De Koker, Bruno G. De Geest – "pH-Degradable Imidazoquinoline-Ligated Nanogels for Lymph Node Focused Immune Activation", Proceedings of the National Academy of Sciences 2016, 113, 8098-8103.

Magdalena Obarzanek-Fojt

PhD, Novartis Pharma AG

Magdalena holds a PhD from a University of Lausanne. She formerly worked at EMPA, St. Gallen – the Swiss Federal Laboratories for Materials Science and Technology.

During her career, she worked in multiple projects related to drug product formulation, tissue engineering, 3D in vitro systems and drug delivery systems.
After joining Novartis in 2015, she focused on cell and viral vector drug products pharmaceutical development. Magdalena contributed to multiple IND/IMPD filling including BLA filing for Kymriah, the first approved ATMPs in the US.
She is currently Drug Product Leader within Cell and Gene Technical Development at Novartis.
Magdalena is a member of APV Drug Delivery Focus Group, as an expert for ATMPs.

Andrew Owen

Professor of Pharmacology

Andrew Owen is Director of the Centre of Excellence in Long-acting Therapuetics (CELT) and Professor of Molecular and Clinical Pharmacology at the University of Liverpool, UK. He is Chair of the British Society for Nanomedicine, a Fellow of the Royal Society of Biology, a Fellow of the British Pharmacological Society, and Fellow of the Learned Society of Wales. His clinical and basic research focuses on understanding mechanisms that underpin inter-patient variability in pharmacokinetics and pharmacodynamics. In recent years a major emphasis has been to employ knowledge of these mechanisms to accelerate the translation of nanomedicine candidates to clinical applications. He has published over 220 original publications, is co-inventor of patents relating to drug delivery and is Director and CSO for Tandem Nano Ltd (www.tandemnano.com). PKTK (www.PKTK.co.uk).

RECENT PUBLICATION

- Hobson JJ, Al-Khouja A, Curley P, Meyers D, Flexner C, Siccardi M, Owen A, Meyers CF, Rannard SP. Semi-solid prodrug nanoparticles for long-acting delivery of water-soluble antiretroviral drugs within combination HIV therapies. Nat Commun. 2019 Mar 29;10(1):1413. doi: 10.1038/s41467-019-09354-z.
- Long-acting injectable atovaquone nanomedicines for malaria prophylaxis. Bakshi RP, Tatham LM, Savage AC, Tripathi AK, Mlambo G, Ippolito MM, Nenortas E, Rannard SP, Owen A, Shapiro TA. Nat Commun. 2018 Jan 22;9(1):315. doi: 10.1038/s41467-017-02603-z.
- Accelerated oral nanomedicine discovery from miniaturized screening to clinical production exemplified by paediatric HIV nanotherapies. Giardiello M, Liptrott NJ, McDonald TO, Moss D, Siccardi M, Martin P, Smith D, Gurjar R, Rannard SP, Owen A. Nat Commun. 2016 Oct 21;7:13184. doi: 10.1038/ncomms13184.
- Antiretroviral solid drug nanoparticles with enhanced oral bioavailability: production, characterization, and *in vitro-in vivo* correlation. McDonald TO, Giardiello M, Martin P, Siccardi M, Liptrott NJ, Smith D, Roberts P, Curley P, Schipani A, Khoo SH, Long J, Foster AJ, Rannard SP, Owen A. Adv Healthc Mater. 2014 Mar;3(3):400-11. doi: 10.1002/adhm.201300280.
- Strengths, weaknesses, opportunities and challenges for long acting injectable therapies: Insights for applications in HIV therapy. Owen A, Rannard S. Adv Drug Deliv Rev. 2016 Aug 1;103:144-56. doi: 10.1016/j.addr.2016.02.003.

Marisa Papaluca Amati

Prof Marisa Papaluca, MD, Regulatory Science and Innovation Adviser
Former European Medicines Agency Senior Scientific Adviser
Visiting Professor, Imperial College London School of Public Health
Expert to WHO and to the European Commission DG Research & Innovation
Member of the Precision Nanomedicine Editorial Board

Marisa, graduated MD at the State university La Sapienza in Rome (Italy) and then spent few years (1978-1994) in clinical immunology scientific research and clinical activity as internal medicine specialist in academic clinical environment. Marisa joined in 1984 the Pharmaceuticals Department of the Italian Ministry of Health as medical director pioneering regulatory science supporting innovations including the Italian database for Adverse Drug Reactions evaluation (1986), the guideline for Drugs Assessment reports (1989), the Office for Centralised Community Procedures (OCCP) to strengthen participation to both EU activities (1990) and the International Conference on Harmonisation (ICH); the first multinational scientific advice for large clinical trials in EU (1993).
Marisa joined the European Medicines Agency in October 1994. Contributing to Agency foundations she drafted and implemented the initial EMA centralised procedures and European Public Assessment Reports (EPARs). Appointed as the scientific secretary of the Biotechnology Working Party Marisa has been internationally leading EMA regulatory science in pharmacogenetics, cell and gene therapies, nanotechnology. Marisa launched in 2000 the first EU regulatory conference in Pharmacogenetics, and in 2001 "safe harbour" platforms to stimulate early dialogue with innovators and secure regulatory preparedness: the EMA's Innovation Task Force (ITF) and the Business Pipeline (https://www.ema.europa.eu/documents/leaflet/business-pipeline_en.pdf). Since 2003 Marisa led the novel expert groups on Gene and Cell therapies, in 2005 launch of the Biosimilars policy with profound impact on availability of biologicals to patients and in 2007 the Expert Group on Nanotechnology-enabled medicines and the Qualification process for novel biomarkers, today important support mechanism for innovation in medicines development (https://www.ema.europa.eu/documents/regulatory-procedural-guideline/qualification-novel-methodologies-drug-development-guidance-applicants_en.pdf).
Marisa has been involved in a number of activities at the forefront of Patient-centred and Personalised medicine and on the international landscape she worked closely with colleagues from the US FDA CBER and CDER and from the Japan PMDA. After having covered various management positions in July 2015 Marisa was appointed as the Senior Scientific Advisor of the European Medicines Agency and played a major role in the establishment of the European Innovation Offices Network (EU-IN), the methodology for the pilot EMA Regulatory Science Observatory and the elaboration of the Regulatory Science Strategy 2025.
Retired from EMA in April 2019, Marisa holds a position as Visiting Professor at Imperial College London School of Public Health, provides expert advice to the WHO and to the European Commission DG Research & Innovation and is member of the Precision Nanomedicine Editorial board.

1 Marisa acted as deputy head of sector Quality of Medicines and secretary to the Biotechnology Working Party, deputy head of sector Safety and Efficacy and Head of Specialised Disciplines Office (covering pre-clinical drug development, Environmental Risk Assessment clinical pharmacology, clinical trials methodology).
2 Data mining, first filtration, case studies impact assessment on the regulatory standards, validation via modified Delphi approach including partners and stakeholders interviews public consultation.

Donald Parsons

Dr. Donald Parsons is Vice President, Early Technical Development and Lipid Nanoparticle Process Development at Moderna Therapeutics in Norwood, Massachusetts, USA. The research of his team focuses on the fundamental process science behind the manufacture of lipid nanoparticles for mRNA delivery; and the development and scaleup of these processes. Additionally, he plays a matrix leadership role coordinating CMC activities for Moderna's early-phase clinical pipeline; as well as leading Moderna's small molecule process chemistry efforts. Prior to his tenure at Moderna, Don spent six years with BIND Therapeutics in Cambridge, Massachusetts, where he led analytical development and process chemistry functions supporting the development and clinical translation of small molecule-loaded polymeric nanoparticles as Vice President, Pharmaceutical Development. Don has extensive experience in the clinical translation of complex drug delivery systems, including process development, analytical characterization, and application of Quality by Design principles to these systems.

Giorgia Pastorin

Giorgia Pastorin received her MSc degree in Pharmaceutical Chemistry and Technology in 2000 and her Ph.D. in 2004 from the University of Trieste (Italy). She spent two years of her postDoc at the CNRS in Strasbourg (France), where she specialized on drug delivery.
She joined the National University of Singapore (NUS) in June 2006, as Assistant Professor in the Department of Pharmacy-Faculty of Science. She was promoted to Associate Professor in 2011, Assistant Head in 2014 and Deputy Head in 2016; she is currently Assistant Dean (Research) at the Faculty of Science and Director of the Pharmaceutical Innovation and Research Center in NUS.
Her main research interests include production, characterization & exploitation of cell-derived nanovesicles (CDNs) as novel biocompatible delivery systems and targeted drug delivery, through strategic functionalizations of nanomaterials and pro-drugs.
She is the Editor of two books related to drug delivery and author in more than 120 articles on internationally peer-reviewed journals including Nature Nanotechnology, Biomaterials, Angewandte etc.
For the work performed by her BioNano-Technology group in NUS, she received the Young Scientist Award (2015).

Anil Patri

Dr. Anil Patri serves as the Chair, Nanotechnology Task, and as the Director of Nanocore, National Center for Toxicological Research, US Food and Drug Administration (US FDA). Nanocore conducts nanotechnology regulatory science research to understand material characteristics, safety, and efficacy through internal research projects in collaboration with product Centers. Nanocore also offers staff training and develops consensus standards through stakeholder collaboration. Dr. Patri serves on the U.S. National Nanotechnology Initiative (NNI) NSET Subcommittee and NEHI working group for US government inter-agency coordination. He is as member of ISO TC229 and ASTM E56 to facilitate standards development and serves on editorial and scientific advisory boards.

Dan Peer

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Dan Peer is a Professor and the Director of the Laboratory of Precision NanoMedicine at Tel Aviv University (TAU). He is currently the Vice Dean for Research at the George S. Wise Faculty of Life Sciences. From 2016, He is the Chair of Tel Aviv University Cancer Biology Research Center; the biggest Cancer Center in Israel that includes 17 affiliated hospitals and from 2017, the Founding and Managing Director of the SPARK program of Translational Medicine at TAU.
Prof. Peer's work was among the first to demonstrate systemic delivery of RNA molecules using targeted nanocarriers to the immune system and he pioneered the use of RNA interference (RNAi) in immune cells. In addition, his lab was the first to show systemic, cell specific delivery of modified mRNA to cells to induce therapeutic gene expression of desired proteins within the immune system that has enormous implications in cancer and inflammation.
Prof. Peer has more than 130 pending and granted patents. Some of them have been licensed to several pharmaceutical companies and one is currently under registration (as a new drug in inflammatory bowel disease). In addition, based on his work, five spin-off companies were generated aiming to bring innovative personalized medicine into clinical practice.
Prof. Peer received more than 30 awards and honors and he serves on the scientific advisory board of more than 10 companies, and on the editorial board of more than 15 journals. He is an associate Editor of the Journal of Controlled Release. Prof. Peer is a past President of the Israeli Chapter of the Controlled Release Society, and a Past Member of the Board of the Israel Young Academy of Science.

Camille Peitsch

Ph.D., Research & IP Manager at InnoMedica

Grew up in the Swiss French Alps near les Diablerets. She studied molecular biology with a specification in structural biology at the University of Basel. After that, she performed a PhD at the Institute of Anatomy at the University of Bern in Benoît Zuber's Group where she developed a method to study membrane fusion during calcium-dependent exocytosis at a structural level. In order to tackle the very fast process of exocytosis, she used cutting edge techniques in the field of correlative light and electron microscopy more specifically using Cryo Transmission Electron Microscopy and Tomography. Towards the end of the PhD she participated in the foundation of the personalized teaching company: Teachy GmbH. Since then she has been member of its advisory board. Right after finalizing her PhD early 2017 she started to work at InnoMedica as a R&D scientist providing cryo-TEM images of InnoMedica's liposomal formulations and working on its innovative pipeline. More specifically she focused on further developing Talineuren whose discovery had just been made. Together with patent lawyers she could secure patentable content for the liposomal formulation crossing the blood brain barrier and support patent application by the end of 2017. Since then she has been Talineuren's product manager while still working on the other pipeline products such as TaliTrace and TaliTaxel. In 2019 Camille Peitsch took the role as Research and IP Manager while continuously pursuing her tasks as a Talineuren's product manager.

Guotao Peng

Guotao Peng, Ph.D.
Division of Molecular Toxicology,
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I received my B.Sc. in Environmental Science from Sichuan University (China). I obtained my Ph.D. in 2017 at Fudan University (China) focusing on the mechanistic understanding of toxic cyanobacterial blooms, integrating the research at University of Tennessee (USA) as a visiting Ph.D. student.
From March 2017 to February 2019, I worked as a postdoctoral fellow with Prof. Sijie Lin at Tongji University (China) focusing on environmental health and safety aspects of nanomaterials. My research has mainly involved the use of zebrafish as an *in vivo* model for various nanotoxicological studies. Specifically, I have examined the toxicities of metal oxide nanoparticles using different developmental stages of zebrafish and demonstrated that the zebrafish larval skin could be used as an analogous model of animal lung for inflammation studies (Differential Effects of Metal Oxide Nanoparticles on Zebrafish Embryos and Developing Larvae. Environ. Sci. Nano 2018, 5, 1200-1207). In addition to the study concerning the environmental implications of nanomaterials, I have also been collaborating with Dr. Pu Chun Ke from Monash University in Australia, to explore the biomedical potential of functional nanoparticles against amyloid diseases and establish zebrafish as an alternative amyloidogenesis model to the conventional mouse models that are no longer sufficient (Inhibition of Alzheimer's-like Pathogenesis in Zebrafish with A Chaperone-Gold Nanoparticle Dual Strategy. Nat Commun. 2019, 10, 3780). From March 2019, I work as a postdoctoral fellow with Prof. Bengt Fadeel at Karolinska Institutet in Stockholm (Sweden) in the frame of the GRAPHENE Flagship project. My research focuses on immune interactions of graphene-related materials using *in vitro* and *in vivo* models.
In 2018, I won a research grant from the China Postdoctoral Science Foundation focusing on using zebrafish for nanotoxicology studies. In the same year, I was awarded the best oral presentation for junior researcher in the 9th International Nanomedicine Conference held in Sydney, Australia.

Jai Prakash

Professor (Adjunct)

Jai Prakash is heading the group of Targeted Therapeutics and Nanomedicine at the Technical Medical Centre, University of Twente, The Netherlands. His research focus is to design novel targeted (nano) therapeutics against fibrotic diseases and fibrotic cancers.
He is a pharmaceutical and entrepreneurial scientist with a strong background in developing novel targeted therapeutics against fibrosis and tumor stroma. His research is highly multi-/inter-disciplinary integrating peptide technology, nanomedicine, biology of cancer and fibrosis, as well as bioengineering fields. He obtained PhD (cum laude) in 2006 from University of Groningen (Netherlands) in the field of drug targeting to treat renal fibrosis under the supervision of Prof. Klaas Poelstra. Thereafter, he worked as a Vice President – Preclinical Research at BiOrion Technologies with a joint position at the University of Groningen. During this period, he developed several products, which are being translated by BiOrion. Besides that, he co-supervised PhD students on the topics related to cell-specific targeting to liver fibrosis. In 2011, he joined the group of Prof. Arne Ostman atKarolinska Institutet in Stockholm (Sweden) as Forskarassistent (Assistant Professor) in the Department of Oncology-Pathology. In 2012, he was appointed as a Tenure track Assistant Professor at University of Twente to set up his new research line. He was then promoted to Associate Professor in 2015 and then to Adjunct Professor in 2018. In 2019, he joined the School of Engineering and Applied Sciences at Harvard University as a visiting professor for his sabbaticals. He has published >75 peer-reviewed publications and (co)-inventor on 5 international patents. Based on his invention, he founded ScarTec Therapeutics BV., a spin-off company, focusing on the development of novel therapeutic technologies against skin scars and cancers with high stroma.

RECENT PUBLICATION

- Kuninty, P. R., Bansal, R., S., D. G., Mardhian, D. F., Schnittert, J., van Baarlen, J., Storm, G., Bijlsma, M., van Laarhoven, Metselaar, J. M., Kuppen, P. J. K., Vahrmeijer, A., Ostman, A., Sier, C. F. M., Prakash J. (2019) ITGA5 inhibition in pancreatic stellate cells attenuates desmoplasia and potentiates efficacy of chemotherapy in pancreatic cancer. Science Advances Sep 4;5(9):eaax2770. doi: 10.1126/sciadv.aax2770.
- Heinrich MA, Bansal R, Lammers T, Zhang YS, Schiffelers RM, Prakash J. (2019) 3D-Bioprinted Mini-Brain: A Glioblastoma Model to Study cellular interactions and therapeutics. Advanced Materials. 31(14):e1806590. doi: 10.1002/adma.201806590.
- Mardhian D.F., Vrynas A, Storm G, Bansal R, Prakash J. (2020) FGF2 engineered SPIONs attenuate tumor stroma and potentiate the effect of chemotherapy in pancreatic 3D heterospheroidal models. Nanotheranostics 1;4(1):26-39.
- Schnittert J, Bansal R, van Baarlen J, Ostman A, Prakash J. (2019) Integrin alpha11 in pancreatic stellate cells regulates tumor stroma interaction in pancreatic cancer. FASEB J May;33(5):6609-6621.
- Mardhian D, Storm G, Bansal R, Prakash J. (2018) Nano-targeted relaxin impairs fibrosis and tumor growth in pancreatic cancer and improves the efficacy of gemcitabine *in vivo*. J Control Release. 28;290:1-10

Adriele Prina-Mello

Dr Adriele Prina-Mello, is the James Ussher Assistant Professor in Translational Nanomedicine and LBCAM director at Trinity Translational Medicine Institute. Associate Director of Research at the School of Medicine, Trinity College Dublin.
Focused on the translation nanomedicines and medical devices for cancer, with a particular interest in theranostics treatment and medical technologies. Key research is focused on identifying and developing new nanotechnology-enable medical products for clinical translation. Core Expert Team of the European Nanomedicine Characterisation Laboratory (EUNCL), Key Expert of the REFINE project aimed at developing a Regulatory Science Framework for Nanomedicine and Core partner in the Open-Innovation-Test-Bed (OITB) Safety Testing In The Life Cycle Of Nanotechnology-enabled Medical Technologies for Health (SAFE-N-MEDTECH). Active partner in several large H2020 projects such as EXPERT and BIORIMA project and more recently INNOV4COV.
Dr Prina-Mello is extensively involved in several educational training and innovation initiatives at national and international level.

Benjamin Rengstl

Head of Immunoreceptor Therapy,
BioNTech Cell & Gene Therapies GmbH

Dr. Rengstl develops cell- and RNA-based immunotherapies and further takes technology agnostic approaches to create new strategies for controlled modulation of the immune system. Dr. Rengstl began specializing in this area during his postdoctoral training at the Medical School of Goethe University Frankfurt, where he developed chimeric antigen receptor (CAR)-engineered T-cell therapies against lymphomas and got trained in clinical pathology. In 2017, he joined BioNTech SE located in Mainz to develop a clinical CAR-T candidate for treatment of solid tumors. To improve CAR-T therapy, his team pioneered an *in vivo* expansion concept based on a liposomally formulated RNA vaccine (CARVac) for systemic delivery of CAR antigen. A FIH clinical trial assessing BioNTech´s novel CLDN6-CAR in combination with CARVac will be opended this year. Furthermore, Dr Rengstl is leading programs on next generation cell & gene therapies that besides oncology focus on infectious disease.

RECENT PUBLICATION

- Reinhard K1*, Rengstl B1*, Oehm P1*, Michel K1, Billmeier A1, Hayduk N1, Klein O1, Kuna K1, Ouchan Y1, Wöll S1, Christ E1, Weber D2, Suchan M2, Bukur T2, Birtel M1, Jahndel V1, Mroz K1, Hobohm K1, Kranz L1, Diken M2, Kühlcke K1, Türeci Ö1#, Sahin U1,2,3#. An RNA vaccine drives expansion and efficacy of claudin-CAR-T cells against solid tumors. Science. 2020 Jan 24;367(6476):446-453. doi: 10.1126/science.aay5967. Epub 2020 Jan 2.

Bernd Riebesehl

Dr. Bernd Riebesehl is Senior Technical Project Leader in the Pharmaceutical Development Parenteral, Topical, Ophthalmic Dosage Forms of Novartis Pharma AG, Basel, Switzerland since 2008.

At Novartis he is leading the Global Pharmaceutical Innovation Committee and the early technical development of several parenteral drug products. Externally Dr. Riebesehl has been serving as Advisory Board Member of the European Society of Clinical Nanomedicine, and chaired drug delivery sessions for the Section Drug Delivery of International Association for Pharmaceutical Technology (APV).
He completed his thesis in Pharmaceutical Technology at the Technical University of Braunschweig. 1992 he started his industrial career at Lilly Forschung GmbH in Hamburg leading several teams for preformulation, early phase development and formulation development. In his role as Research Advisor in Pharmaceutical R&D he led several initiatives enabling the formulation of poorly soluble drugs. In 2007 he became Director of Pharmaceutical Development at Speedel Experimenta AG, Basel.

Cristianne Rijcken

Dr. Cristianne Rijcken is the founder of Cristal Therapeutics, and serves as Chief Scientific Officer of the company. Dr. Rijcken's PhD thesis provided a strong basis for Cristal Therapeutics and she was awarded multiple grants and prizes including the Simon Stevin Gezel Award in 2008 and the Knowledge for Growth Inspiring Young Scientist Award in 2014. She is (co-) author of ~ 40 scientific publications and co-inventor of all patents and patent applications of Cristal Therapeutics. Cristianne is selected as Limburg Businesswoman of the Year 2017 because of her innovative mind-set, the perseverance upon translational activities and her entrepreneurial attitude. Dr. Rijcken is pharmacist by training and holds a PhD degree in Pharmaceutics from Utrecht University (The Netherlands).

Matthias Rösslein

Matthias W. Rösslein (born 30. April 1962) has studied chemistry at the University of Basel from 1981 to 1985. Here, at the Institute of Physical Chemistry, he also got his PhD degree with "Summa cum laude" in 1989. Afterward he spent 22 months as a Postdoc at the University of Chicago. From 1991 to 1995 he was then an assistant professor at the Physical-Chemical Institute of the University Zürich. Since 1996 Matthias Rösslein has a permanent position at Empa, Swiss Laboratories for Materials Science and Technology. In 2006 he was appointed a position as 'Senior Scientist'. Thenceforward he specialized as one of the experts in 'evaluation of measurement uncertainty and metrology'. First the main focus of his work was on applying these principles in analytical chemistry. Also, in 2006 he joined the Empa laboratory for "Particles-Biology Interaction" focusing on two major subjects: the standardization of *in vitro* assays to elucidate the effect of nanoparticles on different cell types as well as next generation sequencing in a very close collaboration with Marc Salit and his group at NIST. Since 2008 he has the status as a foreign guest researcher at NIST with regular visits and par-

ticipations in a number of joined projects. He was awarded in 2016 with a "MML Accolades 2016 Associate". Most of his projects focus on the standardization of basic measurements techniques used for *in vitro* assays.
As part of the H2020 project – EUNCL he has supervised in close collaboration with Dr. Jennifer Grossmann of the NCI-NCL the methods and assays transfer from NCI-NCL to the EUNCL in 2015 and parts of 2016. He evaluated and verified the entire validation of the transfer of the basic NCI-NCL characterisation cascade. Since then he monitors the performance of the contributing partner laboratories and reviews all relevant work-related documents. Furthermore, he is a permanent member of the core expert team of the EUNCL. The goal of this project is the preclinical safety assessment of novel nano-medication before go into clinical trials. This translational research involves physical and chemical assessment of the nano-medication, which is the flowed by *in vitro* haematology, immunology and toxicology evaluation. This is followed by an *in vivo* toxicology verification. Within this highly complex interdisciplinary team-work Matthias Rösslein is responsible for the quality and correctness of all the results listed in the final report. The most recent H2020 project – Refine, where he leads a work package focus on the regulatory framework of nanobiomaterials.

Barbara Rothen-Rutishauser

Prof. Dr. Barbara Rothen-Rutishauser has received her Ph.D. in 1996 in cell biology at the Swiss Federal Institute of Technology (ETH) in Zurich. From 1996 to 2000 she held a post-doctoral position in Biopharmacy at the Institute of Pharmaceutical Sciences at the ETH and in 2000 she joined Prof. Peter Gehr's research group at the University of Bern, Switzerland as a postdoc. After promotion to group leader in 2006 she completed her habilitation in cell biology in 2009. Prof. B. Rothen-Rutishauser is an expert in the field of cell-nanoparticle interactions in the lung, with a special focus on 3D lung cell models. Since 2011 she is the co-chair in BioNanomaterials at the Adolphe Merkle Institute, University of Fribourg, Switzerland, the position is shared equally with Prof. Alke Fink. The research group's activities stretch over many fields from material synthesis and characterization to biological responses and hazard assessment. Prof. Rothen-Rutishauser has published more than 270 peer-reviewed papers and is an associate editor of the journal "Particle and Fibre Toxicology". In 2013 the Swiss National Science Foundation (SNSF) awarded a National Competence Centre of Research (NCCR) on Bio-Inspired Materials to the UNIFR, which includes Prof. Rothen-Rutishauser, as a module and project leader. Prof. Rothen-Rutishauser also serves as a Faculty Delegate for Women and Young Researchers in this NCCR.

Kirsten Sandvig

Prof. Kirsten Sandvig is associated with Dept. of Biosciences, University of Oslo, Norway and she is heading a research group in Department of Molecular Cell Biology, Institute for Cancer Research, The Norwegian Radium Hospital, Oslo University Hospital. The Norwegian Radium Hospital is the main cancer hospital in Norway. Sandvig group, counting ~15 members from different countries, is interested in the mechanisms of endocytosis, intracellular transport and secretion. In some of our studies we are using protein toxins such as ricin and Shiga toxin, which are well established as markers for studies of membrane traffic, and which can be used as agents in cancer diagnosis and therapy. Our expertise is also applied to investigate uptake of nanoparticles, and we obtained a large grant (Biodegradable nanoparticles in cancer diagnosis and therapy) from the Norwegian Research Council to build national competence in nanomedicine (running to spring 2019). This project involved collaboration between 10 Norwegian research groups covering synthesis of nanoparticles, *in vitro* and *in vivo* biology studies, *in vivo* imaging and clinical studies. In addition, international collaboration is included. The Sandvig group was also involved in an INNO INDIGO granted project, which lasted until autumn 2019. INNO INDIGO is an innovation-driven initiative for the development and integration of Indian and European research. The Sandvig group has future support to study nanoparticles *in vitro* and *in vivo* from the Norwegian Cancer Society (2020-2022). We also characterize exosomes from prostate cancer cells and prostate cancer patients with the goal of detecting biomarkers. Our research spans all the way from basic to translational medicine, including innovation. We aim at providing a rational basis for diagnosis, treatment and prevention of disease. The group has extensive national and international collaboration.
EDUCATION: M.Sci. from The Technical University of Norway, Trondheim; Ph.D. from the Medical Faculty, University of Oslo, Norway. Research visits abroad at University of Michigan and at the biological laboratories, Harvard Cambridge, Mass. USA.
SCIENTIFIC ACTIVITY: Published more than 340 articles and supervised a large number of Ph.D. students and master students. Sandvig has been invited as plenary speaker at more than 100 international meetings, and the work is heavily cited, Hirsch index is 73.
AWARDS AND HONOURS: Anders Jahres Medical Prize for young researchers, 1989 (first woman to receive this prize); The Norwegian Research Councils research prize, 1990; Member of the Norwegian Academy of Science and Letters, 1993; Stiansens Biomedical Research Prize, 1995; King Olav V´s Cancer Research Prize, 1998; Member of EMBO (European Molecular Biology Organization), 1998; Member of Academia Europea from 2002; Honorary Doctor at the University of Copenhagen, Denmark, 2007; Member of the American Academy of Microbiology, 2010; The Fridjof Nansen Award for outstanding research in science and medicine, 2014; Oslo University Hospital Prize for excellent research, 2017.

Hélder A. Santos

Full Professor
Drug Research Program, Division of Pharmaceutical Chemistry and Technology, Faculty of Pharmacy, and Helsinki Institute of Life Science, University of Helsinki, Finland

Prof. Santos (D.Sc. Tech., Chem. Eng.; www.helsinki.fi/~hsantos) is a Full Professor in Pharmaceutical Nanotechnology, Head of the Nanomedicines and Biomedical Engineering Group, Director of the Doctoral Program in Drug Research at the University of Helsinki, and the Director of FinPharmaNet in Finland. He is also a Fellow Member of the Helsinki Institute of Life Science (HiLIFE), and former World Portuguese Network Adviser for Science. Prof. Santos research interests include the development of nanoparticles/nanomedicines for biomedical applications. Prof. Santos is co-author of more than 300 publications (+9500 citations; h-index = 58), 34 book chapters and more than 258 conference proceedings/abstracts. He has given over 160 invited talks at prestigious conferences, workshops, universities, general public events, etc., around the world. Prof. Santos has received a number of prestigious awards and grants, such as the "Talent Prize in Science" attributed by the Portuguese Government in 2010, the European Research Council Starting Grant in 2013 and ERC Proof-of-Concept in 2018, the Young Researcher Award in 2013 attributed by the Faculty of Pharmacy at the University of Helsinki, the Academy of Finland Award for Social Impact in 2016, and honor nomination for the USERN Prize in Biological Sciences in 2017.

RECENT PUBLICATION

- Manlio Fusciello, Flavia Fontana, Siri Tähtinen, Cristian Capasso, Sara Feola, Beatriz Martins, Jacopo Chiaro, Karita Peltonen, Leena Ylösmäki, Erkko Ylösmäki, Firas Hamdan, Otto K. Kari, Joseph Ndika, Harri Alenius, Arto Urtti, Jouni T. Hirvonen, Hélder A. Santos*, Vincenzo Cerullo*, "Artificially Cloaked Viral Nanovaccine for Cancer Immunotherapy", Nature Commun. 2019, in press.
- Tuying Yong, Xiaoqiong Zhang, Nana Bie, Hongbo Zhang, Xuting Zhang, Fuying Li, Abdul Hakeem, Jun Hu, Lu Gan*, Hélder A. Santos*, Xiangliang Yang*, "Tumor Exosome-Based Nanoparticles are Efficient Drug Carriers for Chemotherapy", Nature Commun. 2019, 10(1), 3838.
- Hongbo Zhang, Wenguo Cui, Xiangmeng Qu, Huayin Wu, Liangliang Qu, Xu Zhang, Ermei Mäkilä, Jarno Salonen, Yue-Qi Zhu*, Zhou Yang, Dong Chen, Hélder A. Santos, Mingtan Hai*, David A. Weitz*, "Photothermal Responsive Nanosized Hybrid Polymersome as Versatile Therapeutics Co-Delivery Nanovehicle for Effective Tumor Suppression", Proc. Natl. Acad. Sci. U.S.A. 2019, 116(16) 7744–7749.
- Flavia Fontana, Manlio Fusciello, Christianne Groeneveldt, Cristian Capasso, Jacopo Chiaro, Sara Feola, Zehua Liu, Ermei M. Mäkilä, Jarno J. Salonen, Jouni T. Hirvonen, Vincenzo Cerullo*, Hélder A. Santos*, "Biohybrid Vaccines for Improved Treatment of Aggressive Melanoma with Checkpoint Inhibitor", ACS Nano 2019, 13(6), 6477–6490.
- Zehua Liu, Yunzhan Li, Wei Li, Chen Xiao, Dongfei Liu, Chao Dong, Ming Zhang, Ermei Mäkilä, Marianna Kemell, Jarno Salonen, Jouni T. Hirvonen, Hongbo Zhang*, Dawang Zhou*, Xianming Deng*, Hélder A. Santos*, "Multifunctional Nanohybrid Based on Porous Silicon Nanoparticles, Gold Nanoparticles and Acetalated Dextran for Liver Regeneration and Acute Liver Failure Theranostics", Adv. Mater. 2018, 30(24), 1703393.

Bruno Sarmento

Bruno Sarmento is Principal Investigator and Group Leader of the Nanomedicines and Translational Drug Delivery group at Institute of Biomedical Engineering/Institute for Investigation and Innovation in Health (INEB/i3S), University of Porto, Portugal and Assistant Professor at IUCS/CESPU.

His current research is focused on the development of functionalized nanomedicines and materials and their application in the pharmaceutical and biomedical fields. In particular, he is interested in the establishment of nanoformulations of advanced functional biomaterials and understand their interaction with cells and biological surfaces, with interest in diabetes, cancer and infectious diseases. He has also specialized in mucosal tissue engineering models to validate functionalized nanomedicines and to perform *in vitro/in vivo* correlation.

He published more than 350 papers in international peer reviewed (ISI) journals (H index 50), 54 book chapters and more than 350 proceedings. He edited 5 books in the field of nanomedicine, biomaterials and biological barriers.

Bruno Sarmento was the Chair of the Nanomedicines and Nanoscale Drug Delivery Focus Group of the Controlled Release Society, and is member of the Board of the Spanish-Portugal Local Chapter of the Controlled release Society. He is editor of European Journal of Pharmaceutical Sciences, and member of the Editorial Advisory Board of 11 international journals, including the Journal of Controlled Release, Pharmaceutics and Expert Opinion on Drug Delivery. He has acted as referee for top-ranked journals in his area of expertise, and for international funding agencies: FNRS (BE), Inserm (FR), CNCS (RO), FCT (PT), Foncyt (ARG), CNPq (BR) and European Commission.

Email Id: bruno.sarmento@ineb.up.pt

Mater. 2018, 30(24), 1703393.

Raymond Schiffelers

Professor of nanomedicine
University Medical Center Utrecht
Utrecht, The Netherlands
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Raymond Schiffelers studied Bio-Pharmaceutical Sciences at Leiden University (1990-1995). After an industrial traineeship at SmithKline Beecham Pharmaceuticals (UK) he did his PhD in medical microbiology at Erasmus University Rotterdam on liposomal targeting of antimicrobial agents (1996-2001). Subsequently he became post-doc at Utrecht University working on liposomes targeting tumor vasculature. In 2002-2003, at Intradigm Co (USA) he expanded his tumor vasculature-targeting work with polymers for delivery of siRNA. After his return to Utrecht University he became assistant and then associate professor. He received an ERC Consolidator Grant in 2010 to investigate extracellular vesicles as biological drug delivery systems. After he moved to the Laboratory for Clinical Chemistry & Hematology of the University Medical Center Utrecht in 2011 he became professor of nanomedicine working on bio-inspired and synthetic drug delivery systems. He coordinates two H2020 projects on this topic, B-SMART and EXPERT, is editor for the International Journal of Pharmaceutics, Journal of Controlled Release and Journal of Extracellular Vesicles, and is founder of EXCYTEX-an extracellular vesicle-based company.

Avi Schroeder

Avi Schroeder is an Associate Professor of Chemical Engineering at the Technion – Israel Institute of Technology where he heads the Laboratory for Targeted Drug Delivery and Personalized Medicine Technologies (https://www.schroederlab.com/).

Dr. Schroeder conducted his Postdoctoral studies at the Massachusetts Institute of Technology, and his PhD jointly at the Hebrew and Ben Gurion Universities.

Avi is the recipient of more than 30 national and international awards, including named a KAVLI Fellow, the Intel Nanotechnology-, TEVA Pharmaceuticals-, and the Wolf Foundation Krill Awards. Avi is the author of more than 50 research papers inventor of 19 patents and co-founder of several startup companies based on these discoveries.

Schroeder is a member of Israel Young National Academy of Sciences, and the President of the Israel Institute of Chemical Engineers.

Stefan Schulz

Stefan Schulz, MD, has been a full professor at the Medical University of Graz since 2010. He is also a lecturer at the University of Freiburg and holds a part-time role as head of medical research projects at the German start-up company Averbis GmbH.

After his doctoral thesis in theoretical medicine and two years of clinical practice he specialized in Medical Informatics (2000). His research has focused on biomedical terminologies, ontologies, electronic health records and technologies for medical language processing using artificial intelligence methods. In Graz he constituted a research group, which

played a leading role in several EU projects (SemanticHealthNet, SEMCARE, ASSESS-CT, PRECISE4Q) and hosted international events (ICBO 2012, FOIS 2012, JOWO 2019). Within the Austrian CBmed Biomarker research center, Stefan Schulz initiated in 2016 a large international consortium with a strong industry focus, which aims at information extraction from clinical routine data. Stefan Schulz has contributed to standards development at SNOMED International and at the WHO (ICD, ICF). He is author of over 250 peer-reviewed publications and has received several awards.

Simó Schwartz

Director CIBBIM-Nanomedicine (Molecular Biology and Biochemistry Research Center for Nanomedicine). Barcelona Hospital Campus. Passeig de la Vall d'Hebron, 119-129 - 08035 Barcelona, Spain

Simó Schwartz Jr, MD, PhD, (M) is the Director of CIBBIM-Nanomedicine (www.cibbim.eu), a research center focused on new biomedical nanotechnology-based applications. Member of the Science Advisory Board of the Vall d'Hebron Research Institute (VHIR) and recently appointed Director Assistant in Basic Research. He was appointed as member of the Advisory Board of NANOCAN, Southern Denmark University, and deputy director of CIBER-BBN, a center from the Spanish Health Institute Carlos III (ISCIII) which gathers a total of 50 research groups, of national excellence in the field of nanotechnology and nanomedicine. Dr Schwartz was also Co-founder and Science Advisor of ARGON Pharma SL, and former member of the editorial Board of the journals Nanomedicine-NBM and Eur. J. Nanomedicine. External science advisor of the European Nanomedicine Characterization Laboratory (EU-NCL) has acted as science consultant of SOM BIOTECH and CELGENE. Dr Schwartz Jr holds 16 patents, most transferred to leading companies of the biotech and pharma sectors and coauthors more than 90 papers in high impact factor journals. Recently appointed as editor of Precision Nanomedicine journal, he also acts as coordinator and partner of several research projects directly related with the production and validation of therapeutic drug delivery systems. Among them, international and EU projects involving SME's and large Pharma companies. Acting as coordinator for technology transfer at CIBER-BBN, he was recently appointed as President of the European Society of Nanomedicine and Executive Board member of the International Society of Nanomedicine.

RECENT PUBLICATION

- Montero S, Seras-Franzoso J, Andrade F, Martinez-Trucharte F, Vilar-Hernández M, Quesada M, Xandri H, Arango D, Abasolo I, Rafael D, Schwartz S Jr. Intracellular Delivery of Anti-SMC2 Antibodies against Cancer Stem Cells. Pharmaceutics. 2020 Feb 21;12(2). pii: E185. doi: 10.3390/pharmaceutics12020185. PMID:32098204
- Pesarrodona M, Sánchez-García L, Seras-Franzoso J, Sánchez-Chardi A, Baltá-Foix R, Cámara-Sánchez P, Gener P, Jara JJ, Pulido D, Serna N, Schwartz S Jr, Royo M, Villaverde A, Abasolo I, Vazquez E. Engineering a Nanostructured Nucleolin-Binding Peptide for Intracellular Drug Delivery in Triple-Negative Breast Cancer Stem Cells. ACS Appl Mater Interfaces. 2020 Feb 5;12(5):5381-5388. doi: 10.1021/acsami.9b15803. Epub 2020 Jan 21. PMID:31840972
- Pesarrodona M, Jauset T, Díaz-Riascos ZV, Sánchez-Chardi A, Beaulieu ME, Seras-Franzoso J, Sánchez-García L, Baltà-Foix R, Mancilla S, Fernández Y, Rinas U, Schwartz S Jr, Soucek L, Villaverde A, Abasolo I, Vázquez E. Targeting Antitumoral Proteins to Breast Cancer by Local Administration of Functional Inclusion Bodies. Adv Sci (Weinh). 2019 Jul 24;6(18):1900849. doi: 10.1002/advs.201900849. eCollection 2019 Sep 18. PMID:31559131
- Giannotti MI, Abasolo I, Oliva M, Andrade F, García-Aranda N, Melgarejo M, Pulido D, Corchero JL, Fernández Y, Villaverde A, Royo M, García-Parajo MF, Sanz F, Schwartz S Jr. Highly Versatile Polyelectrolyte Complexes for Improving the Enzyme Replacement Therapy of Lysosomal Storage Disorders. ACS Appl Mater Interfaces. 2016 Oct 5;8(39):25741-25752. Epub 2016 Sep 22. PMID:27610822
- Cabrera I, Abasolo I, Corchero JL, Elizondo E, Gil PR, Moreno E, Faraudo J, Sala S, Bueno D, González-Mira E, Rivas M, Melgarejo M, Pulido D, Albericio F, Royo M, Villaverde A, García-Parajo MF, Schwartz S Jr, Ventosa N, Veciana J. α-Galactosidase-A Loaded-Nanoliposomes with Enhanced Enzymatic Activity and Intracellular Penetration. Adv Healthc Mater. 2016 Apr 6;5(7):829-40. doi: 10.1002/adhm.201500746. Epub 2016 Feb 18. PMID:26890358

Christopher Scott

Professor Chris Scott is currently Director of the Centre for Cancer Research and Cell Biology at Queen's University Belfast. Following a primary degree in Biochemistry he undertook a PhD and post doctoral training at Queen's in molecular enzymology. In 2001, Chris was one of the founding members of QUB spinout company Fusion Antibodies Ltd., before returning to Queen's in 2003 to take up an academic position. Chris is internationally renowned for his work in development of antibody and nanomedicine-based therapies for the treatment of cancer and other conditions. Work in his laboratory is funded by agencies such as Medical Research Council, HSCNI, and various industrial sources such as AstraZeneca and Immunocore. He also held a Royal Society Industrial Fellowship with GSK from 2012-15, and won the Vice Chancellor's Prize for Innovation in 2015 with his group's work on developing a novel nanomedicine for the treatment of sepsis and other inflammatory conditions. Chris is actively involved in nanomedicine across the UK and is a Trustee of the British Society of Nanomedicine.

SELECTED PUBLICATIONS

- Development of an advanced nanoformulation for the intracellular delivery of a caspase-3 selective activity-based probe. Cogo F, Poreba M, Rut W, Groborz K, Smyth P, Johnston MC, Williams R, Longley DB, Burden RE, Salvesen GS, Drag M, Scott CJ. Nanoscale. 2019 Jan 3;11(2):742-751.
- Clearance of intracellular Klebsiella pneumoniae infection using gentamicin-loaded nanoparticles. Jiang L, Greene MK, Insua JL, Sa Pessoa J, Small DM, Smyth P, McCann A, Cogo F, Bengoechea J, Taggart CT, Scott CJ. J Controlled Release 2018 Jun 10;279:316-325.
- Forming next-generation antibody–nanoparticle conjugates through the oriented installation of non-engineered antibody fragments. Greene MK, Richards DA, Nogueira IJ, Campbell K, Smyth P, Fernández M, Scott CJ, Chudasama V. Chemical Science, 2018,9, 79-87
- Targeting Siglecs with a sialic acid-decorated nanoparticle abrogates inflammation (2015). Spence S, Greene MK, Fay F, Hams E, Saunders SP, Hamid U, Fitzgerald M, Beck J, Bains BK, Smyth P, Themistou E, Small DM, Schmid D, O'Kane CM, Fitzgerald DC, Abdelghany SM, Johnston JA, Fallon PG, Burrows JF, McAuley DF, Kissenpfennig A, Scott CJ. Sci Transl Med. 2015 Sep 2;7(303):303ra140. doi: 10.1126/scitranslmed.aab3459.
- Oriented attachment of VNAR proteins, via site-selective modification, on PLGA-PEG nanoparticles enhances nanoconjugate performance. Nogueira JCF, Greene M, Richards DA, Furby AO, Steven J, Porter A, Barelle C, Scott CJ, Chudasama V. 2019. Chem. Commun., 2019,55, 7671-7674

Varda Shalev

Prof. Varda Shalev, MD MPH, is the head of the KSM Kahn - Sagol - Maccabi Research & Innovation Institute, and a faculty member at the Tel-Aviv University School of Public Health (TAU SPH). Side by side to her senior level roles, Prof. Shalev is an active primary care physician in Maccabi Healthcare Services (MHS) sick fund.

With an MD degree from Ben-Gurion University Medical School, she completed her residency in family medicine and earned an MPA in Health Public Administration at Clark University. After a two-year fellowship in medical informatics at the Johns Hopkins University Hospital, Prof. Shalev established the Medical Informatics Department at Maccabi and was responsible for planning and developing its computerized systems encompassing data from two million members and 9000 care providers. She has pioneered the development of multiple disease registries to support chronic disease management. Prior to her current position, Prof. Shalev has served as the director of Primary Care Division at MHS and implemented several structural reforms in the provision of care.

Prof. Shalev's research interests are epidemiology, medical informatics and predictive analytics. She is a member of the European Health Telematics Association and the American Medical Informatics Association. Prof. Shalev teaches regularly at the TAU SPH graduate school in the areas of big-data and medical informatics. She has authored or co-authored over 200 publications in peer-reviewed journals

Daniel J Siegwart

Associate Professor, University of Texas Southwestern Medical Center

Dr. Daniel J. Siegwart is an Associate Professor in Department of Biochemistry (primary) and the Simmons Comprehensive Cancer Center (secondary) at UT Southwestern Medical Center. He received a B.S. in Biochemistry from Lehigh University (2003), and a Ph.D. in Chemistry from Carnegie Mellon University (2008) with University Professor Krzysztof Matyjaszewski. He also studied as a Research Fellow at the University of Tokyo with Professor Kazunori Kataoka (2006). He then completed a Postdoctoral Fellowship at MIT with Institute Professor Robert Langer and Professor Daniel G. Anderson (2008-2012). The central goal of the Siegwart Lab is to use materials chemistry to solve challenges in disease therapy and diagnosis. An array of coding and non-coding RNAs can now be used as therapeutics (siRNA, miRNA, tRNA, mRNA, CRISPR RNAs) because they are able to manipulate and edit expression of the essential genes that drive disease development and progression. Although great advances have been made in the delivery of short RNAs, the ideal chemical and formulation composition is largely unknown for longer RNA cargo. The Siegwart Lab aims to discover and define the critical physical and chemical properties of synthetic carriers required for therapeutic delivery of small (e.g. ~22 base pair miRNA) to large (e.g. ~5,000 nucleotide mRNA) RNAs. The Siegwart lab has a major focus on CRISPR/Cas gene correction of genetic diseases. Their research is grounded in chemical design and takes advantage of the unique opportunities for collaborative research at UT Southwestern.

RECENT PUBLICATION

- "Dendrimer-based lipid nanoparticles deliver therapeutic FAH mRNA to normalize liver function and extend survival in a mouse model of Hepatorenal Tyrosinemia Type I." Qiang Cheng, Tuo Wei, Yuemeng Jia, Lukas Farbiak, Kejin Zhou, Shuyuan Zhang, Yonglong Wei, Hao Zhu, and Daniel J. Siegwart.* Advanced Materials, 2018, 30, 1805308.
- "High-contrast fluorescence detection of metastatic breast cancer including bone and liver micrometastases via size-controlled pH-activatable water-soluble probes." Hu Xiong, Hao Zuo, Yunfeng Yan, Gino Occhialini, Kejin Zhou, Yihong Wan, and Daniel J. Siegwart* Advanced Materials, 2017, 29, 1700131.
- "Non-viral CRISPR/Cas gene editing *in vitro* and *in vivo* enabled by synthetic nanoparticle co-delivery of Cas9 mRNA and sgRNA." Jason B. Miller, Shuyuan Zhang, Petra Kos, Hu Xiong, Kejin Zhou, Sofya S. Perelman, Hao Zhu, and Daniel J. Siegwart* Angewandte Chemie International Edition, 2017, 56, 1059-1063.
- "Functional polyesters enable selective siRNA delivery to lung cancer over matched normal cells." Yunfeng Yan, Li Liu, Hu Xiong, Jason B. Miller, Kejin Zhou, Petra Kos, Kenneth E. Huffman, Sussana Elkassih, John W. Norman, Ryan Carstens, James Kim, John D. Minna, and Daniel J. Siegwart.* Proceedings of the National Academy of Sciences, U.S.A., 2016, 113, E5702–E5710.
- "Modular degradable dendrimers enable small RNAs to extend survival in an aggressive liver cancer model." Kejin Zhou, Liem H. Nguyen, Jason B. Miller, Yunfeng Yan, Petra Kos, Hu Xiong, Lin Li, Jing Hao, Jonathan T. Minnig, Hao Zhu, and Daniel J. Siegwart.* Proceedings of the National Academy of Sciences, U.S.A., 2016, 113, 520-525.

Dmitri Simberg

Dr. Simberg received his Ph.D. in Biochemistry at the Hebrew University of Jerusalem, Israel. His thesis in the laboratory of Professor Chezy Barenholz was on biochemical and biophysical mechanisms of lipofection using cationic lipids *in vitro* and *in vivo*. After receiving the PhD, he did a 2-year postdoctoral study on tumor targeting of iron oxide nanoparticles in the laboratory of Professor Erkki Ruoslahti at the Burnham Institute, La Jolla in California. This was followed by a 1-year postdoctoral research at the Department of Radiology, University of California San Diego. Between 2008-2013 Dr. Simberg was a project scientist at the Center for Cancer Nanotechnology Excellence at UCSD, where he developed his research program in nano-bio interface and immune recognition of nanomaterials. In 2013, Dr. Simberg joined the faculty of the Skaggs School of Pharmacy, University of Colorado. Dr. Simberg is the corresponding author or coauthor of over 75 research papers, reviews, perspectives and book chapters, and a recipient of funding totaling over $10M. His current research interests are focused on the development of nanoparticles and cells for drug delivery and imaging, mechanisms of complement activation by nanomedicines, and mechanisms of accumulation of drug carriers in the skin and tumors.

Gert Storm

Full professor
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Gert Storm, professor Targeted Drug Delivery at the Department of Pharmaceutics (80% employment), obtained his Ph.D. degree in 1987 at the Utrecht University. In 1988-1989, he was a visiting scientist at Liposome Technology Inc. in Menlo Park, USA, and visiting assistant professor at the School of Pharmacy, UCSF, San Francisco. In 1990-1991, he became senior research scientist at Pharma Bio-Research Consultancy B.V. in Zuidlaren, The Netherlands. During this

period he contributed to the design, co-ordination and evaluation of clinical pharmacological studies. In September 1991 he took up his position at the Utrecht University. He is honorary professor (Biomacromolecular Drug Delivery) at the University of Copenhagen. From 2012 on, he is also professor (Targeted Therapeutics) at the MIRA institute of the University of Twente (20% employment). Furthermore, he keeps a position (seconded) at the University Medical Center Utrecht (UMCU) (Division Imaging).

RESEARCH

The design, characterization and (pre)clinical testing of targeted nanomedicine formulations is the core activity. Additionally, the implementation of imaging-guided drug delivery protocols (using e.g. SPECT, MRI, PET) is a major research objective. Over the last decade, clinical translation of academic results has become a vital element of Prof. Storm's ambition, as exemplified by the clinical development of liposomal corticosteroids (by the creation of the spin-off company Enceladus Pharmaceuticals BV in 2005), the clinical breast cancer study at the UMCU evaluating image-guided targeted doxorubicin delivery with hyperthermia (follow-up of the HIFU-CHEM project), his involvement in public-private partnership projects, i.e. EC (coordinator Meditrans (FP6), partnering in 4 FP7 projects and 2 Horizon2020 projects), CTMM (PI HIFU-CHEM), NanoNextNL (director Program Drug Delivery), TI-Pharma and AgentschapNL. Over the years, he also obtained significant funding for applied research from STW and several large industries (e.g. Astellas, Astra Zeneca, Novartis). He is on the Board of the CLINAM (European Foundation for Clinical Nanomedicine) organization in Basel, and the PRC (Phospholipid Research Center) in Heidelberg. Form 2014 on, he is every year included in the lists of The World's Most Influential Scientific Minds of Thomson Reuters (Highly Cited Researchers) and Clarivate Analytics.

Tore Skotland

Senior scientist

Tore Skotland is a biochemist by training and received his PhD from the University of Bergen, Norway in 1980. After 11 years at the university studying protein chemistry and enzymology, he moved to pharmaceutical R&D (Nycomed AS, Oslo, Norway) in 1983. He stayed within the same field of research for 26 years in one of the world leading companies developing contrast agents for medical imaging; Nycomed was bought by Amersham in 1997 and Amersham was bought by GE Healthcare in 2003. During the last 20 years in pharmaceutical R&D he was heading work to describe the biodistribution, metabolism and excretion of all types of contrast agents (water soluble as well as particle based) for CT, MRI, ultrasound, SPECT, PET and optical imaging. He has been involved in bringing 5 products to the marked (including 2 particle-based) and another 5 products into clinical trials (also including 2 particle-based). Skotland is the first or last author of publications related to all these 10 products. He is co-author of more than 120 publications and is used as referee for many journals in the field of bioanalysis, metabolism, biochemistry, nanomedicine and contrast agents for medical imaging.

Skotland is since 2009 a senior researcher at the Department of Molecular Biology at The Norwegian Radium Hospital, the main cancer hospital in Norway, being part of Oslo University Hospital. He is there a member of a group studying exosomes, endocytosis and intracellular transport of protein toxins and nanoparticles. This group was heading a 5-year national competence building project in Norway ending in April 2019. The project title was "Biodegradable nanoparticles for cancer diagnosis and therapy". Skotland was co-ordinating the *in vivo* studies in this project, which had members from academia, university hospitals, research institutes and pharmaceutical industry. The 10 groups involved have expertise in nanoparticle syntheses and characterization, *in vitro* studies of cellular uptake and intracellular transport, immunology studies, and studies using small animals with xenograft models, including use of different *in vivo* imaging modalities such as MRI, PET/CT and fluorescence. Clinicians were also involved.

MOST IMPORTANT PUBLICATIONS IN THE FIELD OF NANOPARTICLE RESEARCH:

- Skotland T, Iversen TG, Sandvig K: New metal-based nanoparticles for intravenous use: requirements for clinical success with focus on medical imaging. Nanomedicine: NBM 6 (2010) 730-737.
- Iversen TG, Skotland T, Sandvig K: Endocytosis and intracellular transport of nanoparticles: Present knowledge and need for future studies. Nano Today 6 (2011) 176-185.
- Skotland T, Iversen TG, Sandvig K: Development of nanoparticles for clinical use. Nanomedicine (Future Medicine) 9 (2014) 1295-1299.
- Skotland T: Injection of nanoparticles into cloven-hoof animals: Asking for trouble. Theranostics 7 (2017) 4877-4878.
- Szwed M et al.: Small variations in nanoparticle structure dictate differential stress responses and mode of cell death. Nanotoxicology 13 (2019) 9-18.

Alejandro Sosnik

Prof. Alejandro Sosnik received his Pharmacy degree from the Faculty of Pharmacy and Biochemistry of the University of Buenos Aires in 1994, carrying out his professional stage in the pharmacy of a public hospital and volunteering afterwards in the pharmacy of a public pediatric hospital in Buenos Aires for more than one year. During his undergraduate studies, he was also teaching assistant in analytical chemistry and organic chemistry. After two years as junior research scholar of the University of Buenos Aires in the field of organic chemistry (1993-5), he worked as research pharmacist in the Department of Chemistry of the Argentine regulatory agency (equivalent to the US-FDA), a dependency of the Ministry of Health of Argentina (1996). In early 1997, he emigrated to Israel where after obtaining the pharmacist license, he continued his graduate studies, receiving M.Sc. (equivalency, 1998) and Ph.D. degrees in applied chemistry (polymeric biomaterials) from the Casali Institute of Applied Chemistry (The Hebrew University of Jerusalem, Israel, 2003) under the supervision of Prof. Daniel Cohn. In 2003-6, Prof. Sosnik spent a postdoctoral in the laboratory of Professor Michael Sefton (Institute of Chemical Engineering and Applied Chemistry/ Institute of Biomaterials and Biomedical Engineering, University of Toronto, Canada) working in the development of hybrid matrices for cell culture and tissue engineering. Between 2006 and 2013, Prof. Sosnik was Assistant Professor (tenure) of Pharmaceutical Technology at the Faculty of Pharmacy and Biochemistry (University of Buenos Aires) and Investigator of the National Science Research Council of Argentina (CONICET, tenure). In this period, he established a research group that worked at the interface of drug crystallization and processing, biomaterials science, nanotechnology and microtechnology, drug delivery and therapeutics. In this context, he supervised three junior staff scientists (CONICET), five postdocs (CONICET) and four Ph.D. theses at the Faculty of Pharmacy and Biochemistry of the University of Buenos Aires. Prof. Sosnik established the "Ibero-American Network of New Materials for the Design of Advanced Drug Delivery Systems in Diseases of High Socioeconomic Impact" (RIMADEL) of the CYTED Program that gathered eleven research groups and companies of Spain, Portugal, Mexico, Cuba, Colombia, Brazil and Argentina and over 75 scientists and served as its international coordinator in the period 2011-2013. He also served as advisor of several Argentine pharmaceutical companies in scientific, technical and intellectual property issues. Owing to its multidisciplinary background and expertise at

the interface of drug research and development and polymeric biomaterials, in 2014, Prof. Sosnik was appointed Associate Professor of the Department of Materials Science and Engineering of Technion-Israel Institute of Technology where he founded the Laboratory of Pharmaceutical Nanomaterials Science and heads the BSc program in Materials Engineering and Biology. He currently supervises one postdoc, nine graduate students and six undergraduate students. In the last two years, he was awarded the Marie Reintegration Grant of the European Commission for the period 2014-2018 and other competitive Israeli and European research grants. His current research lines comprise drug self-assembly and crystallization phenomena and processing, polymer and macromolecular chemistry, biomaterials science, colloidal chemistry (drug and polymer self-assembly), mucoadhesive drug delivery systems, nanomedicine (drug encapsulation, release and targeting), therapy of poverty-related diseases (HIV, tuberculosis), pediatric cancer, intestinal diseases and pharmacokinetics (oral, inhalatory and intranasal administration routes) in both preclinical and clinical trials. he has served and serves as evaluator for more than twenty national and international research funding agencies and universities. Prof. Sosnik is co-author of over 150 peer-reviewed articles, reviews, editorials and book chapters in areas of biomaterials sciences, tissue engineering, pharmaceutical research and development and innovation, drug delivery and co-inventor several patents and patent applications related to biomedical and pharmaceutical innovation.

Georgios A Sotiriou

Dr. Georgios A. Sotiriou is an Assistant Professor in the Department of Microbiology, Tumor and Cell Biology (MTC) at Karolinska Institutet (KI). He directs the Bionanomaterial Technology Laboratory in MTC that is located in the BioClinicum Research Building at the Karolinska University Hospital, promoting interdisciplinary collaborations and guaranteeing the clinical relevance of the medical challenges that his lab aims to address. The key expertise of his lab lies on flame aerosol engineering of smart nanoscale materials and devices aiming superior performance in biomedical applications. The main target of his research program is to address societal and clinical needs by developing the next generation of nano-enabled molecular diagnostic and therapeutic (theranostic) systems towards their employment in precision nanomedicine. The focus lies on nanoparticle-biomolecule conjugates for their integration in functional systems exploiting both the responsive properties of nanomaterials in the presence of target analytes but also external stimuli, acting as transducer elements towards the diagnosis and on-demand therapeutic interventions. The systematic approach for the investigation of the theranostic capabilities of smart nanostructured materials provides knowledge and insight into the fundamental physicochemical and molecular processes assisting in rapid translation into clinics. Dr. Sotiriou received a Diploma in Applied Physics (2006) from the National Technical University of Athens, Greece and he continued his postgraduate studies at ETH Zurich, Switzerland where he received a MSc in Micro- and Nanosystems (2008) and later on his PhD from the Particle Technology Laboratory (2011). He carried out postdoctoral research stays in Harvard University (2013-2015, Center for Nanotechnology and Nanotoxicology) and ETH Zurich (2015-2016, Drug Formulation and Delivery Lab) before joining KI. His research has been recognized internationally by several awards including the 2011 AIChE Bionanotechnology Graduate Student award, the 2012 Best PhD Thesis Award from the Swiss Chemical Society, the 2012 ETH Medal for Outstanding Dissertation, the 2013 Hilti Award for Innovative Research, the ERC Starting Grant in 2017, the Young Faculty Award from the Mayo Clinic in 2018, the Young Investigator Award from MTC at KI in 2019 and his appointment as Future Research Leader from the Swedish Foundatin for Strategic Research (SSF) in 2020.

Hulda Swai Shaidi

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A goal-oriented and results-driven professional who has a proven ability to independently formulate, initiate and execute research projects and implement scientific research programmes, both in public and privat e sectors. Experience in sourcing funds both locally and internationally with a remarkable track record in initiating and maintaining collaborations, networks, synergies, exchange programs and sabbaticals. Display astounding work ethics. Exhibits excellent leadership/ managerial, strategic, organizational, interpersonal and communication skills. Able to work as an individual and /or part of a team. Renowned speaker in both local and international forums. Received several honours, prizes and excellence awards for achievement s. resourceful, highly organised and accomplished.

PUBLICATION

- L Kalombo, H Swai, B Semete, L Katata, P Chelule. Nanoparticle carriers for drug administration and process for producing the same. Patent Application Ref: PCT/IB2008/000012)
- B Semete, M Chuene, and H Swai, RNA aptamers specific to the macrophage mannose receptor for targeted delivery of therapeutics or diagnostic agents to macrophage or dendritic cells. (Invent ion Disclosure Ref: “PA152171-P Aptamer targeted drug delivery”)
- Dual application of Mycolic acids: (a) as ligand onto anti-tuberculosis drug-loaded nanoparticles for targeting infected sites and (b) use of antibody immunosorbent in TB serological diagnostic assay (in preparation)
- L Kalombo, J R Zeevaart, H Swai. Solid lipid nanoparticle radiopharmaceutical compositions
- L Kalombo, P Nkuna, P Melariri, H Swai. Microemulsion formulation and method of manufacture

Janos Szebeni

János Szebeni, MD, PhD, DSc, Med. Habil., immunologist, co-founder and CEO of SeroScience Ltd. is also director of the Nanomedicine Research and Education Center at Semmelweis University in Budapest and full professor of immune biology at Miskolc University in Miskolc, Hungary. He has held various guest professor and scientific positions in Hungary and the United States where he lived for 22 years. In the US he worked, among others, at the University of Arizona, NCI/NIH, Walter Reed Army Institute of Research, Harvard University. His research on various themes in hematology, membrane biology and immunology resulted in over 200 publications including peer-reviewed papers and book chapters, and also 3 patents. He is sole or co-editor of the books “The Complement System: Novel Roles in Health and Disease” (Kluwer, 2004) and “Im-

mune Aspects of Biopharmaceuticals and Nanomedicines" (Pan Stanford Series on Nanomedicine Vol. 3, 2018). H-index: 46, citations >8568. He was primary investigator in more than a dozen European and Hungarian research grants and over 30 CRO projects over the past 15 years. Dr. Szebeni is a regular speaker at international conferences and seminars with over 60 presentations in the past five years. He is also an ad hoc consultant for the United States Food and Drug Administration (FDA). Three fields stand out where he has been most active: artificial blood, liposomes and the complement system. His original work led to the CARPA concept, i.e., that complement activation underlies numerous drug-induced (pseudo)allergic infusion reactions. CARPA has been included in a recent European Medicines Agency (EMA) guideline as a recommended preclinical safety test.

Wouter Tonnis Müllers

Wouter Tonnis studied Pharmacy and did his PhD in Pharmaceutical Technology at the University of Groningen, The Netherlands. In 2014 he joined Janssen in the role of Scientist working on Formulation Development of Vaccines. Afterwards, he joined Bayer in 2017 as Pharmaceutical Technology Scout looking for external formulation and drug delivery technologies fitting to internal needs

Enza Torino

Enza Torino graduated in Chemical Engineering from the University of Salerno (Italy) in 2006. Lifetime goal of her research interests has always been obtaining of nanostructures and the exploitation of their fascinating properties. Since her bachelor degree, she worked on carbon nanotubes to increase polymer strength and later on, during her master degree, she used the thermodynamics to improve the characteristic of the nanoparticles in the pharmaceutical field (size, shape, and charge). Dr. Torino gained a Ph.D. in Chemical Engineering on the development of novel technologies for nanoparticle production. She addressed to the study, characterization, and development of new processes and materials, at University of Salerno (ITALY) – Supervisor Prof Ernesto Reverchon- Thesis Title: Nanoparticles Production by SUPERCRITICAL-CO2- Her last ten years of research have always been devoted to the nanotechnologies in the medical field. Starting from her background in chemical engineering, she was involved in a project for the pharmaceutical industry in Switzerland to design a process to increase the bioavailability of several drugs and later spent part of her Ph.D. to study how nanoparticles can be modified using surfactants to enhance their delivery properties in a biological environment. Indeed, during her PhD Enza Torino also worked as visiting scientist at University of Texas at Austin – Texas (USA), studying "Research on Colloidal systems: emulsion and microemulsion formation and stability for pharmaceutical and energy applications – supervisors Prof. Keith P. Johnston – and she was also involved in a Collaboration project on EOR (Enhanced Oil Recovery) supported by Dow Chemicals and Petroleum and Chemical Engineering Department at UT at Austin (TX). After her Ph.D., she worked as Guest Scientist at the "School in Advanced Optical Technologies" (SAOT) established at the University of Erlangen-Nuremberg - Department of Chemical and Bioengineering within the framework of the Excellence Initiative of the German Federal and State Governments. Here, she studied the mechanism of precipitation involved on in drug nanoparticles production by Supercritical Antisolvent technique using on situ laser diagnostic technique and the Control and manipulation of pharmaceutical emulsions to produce nanospheres or nanocapsules by Microfluidics. From 2010 to 2016 she worked as Post Doc Researcher at Italian Institute of Technology – Center for Advanced Biomaterials for Health Care- coordinated by Prof. Paolo Antonio Netti – at Theranostic Engineered Nanoshuttle (TeNs) Platform, where she designs new processes to obtain novel polymer-based engineered nanoshuttles for *in vivo* application in diagnostic and therapy. She is currently working as a researcher at the University of Naples "Federico II" at the Department of Chemical, Materials and Production Engineering on the design of multimodal imaging nanoparticles for theranostics and responsible of the Master Degree course on "Diagnostic Devices and Drug Delivery". She is also leading 3 spin-off and start up projects in Life Sciences based on patented technologies.Currently, Enza Torino is also Principal Investigator of the project "Theranostic nanoparticles based approach targeting a set of microRNAs in drug resistant thyroid and breast cancers" financed by PRIN - Research Projects of National Relevance -MIUR - Ministry of Education University and Research.

Katherine Tyner

Associate Director for Science (acting)

Dr. Katherine Tyner is the Associate Director of Science in the Immediate Office of the Office of Pharmaceutical Quality (OPQ), Center for Drug Evaluation and Research at the United States Food and Drug Administration (FDA). As Associate Director, Dr. Tyner leads the OPQ Science Staff in coordinating the intersection between science, review, and policy in OPQ as well as facilitating interactions between other CDER offices and FDA Centers. She received her PhD in Chemistry from Cornell University and joined the Food and Drug Administration in 2007 as a chemist specializing in nanotechnology. While at the FDA, Dr. Tyner has investigated the quality, safety, and efficacy of complex drug products including drug products containing nanomaterials, and she currently leads the CDER nanotechnology working group and is active in other CDER and FDA nanotechnology initiatives. Dr. Tyner is the author of multiple book chapters and journal articles concerning the appropriate characterization and biological impact of nanoparticle therapeutics and other complex products.

RECENT PUBLICATION

- D'Mello S…Tyner KM*. FDA/CDER analysis of drug products containing nanomaterials, Nature Nanotechnology 12(6) 523-529 (2017).
- Tyner KM… Cruz CN. How Has CDER Prepared for the Nano Revolution? A Review of Risk Assessment, Regulatory Research, and Guidance Activities. AAPS J. 19(4) 1071-1083 (2017).

Mark Van Eldijk

Group leader Nanomedicines

Mark van Eldijk is a group leader and project manager at Ardena API & Nanomedicine Development and Manufacturing. In 2016, he joined ChemConnection, which is now part of Ardena. Together with his team, he is responsible for development and manufacture of nanomedicine products, including polymeric nanoparticles and iron oxide nanoparticles. Often these nanomedicine products consist of complex nanoparticles functionalized with

biomolecules and/or APIs. Before joining Ardena, Mark was a post-doctoral research fellow at California Institute of Technology. He obtained a PhD in Bio-organic Chemistry from Radboud University Nijmegen, where he worked on the design and preparation of protein-based nanoparticles. He has published more than 20 papers in peer-reviewed journals.

RECENT PUBLICATIONS

- B.M. Babin, L. Atangcho, M.B. van Eldijk, M.J. Sweredoski, A. Moradian, S. Hess, T. Tolker-Nielsen, D.K. Newman, D.A. Tirrell, Selective Proteomic Analysis of Antibiotic-Tolerant Cellular Subpopulations in Pseudomonas aeruginosa Biofilms, mBio, 2017, 8, e01593-17
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Peter van Hoogevest

Head Scientific Department, Lipoid GmbH, Ludwigshafen am Rhein, Germany

Peter van Hoogevest, is a pharmacist by training (Utrecht University in The Netherlands), who got his PhD degree in biochemistry 1984 at the Utrecht University in The Netherlands. In 1994 he received the degree of Privat Dozent (adjunct professor) in pharmacy at the University of Basel, Switzerland.

His industrial career started at the Biovet Group of the Animal Health Division of Ciba-Geigy Ltd. (Basel) in 1984. Shortly thereafter he obtained a position at the Novel Dosage Form Department of Pharmaceutical Development of the Pharmaceuticals Division of Ciba-Geigy Ltd. After having several positions at this department at Ciba Ltd. and Novartis Ltd. he founded in 1998 together with colleagues of the Pharmaceutical Development Department and reputed industrial managers and scientists the company ADD Advanced Drug Delivery Technologies (Muttenz, CH) and became CEO of this company and was member of the Board of Directors. In 2000 he joined Phares Drug Delivery AG (Muttenz, CH), a company specialized in the delivery of poorly water soluble drug substances, as Managing Director and COO and member of the Board of Directors. Since 2012 he is Managing Director of the Phospholipid Research Center, Heidelberg and Head of the Scientific Department (including the Development Department) of Lipoid GmbH, Ludwigshafen am Rhein, Germany.

His drug delivery expertise especially in the (phospho)lipid research and development area is underscored by 75 scientific publications, including 8 book chapters, 33 symposium posters, co-promotion of 48 PhD Theses, 13 patents and 45 patent applications.

RECENT PUBLICATIONS

- Van Hoogevest. P., Luciani, P., Recent Advances in the Use of Phospholipid Excipients in Local or Injectable Depot Formulations, Pharm. Ind. (2018), 8, 1104.
- Van Hoogevest, P., Fahr, A., Phospholipids in Cosmetic Carriers, In: "Nanoscience and Cosmetics", Eds. J. Cornier, C.M. Keck, M van de Voorde, Springer Nature Switzerland AG (2019)
- Crommelin, D.J.A., van Hoogevest, P., Storm, G., The role of liposomes in clinical nanomedicine development. What now? Now what? J. Control. Release, (2020), 318, 256-263.

Maria Vicent

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Dr. María J. Vicent received her Ph.D. degree in 2001 in chemistry after her research on solid supports from the Universitat Jaume I (Castellon, Spain) after several scientific stays in the laboratory of Prof. Fréchet's lab. at the University California (Berkeley, USA). María then moved into more biomedically-oriented research, initially with the Spanish company Instituto Biomar S.A., and subsequently at the Centre for Polymer Therapeutics at the University of Cardiff (UK) with Prof. R. Duncan after receiving a Marie Curie Postdoctoral Fellowship in 2002. In 2004, María joined the Centro de Investigación Príncipe Felipe (CIPF, Valencia, Spain) as a research associate through a Marie Curie Reintegration contract and was promoted to her current position as the head of the Polymer Therapeutics Laboratory at CIPF in 2006. María is currently responsible for the Screening Platform one of the Specialist Sites in the EU-OPENSCREEN European Research Infrastructure Consortium (ERIC) and coordinates the Advanced Therapies Program at the CIPF.

María's research group focuses on the development of novel nanopharmaceuticals for different therapeutic and diagnostic applications - in particular the application of Polymer Therapeutics in unmet clinical needs. María has been funded by both national and European grants (several acting as coordinator, including an ERC Consolidator grant-MyNano and ERC-PoC-POLYIMMUNE, Fund Health La Caixa-NanoPanTher) from academia as well as industry. María has received several prizes, including the IVth and the IXth Idea Awards, and she has been elected as member of American Institute for Medical and Biological Engineering (AIMBE) College of Fellows Class of 2019. María has co-authored >115 peer-reviewed papers and ten patents. Three patents have been licensed to the pharmaceutical industry and a third used as the foundation for the founding of the spin-off company 'Polypeptide Therapeutic Solutions S.L.' (Valencia, Spain) in 2012. María was the President of the SPLC-CRS up to 2013 and the chairperson in key conferences in the nanomedicine field, such as the International Symposium on Polymer Therapeutics and the annual Controlled Release Society meeting in 2019. María is also the executive editor of Adv. Drug Deliv Rev, the associate editor of NanoMedicine: NBM and DDTR, and a member of the editorial boards of key journal in the field including, J. Control Rel., Polymer Chemistry, Biomaterial Sciences, and Mol. Pharmaceutics.

Viola Vogel

Laboratory of Applied Mechanobiology, Institute of Translational Medicine, Department of Health Sciences and Technology, ETH Zurich, Switzerland

Viola Vogel is Professor of Applied Mechanobiology in the Department of Health Sciences and Technology (D-HEST) at the ETH Zurich and chaired D-HEST from 2018-2020. She holds a PhD in Physics from the University of Frankfurt (1987) and conducted her

research at the Max-Planck Institute for Biophysical Chemistry in Göttingen (1980-88) for which she received the Otto-Hahn Medal (1988). After her postdoctoral studies in the Department of Physics at UC Berkeley in nonlinear optics, she started her academic career at the University of Washington Seattle in Bioengineering (1990-2004) and was the founding Director of the Center for Nanotechnology (1997-2003). When moving to ETH Zurich in 2004, she initially joined the Department of Materials and then co-founded D-HEST (2012). With her background in Physics and Bioengineering, she pioneered the rapidly growing field of Mechanobiology and its medical applications, as she discovered many structural mechanisms how mechanical forces can turn proteins into mechano-chemical switches. Such mechanisms are exploited by bacteria, as well as by mammalian cells and tissues to sense and respond to mechanical forces, and if abnormal, can cause various diseases. Her research was recognized by major awards, including an ERC Advanced Grant on "Proteins as Mechano-Chemical Switches" (2008-13), the International Solvay Chair in Chemistry Brussels 2012. She serves on various international advisory boards in the fields of nanotechnology and bioengineering, including on the White House panel that finalized the US National Nanotechnology Initiative under the Clinton administration (1999), as well as for the Max-Planck Society, A*STAR and CREATE in Singapore and the Wyss Institute, Boston. She was awarded an Honorary Doctor of Philosophy from Tampere University, Finland (2012), she served on the Board of Regents of the Ludwig Maximilian University in Munich (2011-19), on the Board of Trustees of the Gordon Research Conference Organisation since 2018, and is an Einstein Fellow at the Charité Berlin since 2017. She is an elected member of the National Academy of Engineering USA (NAE), the National German Academy Leopoldina since 2018 and of the Berlin-Brandenburg Academy of Sciences since 2019. She is Member of the Jury of the Queen Elizabeth Prize for Engineering since 2014.

Matthias Wacker

Matthias G. Wacker is Associate Professor in the Department of Pharmacy of the National University of Singapore (NUS). Initially, he studied Pharmacy at Goethe University in Frankfurt (Germany) where he obtained his doctoral degree in pharmaceutical technology. As a post-doc and group leader he has joined Jennifer Dressman and Jörg Kreuter in the Institute of Pharmaceutical Technology, Goethe University. There he accomplished his habilitation exploring the 'Rational Formulation Design of Nanocarrier Devices' and was awarded the venia legendi in pharmaceutical technology. Prior to joining NUS, he was heading the Department of Pharmaceutical Technology and Nanosciences of the Fraunhofer-Institute for Molecular Biology and Applied Ecology in Frankfurt. Currently, he serves the European Journal of Pharmaceutics and Biopharmaceutics and the Journal of Pharmacy and Pharmacology of the Royal Pharmaceutical Society as an editorial board member. Also, he is scientific advisor to the editors of the Journal of Pharmaceutical Sciences and was guest editor for the Beilstein Journal of Nanotechnology and Frontiers in Chemistry.
In recognition of his research excellence, he was honored with the Eudragit® Best Paper Award (2014) and the Phoenix Pharmaceutics Science Award (2017). From 2020-2025, he is member of expert panel on New Advancements in In-Vitro Performance Testing of the United States Pharmacopeia.

RECENT PUBLICATIONS

- Hering I, Eilebrecht E, Parnham MJ, Günday-Türeli N, Türeli AE, Weiler M, Schäfers C, Fenske M, Wacker MG (2020); Evaluation of potential environmental toxicity of polymeric nanomaterials and surfactants, Environ. Toxicol. Pharmacol. (10.1016/j.etap.2020.103353)
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- Marques MRC, Choo Q, Ashtikar M, Rocha TC, Bremer-Hoffmann S, Wacker MG (2019); Nanomedicines – Tiny particles and big challenges, Adv. Drug Del. Rev. (10.1016/j.addr.2019.06.003)
- Jablonka L, Ashtikar M, Gao G, Jung F, Thurn M, Preuß A, Scheglmann D, Albrecht V, Röder B, Wacker MG (2019); Advanced in silico modeling explains pharmacokinetics and biodistribution of temoporfin nanocrystals in humans, J. Control. Rel. (10.1016/j.jconrel.2019.06.029)
- Feczkó T, Piiper A, Ansar S, Blixt FW, Ashtikar M, Schiffmann S, Ulshöfer T, Parnham MJ, Harel Y, Israel LL, Lellouche JP, Wacker MG (2018); Stimulating brain recovery after stroke using theranostic albumin nanocarriers loaded with nerve growth factor in combination therapy, J. Control. Rel. 293: 63-72 (10.1016/j.jconrel.2018.11.017)

Andreas Wagner

Dr Andreas Wagner is currently the Head of Liposome Technology at Polymun Scientific GmbH in Klosterneuburg, Austria. He has significant expertise in development and optimization of liposomal drug products. Over the last 15 years, his group guided approx. 15 different liposomal drug products into clinical trials. He studied Biotechnology in Vienna, Austria and earned his Master and Ph.D. degrees in the field of liposomology at the Institute of Applied Microbiology supervised by Prof. Hermann Katinger and Prof. Karola Vorauer-Uhl. Dr Andreas Wagner is listed as inventor on several patents, like the liposome technology and some product patents of liposomal formulations and he has published several peer reviewed articles dealing with liposomes, the technology, products thereof and their application in preclinical and clinical studies. Since 2001, he built up the liposome technology unit at Polymun Scientific GmbH.
Polymun Scientific is a private Austrian company, located in Klosterneuburg, offering contract development and manufacturing of biopharmaceuticals as well as development and production of liposomal formulations. Its patented liposome/LNP technology allows efficient manufacturing of constantly high quality in small and large scale. Over the last 10 years, Polymun has guided more than 15 liposomal formulations into clinical trials, amongst them DNA and different kinds of RNA formulations. Polymun is an FDA- and EMA-inspected manufacturer conducting several own R&D projects. For more information, please visit www.polymun.com

Wang Julie Tzu-Wen

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Dr Julie Wang obtained her BSc Degree in Biomedical Technology and MSc in Biomedical Engineering at the National Taiwan University in 2002 and 2004. She obtained her PhD in Photobiology in Cancer Therapeutics at the Department of Surgery and Interventional Science, University College London (Dr AJ MacRobert & Prof.

SG Bown) in 2010. Her post-doctoral research moved to the field of nanomedicine after joining UCL School of Pharmacy in 2011 and then King's College London (KCL) (PI: Prof Al-Jamal) in 2012. She is currently a senior research and teaching fellow in Nanomedicine at the Institute of Pharmaceutical Science at KCL, and also a visiting scientist at the Centre for Molecular Oncology at Queen Mary University of London. Her research focus is on the pre-clinical translation of nanomedicines for imaging, drug delivery for cancer and brain diseases. She has utilised a range of nanoparticulate systems including dendrimers, polymeric nanoparticle, liposomes and carbon nanotubes (CNTs), mostly designed for cancer and brain delivery.

RECENT PUBLICATIONS

- JT-W Wang, R Klippstein, M Martincic, E Pach, R Feldman, M Sefl, Y Michel, JK Sosabowski, M Kalbac, T Da Ros, C Ménard-Moyon, A Bianco, I Kyriakou, D Emfietzoglou, J-C Saccavini, B Ballesteros, KT Al-Jamal and G Tobias. (2019) Neutron activated samarium-153 encapsulated single- and multi-walled carbon nanotubes for *in vivo* imaging and tumour radiotherapy. ACS Nano. (In press; doi: 10.1021/acsnano.9b04898)
- FN Faruqu, JT-W Wang, L Xu, L McNickle, EM-Y Chong, A Walters, M Gurney, A Clayton, LA Smyth, R Hider, JK Sosabowski, KT Al-Jamal (2019) Membrane radiolabelling of exosomes for comparative biodistribution analysis in immunocompetent and immunodeficient mice – a novel and universal approach. Theranostics. 9(6): 1666-1682
- NO Hodgins, JT-W Wang, KT Al-Jamal. (2017) Nano-technology based carriers for nitrogen-containing bisphosphonates delivery as sensitisers of γδ T cells for anticancer immunotherapy. Advanced Drug Delivery Reviews. 114:143-160
- KT Al-Jamal, J Bai, JT-W Wang, A Protti, P Southern, L Bogart, H Heidari, X Li, A Cakebread, D Asker, WT Al-Jamal, A Shah, S Bals, J Sosabowski, QA Pankhurst. (2016) Magnetic drug targeting: Preclinical *in vivo* studies, mathematical modeling, and extrapolation to Humans. Nano Letters. 16(9):5652–5660
- J Bai*, JT-W Wang*, K-C Mei, WT Al-Jamal, KT Al-Jamal (2016) Real-time monitoring of magnetic drug targeting using fibered confocal fluorescence microscopy. Journal of Controlled Release. 244;B:240–246

Frank F. Weichold

Dr. Weichold is Senior Science Advisor for the office of the Chief Scientist and the Office of the Commissioner at the US-Food and Drug Administration. The expertise he brings to the regulatory agency builds on his ability to advance, coordinate and integrate scientific resources for FDA by addressing mission critical scientific regulatory challenges in a global environment. The FDA Centers of Excellence in Regulatory Science and Innovation (CERSI) network has been built under Dr. Weichold's leadership in collaboration with academic institutions to leverage scientific expertise, resources and capacity toward FDA's mission. He represents FDA at the Maryland Life Science Advisory Board and at the NIH National Center for Advancing Translational Sciences. He also chaired the FDA Senior Science Council and he was leading strategic partnership development and technology transfer. Health data liberation, value generation and knowledge management in the public health sector are the focus of his current work.

Dr. Weichold's experience includes execution of strategic and operational initiatives across the sciences' value chain. Dr. Weichold has led the development of international collaborations and public private partnerships for discovery and early medical product development, implemented global operating and development models, and executed large-scale business model transformations. He has accumulated more than a decade of industrial research and medical product development experience while leading teams in Clinical Pharmacology, DMPK, as a Director at MedImmune LLC, and AstraZeneca. Prior, he directed research and clinical development of vaccines at the Aeras Foundation (founded by The Bill and Melinda Gates Foundation).

As a tenured Professor in the University of Maryland system, he developed and managed independent research programs and trained graduate students. He also held faculty positions at the University of Maryland Biotechnology Institute to study signal transduction pathways that affect immune responses, as well as at the Humboldt University, Berlin (Germany) to teach and study microbial immune modulation. During the five years of postdoctoral education, Dr. Weichold worked at the National Institutes of Health in Bethesda, Maryland, first at the National Cancer Institute where he researched immune pathologies in HIV infection, then at the Hematology Branch of the National Heart Lung and Blood Institute where bone marrow pathologies, transplantation immunology and gene therapy were the focus of his clinical research studies. His medical practice and clinical experience include Infectious Diseases and Immunology/Rheumatology.

Marieluise Wippermann

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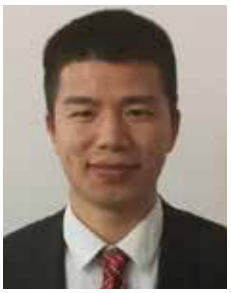
Since 2000: CEO, TECOmedical AG Switzerland
1997–2000: Managing Director, CH-Werfen Group, Spain
1988–1997: Vice President International, Nichols Institute, USA
1983–1988: Head of development and production, Eurodiagnostics, The Netherlands
1983: School of economics, Basel, Switzerland
1979–1983: Head of development and production, Bühlmann Laboratories AG, Switzerland
1976–1978: Research scientists, Institute of Biochemistry, University of Hamburg, Germany
1973–1976: Research scientists, Dep. of Internal Medicine, University of Zurich, Switzerland
1973: Degree as Chemistry Engineer



Lin Yang

Lin Yang, a PhD candidate in Helmholtz Center Munich and Technical University of Munich since October 2015. He is currently working in Dr. Otmar Schmid group (Comprehensive Pneumology Center/Institute of Lung Biology and Disease) for investigating the biodistribution, biokinetics, and bioactivity profile of pulmonary delivered drugs or novel-designed nanomedicine/nanoparticle (NM/NPs) *in vivo* for improved inhalation therapy. The research goal is to translate new advances of aerosol delivery, biological insights, and engineering breakthroughs into the biomedical and technological innovations accelerating the development of novel inhaled drugs or NM/NPs for the treatment of lung diseases. To this end, the pulmonary delivery and fate of drugs or NM/NPs in murine lungs *in vivo* has been successfully unveiled by leveraging multimodal cutting-edge imaging techniques including *in vivo* phase contrast X-ray imaging, *in vivo* imaging system (IVIS), and ex vivo tissue-cleared three-dimensional light sheet fluorescent microscopy. He received bachelor and master degrees in biotechnology (2012) and microbiology (2015) from Nanchang University. During his master period, the biodistibution, toxicokinetics, and toxicology of engineered nanoparticles/nanomedicines in adult and/or pregnant murine

animals have been conducted. Until now he has published more than 20 peer-reviewed SCI papers and 8 SCI papers as the first author in high-impact scientific journals like ACS Nano, Small, Journal of Controlled release, Nanoscale, Journal of Hazardous Materials, and Scientific Reports, etc. He has been awarded several outstanding prizes in well-renowned international conferences such as a "first prize" at 11th CLINAM conference 2018, a "poster prize" at the Nanotox 2018-9th International Conference on Nanotoxicology, and a student travel grant at Nano Today conference 2019. He has also received several scholarships such as "Graduate National Scholarship" awarded by Ministry of Education (MOE) of the People's Republic of China (PRC) in 2014, and 3 times Second-class scholarship from 2009 to 2012 awarded by Nanchang University, and was also awarded as "Outstanding postgraduates" in 2015.

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REPRESENTATIVE PUBLISHED PAPERS:

- Yang, L, Feuchtinger, A, Möller, W, et al. Three-Dimensional Quantitative Co-Mapping of Pulmonary Morphology and Nanoparticle Distribution with Cellular Resolution in Nondissected Murine Lungs [J]. ACS Nano 2019, 13 (2): 1029-1041.
- Yang, L, Gradl, R, Dierolf, M, Schmid O. et al. Multimodal precision imaging of pulmonary nanoparticle delivery in mice: Dynamics of application, spatial distribution, and dosimetry [J]. Small 2019, DOI: 10.1002/smll.201904112 .
- Yang L, Kuang H, Zhang W, et al. Quantum dots cause acute systemic toxicity in lactating rats and growth restriction of offspring[J]. Nanoscale, 2018, 10(24):11564-77.
- Yang L, Kuang H, Zhang W, et al. Comparisons of the biodistribution and toxicological examinations after repeated intravenous administration of silver and gold nanoparticles in mice[J]. Scientific Reports, 2017, 7(1): 3303.
- Yang L, Kuang H, Zhang W, et al. Size dependent biodistribution and toxicokinetics of iron oxide magnetic nanoparticles in mice [J]. Nanoscale, 2015, 7(2): 625-636.

Alexander Zelikin

Alexander N. Zelikin finished his education in polymer chemistry in Moscow State University (Russia) in 2003, and subsequently held research positions at MIT, Cornell University, and the University of Melbourne. In 2009, he joined Aarhus University (Aarhus, Denmark) where he lectures in medicinal chemistry. His research interests include polymer chemistry, materials science, medicinal chemistry (prodrug design), and artificial biology.

Rudolf Zentel

Prof. Rudolf Zentel studied chemistry at the Johannes Gutenberg-University Mainz (Germany) and received his PhD in 1983. During this time he got introduced to the concept of polymers for pharmaceutical applications in the lab of Prof. Helmut Ringsdorf. After a postdoctoral stay in Freiburg (Germany), and research stays at the "IBM Almaden Research Center" in San Jose (USA, 1989-1990) and in Düsseldorf (1990-1992) he got his first professorship in Mainz in 1992. Central topics of his research are self-organizing systems, materials for optoelectronics and - recently again – well defined biocompatible polymers as nanocarriers for bioactive agents.

RECENT PUBLICATIONS

- In Vivo Gene-Silencing in Fibrotic Liver by siRNA-Loaded Cationic Nanohydrogel Particles, L. Kaps, L. Nuhn, et al., Adv. Health Care Materials, 4, 2809 (2015)
- Polymeric Selectin Ligands Mimicking Complex Carbohydrates: From Selectin Binders to Modifiers of Macrophage Migration, K. E. Moog, M. Barz, et al., Angew. Chem. Int. Ed. 56 (5), 1416 (2017);
- SiRNA-mediated In Vivo Gene Knockdown by Acid-Degradable Cationic Nanohydrogel Particles , N. Leber, L. Kaps, et al., J. Controlled Release 248, 10 (2017)
- α-Mannosyl-Functionalized Cationic Nanohydrogel Particles for Targeted Gene Knockdown in Immunosuppressive Macrophages , N. Leber, L. Kaps, et al., Macromol. Biosci. 19, 1900162 (2019)
- HPMA-based Nanoparticles for Fast, Bioorthogonal iEDDA Ligation, S. Kramer, D. Svatunek, I. Alberg, et al.,Biomacromolecules 20, 3786 (2019)

María José Alonso

María José Alonso's lab has pioneered numerous discoveries in the field of Nanopharmaceutical Technology and nanomedicine. She has coordinated several research consortia financed by the WHO, the Gates Foundation and the European Commission. Currently, she is involved in 7 international projects. She is the author of over 280 scientific contributions with more than 17,200 cites (H factor 72) and the inventor of 22 patent families. Because of the quality of her scientific articles she has been among the TOP TEN in Pharmacology (Times Higher Education international ranking, 2010). She has served to the Release Society (CRS) for 15 years and she is currently Past President of the Controlled Release Society (CRS). She is also Editor-in-Chief of the Drug Delivery and Translational Research, an official journal of the CRS, and she is part of the editorial board of 11 journals. In 2006-10, she was the Vice-rector of Research and Innovation of the USC. She has received 29 awards, among them the Founders Award of the CRS. She is a fellow of the American Institute for Medical and Biological Engineering (AIMBE) and a Fellow of the Controlled Release Society, a member of three Academies in Spain and a member of the US National Academy of Medicine (NAM).

CLINAM

European Foundation for Clinical Nanomedicine

12/2020

Basel, October 26–28

CURRICULA VITAE POSTERS

Hend Mohamed Abdel-Bar

Schlumberger Research Fellow at King's College London & Associate Professor of Pharmaceutics, Faculty of Pharmacy, University of Sadat City

Hend Mohamed Abdel-Bar joined the National Organization of Drug control and research, the agency responsible for drug quality control in Egypt, as a quality control specialist (2014-2017). In 2016, she was appointed as a Lecturer at the University of Sadat City, Egypt. Her current research interest is controlled release systems, nanotechnology, experimental design, formulation optimization, lipid-based systems, nanocomposites, topical drug delivery, ocular delivery systems, intra-nasal drug delivery systems, gene delivery, brain and tumor targeting. In 2018 till present, she joined Al-Jamal's lab as a Newton then Schlumberger Research Fellow.

RECENT PUBLICATION

- Mohamed Hamdi, **Hend Mohamed Abdel-Bar**, Enas El-mowafy, Khuloud T. Al-Jamal, Gehanne A. S. Awad. An integrated vitamin E-coated polymer hybrid nanoplatform: A lucrative option for an enhanced in vitro macrophage retention for an anti-hepatitis B therapeutic prospect. *PLoS One*. 2020; 15(1):e0227231.
- Eman Sadder El-Leithy, **Hend Mohamed Abdel-Bar**, Raghda Abdel-Moneum. Folate-Chitosan Nanoparticles Triggered Insulin Cellular Uptake and Improved In vivo Hypoglycemic Activity. *International journal of Pharmaceutics*. 2019; 571:118708.
- Dalia El Baihary, Rihab Osman, **Hend Mohamed Abdel-Bar**, Omaima A. Sammour. Pharmacokinetic/pulmokinetic analysis of optimized lung targeted spray dried ketotifen-dextran core shell nanocomplexes–in-microparticles. *International Journal of Biological Macromolecules*, 2019: 139: 678-687.
- **Hend Mohamed Abdel-Bar**, Rania Abd el Basset Sanad. Endocytic pathways of optimized resveratrol cubosomes capturing into human hepatoma cells. *Biomedicine & Pharmacotherapy* 2017; 93: 561-569.
- Rania Abdel-Basset Sanad, **Hend Mohamed Abdel-Bar**. Chitosan–Hyaluronic acid composite sponge scaffold enriched with Andrographolide-loaded lipid nanoparticles for enhanced wound healing. *Carbohydrate Polymers* 2017; 173: 441-450.

Seyedeh Hoda Alavizadeh

(Pharm. D, Ph.D.)
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I was born in Iran on August 14, 1986. I'm Pharm. D graduate (2004-2010) from Pharmacy school, Mashhad University of Medical Sciences (MUMS). I got my Ph. D in Pharmaceutical Nanotechnology (2011-2016) from the same school under supervision of professor Mahmoud Reza Jafari. I started my professional career as an assistant professor in the Department of Pharmaceutical Nanotechnology, School of Pharmacy, MUMS following my graduation (2016-now). During my Ph. D I worked on various cisplatin liposomal formulations including targeted and thermo-responsive formulations. My research focuses on smart nanomaterials including liposome, iron and gold nanoparticles for the delivery of chemotherapeutics to the tumor by exploiting cancer microenvironment features. I also work on developing phytochemicals for their anti-oxidant and anti-cancer properties. We have several Ph. D students working on different stimuli-responsive carriers including thermo-responsive, pH & REDOX-sensitive targeted liposome for cancer therapy. Since the focus of my Pharm.D thesis was on Leishmania, I continued my collaboration in this area in our group on drug or vaccine development for this infection.

I have previously participated in conferences including ILS/LRD Liposome Advances Conference, Athens 2017, 8th CLINAM and 10th CLINAM Conferences, in the latter I won the poster prize in basic nanomedicine category. I have won the scholarship from DAAD for the ProGRANT proposal writing course 2018 and one of my review papers on Saffron was highly cited in 2016 as reported by Essential Science Indicator of Thompson Reuter ISI.

Some of my recent international publications are as follows:

PEER-REVIEWED PUBLICATION:

- Jaafari MR*, Hatamipour M, Alavizadeh SH, Abbasi A, Saberi Z, Rafati S, et al. (2019): Development of a topical liposomal formulation of Amphotericin B for the treatment of cutaneous leishmaniasis. International Journal for Parasitology: Drugs and Drug Resistance.
- Hatamipour, H., Sahebkar AH., Alavizadeh, SH., Dorri, M., Jaafari, MR.* (2019): Novel nanomicelle formulation to enhance bioavailability and stability of curcuminoids. Iranian journal of basic medical sciences. 282-289 pp.
- Iman, M., Huang, Z., Alavizadeh, SH, Szoka, Jr FC., Jaafari, MR.* (2017): Biodistribution and In Vivo Antileishmanial Activity of 1, 2-Distigmasterylhemisuccinoyl-sn-Glycero-3-Phosphocholine Liposome-Intercalated Amphotericin B. Antimicrobial agents and chemotherapy. e02525-16 pp.
- Alavizadeh, SH., Gheybi, F., Nikpoor, AR., Badiee, A., Golmohammadzadeh, S., Jaafari, MR.* (2017): Therapeutic Efficacy of Cisplatin Thermosensitive Liposomes upon Mild Hyperthermia in C26 Tumor Bearing BALB/c Mice. Molecular Pharmaceutics. 712-721pp.

REFEREES:

Mahmoud Reza Jaafari, Ph.D.Professor, School of Pharmacy, Mashhad University of Medical Sciences (MUMS)Mashhad – Iran, Email: jafarimr@mums.ac.ir

Prof. Avi Schroeder, PhD,
Associate Professor of Chemical Engineering Laboratory for Targeted Drug Delivery and Personalized Medicine Technologies Technion - Israel Institute of Technology, Email: avids@technion.ac.il

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Mona Alibolandi received her Ph.D. degree majoring in medical biotechnology in 2015 from Mashhad University of Medical Sciences, Iran. Since 2015, she started her career as an assistant professor at the Pharmaceutical Biotechnology Department at the School of Pharmacy, Mashhad University of Medical Sciences, Iran. From 2016 up to now, she served as the Head of Nude Mice and Nanomedicine Laboratory at Mashhad University of Medical Sciences,

Iran. Her research topic focuses on the design of smart hybrid material nanostructures for targeted drug and gene delivery purposes. She is also interested in design and fabrication of polymersomes and their application in cancer research.

EDUCATION

- PhD of Medical Biotechnology, Mashhad University of Medical Sciences, Mashhad, Iran. Graduated 2015. **The thesis title: "Synthesis of nanopolymerosome-anti EpCAM aptamer bioconjugates for delivery of doxorubicin into cancer stem cells and evaluating them in vitro and in vivo characteristics"**
- MSc in Microbial Biotechnology, Science and Research University of Tehran. Tehran, Iran. Graduated 2012. **The thesis title: "High level recombinant expression and purification of human basic fibroblast growth factor"**

SELECTED PUBLICATIONS

- Shahriari M, Zahiri M, Abnous K, Taghdisi SM, Ramezani M, Alibolandi M*. Enzyme responsive drug delivery systems in cancer treatment. J Control Release. 2019 Jul 8; 308:172-189. (IF: 7.8).
- Zahiri M, Babaei M, Abnous K, Taghdisi SM, Ramezani M, Alibolandi M*. Hybrid nanoreservoirs based on dextran-capped dendritic mesoporous silica nanoparticles for CD133-targeted drug delivery. J Cell Physiol. 2019 Jul 5. (IF: 4.6).
- Oroojalian F, Babaei M, Taghdisi SM, Abnous K, Ramezani M, Alibolandi M*. Encapsulation of Thermo-responsive Gel in pH-sensitive Polymersomes as Dual-Responsive Smart carriers for Controlled Release of Doxorubicin. J Control Release. 2018 Oct 28; 288:45-61. (IF: 7.8).
- Mohammadi M, Taghavi S, Abnous K, Taghdisi SM, Ramezani M, Alibolandi M*. Hybrid Vesicular Drug Delivery Systems for Cancer Therapeutics. Advanced Functional Materials. 2018, 1802136. (IF: 15.63).
- Alibolandi M, Mohammadi M, Taghdisi SM, Abnous K, Ramezani M. Synthesis and preparation of biodegradable hybrid dextran hydrogel incorporated with biodegradable curcumin nanomicelles for full thickness wound healing. Int J Pharm. 2017 Oct 30; 532(1):466-477. (IF: 4.2).
- Alibolandi M, Abnous K, Mohammadi M, Hadizadeh F, Sadeghi F, Taghavi S, Jaafari MR, Ramezani M. Extensive preclinical investigation of polymersomal formulation of doxorubicin versus Doxil-mimic formulation. J Control Release. 2017 Oct 28; 264:228-236. (IF: 7.8).

Leila Arabi

Dr. Leila Arabi is an Assistant Professor of Pharmaceutical Nanotechnology, School of Pharmacy in Iran. She holds Doctor of Pharmacy and Ph.D. (summa cum laude) from Mashhad University of medical sciences, Iran. She had the one-year PhD internship during 2012-2013 at University Hospital Basel, Switzerland. Following her visit to several labs and meetings with academics in the US and Europe, she relocated back to Iran.

Her research is focused on developing nanoscale drug delivery systems with particular emphasis on developing liposomes for targeted cancer drug delivery, combination therapy, cancer immunotherapy, and gene therapy. Her researches have been recognized as highlight from the Controlled Release Society (CRS), and has led to several publications and research presentations in different nanomedicine conferences. She is currently the Communication chair of immune-Delivery focus group of CRS and serves as an ambassador in CRS Young Scientist Committee.

As an Assistant Professor with a demonstrated history of working in the hospital & health care, her goal is to link the fields of Pharmaceutical Nanotechnology to cancer Biology and immunology to improve therapeutic efficacy of conventional cancer therapies. She is also interested in empowering the role of women in science and gender parity in STEM fields.

Nerea Argarate

R&D Project Manager
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She obtained her PhD in Pharmacy (University of Basque Country, 2010). Her research is focused on the materials for Health. She has participated in autonomic, national and European projects. From 2002 to 2008 she worked between the laboratories of AZTI (Basque Country) and CSIC (group of Nanobiotechnology, Nb4D group, IQAC-CSIC). She worked in the research of novel diagnostic methods for Food Safety and Quality within the context of Spanish and European Consortium Projects. Moreover, in 2008, she was enrolled as Researcher of the CIBER-BBN at INASMET (since 2011 Tecnalia Research and Innovation, Basque Country) where she worked on new challenging research objectives related to Biomaterials for health. Her main areas of interest were nanodiagnostic methods based on easy-to-handle Point-of-Care (PoC) devices and therapeutic nanoconjugates for controlled release systems. Since 2016, she works for the Management Area of CIBER-BBN as a Project Manager.

Brahamdutt Arya

Brahamdutt Arya presently working as Senior Research Fellow under the supervision of Dr Surinder P. Singh at CSIR-National Physical Laboratory, New Delhi, India. He received his Bachelor degree in Chemistry honors From M. D. U. Rohtak (2012) and Master degree with specialization in Organic Chemistry from University of Delhi, New Delhi, India (2014). He has worked on project entitled "Suzuki- Miyura coupling reactions- A Novel approach towards natural products synthesis" as a part of his master's degree. He has also worked as assistant professor of Chemistry at Pt. N. R. S. Government College, Rohtak and taught the stereochemistry, basic organic chemistry, organometallic chemistry and bioorganic chemistry subjects (2014-15). He is Young Investigation Member and Ambassador of European Association for Cancer Research (EACR) and Associate member of American Association for Cancer Research (AACR). Presently, being a material chemist he is exploring the fabrication of novel nanomaterials for improved and personalized clinical nanomedicine. On the other hand, with his team he is developing the Indian Standard Reference Materials for Gold, Silver, SiO2 nanoparticles. For the doctoral degree, his major area of work consists of synthesis of monodispered and highly size specific synthesis of Gold and Graphene oxide based multifunctional nanomaterials. He is working on various aspects of the nanomaterial – bio interactions by exploring their toxicological, bioimaging, cellular internalization, pharmacokinetics studies. His ultimate objective is to explore and enhance the present knowledge

of clinical and personalized nanomedicine. As a research fellow, he is currently working on Graphene oxide-Chloroquine nanoconjuagte and studied its antiproliferative mechanism on A549 lung cancer cell lines through autophagy modulation and DNA damage, along with target application of this novel nanoconjugate.

PUBLICATIONS:

- Book chapter with title "Green synthesis of Ag NPs from spinacia oleracea leafs and their antimicrobial efficacy against human pathogenic microbes", in book Engineering Practices for Agricultural Production and Water Conservation, ISSN/ISBN no. 9781771884518, Apple Academic Press, Taylor and Francis group, New Jersey, USA.
- Research article with title "Graphene oxide-chloroquine nanoconjugate induce necroptosis cell death in A549 cells through autophagy modulation", Braham Dutt Arya, Sandeep mittal, Prachi Joshi Jaime R. Vick, Alok Panday, Surinder P. Singh, published in Nanomedicine – London, ISSN number: 1743-5889, DOI: 10.2217/nnm-2018-0086.
- Research article with title "Synthesis and application of PHT- TiO2 nanohybrid for amperometric glucose detection in undiluted human saliva sample", Sachin Kadian, Braham Dutt Arya, Sumit Kumar, S. N. Sharma, R. P. Chauhan, Ananya Srivastava , Pranjal Chandra , Surinder P. Singh published in Elecroanalysis-Wiley, ISSN number: 1521-4109, DOI: 10.1002/elan.201800207.
- Research article with title "Biophysical Characterization and Drug Delivery Potential of Exosomes from Human Wharton's Jelly-Derived Mesenchymal Stem Cells", Neha Chopra, Braham Dutt Arya, Namrata Jain, Poonam Yadav, Saima Wajid, Surinder P. Singh , Sangeeta Chaudhury, published in ACS Omega, ISSN no: 2470-1343, DOI: 10.1021/acsomega.9b01180
- Research article with title "GO-Chl induce DNA damage response in A549 lung cancer cells through p62/SQSTM1", Braham Dutt Arya, Sandeep mittal, Prachi Joshi, Jaime R. Vick, Alok Panday, Surinder P. Singh. (In process)

Ashish Avasthi

Ashish Avasthi is currently a Marie Sklodowska-curie early stage researcher at BIONAND in Malaga, Spain. Here, he is working on his PhD project titled magnetic nanoparticles as tumor theranostics with his supervisor Dr. Maria Luisa Garcia Martin. Prior to this research he was working as junior research fellow in Indian Institute of Technology (IIT) Ropar with Dr. Yashveer singh where he worked on early diagnosis and treatment of ductal carcinoma in-situ (DCIS) using drug loaded nanoparticles as well as liposomes. He got his bachelors and masters (dual degree) from centre for converging technologies (CCT) at University of Rajasthan, Jaipur, where he majored in nanotechnology and minored in biotechnology & bioinformatics. During his short career he has done several internships with leading research groups in India such as Prof. B. Jayaram and Prof. Sangeeta Kale. He published a few research articles as well as presented his research in multiple conferences.

PUBLICATIONS

- Ghosh, Sougata, et al. "Diosgenin functionalized iron oxide nanoparticles as novel nanomaterial against breast cancer." Journal of nanoscience and nanotechnology 15.12 (2015): 9464-9472.
- Pozo-Torres, Esther, et al. "Clickable Iron Oxide NPs Based on Catechol Derived Ligands: Synthesis and Characterization" Soft Matter 2020 submitted
- Avasthi, Ashish, et al. "Magnetic Nanoparticles as MRI Contrast Agents" Topics in current chemistry 2020 submitted.

Maryam Babaei

Maryam Babaei was born in 1980 in Iran. She received BSc in applied chemistry from Tehran University. She obtained her PhD degree in organic chemistry from Ferdowsi University of Mashhad in 2017 under the supervision of Prof. Mohammad Ramezani and Prof. Hossein Eshghi and the thesis title was "Organic Polycation Derivatization of Size-selected Silica-based Mineral Fibers as Transfection Agents".

During her PhD study, she focused on development of silica-based nanomaterials for gene and drug delivery to cancer cells and she published two articles in the journal of "Cancer Gene Therapy" and "Nanomedicine - Future Medicine" journal.

From 2017 up to now, she worked as a postdoctoral researcher in Pharmaceutical Research Center at the School of Pharmacy, Mashhad University of Medical Sciences, Iran. In the last two years she published 4 articles in high impact journals such as" Controlled release". Her research interest focuses on the development and synthesis of polymers, magnetic nanoparticles, quantum dots and silica nanoparticles as targeted nanoplatform for cancer diagnosis and therapy.

Tamás Bakos

I completed both my BSc and MSc studies at Budapest University of Technology and Economics bioengineer program applied biotechnology specialisation between 2013 and 2019. During my studies I gathered my professional experiences in the field of molecular biology at Hungarian Academy of Sciences Institute of Enzymology. After I finished my studies I started working for Autovaccine Ltd as a biologist. My further experiences include management and quality control. I'm currently a PhD student at Doctoral School of Basic and Translational Medicine Nanomedicine Research and Education Center at Semmelweis University. My research topic is „Pathophysiology of the complement system, with particular focus on its role in drug-induced allergic reactions". I design and perform both *in vivo* and *in vitro* experiments related to CARPA (Complement Activation Related Pseudo Allergy) using pig and rat models to expand the knowledge about the reaction to nanomedicines.

PUBLICATIONS:

Title: A unified and modular vector set for expression screening in bacteria, yeast, insect and mammalian cells optimized for small and medium laboratories.

Authors: Márk Somogyi, Tamás Szimler, Attila Baksa, Barbara Végh, Tamás Bakos, Katalin Paréj, Csaba Ádám, Áron Zsigmond, Márton Megyeri, Éva Gráczer, Péter Závodszky, István Hajdú, László Beinrohr

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I am a PhD Student in "Bioengineering and Robotics" at the university of Genoa. Since November 2017, I am carrying out the doctoral activities in the Laboratory of the Nanotechnology for Precision Medicine with Professor Paolo Decuzzi at the Istituto Italiano di Tecnologia. My research activities have focused on developing microfluidic vasculature on-a-chip in order to assess the extravascular and vascular transport behaviors of nanomedicines. Through these activities, I love developed my skills in fabrication techniques, including nano/micro-fabrication, soft lithography techniques, as well as cell cultures, microscopy imaging and post-process of experimental data.
I achieved my MSc Degree in Medical Biotechnology and Nanobiotechnology in December 2016 presenting an experimental thesis titled "New bio compost: growth of nanostructured protein film on graphene oxide". During my master program, I was involved in the synthesis and characterization of the new biocomposite material obtained by the sequential deposition of fibronectin onto graphene oxide nanosheets. This project was developed in collaboration with my M Sc. Supervisor Prof. Rosaria Rinaldi and my Tutor Alessandra Aloisi (Università del Salento).

PUBLICATIONS:

- Palomba R, Palange AL, Rizzuti IF, Ferreira M, Cervadoro A, Barbato MG, Canale C, Decuzzi P. Modulating Phagocytic Cell Sequestration by Tailoring Nanoconstruct Softness. ACS Nano. 2018 Feb 27;12(2):1433-1444. doi: 10.1021/acsnano.7b07797. Epub 2018 Jan 16. PubMed PMID: 29314819.

Tobias Alexander Bauer

PhD Student
After being trained as a chemical laboratory assistant at Sanofi-Aventis Deutschand GmbH, Tobias studied chemistry at Johannes Gutenberg University Mainz and University of Toronto. He received his B.Sc. after focusing on post-polymerization modification of polythiophene during his bachelor's thesis with Prof. Dr. R. Zentel in 2015. In 2017, he graduated his M.Sc. and was awarded the Fritz-Henkel-Prize for his master's thesis on core cross-linked polymeric micelles under the supervision of Dr. M. Barz. His current research as a doctoral candidate with Dr. M. Barz focuses on the design of stimuli-responsive core cross-linked micelles for mono- and combination therapy. For his doctural studies, Tobias acknowledges the HaVo-Stiftung for funding the Max-Planck-Graduate Center for complementary training and support.

Salime Bazban-Shotorbani

Salime Bazban-Shotorbani is currently in the last year of her PhD studies at Department of Health Technology, Denmark Technical University, Lyngby, Denmark. She is also a visiting PhD student at Department of Chemistry, Imperial College London, London, UK. Salime is a member of BioNanoMat group, under supervision of Dr. Nazila Kamaly. She is a biomedical engineer and her PhD involves the development of static and dynamic cell-based screening platforms that allows the study of a variety of nanoparticles across an inflamed endothelium. Using a library of targeted nanoparticles engineered with different physicochemical properties, the exact nature of their interactions with endothelial cells under static and flow conditions have been investigated. These mechanistic insights will aid in the design and development of optimal nanotherapeutics aimed at chronic diseases involving inflamed endothelia such as atherosclerosis.
Salime holds two BSc degrees in Biomedical Engineering and Polymer Engineering from Amirkabir University of Technology, and a MSc degree in Biomaterial Engineering from Amirkabir University of Technology, Tehran, Iran. Her BSc studies were focused on the theoretical modelings and applications of polymers in biomedical engineering. In her master thesis, she designed and developed smart nanogels for stimuli-responsive drug delivery systems.

Marilena Bohley

The presenting author studied pharmacy at the Julius-Maximilians-University Wuerzburg, Germany from 2011-2015. In 2017, after practical training in pharmacy and industry, she joined the group of Achim Goepferich, Department of Pharmaceutical Technology, University Regensburg as a Ph.D. student.

LIST OF PUBLICATIONS

- Bohley, Marilena; Birch, Emily; Baumann, Felix; Dillinger, Andrea; Tamm, Ernst; Goepferich, Achim: Design of dye and superparamagnetic iron oxide nanoparticle loaded lipid nanocapsules with dual detectability *in vitro* and *in vivo*. In: International Journal of Pharmaceutics 2020,585, 119433
- Bohley, Marilena; Haunberger, Alexandra; Goepferich, Achim: Intracellular availability of poorly soluble drugs from lipid nanocapsules. In: European Journal of Pharmaceutics and Biopharmaceutics 2019,139, 23-32
- Bohley, Marilena; Goepferich, Achim (2019): Intracellular availability of poorly soluble drugs from lipid nanocapsules. Controlled Release Society German Chapter Meeting, Leipzig, Germany, 2019.
- Bohley, Marilena; Goepferich, Achim (2019): RGD-peptide grafted Lipid Nanocapsules (LNC) for drug delivery to the posterior eye. Controlled Release Society Annual Meeting & Exposition, Valencia, Spain, 2019.

Maximilian Brückner

Ph.D. candidate in the group of Prof. Katharina Landfester - Department of Physical Chemistry of Polymers at the Max-Planck-Institute for Polymer Research in Mainz. From 2012 to 2016, I studied Biotechnology at the University of Applied Sciences in Darmstadt. The main focus of the study programme was on basics in engineering science as well as subject-specific basics in genetic engineering, cell cultivation, and enzyme technology. For my Bachelor thesis at the University Medical Center in Mainz, I investigated the effect of glaucoma-relevant antibodies on the expression of GLAST and the activity of glutamine synthetase in primary Müller cells and retinal cultures. For my Master, I studied Physical Cell Biology at the Goethe University in Frankfurt. This international study programme is based on the modern concepts and methods of cellular and physiological biology in research and development. In 2018, I wrote my Master thesis at the Max-Planck-Insitute for polymer research in the group of Prof. Mailänder. During that time I functionalized magnetic nanocarriers with modified monoclonal antibodies under two different conjugation strategies to compare the targeting efficiency of the conjugates *in vitro* on dendritic cells. In December 2018, I fully joined the Landfester group for my Ph.D. which is subject to bioconjugation of nanocarriers with antibodies and derivatives to create dual- or multi-functional nanocarriers. Furthermore, I joined the Collaborative Research Centre SFB 1066 "Nanodimensional polymeric therapeutics for tumor therapy" where I am working on the subject: attachment of antibodies on the artificial protein corona.

RECENT PUBLICATION

- Monitoring of Cell layer Integrity with a Current-Driven Organic Electrochemical Transistor (third autor)
- How washing media influences the composition of the protein corona on nanocarriers formed in blood plasma and serum (shared first autor, almost submitted)
- The conjugation strategy affects antibody orientation and targeting properties of nanocarriers (shared first autor, almost submited)

Alexandra Bukchin

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I was born on 24th March, 1988 in Moscow, Russia. At the age of 7 my family and I made the Aliya and came to Haifa, Israel where I live until this day. As all 18 year-olds in Israel, right after school, we are called to serve in the Israel defense forces. Thus on February 2007 I have started my military service in the Israeli Navy in Rosh Ha'Nikra, which is on the Lebanon border. I was in charge upon 6 more girls per shift and together we closely monitored the sea border day and night.
At 2009, after the military service I have started studying towards my first double degree in material engineering and chemistry at the Department of Materials Science and Engineering at the Technion – Israel Institute of Technology. During my studies I have worked at Israel Schechter's analytical chemistry lab at the Department of Chemistry.
After that I have pursued my M.Sc in the Department of Materials Science and Engineering under the supervision of Prof. Alejandro Sosnik. Finally, I am now at my last year of my PhD at Alejandro Sosnik's lab. My current thesis is about polymeric nanocarriers modified with shuttle peptides for drug targeting to the central nervous system. This is a project we collaborate with Angel Carcaboso, also my co-supervisor in the thesis. Angel works at the pediatric Hospital Sant Joan de Deu in Barcelona, Spain. I have traveled there number of times to continue out fruitful collaboration.
As part of my PhD work in the faculty I am a teacher assistant for solid mechanics and introduction to materials science in our faculty as well as partial differential equations and calculus in the Department of Mathematics.
I was a part of the organizing committee in the 3rd International Conference on Biological and Biomimetic Adhesivs in Israel.
I was honored to receive The Miriam and Aaron Gutwirth memorial fellowship for excellent achievements in research and study for 2018-2019 academic year. As well as the Rozen prize for excellency in study, research and teaching for 2018.

PUBLICATIONS:

- A. Bukchin, N. Kuplennik, Á.M. Carcaboso and A. Sosnik," Effect of growing glycosylation extents on the self-assembly and active targeting*in vitro* of branched poly(ethylene oxide)-poly(propylene oxide) blockcopolymers ", Applied Materials Today 11 (2018) 57-69.
- A. Bukchin, G. Pascual-Pasto, M. Cuadrado-Vilanova, H. Castillo-Ecija, C. Monterrubio, N.G. Olaciregui, M. Vila-Ubach, L. Ordeix, J. Mora, Á.M. Carcaboso and A. Sosnik "Glucosylated Nanomicelles Target Glucose-Avid Pediatric Patient-Derived Sarcomas", Journal of Controlled Release 276 (2018) 59-71.

Loïc Burr

Loïc Burr, PhD, is Research and Development Engineer at CSEM since 2017 and is a specialist in nanomaterials fabrication, detection and characterization. He graduated with an Engineer diploma from the European Engineering school for Chemistry Polymer and Materials in 2012 and graduated with a Master diploma from the University of Strasbourg with Material Sciences specialization in 2012. He then performed his PhD studies at University of Darmstadt and GSI Helmholtz Center for Heavy Ion Research GmbH in Germany, on the topic of "Ion-track technology based synthesis and characterization of gold and gold alloys nanowires and nanocones".
His current focus is the development of new method for nanomaterials characterization as well as the development of automated risk assessment and point-of-care devices. He is participating in several European projects such as ACEnano (risk assessment of nanomaterials), HEDIMED (exposome influence on immune mediated diseases) and FoodSmartPhone (portable risk assessment in food matrices).

KEY PUBLICATIONS:

- Burr, L.; Schubert, I.; Sigle, W.; Trautmann, C.; Toimil-Molares, M. E. Surface Enrichment in Au–Ag Alloy Nanowires and Investigation of the Dealloying Process. J. Phys. Chem. C 2015, 119 (36), 20949– 20956, DOI: 10.1021/acs.jpcc.5b05596.

Fanny Caputo

Dr. Fanny Caputo (f), PhD, is working as Research Scientist at SINTEF since November 2019. She has an undergraduate education in Materials Science and a PhD in Materials for Health Environment and Energy (2015). In 2015 she joined as Research Scientist CEA-Grenoble (France) where for 4 years she led the physical-chemical character-

ization of Nanopharmaceuticals, for the European Nanomedicine Characterisation Laboratory (EUNCL H2020). Since June 2019 she is the chair of the "Safety and Characterisation" working group of the European Technology Platform of Nanomedicine (ETPN), and she is also the contact point for EUNCL. She had been participated with scientific and management roles to multiple H2020 projects focused on the pre-clinical clinical characterisation of nanomedicine and on the development of a nanomedicine regulatory framework (e.g. H2020 EUNCL, EXPERT, REFINE). She is also currently involved in the standardisation of physical-chemical methods of nanoparticle systems, being a member of the ASTM E56 committee.

RELEVANT PUBLICATIONS

- Hon S. Leong, .., Caputo F., .., Pastore C., On the issue of transparency and reproducibility in nanomedicine, Nature Nanotechnology, 14, 629–635 (2019).
- Gioria S., Caputo F., Mehn D. Nano-enabled medicinal products: time for an international advanced community? Nanomedicine (Lond), 14(14):1787-1790 (2019).
- Caputo F., Clogston J., Calzolai L., Rösslein M., Prina-Mello A., Measuring particle size distribution of nanoparticle enabled medicinal products, the joint view of EUNCL and NCI-NCL. A step by step approach combining orthogonal measurements with increasing complexity. Journal of Controlled Release, 299, 31-43 (2019).
- Caputo F., Arnould A., Bacia M., Ling W.L., Rustique E., Texier I., Prina Mello A., Couffin A-C., Measuring Particle Size Distribution by Asymmetric Flow Field Flow Fractionation: A Powerful Method for the Preclinical Characterization of Lipid-Based Nanoparticles, Mol. Pharmaceutics, 16 (2), 756–767 (2019).
- Gioria S., Caputo F., Urbán P., Maguire C.M., Bremer-Hoffmann S., Prina-Mello A., Calzolai L., Mehn D., Are existing standard methods suitable for the evaluation of nanomedicines: some case studies. Nanomedicine (Lond). 13, 539-554 (2018).

Estela O. Carvalho

Estela O. Carvalho is a PhD student in Materials Engineering at the University of Minho, Portugal, affiliated to the Center of Physics of the University of Minho and Porto. She has a degree in Genetics and Biotechnology (2015) at UTAD, Portugal, and a master's degree in Biophysics and Bionanosystems at the University of Minho (2018). As part of her master's degree, she joined the Department and Center of Physics at UM in order to develop research in the area of piezoelectric materials for tissue engineering applications. During this time she also performed a research stay at the Centre for Biomaterials and Tissue Engineering (CBIT), Valencia, Spain This work resulted in a scientific contribution on "Human mesenchymal stem cells growth and osteogenic differentiation on piezoelectric poly(vinylidene fluoride) microsphere substrates" – 10.3390/ijms18112391).

After successful finish of her master degree, she worked as researcher in an EuroNanoMed funded project (ENMed/0049/2016) entitled: "Multiplex point-of-care device for lung disease biomarkers in sputum" – "LungCheck"- aiming to develop a bioanalytical device based on sputum sampling and rapid multiplex biomarker analysis, for the early diagnosis of lung cancer. The results on this work were presented at the EuroNanoMed III Training Workshop & Review Seminar for funded projects & NSC5 Meeting in Bratislava, Slovakia, where she won the prize of Best Poster award.

In her PhD work, the research interest is focused in the use of smart materials with controlled structures, morphologies and dimensionalities, for tissue engineering and biomedical applications, with published works "Tailoring Bacteria Response by Piezoelectric Stimulation" – 10.1021/acsami.9b05013, and "Tailoring electroactive poly(vinylidene fluoride-co-trifluoroethylene) microspheres by a nanoprecipitation method" – 10.1016/j.matlet.2019.127018).

Carole Champanhac

Postdoctoral researcher

Carole did her Bachelor's degree at the University of Bordeaux, France until 2011 with a major in Chemistry. She then moved to the University of Florida, USA. She did her PhD under the supervision of Prof. Dr Weihong Tan in the field of aptamer and cellular targeting. She was awarded her doctorate in August 2011. She is currently working as a postdoctoral researcher at the Max-Planck Institute for Polymer Research in the group of Prof. Dr. Volker Mailander. Her current research focuses on the cellular uptake of nanocarriers.

- Champanhac, C; Simon, J; Mailänder, V; Landfester, K. Timing of Heparin Addition to the Biomolecular Corona Influences the Cellular Uptake of Nanocarriers. Biomacromolecules 2019, 20, 10, 3724-3732.

Marco Cordani

I obtained my master's degree in molecular biology, with the maximum score, at University of Parma, Italy. In 2011 I was awarded with an undergraduate fellowship for a short stay (2 months) at Spanish National Center for Cancer Research (CNIO), Madrid. There, I have been trained in molecular biology and biochemistry in the laboratory of Structural Biology directed by Dr. Daniel Lietha. Between 2014 to 2016 (3 years), I was enrolled in the doctoral school of Molecular Biomedicine, at University of Verona, Italy. During these years, I elucidate the molecular mechanisms by which oncogenic mutant p53 proteins regulate signaling pathways involved in chemoresistance and cancer progression, with a specific focus on autophagy and ROS regulation (Fiorini et al, Biochim Biophys Acta. 2015 Jan;1853(1):89-100; Cordani et al. Mol Oncol. 2016 Aug;10(7):1008-29; Cordani et al, Br J Cancer. 2018 Oct;119(8):994-1008). During my doctoral period I had the opportunity to undergo training and perform collaborative work at Spanish National Center for Cardiovascular Research, Madrid (CNIC) for a total of 21 months, where I acquiring a broad background in cell biology and expertise in multiple state-of-the-art techniques related to life science. At CNIC, in the laboratory directed by Prof. Miguel Angel Del Pozo, I worked in a project aimed to understand how cancer cells respond to mechanical stimulus using novel High Content Screening and state-of-the-art imaging techniques (Bravo-Cordero JJ*, Cordani M* et al. J Cell Sci. 2016 Apr 15;129(8):1734-49). On May 2017 I defended my PhD thesis with the maximum qualification.

In 2017, I obtained my first postdoctoral fellowship to work in the laboratory of translational oncology directed by Prof. Gema Moreno-Bueno, at Autonomous University of Madrid (UAM), Spain. During this research stay (12 months), I worked in a project titled: in a project titled: "Targeting Gasdermin-B to overcome chemoresistance in Her2-positive cancers".

Since April 2018 I am postdoctoral researcher at IMDEA Nanociencia, Madrid, where I am working in the laboratory of nanobiotechnology directed by Prof. Álvaro Somoza on the development of CRISPR-based genome editing approaches and novel nanomaterials for drug delivery for cancer therapy.

Although the early stage of my career (< 3 years from PhD), I published more than 20 articles in peer-reviewed journals, 11 in Quartil-1 and 2 in Decil-1 (JCR), with a H-index of 11 (Google Scholar) and > 390 citations totals in only 5 years (2015-2019). During my career I has been granted with 8 highly competitive fellowships and I participated in 4 national/local projects founded thorough competitive calls. I was appointed as Guest Editor for several recognized

journals, including Frontiers in Pharmacology, Nanomaterials and Oxidative Medicine and Cellular Longevity. I am holding peer-reviewer duty for many journals, including ACS Applied Nano Materials, Cancers and Cell Death & Disease. Moreover, I was involved in the supervision of two talented master students.

RECENTS PUBLICATIONS:

- Rigotti T, Asenjo J, Martín-Somer A, Milán Rois P, Cordani M, Díaz-Tendero S, Somoza A, Fraile A, Alemán J. Asymmetric [3+2] Cycloaddition Reactions of Azomethine Ylides to 8-Alkenyl-BODIPYs: Synthesis of New Biological Sensors. Adv. Synth. Catal. 10.1002/adsc.201901465
- Cordani M et al. Mutant p53 blocks SESN1/AMPK/PGC-1α/UCP2 axis increasing mitochondrial O2-· production in cancer cells. British Journal of Cancer. 119(8):994-1008.
- Cordani M, et al. Mutant p53 proteins counteract autophagic mechanism sensitizing cancer cells to mTOR inhibition. Molecular Oncology, 10(7):1008-29
- Bravo-Cordero JJ*, Cordani M*, et al. A novel High Content Analysis tool reveals Rab8-driven actin and FA reorganization through RhoGTPases and calpain/MT1. J Cell Science, 129(8):1734-49
- Fiorini C, Cordani M, et al. Mutant p53 stimulates chemoresistance of pancreatic adenocarcinoma cells to gemcitabine. Biochimica et Biophysica Acta Mol Cell Research, 1853(1):89-100

Noemi Stefania Csaba

PhD

Associate Professor at the Dept. of Pharmacology, Pharmacy and Pharmaceutical Technology of the University of Santiago de Compostela and principal investigator of the Center for Research in Molecular Medicine and Chronic Diseases. Author of > 40 Q1 original and review articles (H index 25), several book chapters and one book as editor. Co-inventor of 6 patent families, two licensed and all extended and published as PCT.

Since 2017 leader of the group "Natural Polymers and Biomimetics", with main research interest in the formulation of drug-loaded nanocarriers for therapeutic applications and antigen-loaded nanosystems for vaccination and antigen-specific immunotherapy, with delivery strategies specifically targeted to mucosal surfaces.

Christian Czysch

PhD student

Christian Czysch studied biomedical chemistry at the Johannes Gutenberg University, Mainz (Germany), and received his master's degree in 2018. During his studies he worked at the laboratories of Prof. Rudolf Zentel and Prof. Holger Frey, gaining first insights in polymer chemistry in the context of bioapplication. For his PhD studies he joined the group of Dr. Lutz Nuhn at the Max Planck Institute for Polymer Research working on "Functional Polycarbonates for Drug Delivery". Christian is member and student speaker of the Integrated Research Training Group within the Collaborative Research Center 1066, Mainz (CRC 1066).

RECENT PUBLICATION:

Verkoyen, P., Johann, T., Blankenburg, J., Czysch, C., & Frey, H. (2018). Polymerization of long chain alkyl glycidyl ethers: a platform for micellar gels with tailor-made melting points. Polymer Chemistry, 9(44).

Richard da Costa Marques

Ph.D. Student

I am a doctoral student in the research group of Prof. Dr. Volker Mailänder and the department of Prof. Dr. Katharina Landfester at the Max Planck Institute of Polymer Research. My scientific studies focus on the intracellular trafficking of nanocarriers and LC-MS proteomics to study protein-nanocapsule interactions.

I studied biochemistry at the Goethe University Frankfurt from 2012 to 2016 with an emphasis on membrane proteins and biophysical chemistry. I wrote my bachelor's thesis at the Paul Ehrlich Institute in Langen about the cellular uptake of liposomal formulations containing mycobacterial lipid antigens.

After obtaining my bachelor's degree, I completed a master's program in biomedicine from 2016 to 2019 at the Johannes Gutenberg University Mainz. The program focused on immunology and molecular medicine. I carried out my master's thesis at the Max Planck Institute of Polymer Research, revolving around the interactions of bone-implant materials and angiogenic platelet proteins. At the end of my master's program, I underwent a scholarship-funded, three-month internship in Tokyo, Japan at Dr. Kei Yura's research group at the Ochanomizu University. Here, I learned and performed computational biology methods to study protein-DNA interactions. In April 2019, I fully joined the department of physical chemistry of polymers at the Max Planck Institute of Polymer Research as a Ph.D. student under the supervision of Prof. Dr. Katharina Landfester and Prof. Dr. Volker Mailänder, as my group leader. The group's research focuses on nanocarriers for biomedical therapy and protein-nanocarrier interactions. Furthermore, I joined the Collaborative Research Center 1066 and take part in the integrated research training group in which we work to provide nanoscale solutions to immunotherapeutics and drug delivery.

Ali Dehshahri

Dr. Ali Dehshahri is currently associate professor of pharmaceutical biotechnology at Shiraz University of Medical Science, Iran. He obtained his PhD at Mashhad University of Medical Sciences, Iran. In his thesis, he investigated the role of polymer amine content on its efficiency for gene delivery. As a distinguished Ph.D.student, he was awarded a short-term research grant from the Iranian Ministry of Health to pursue his investigation at LMU, Munich, Germany under the supervision of Prof. Ernst Wagner on polymeric nanoparticles for siRNA delivery. In 2014, Dehshahri accepted an Associate Professor position at Shiraz University of Medical Sciences, where he has been since that time.

Valentina Di Francesco

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I am a PhD Student in "Bioengineering and Robotics" at the university of Genoa. I am carrying out the doctoral activities at the

laboratory of the Nanotechnology for Precision Medicine of Professor Paolo Decuzzi at Istituto Italiano di Tecnologia, since November 2017. My research activities have focused on the design, preparation, physicochemical characterization, *in vitro* and *in vivo* evaluation of Supramolecular Nanotherapeutics for systemic delivery of bioactive compounds for cardiovascular disease, in particular Atherosclerosis. Methodologically speaking, I learnt new fabrication techniques for particles preparation with a priori defined size and shape combinations based on specific pathology and administration route.
I achieved my MSc Degree in Chemistry and Pharmacology Technology in at the "G. d'Annunzio" University of Chieti – Pescara in March 2016, with a thesis on "Therapeutic non phospholipid vesicles for neuronal anticancer treatment". During my master, I was involved in the design, synthesis and characterization of different supramolecular vesicular aggregates (SVAs) using hydrophilic surfactants and lipids. Chemotherapeutic, showing different physical-chemical properties, i.e. temozolomide (TMZ) was selected as drug candidates. TMZ-loaded innovative niosomes were design as potential nanomedicine for the treatment of brain cancer, glioblastoma multiforme, *in vitro*. This project was developed in collaboration with my M Sc. Supervisor Prof. Luisa Di Marzio (University of Chieti-Pescara "G.d'Annunzio").

Chengchen Duan

D.Phil. Student
Nuffield department of Women's and Reproductive Health, Oxford University John Radcliffe Hospital, Headington, Oxford
Chengchen Duan obtained his Msc degree in Biotechnology Research Extensive degree from University of Queensland, Australia in 2018 and BS degree in Biotechnology from Jinan University, China in 2016. He is now a second-year D.Phil. student in University of Oxford. His D.Phil. study is fully funded by University of Oxford-China Scholarship Council Scholarships covering 100% of his tuition and living expenses. He has been working with Dr Helen Townley in the Nuffield Department of Women's and Reproductive Health, Medical Science Division, University of Oxford since 2018. His current research focuses on developing a unique dual functional theranostic nanoprobe which could kill the cancer cells efficiently and real-time monitor the therapeutic effect non-invasively.

PUBLICATIONS:

- Duan, C., Liang, L., Li, L., Zhang, R. and Xu, Z.P., 2018. Recent progress in upconversion luminescence nanomaterials for biomedical applications. Journal of Materials Chemistry B, 6(2), pp.192-209.
- White, B.D., Duan, C. and Townley, H.E., 2019. Nanoparticle Activation Methods in Cancer Treatment. Biomolecules, 9(5), p.202. [Co-First Author]
- Liu, J., Duan, C., Zhang, W., Ta, H., Yuan, J., Xu, Z., Zhang, R., 2019. Responsive Nanosensor for Ratiometric Luminescence Detection of Hydrogen Sulfide in Inflammatory Cancer Cells. Analytica Chimica Acta. [Co-First Author] [In Review]
- Du, Z., Song, B., Zhang, W., Duan, C., Wang, Y. L., Liu, C., ... & Yuan, J. (2018). Quantitative Monitoring and Visualizing of Hydrogen Sulfide *in vivo* by A Ruthenium (II) Complex-based Luminescence Probe with New Recognition Moiety. Angewandte Chemie (International ed. in English), 57(15), 3999-4004.
- Chen, W., Zuo, H., Li, B., Duan, C., Rolfe, B., Zhang, B., Mahony, T.J. and Xu, Z.P., 2018. Clay Nanoparticles Elicit Long-Term Immune Responses by Forming Biodegradable Depots for Sustained Antigen Stimulation. Small, 14(19), p.1704465.

Zeinab Farhadi Sabet

Ph.D. student

Zeinab Farhadisabet is a PhD student at National Center for Nanoscience and Technology.
She graduated from Shahid Beheshti Universtiy with bachelor's degree (Iran 2015), and continued her postgraduated studies at National Center for Nanosceince and Technology at Chinese Academy of Science (China, 2015-2017), after that she accepted in PhD qualification exam at National center for Nanoscience and Technology and won Cas-Twas scholarship as a PhD student (china, 2018-now).
Her main interest are about self-assembly of short peptide toward cancer therapy, conjugation of small molecule, inhibitor or cancer cell drug to peptide, targeting cancer cell and pericellular or intracellular self-assembly.
She already work on hypoxia targeting of cancer cell by inhibiting one of the hypoxia enzyme.

RECENT PUBLICATION

- [Co-first author] New Power of Self-assembling Carbonic Anhydrase Inhibitor: Short Peptide Constructed Nanofibers Inspire Novel Hypoxic Cancer Therapy, 2019 science advances. [IF: 12.8]
- [Co-author] Corona of Thorns: The Surface Chemistry-Mediated Protein Corona Perturbs the Recognition and Immune Response of Macrophage, 2019 ACS Applied Materials & Interfaces [IF: 8.45]
- [Co-author] Dopamine Delivery via pH-Sensitive Nanoparticles for Tumor Blood Vessel Normalization and An Improved Effect of Cancer Chemo-therapeutic Drugs, 2019 Advanced Healthcare Materials. [IF 6.27]

Farid N Faruqu

Farid graduated from the University of Cambridge with both a BA and MSci in Biochemistry in 2014. During his MSci studies, he looked into the involvement of the Rho-family of small G-proteins in tumourigenesis and metastasis of cancer. Upon graduation, he did an internship with Cancer Research Malaysia, a non-profit research institute in Malaysia. There, he was involved in the screening of locally sourced natural compounds as potential cancer therapeutics to target cytidine deaminases and synthethic lethality pathways. He was awarded a scholarship by the Malaysian Government to pursue his PhD at the Institute of Pharmaceutical Sciences, King's College London in 2015. He is now working on developing exosomes as delivery systems for regenerative therapy in liver diseases.

Marie-Luise Frey

PhD student
Marie started her chemical career in 2011, studying Biomedical Chemistry at Johannes Gutenberg University in Mainz. After finishing her bachelor thesis in the group of Prof. Dr. Siegfried Waldvogel, she obtained her Bachelor degree in 2014. She continued her studies in Biomedical Chemistry and spent a semester abroad

at the Amsterdam Institute for Molecules, Medicine and Systems (AIMMS) at Vrije Universiteit Amsterdam under supervision of Dr. Maikel Wijtmans where she gathered more experience in medicinal chemistry. She did her Master thesis in the group of Jun.-Prof. Dr. Peter Wich at the Institute for Pharmacy and Biochemistry of Johannes Gutenberg University Mainz, working on chemical modifications of dextran for novel nanoparticulate materials, which she finished in 2017. After a short stay as research assistant in the group of Jun.-Prof. Dr. Ute Hellmich in the department of biochemistry, she started her PhD in the group of Prof. Dr. Katharina Landfester at the Max Planck Institute for Polymer Research in Mainz. Currently she is working on the surface modification of hydroxylethyl starch- and protein-based nanocapsules for targeted drug delivery. As a part of the interdisciplinary research training group of the Collaborative Research Center "Nanodimensional polymer therapeutics for tumor therapy" (SFB 1066) she is working closely together with collaborators of the department of dermatology of the University Medical Center in Mainz.

RECENT PUBLICATION

Guindani, C.; Frey, M.-L.; Simon, J.; Koynov, K.; Schultze, J.; Ferreira, S. R. S.; Araújo, P. H. H.; de Oliveira, D.; Wurm, F. R.; Mailänder, V.; Landfester, K.: Covalently Binding of Bovine Serum Albumin to Unsaturated Poly(Globalide-Co-ε-Caprolactone) Nanoparticles by Thiol-Ene Reactions. Macromolecular Bioscience (2019)

Meiyu Gai

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T: +49 1774450501
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Affiliation: Max Planck Institute for Polymer Research
Personal website: http://www.mpip-mainz.mpg.de/5525527/MG
https://www.researchgate.net/profile/Meiyu_Gai

Dr. Meiyu Gai studied Biomedical Materials as a PhD student at Queen Mary University of London (London, United Kingdom) in Sep. 2014, and the PhD degree was awarded in Sep. 2018. Her PhD thesis was titled "Design and Fabrication of Micro/Nano-chamber Arrays for Drug Encapsulation and Controlled Release" and was realized in the group of Professor Gleb B. Sukhorukov. Following her PhD, she joined Professor Katharina Landfester's research group at the Max Planck Institute for Polymer Research (Mainz, Germany) as postdoctoral researcher in Sep. 2018. Meiyu is currently working in the EU project "Ves4Us", which is focused on developing a radically new platform for the efficient production and functionalization of extracellular vesicles (EVs) to enable their exploitation as tailor-made products in the fields of nanomedicine. https://ves4us.eu/

FEATURED RESEARCH:

- Gai, M. et al. A bio-orthogonal functionalization strategy for site-specific coupling of antibodies on vesicle surfaces after self-assembly. Polym. Chem. 527–540 (2019). doi:10.1039/c9py01136f
- Gai, M., Frueh, J., Kudryavtseva, V. L., Yashchenok, A. M. & Sukhorukov, G. B. Polylactic Acid Sealed Polyelectrolyte Multilayer Microchambers for Entrapment of Salts and Small Hydrophilic Molecules Precipitates. ACS Appl. Mater. Interfaces acsami.7b03451 (2017). doi:10.1021/acsami.7b03451
- Gai, M. et al. Patterned Microstructure Fabrication: Polyelectrolyte Complexes vs Polyelectrolyte Multilayers. Sci. Rep. 6, 1–6 (2016).
- Gai, M. et al. Polylactic acid nano- and microchamber arrays for encapsulation of small hydrophilic molecules featuring drug release via high intensity focused ultrasound. Nanoscale 9, (2017).
- Gai, M. et al. In-situ NIR-laser mediated bioactive substance delivery to single cell for EGFP expression based on biocompatible microchamber-arrays. J. Control. Release asap, 84–92 (2018).

Eduard Gatin

Lecturer, Ph.D
University of Bucharest, Faculty of Physics, Department Science Materials, P.O.Box; MG 11, Bucharest-Magurele, Romania; University of Medicine 'Carol Davila', Faculty of Medicine, Biophysics Department, Blv. Eroii Sanitari 8, Sect.5, Bucharest, Romania.

Me Eduard Gatin, Physicist Education, research in polymer and materials science, dental materials University of Bucharest, Faculty of Physics (1994 – present, Assistant / Lecturer) Ph. D Biology & Physiology. From 2010 – present, Lecturer – Biophyscs Department University of Medicine "Carol Davila", Faculty of Medicine. Area of interest: polymer membranes for blood filtration. I continued with research in material science - polymers, advanced nano materials, ceramics, metal alloys, corrosion, dental materials and tissue regeneration. Laboratory Research activity: Materials structure, physical / chemical properties, dental enamel, bacteria activity, polymer resin composites, dental ceramics, metal alloys and corrosion studies. Techniques skills: RAMAN spectroscopy (improved by SERS), SEM, EDX. In 2013 I succeded to propose a method for quality evaluation of dental enamel by Raman method, to be applied " *in vivo*". In 2015, the patent registration certificate was issued. It was started a study " Introducing RAMAN technique to Periodontology" (*in vivo* application). It is the present target, cooperation with Semmelweis University Budapest, Faculty of Dentistry.

PUBLICATIONS:

- E. Gatin et al, 'Evaluation of the quality of local butters: A new approach based on Raman spectroscopy and supported by classical pycnometer method', Food Science and Technology International (2019) 1082013219871118 in press;
- E. Gatin et al, 'Raman Spectroscopy: Application in Periodontal and Oral Regenerative Surgery for Bone Evaluation', IRBM 40 (5) 2019;
- E. Gatin et al, 'Dental enamel quality and black tooth stain: A new approach and explanation by using Raman and AFM techniques', Part Sci Tech 33 (4) 2015.

Lukas Gerken

After obtaining his bachelor degrees in Physics from the University of Cologne and Sports and Performance at the German Sports University Cologne, Lukas moved to Vienna to pursue his master degree in Biomedical Engineering at the Vienna University of Technology. During this time, he connected, via an exchange, to ETH Zürich and the Swiss Federal Laboratories for Materials Science and Technology (Empa) in St. Gallen, where he conducted his master thesis in the Lab of Prof. I.K. Herrmann. Since 2018 he is a PhD student in the Nanoparticle Systems Engineering Lab of Prof. Herrmann at ETH Zürich, where he studies the synthesis and application of nanoparticles in the field of radiotherapy.

PUBLICATIONS:

- Gerken, L. R. H., Keevend, K., Zhang, Y., Starsich, F. H. L., Eberhardt, C., Panzarasa, G., Matter, M. T., Wichser, A., Boss, A., Neels, A., & Herrmann, I. K. (2019). Lanthanide-Doped Hafnia Nanoparticles for Multimodal Theranostics: Tailoring the Physicochemical Properties and Interactions with Biological Entities. ACS Applied Materials & Interfaces, 11(1), 437–448. https://doi.org/10.1021/acsami.8b20334
- Anthis, A. H. C., Matter, M. T., Keevend, K., Gerken, L. R. H.,

Scheibler, S., Doswald, S., Gogos, A., & Herrmann, I. K. (2019). Tailoring the Colloidal Stability, Magnetic Separability, and Cytocompatibility of High-Capacity Magnetic Anion Exchangers. ACS Applied Materials & Interfaces, 11(51), 48341–48351. https://doi.org/10.1021/acsami.9b16619
- Keevend, K., Puust, L., Kurvits, K., Gerken, L. R. H., Starsich, F. H. L., Li, J.-H., Matter, M. T., Spyrogianni, A., Sotiriou, G. A., Stiefel, M., & Herrmann, I. K. (2019). Ultrabright and Stable Luminescent Labels for Correlative Cathodoluminescence Electron Microscopy Bioimaging. Nano Letters, 19(9), 6013–6018. https://doi.org/10.1021/acs.nanolett.9b01819

Michał Gorzkiewicz

Michał Gorzkiewicz received his M.Sc. title in biotechnology from the Technical University of Lodz (Poland) in 2012. After graduation, he conducted research at the Institute of Medical Biology of Polish Academy of Sciences (Lodz, Poland) and the University Paris-Sud INSERM UMR-S757 (Orsay, France). Currently he is a PhD candidate and an employee of the University of Lodz at the Department of General Biophysics in the team of prof. Barbara Klajnert-Maculewicz. He has published 17 articles and 2 book chapters. He is a two-time winner of Minister of Science and Higher Education Scholarship for outstanding achievements for PhD students. In 2019, he has been recognized as one of the hundred most talented young Polish scientists, being granted the prestigious START scholarship of the Foundation for Polish Science. Michał Gorzkiewicz conducts research on the edge of biophysics, molecular biology, chemistry and nanotechnology, regarding the immunomodulatory properties of nanoparticles and the possibility of application of these compounds as carriers of genetic material, anticancer and antiinflammatory drugs.

RECENT PUBLICATIONS:

- Gorzkiewicz M., Klajnert-Maculewicz B. (2017), Dendrimers as nanocarriers for nucleoside analogues, European Journal of Pharmaceutics and Biopharmaceutics, 114:43-56. 5-year IF = 5.11
- Gorzkiewicz M., Buczkowski A., Appelhans D., Voit B., Pułaski Ł., Pałecz B., Klajnert-Maculewicz B. (2018), Poly(propyleneimine) glycodendrimers non-covalently bind ATP in a pH-and salt-dependent manner-model studies for adenosine analogue drug delivery, International Journal of Pharmaceutics, 544(1):83-90. 5-year IF = 4.42
- Gorzkiewicz M., Sztandera K., Jatczak-Pawlik I., Zinke R., Appelhans D., Klajnert-Maculewicz B., Pulaski Ł. (2018). Terminal sugar moiety determines immunomodulatory properties of poly(propyleneimine) glycodendrimers, Biomacromolecules, 19(5):1562-1572. 5-year IF = 5.83
- Gorzkiewicz M., Jatczak-Pawlik I., Studzian M., Pułaski Ł., Appelhans D., Voit B., Klajnert-Maculewicz B. (2018), Glycodendrimer nanocarriers for direct delivery of fludarabine triphosphate to leukemic cells: improved pharmacokinetics and pharmacodynamics of fludarabine, Biomacromolecules, 19(2):531-543. 5-year IF = 5.83
- Gorzkiewicz M., Deriu M.A., Studzian M., Janaszewska A., Grasso G., Pułaski Ł., Appelhans D., Danani A., Klajnert-Maculewicz B. (2019), Fludarabine-specific molecular interactions with maltose-modified poly(propyleneimine) dendrimer enable effective cell entry of the active drug form: comparison with clofarabine, Biomacromolecules, 20(3):1429-1442. 5-year IF = 5.83

Shanshan Guo

Shanshan Guo received her bachelor degree in Bioinformatics in 2012 from the Huazhong University of Science and Technology in China. She got Ph.D. in Physical Chemistry in 2018 under supervision of Professor Guangjun Nie from the National Center for Nanoscience and Technology in China. She spent two years (2015-2017) as a visiting PhD student supervised by Professor Greg Anderson in the Iron Metabolism Laboratory of QIMR Berghofer Medical Research Institute in Australia. She was focus on the mechanism of iron metabolism and treatment of iron related disorders by nanotechnology at the beginning of her PhD career. At the end of her PhD study, she showed great interest in developing nano-tools to treat the glycolipid-related diseases. Since 2019, she has joint Dr. Chun-xia Yi's group as a postDoc at the Department of Endocrinology and Metabolism of Amsterdam University Medical Centers (UMC) in the Netherlands.
Her current research interests include reprogramming of the microglia metabolism by nanotechnology, the pharmacokinetics of nanoparticles in the brain, the translational studies on human brain microglia related disorders, mechanism and treatment of iron related disorders and liver diseases using nano-drugs.

PUBLICATION

- Guo S, Liu G, Frazer D.M, Anderson G.J, Nie G, et al. Polymeric Nanoparticles Enhance the Ability of Deferoxamine To Deplete Hepatic and Systemic Iron. Nano Letters 2018. 18(9): 5782-5790.
- Guo S, Frazer DM, Anderson GJ. Iron homeostasis: transport, metabolism, and regulation. Current Opinion in Clinical Nutrition and Metabolic care 2016. 19(4):276-81.
- Guo S, Wang L, Li X, Nie G, Li M, Han B. Identification of a novel UROS mutation in a Chinese patient affected by congenital erythropoietic porphyria. Blood Cells, Molecules &Diseases. 2014. 52(1):57-8.
- Liu G, Guo S, Anderson GJ, Camaschella C, Han B, Nie G. Heterozygous missense mutations in the GLRX5 gene cause sideroblastic anemia in a Chinese patient. Blood 2014. 124(17):2750-1.
- Liu G, Guo S, Kang H, Zhang F, Hu Y, Wang L, et al. Mutation spectrum in Chinese patients affected by congenital sideroblastic anemia and a search for a genotype-phenotype relationship. Haematologica 2013. 98(12):e158-60.

Halamoda-Kenzaooui Blanka

Blanka Halamoda-Kenzaoui
Scientific Project Officer,
Consumer Products Safety Unit
European Commission Joint
Research Centre, Ispra, Italy

Blanka Halamoda-Kenzaoui (PhD) is Scientific Project Officer working in the area of safety of nanomaterials at the Consumer Products Safety Unit of the European Commission's Joint Research Centre (JRC). She has an expertise in nanotechnology, *in vitro* toxicology and method development.
She has received a Master degree in pharmaceutical sciences at Wroclaw Medical University (Poland) and a PhD in Life Sciences at University of Lausanne (Switzerland), for her work in the area of nanotoxicology. She has taken part in several projects related to the assessment of safety and compatibility of nanomedicine formulations and manufactured nanomaterials. In 2013 she joined the JRC for a post-doctoral project focusing on the influence of the

physicochemical properties of nanoparticles on their biological effect. Currently she is working in the area of the regulatory science for nanotechnology-enabled health products.

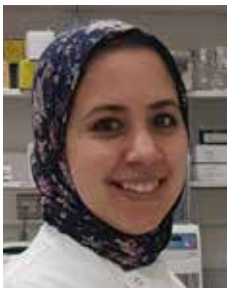
RECENT PUBLICATIONS:

- Halamoda-Kenzaoui, B., Box, H., van Elk, M., Gaitan, S., Geertsma, R. E., Gainza Lafuente, E., … Bremer-Hoffmann, S.. Anticipation of regulatory needs for nanotechnology-enabled health products-The REFINE White Paper. Publications Office of the European Union, 2019, EUR 29919.
- Halamoda-Kenzaoui, B., Baconnier, S., Bastogne, T., Bazile, D., Boisseau, P., Borchard, G., … Bremer-Hoffmann, S. Bridging communities in the field of nanomedicine. Regulatory Toxicology and Pharmacology, 2019, 106, 187–196. https://doi.org/10.1016/J.YRTPH.2019.04.011
- Halamoda-Kenzaoui B, Holzwarth U, Roebben G, Bogni A, Bremer-Hoffmann S. Mapping of the available standards against the regulatory needs for nanomedicines. Wiley Interdiscip Rev Nanomed Nanobiotechnol. 2018, 11(1):e1531.
- Halamoda-Kenzaoui B, Bremer-Hoffmann S. Main trends of immune effects triggered by nanomedicines in preclinical studies. Int J Nanomedicine. 2018, 17;13:5419-5431.
- Bremer-Hoffmann S, Halamoda-Kenzaoui B, Borgos SE. Identification of regulatory needs for nanomedicines. J of Interdisciplinary Nanomedicine 2018, 3(1), 4-15.

Alina Heck

PhD

Alina Heck studied biomedical chemistry at the Johannes Gutenberg University, Mainz (Germany), and received her master's degree in 2019. During her studies she could gain first research experience by spending 7 months in the laboratories of Prof. Dr. Winnik at the University of Toronto, Canada. Currently she is doing her PhD in the group of Dr. Lutz Nuhn at the Max Planck Institute for Polymer Research and a member of the Integrated Research Training Group within the Collaborative Research Center 1066, Mainz (CRC 1066). Her PhD topic deals with pH-reversible, Nanogel-mediated Immunodrug Delivery Systems.



Dina Helal

Newton-Musharafa Fellow, PhD student

Dina Helal completed her BSc and MSc in Pharmacy from Ain Shams University, Egypt. She worked as a teaching assistant before being awarded the Newton Mosharafa Scholarship (Host: Khuloud Al-Jamal) to pursue a joint PhD programme between the Institute of Pharmaceutical Science, King's College London and the Faculty of Pharmacy, Ain Shams University, Egypt. Her current project involves developing albumin nanoparticles for glioma therapy and their pre-clincial translation.

RECENT PUBLICATIONS:

- Elena Zaghi, Dina Omar, Jia Sun, Julie Wang and Khuloud Al-Jamal. The development of riluzole nanoparticles for treatment of Amyotrophic lateral sclerosis. In preparion.

Herrero Jorge Moreno

Jorge started his studies in Biomedical Sciences (BSc) in the University of Alcala (Madrid, Spain) in 2010 with majors in Biochemistry and Cellular Biology. He did an Erasmus exchange in 2012 at University of Mainz (Germany) and decided to stay after the Exchange. He continued and finalized his studies in Molecular Biology (BSc). He has a Master of Science in Biomedical Sciences from the University of Mainz, where he focused on immunology, molecular medicine and biochemistry.

He joined BioNTech's RNA Formulation & Drug Delivery Department in 2016 as a master student. Through his master's thesis, he investigated cationic polymer system for gene delivery, focusing on polyethyleimine-RNA systems. After his master, he was offered the chance to start a PhD in BioNTech, focusing on structure-function coherencies of mRNA-polymers for rational design of tailored polymer vehicles. Since 2016, he is author of 2 published patents and 1 pending patent. He has acquired experience in different characterization technics such as DLS, CD, UV, SAXS, SANS. He is trained in different microscopy technics such as dSTORM and confocal microscopy and has extensive experience on different in-vitro assays for the evaluation of biological activity of non-viral delivery systems.

Jorge was born in the Basque Country (Spain). He speaks fluently Spanish, German, English and Basque.

Natkritta Hüppe

PhD student

Natkritta started her bachelor studies in Chemistry at the RWTH Aachen University in 2012. After finishing her bachelor thesis in the group of Prof. Dr. Martin Möller at the DWI-Leibniz Institute for Interactive Materials, she obtained her Bachelor degree in 2016. Natkritta did her following master studies in chemistry majoring in organic synthesis, bioactive compounds, technical chemistry and catalysis. During her master studies she was an intern in Prof. Matthew Moffits research group at the Universtiy of Victoria in Canada and was working on polymer assemblies via microfluidics. Natkritta did her Master thesis in the group of Priv.-Doz. Frederik Wurm at Max-Planck Institute for Polymer Research in Mainz about "Controlled Anionic Polymerization of Cyanoacrylates". She finished her master studies in chemistry in 2018. Right after Natkritta started her PhD in the group of Prof. Dr. Katharina Landfester at the Max Planck Institute for Polymer Research in Mainz. Currently she is working on protein-based nanocapsules via azide-alkyne click chemistry for targeted drug delivery. As a part of the interdisciplinary research training group of the Collaborative Research Center "Nanodimensional polymer therapeutics for tumor therapy" (SFB 1066) she is working closely together with collaborators of the department of dermatology of the University Medical Center in Mainz.

Anne Huppertsberg

PhD Student
Anne Huppertsberg studied Applied Chemistry (B.Sc.) and Bio- and Pharmaceutical Analysis (M.Sc.) at the Hochschule Fresenius, Idstein (Germany) receiving her master's degree in 2018. During her studies she could already gain first research experience by spending an integrated practical semester abroad in the research group of Prof. Dr. Peter Skabara at the University of Strathclyde in Glasgow, Scotland. The research of Skabara's group focuses on the synthesis and investigation of organic semiconductors for photonic devices. To gather further practical experience in the organic and polymer chemistry she wrote her bachelor's thesis in the Performance Materials unit of the Merck KGaA, Darmstadt (Germany).
She is currently a PhD student in the group of Dr. Lutz Nuhn at the Max Planck Institute for Polymer Research as well as a scholar and member of the Max Planck Graduate School (MPGC) and the Graduate Center of the Collaborative Research Center 1066, Mainz (CRC 1066). In her PhD she focuses on the synthesis and investigation of pH-responsive nanogels derived from squaric ester amide-based precursor polymers for drug delivery applications.

Isabell Keil

Isabell Sofia Keil has a Master of Science in Biology from the Justus-Liebig University Giessen with focus on biochemistry, immunology and animal physiology. She graduated in 2018 and subsequently started her PhD at TRON gGmbH at the University Medical Center of the Johannes Gutenberg University Mainz. Her PhD project focuses on the development of nanoparticle-based mRNA carrier systems for transient immunomodulation of immune cells. Her skills include particle formulation and functionalization, *in vitro* work with primary human and murine cells as well as flow cytometry.

Patric Komforth

PhD
Patric Komforth studied chemistry at the Johannes Gutenberg University, Mainz (Germany), and received his master's degree in 2019. He is currently a PhD student in the group of Dr. Lutz Nuhn at the Max Planck Institute for Polymer Research and a member of the Integrated Research Training Group within the Collaborative Research Center 1066, Mainz (CRC 1066). His PhD topic is about redox-responsive depolymerizable Polycarbonates.

RECENT PUBLICATION

- Martin Kluenker, Muhammad Nawaz Tahir, Rene Dören, Mareike Deuker, Patric Komforth, Sergi Plana-Ruiz, Bastian Barton, Sergii I. Shylin, Vadim Ksenofontov, Martin Panthöfer, Nadine Wiesmann, Jana Herzberger, Angela Möller, Holger Frey, Jürgen Brieger, Ute Kolb and Wolfgang Tremel, Iron Oxide Superparticles with Enhanced MRI Performance by Solution Phase Epitaxial Growth, Chem. Mater. 2018, 30, 4277−4288.

Michał Kopka

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EDUCATION

01.10.2016 – until now Student of Medical University of Warsaw

I was graduated from High School No. 3 in Opole in 2016. In the same year I participated 45th National Biology Olympiad and won golden medal. That allowed me to represent Poland at 27th International Biology Olympiad in Vietnam, where I gained silver medal. I also took part in the final stage of 62nd National Chemistry Olympiad.
In 2016 I started studying at Medical University of Warsaw. From the beginning I was especially interested in histology. From the second year of study I was a member of The Histology and Embryology Students' Association where I learned essentials of laboratory work. From July 2018 our research team created Students' Association of Experimental Medicine. In that time I was working in the grant "Impact of N-acetycysteine on surgical wound healing" in a part concerning on genes expression. I was also working in the project that applied molecular navigation system for oncological surgery. Publications form following research are in review. At the same time I was developing a software for automatic morphometric analysis of axons and myelin sheaths.Now we begin experiment that analyses potential of adipose stem cell in regenerative medicine. We also achieved grant from project "Best of the Best! 4.0" financed by Polish Ministry of Science and Higher Education in September 2019. I am also interested in clinical medicine. Since September 2019 I am in Students' Association at Department of Paediatrics, Hematology and Oncology and focus on side effects of L-asparaginase in therapy.
I have presented results of research at international students' conferences in Berlin and Bucharest:

1. Kopka. Michał, Wiktor Paskal, Adriana Paskal, Piotr Pietruski, Kacper Pełka, Albert Stachura, Paweł K. Włodarski, "The molecular response of wounded skin to pre-incisional N-acetylcysteine injection - an animal model of improving skin healing." 4th Medical International Conference for Students MEDICS 2019, 11 – 14.04.2019, Bucharest, Romania
2. Kopka. Michał, Wiktor Paskal, Adriana Paskal, Piotr Pietruski, Paweł K. Włodarski, "A semi-automated morphometric analysis of peripheral nerve image" 30th European Students' Conference 2019 Charité, 25 – 29.09.2019 Berlin, Germany

Silke Krol

Laboratory for personalized medicine,
National Institute of Gastroenterology,
"S. de Bellis" Research, Hospital,
Castellana Grotte, Bari (I)
E-Mail: silke.krol@aol.com

Since 2018 Silke Krol is with IRCCS Ospedaliero Specializzato in Gastroenterologia "Saverio de Bellis" developing novel nanoparticle-based therapeutic and diagnostic approaches for inflammatory bowel disease. From 2010 till 2018 Silke Krol was with Fondazione IRCCS Istituto Neurologico "Carlo Besta" in Milan, Italy heading the laboratory for Nanomedicine. There she studied the transport mechanisms for differently functionalized gold nanoparticles across the blood brain barrier and how this is influenced by blood-derived proteins. Moreover, different novel metallic and non-metallic delivery systems for various other diseases (cardiovascular, prion disease, epilepsy, glioma, lymphomas, viral diseases) were designed for projects funded by Italian and European founda-

tions. In collaboration with Prof. Stellacci in Lausanne, Switzerland, they discovered the role of nanoparticles in vaccine stabilization, antiviral action and enhancer for viral infectivity for gene delivery. From 2016-2018 she worked in parallel for the IRCCS Istituto tumori "Giovanni Paolo II" in Bari, Italy leading the laboratory for translational Nanotechnology with focus on early diagnosis and advanced therapy of cancer.
She studies the application of multifunctional polymer/nanogold based drug or drug delivery systems as well as diagnostic tool for medical applications such as a hand-held point-of-care device for measuring chemotherapeutic drugs in real-time at the bedside of the patient. Moreover, the multilayer-nanocoating was used for encapsulation and immune protection of living cells like e.g. pancreatic islets. She has several pending patents for possible future drugs for prion disease and cancer treatment, viral diseases, and cancer diagnostics.
In 2009 she worked as an expert consultant for the United Nations and serves as external expert reviewer for National projects in France, Italy, Georgia and Greece. She worked as project technical advisor in 3 EU-FP7 projects and was external expert for the evaluation of EU project. She is member of the international advisory committee of the International scientific spring conference in Islamabad, Pakistan. She is member of the editorial board of the journal "Precision Medicine", and associate editor of "Frontiers in Nanobiotechnology". Since 2013 she is adjunct faculty member at the Pakistan Institute of engineering and applied science (PIEAS). Recently she founded a start-up, EnCytos with a team from the University of Twente.

Fredrik Kullenberg

Fredrik Kullenberg has his background in Engineering Nanoscience, which he studied at the Faculty of Engineering at Lund University. He started there in 2011 and received his Master of Science in 2016.
The education is interdisciplinary and includes many subjects, from quantum physics to immunology and neurobiology. During his studies he has, besides the subjects mentioned above, also studied physiology, pharmaceutical chemistry, cell biology and programing.
His master's thesis, Formulation and Characterization of a Liposomal Spray Dried Powder Intended for Inhalation , concerned the development and analysis of a model protein drug. The project was a collaboration between a pharmaceutical company and the University, which gave Fredrik an insight into how research is performed in both academia and in the pharmaceutical industry.
After receiving his master's degree, Fredrik also studied Pharmaceutical Bioinformatics at Uppsala University, which he passed with distinction.
After this, Fredrik spent a year working as a Drug Safety Associate at Sobi, a Swedish pharmaceutical company which specializes in rare diseases and protein pharmaceuticals.
Fredrik's current position is as a PhD student in the Lennernäs group at the Department of Pharmacy, Uppsala University, where he started in October 2017. His PhD project has the preliminary title Functional liposome nanoparticle drug carriers as a theranostic for hepatocellular carcinoma: the role of locoregional targeted drug delivery for interventional therapy. As can be seen in the preliminary title, the project concerns the innovation and development of novel nanoparticle formulations to be used in the treatment of primary liver cancer. So far, the project has mainly consisted of testing currently existing formulations, such as Caelyx®, as a first step to determine the impact of various parameters in different formulations.

Emilie Laprévotte

Emilie Laprévotte has a PhD in biology, with a specialization in oncology, from Paul Sabatier University (Toulouse, France). During her PhD, she developed, in collaboration with Roche Glycart AG, a new combination therapy using an antibody with a human cytokine for the treatment of haematological malignancies. Then she joined OREGA Biotech (Montpellier, France) as project manager where she established the preclinical proof of concept of antibodies targeting the tumor in solid cancers. In 2017, she joined INOFEA AG (Basel, Switzerland), where she established the early proof of concept of enzzen®-therapeutic enzymes. Emilie is now Chief Development Officer at Perseo pharma.

Dorelia Lipsa

Dorelia Lipsa has a Medical Bioengineering diploma followed by a specialisation in Bioactive substances and Biotechnology, both obtained from the University of Medicine and Pharmacy, Romania.
In 2017, she obtained a PhD in Natural Sciences from the Technical University of Münich (Germany), being a Research Fellow at the European Commission Joint Research Centre (EC-JRC), Institute for Health and Consumer Protection (IHCP) in Ispra, Italy.
With over 10 years' experience in a multidisciplinary research field, she is able (1) to set-up *in vitro* exposure devices (e.g. CULTEX) aiming to study the toxicological effects of volatile pollutants on human blood and derived cells; (2) to develop and/or optimise analytical tools for *in vitro* toxicity testing; (3) to evaluate the biocompatibility of drugs excipient and/or smart nanomaterials; (4) to characterise chemical and/or biological substances by using different analytical techniques such as HPLC, LC-MS, GC-MS, GC-FID and *in vitro* assays (e.g. LAL test, ELISA, EIA assay, MAT).
Currently she is a scientific officer at the EC-JRC where she is carrying out research for the PIRAT (Personalised Immunological Risk Assessment Technology) project that aims to develop a blood-on-the-chip device allowing the evaluation of immunogenicity of advanced material in ex vivo model systems.

PUBLICATIONS:

- D. Lipsa, C. Cacho, D. Rembges, J. Barrero, Fresenius Environmental Bulletin, Parlar Scientific Publications, vol. 23, no. 12, p. 3054-3058, 2014, "Fast cell counting – the better cell counting?"
- D. Lipsa, C. Carmen, P. Leva, J. Barrero, M.P. Aguar Fernandez, OMICS international, 2015, "Development of a HPLC-UV method for the simultaneous determination of intercellular glutathione species in human cells"
- D. Lipsa, P. Leva, J. Barrero, C. Mehmet, Toxicology Letters, vol. 262, p. 70-79, 2016 "Inflammatory effects induced by selected limonene oxidation products: 4-OPA, IPOH, 4-AMCH in human bronchial (16HBE14o-) and alveolar (A549) epithelial cell lines
- D. Lipsa, J. Barrero, C. Mehmet, Chemosphere, vol. 191, p. 937-945, 2018, "Exposure to selected limonene oxidation products: 4-oxopentanal (4-OPA), 3-isopropenyl-6-oxo-heptanal (IPOH), 4-acetyl-1-methylcyclohexene (4-AMCH) induce oxidative stress and inflammation in human epithelial cell lines"
- S. Kephalopoulos, S. Bopp, S. dalla Costa, A. Cusinato, D. Lipsa, O. Geiss, "Indoor air monitoring: sharing and accessing data via the Information Platform for Chemical Monitoring (IPCHEM)", accepted by the reviewers

Giovanna Lollo

Dr. Giovanna Lollo is Associate Professor at the Faculty of Pharmacy-ISPB (Institut des Sciences Pharmaceutiques et Biologiques) at the University of Claude Bernard Lyon 1. She carries out research activity, at the frontiers of pharmaceutical technology, physical chemistry, and biology at the LAGEPP UMR CNRS 5007.

She graduated as Pharmacist in 2007 from the University of Naples Federico II (Italy) with the greatest distinction, and she has also obtained the diploma of Hospital Pharmacist from the same University. In 2012, under de supervision of Prof Maria Jose Alonso at the University of Santiago de Compostela (Spain), she held a Ph.D. in Pharmaceutical Technology with a dissertation: "Polyaminoacid nanocapsules as drug delivery systems for antitumor drugs". Then, she joined the MINT (Micro et Nanomédecines Biomimétiques) group at University of Angers (France) where she worked during 3 years as Postdoctoral Scientist developing novel nano-immuno-chemotherapeutic approaches to defeat cancer.

She has authored/ co-authored of several peer reviewed scientific publications in the field of drug delivery and she filed 3 patents. Currently, her research field is focused in the design and development of novel nanosytems to cross biological barriers reaching target pathological sites without compromising healthy tissues.

RECENT PUBLICATIONS:

- Pinton L, Magri S, Masetto E, Vettore M, Schibuola I, Ingangi V, Marigo I, Matha K, Benoit JP, Della Puppa A, Bronte V, Lollo G, Mandruzzato S.; Targeting of immunosuppressive myeloid cells from glioblastoma patients by modulation of size and surface charge of lipid nanocapsules. J Nanobiotechnology. (2020); 18(1):31. doi: 10.1186/s12951-020-00589-3
- Carton F., Chevalier Y., Nicoletti L., Tarnowska M., Stella B., Arpicco S., Malatesta M., Petter Jordheim4, L., Briançon S., Lollo G. Rationally designed hyaluronic acid-based nano-complexes for pentamidine delivery. Int J Pharm. (2019); 568:118526. doi: 10.1016/j.ijpharm.2019.118526
- Lollo G., Matha K., Bocchiardo M., Marigo I., Virgone-Carlotta A., Dehoux T., Rivière C., Rieu JP., Briançon S., Meyer O., and Benoit JP. A novel 5FU derivative encapsulated into lipid nanocapsules for the drug delivery to tumors. J. Drug Target. (2019); 27(5-6):634-645. doi: 10.1080/1061186X.2018.1547733
- Lollo G., Ullio-Gamboa G., Fuentes E., Matha K., Lautram N., Benoit JP., In vitro anti-cancer activity and pharmacokinetic evaluation of curcumin-loaded lipid nanocapsules. Mater. Sci. Eng. C (2018); 91:859-867. doi: 10.1016/j.msec.2018.06.014
- Lollo G., Gonzalez-Paredes A., Garcia-Fuentes M., Calvo P., Torres D. and Alonso M.J., Polyarginine nanocapsules as a new carrier for oral delivery. J. Pharm. Sci., (2017); 106 (2): 611-618. doi. org/10.1016/j.xphs.2016.09.029

Stefan Lyer

STEFAN LYER studied Biology at the Friedrich-Alexander University Erlangen/Nürnberg. After finishing his PhD thesis at the German Cancer Research Center (DKFZ)/ Ruprecht-Karls-University Heidelberg he stayed as a post doc at the Department of Genome Analysis at the DKFZ for another year. In 2008 he moved back to Erlangen starting a post doc position at the group of Prof. Christoph Alexiou at the ENT-Department of the University Hospital Erlangen, which was renamed Section for Experimental Oncology and Nanomedicine (SEON) in 2009. Here, in the beginning he focussed on the application of nanoparticles in cancer therapy. Since 2011 he has been assistant group leader of SEON. Due to the interdisciplinary group he gained insight into different areas of science but still his expertise is focussed on the biological aspects of Magnetic Drug Targeting. His main topic is performing animal experiments in rabbits. Here, he introduced catheter-based application of the nanoparticles in the vicinity of the tumours to treat. He also implemented an arteriosclerotic model in the abdominal aorta of rabbits and a technique of nanoparticle application for the treatment of arteriosclerotic plaques in vessels with such a high flow rate.

RECENT PUBLICATIONS:

- Tietze R, Lyer S, Durr S, Struffert T, Engelhorn T, Schwarz M, et al. Efficient drug-delivery using magnetic nanoparticles - biodistribution and therapeutic effects in tumour bearing rabbits. Nanomedicine-Nanotechnology Biology and Medicine. 2013;9(7):961-71.
- Heid S, Unterweger H, Tietze R, Friedrich RP, Weigel B, Cicha I, et al. Synthesis and Characterization of Tissue Plasminogen ActivatorFunctionalized Superparamagnetic Iron Oxide Nanoparticles for Targeted Fibrin Clot Dissolution. International Journal of Molecular Sciences. 2017;18(9).
- Lyer S, Knopp T, Werner F, Zaloga J, Friedrich R, Trahms L, et al. Multifunctional SPIONs for Theranostics in Cancer. 2018. 2018;4(1).
- Matuszak J, Lutz B, Sekita A, Zaloga J, Alexiou C, Lyer S, et al. Drug delivery to atherosclerotic plaques using superparamagnetic iron oxide nanoparticles. International Journal of Nanomedicine. 2018;13:8443-60.
- Janko C, Ratschker T, Nguyen K, Zschiesche L, Tietze R, Lyer S, et al. Functionalized Superparamagnetic Iron Oxide Nanoparticles (SPIONs) as Platform for the Targeted Multimodal Tumor Therapy. Frontiers in Oncology. 2019;9.

Guillaume Maurin-Pasturel

Guillaume MAURIN-Pasturel was graduated in chemistry and physic-chemistry of materials (specialization in Molecular, Macromolecular and Supramolecular Engineering) in 2012 in the university of Montpellier (France). He obtained his PhD in chemistry and physic-chemistry of materials in 2015 in the university of Montpellier/ Charles Gerhardt Institute (France). During his doctoral thesis he worked on the synthesis and study of molecular-based multifunctional nano-objects for biomedical applications. That consisted in the elaboration of a Gold@Prussian Blue Analogue (PBA) core@shell nano-object presenting optical and magnetic properties, which was the first well-defined example of such combination as nanoparticle in the literature. Such nano-system presents magneto-optic multifunctionality which can be modulated by varying the size of the gold core and/or the thickness of the PBA as the nature of the PBA used. By epitaxial growth of different PBA, it has been possible to implement one or several different PBA layers, permitting to modulate the final properties. Particularly, he designed one nano-object with a second layer of Prussian Blue, and proposed it to pursue a first study previously made in the team. Consequently, such nano-objects were used after loading of radioactive thallium has radiotracers for SPECT-CT-imaging, permitting to significantly change the biodistribution *in vivo* and the activity half-time in comparison with commercial 201TlCl radiotracer. On other hand, the comparison of the magnetic properties with superparamagnetic NiCr PBA NPs, the study of the magnetic properties of core@shell gold@PBA made of the same NiCr PBA revealed a spin-glass behavior. By comparison with simple NiCr PBA NPs of similar size, exhibiting superparamagnetic properties, he illustrated an interesting modification of the magnetic properties of a material because of the use of different architectures. After his PhD, he

made a first postdoctoral contract in 2016 in the Charles Gerhardt Institute in Montpellier (France) for a start-up, OLEOWAYS, developing a new oil for hybrid coating of surfaces. The idea consisted to modified a vegetable oil by click-chemistry to obtain Si-coated vegetable oils able to for hybrid coating on glass or metal surfaces. The coating obtained showed interesting properties as protective coating, and able to modify the resistance properties of cellulose.
Then he went the same year in the university of Aveiro, Portugal, to study the use of luminescence for thermometry. He worked during almost one year and a half on the synthesis and study of Lanthanide MOFs permitting to make pH-sensing and/or thermometry using Eu luminescence. Particularly, he investigated on the bimodal thermometry using both radiometric change of intensity and shift of the Eu emission bands of these compounds. By varying the experimental conditions, he obtained a new phase, which reveals able to be use for bimodal thermometry. The study is still active, and some articles are expected.

In 2018, he obtained an ERC fellowship Horizon 2020 with Dr. Ángel Millán Escolano from ICMA, in Zaragoza, Spain, and moved there, where he is still actually working. This current project consists in improving a system developed in this team with the collaboration of the University of Aveiro consisting of a nano-object permitting to act as both a nanoheater and a nanothermometer to performed local controlled hyperthermia. The main goal lies in determining the feasibility of a new concept of cancer therapy, based on the local intracellular therapy. For this, one of the main activities consists in improving the luminescent properties of the lanthanides' complexes used, as their stability, to obtain better suitable luminescent thermometers for biological applications. In this purpose, he is currently working on a study of various complexes, and particularly about the effect of the nature of the ancillary ligand on the luminescent properties, which still remains not understood in the literature.

LIST OF 5 MAIN ARTICLES:

- G. Maurin-Pasturel, and al., 2019, Dalton Transactions, 48 (18), 6205-6216.
- G. Maurin-Pasturel, and al., 2019, Eu. J. Lipid. Sci. Technol., 121 (4), 1800231.
- G. Maurin-Pasturel, and al., 2017, Inorganic chemistry frontiers, 4, 1737.
- G. Maurin-Pasturel, and al., 2017, Chemistry: An European Journal, Vol. 23, 31, 7483-7496. (Inside Cover)
- G. Maurin-Pasturel and al., 2014, Angewandte Chemie, 53, 3872. (Back Cover)

Carolina Medina-Montano

Hintere Bleiche 61, 55116 Mainz – 01783750382 – gmedinam@students.uni-mainz.de

Mrs Medina-Montano is graduated in Microbiology from the University of Antioquia in Medellin, Colombia. Mrs Medina-Montano came for a couple years to Germany to continue her studies in Biomedicine at the Johannes Gutenberg University in Mainz. Mrs Medina-Montano´s carrier started in Colombia by working in clinical laboratories in different hospitals. In these roles, she discovered her passion about investigation and she decided to move to Germany to obtain her Master degree. After finish her Master degree, she has worked at the University Medical Center in Mainz and Frankfurt, in several topics including tropical medicine, traslational immunology and hemato-oncology.
In December 2019, she started her PhD in Nano-biomedicine in the research laboratory group from Professor Grabbe. She is working on several nanoparticles projects, which include the collaboration with other interdisciplinary research groups at the Johannes Gutenberg University in Mainz, Germany and also with the University of Antioquia in Medellin, Colombia. Through her interdisciplinary and international work, she can also speak three languages: Spanish as mothertongue, English and German.

Florian Meier

Florian Meier holds a PhD in Analytical Chemistry earned from University of Ulm, Germany in 2013 and joined Postnova Analytics in 2014 as Group Leader Research.
In this position, he gained vast experience in the application of various Field-Flow Fractionation (FFF) techniques and related detection systems such as for example Multi Angle Light Scattering, Dynamic Light Scattering or Inductively-Coupled Plasma Mass Spectrometry.
As a passionate researcher in an industrial environment, his research focuses on the characterization of samples in the nano- and micrometer size range (e.g. engineered nanomaterials, micro- and nanoplastics, environmental colloids, proteins, polymers, viruses, nano-enabled pharmaceuticals and many more), thereby exploiting and continuously pushing the limits of multi-detector FFF. In this respect, he was and is involved in several collaborative national and international research projects
Being a designated member of the "Arbeitsausschuss Nanotechnologien" of the German Institute for Standardization (DIN), he enjoys bringing in his FFF-expertise as an appointed expert for the ISO/TC 229 "Nanotechnologies".

LIST OF NATIONAL AND INTERNATIONAL COLLABORATION PROJECTS (EXCERPT)

- NanoCELL, German BMBF, ongoing (project coordinator) https://www.nanopartikel.info/en/projects/current-projects/nanocell
- SubµTrack, German BMBF, ongoing; https://bmbf-plastik.de/en/joint-project/submtrack
- ACEnano, EU Horizon 2020 Programme, ongoing; http://www.acenano-project.eu/
- NanoUmwelt, German BMBF, 2014-2017 (project coordinator); https://www.nanopartikel.info/en/projects/completed-projects/nanoumwelt
- SamrtNano, EU Framework 7 Programme, 2012-2016; https://www.linkedin.com/in/smartnano-project-47496763/

LIST OF PEER-REVIEWED PUBLICATIONS (EXCERPT)

- M. Hesler, L. Aengenheister, B. Ellinger, R. Drexel, S. Straskraba, C. Jost, S. Wagner, F. Meier, H. von Briesen, C. Büchel, P. Wick, T. Buerki-Turnherr, Y. Kohl, "Multi-endpoint toxicological assessment of polystyrene nano- and microparticles in different biological models *in vitro*", Toxicology in Vitro, 2019, 61, 104610.
- D. Müller, M. Nogueira, S. Cattaneo, F. Meier, R. Drexel, C. Contado, A. Pagnoni, T. de Vries, D. Cohen, M. Portugal-Cohen, A.J. deMello, "Integration of inverse Supercritical Fluid Extraction and miniaturized Asymmetrical Flow Field-Flow Fractionation for the rapid analysis of nanoparticles in sunscreens", Analytical Chemistry, 2018, 90(5), 3189-3195.
- Z. You, F. Meier, S. Weidner, "Comparison of Miniaturized and Conventional Asymmetrical Flow Field-Flow Fractionation (AF4) Channels for Nanoparticle Separations", Separations, 4(1), 8-19.
- V. Sogne, F. Meier, T. Klein, C. Contado, "Investigation of Zinc Oxide particles in cosmetic products by means of Centrifugal and Asymmetrical Flow Field-Flow Fractionation", Journal of Chromatography A, 2017, 1515, 196-208.
- K. Eskelin, M. Lampi, F. Meier, E. Moldenhauer, D.H. Bamford, H.M. Oksanen, "Asymmetric flow field flow fractionation methods for virus purification", Journal of Chromatography A, 2016, 1469, 108-119.

Ana Milosevic

Ana Milosevic obtained her MSc degree in Biochemistry at Faculty of Chemistry, University of Belgrade. In 2018 she completed her PhD studies at the Adolphe Merkle Institute, University of Fribourg, in the group of Prof. Barbara Rothen Rutishauser and Prof. Alke Fink where she worked with fluorescently labelled nanoparticles and investigated their interaction with cell and ultimate fate. Currently she is a project manager for the national contactpoint-nano.ch for the safe handling of nanomaterials, regulation and knowledge transfer.

Gergely Milosevits

MD, specialist of pediatrics, research fellow (Nanomedicine Research and Education Center, Institute of Translational Medicine, Semmelweis University, Budapest, Hungary).
After graduating from Semmelweis University in Budapest, dr. Gergely Milosevits started working as a medical doctor at the University's II. Department of Pediatrics, where he became a specialist in pediatrics and the head of the General Outpatient Ward. Recently he has been working as a pediatric GP in Szigetszentmiklós. He is also a research fellow in the laboratory of Professor János Szebeni at the Nanomedicine Research and Education Center in Budapest, Hungary. He is especially interested in flow cytometry, liposomes, exosomes and CARPA.

RECENT PUBLICATIONS:

- Exosomes: potential model for complementstealth delivery systems. Eur. J. Nanomed. 2015; 7(3): 207–218. Gergely Milosevits*, János Szebeni and Silke Krol
- University thesis: Flow cytometric analysis of the physicochemical characteristics and stability of nanopharmaceutical carriers and agents.
- Flow cytometric analysis of supravesicular structures in doxorubicin-containing pegylated liposomes. Chem Phys Lipids. 2012 May;165(4):482-7. Milosevits G, Rozsnyay Z, Kozma GT, Milosevits J, Tömöry G, Robotka H, Rosivall L, Szebeni J.

Nura Adam Mohamed

Dr. Nura Adam Mohamed is currently working as a Postdocoral fellow at Qatar University, Doha-Qatar. She received her Bachelor degree in Biomedical Science from Qatar University (2009) and her Master and PhD degrees from Imperial College of London (2012-2016). Her current postdoctoral fellowship is between Qatar University and Imperial College of London as she is working on a project entitled "A nanomedicine approach to the treatment of pulmonary arterial hypertension", She has previously worked as a research fellow at Qatar University and before that worked as a research assistant in the Shafallah Medical Genetic Centre, Doha-Qatar gaining experience in in recombinant DNA. She is a member of the British Pharmaceutical Society, the British Pharmaceutical Society Advisory Group, the British Nanomedicine Society and holds an honorary research officer position at Imperial College of London. She has been supervising BSc and Master Students from UK and Qatar for their research projects since 2015. She has also won several local and international awards as well as holding funds for her current project. Her main focus is to apply nanotechnology and stem cells applications to improve treatment strategies for cardiovascular diseases. Her disease of interest is Pulmonary Arterial Hypertension (PAH) which is a devastating/incurable disease with available treatments being limited by their half-life and systemic side effects. These limitations can be overcome by the use of the unique nanoparticles, the Metal organic frameworks (MOF)-nanoMIL-89 for the many attractive properties it offers. Her work yield the development of the first PAH drug-MOF conjugate which showed unique pharmacokinetic properties that is now being tested *in vivo* using PAH models for it to be taken all the way to the pre-clinical stages

Marzieh Mohammadi

Marzieh was born in 1989 in Iran. In 2013, She received her Pharm.D. degree and the thesis title was "Targeted delivery of BCL9L siRNA to colon carcinoma stem cells using aptamer- conjugated carbon nanotubes" which was published in international journal of pharmaceutics. Then, she started her PhD in Pharmaceutical Nanotechnology in Mashhad University of Medical Sciences (MUMS), Iran and received her PhD degree in 2018. Her PhD thesis was entitled "Electrospun nanofibers containing BMP2-encapsulated liposomes to promote osteogenic differentiation" under the supervision of Prof. Mohammad Ramezani and Prof. Mahmoud Reza Jaafari. In 2017, she joined a short term visiting scholar program in Harvard-MIT division of health sciences and technology, USA under the supervision of Prof. Khademhosseini.
Marzieh was ranked among top 10 in National medical students Olympiad, 2011 and she was the top graduated student (first rank) of Mashhad pharmacy school based on overall score (18.37 out of 20), 2013. Additionally, she ranked first among PhD candidates for PhD program in pharmaceutical nanotechnology in Iran, 2013. During her PhD program, she published 17 papers (13 original and 4 review) in high impact journals such as Advanced functional materials. Her thesis papers were also published in the journal of Nanomedicine: Nanotechnology Biology and Medicine and Controlled release.
Recently, she started as an assistant professor of pharmaceutics at school of Pharmacy, MUMS, Iran and her current research interest is focused on the design of drug delivery systems and their application in regenerative medicine and cancer therapeutics.

Fotios Mpekris

Dr. Mpekris earned a BS degree in Physics (2012) and a PhD in Biomedical Engineering (2016) from the University of Cyprus. During his doctoral studies, he focused on the development of strategies that remodel the tumor microenvironment to improve drug delivery and thus, therapeutic outcomes. He was initially trained as a biomedical engineer and mathematical modeller, and received experimental training on the biomechanical characterization of solid tumors and other biological tissues and polymers. Subsequently, he was extensively trained in murine tumor models, small laboratory animal handling and surgical procedures as well as in anticancer drug treatments. His research work has been licensed by the Veterinary Services of the Ministry of Agriculture (Republic of Cyprus). These acquired skills and knowledge has enabled him to work comfortably at the interface of mathe-

matical, computational and experimental biology. Since 2016, Dr. Mpekris has been a Postdoctoral fellow at the Cancer Biophysics Laboratory at the University of Cyprus. He has been participated in research projects funded by the European Research Council (336839-ReEngineeringCancer), Research Promotion Foundation of Cyprus (POST-DOC/0718/0084, CancerNanoMED) and the University of Cyprus.
The implementation of his research has led to the publication of a large number of articles in high impact journals and the development of scientific expertise that is internationally competitive. He has co-authored 21 scientific articles in peer-review journals (h-index=10, >550 citations), including 4 publications in PNAS and articles in Cancer Research, ACS Nano, Journal of Controlled Release, Oncotarget and Theranostics. Additionally, he has co-authored 8 papers and/or abstracts in referred conference proceedings and has given 6 podium presentations in International conferences.

RECENT PUBLICATIONS:

- Panagi M., Voutouri C., Mpekris F., Papageorgis P., Martin M.R., Martin J.D, Polydorou C., Kojima M., Ishii G., Kataoka K., Cabral H., Stylianopoulos T. (2020). TGF-β inhibition combined with nanomedicine normalizes the metastasis microenvironment towards anti-tumor immunity. Theranostics 10(4):1910-1922
- Martin J.D., Panagi M., Khan T.T., Wang C., Martin M.R., Voutouri C., Toh K., Papageorgis P., Mpekris F., Suzuki T., Wilheim M., Melo V.A., Polydorou C., Quader S., Norimatsu J., Lanning R.M., Kojima M., Stuber M.D., Styalianopoulos T., Cabral H., Kataoka K. (2019). Dexamethasone increases nanocarrier delivery by normalizing the tumor microenvironment. ACS Nano 13(6):6396-6408 [DOI:10.1021/acsnano. 8b07865]
- Zhao Y., Cao J., Jones D., Zhang Y., Nia H.T., Stylianopoulos T., Mpekris F., Datta M., Sun Y., Wu L., Gao X., Jain R.K., Xu L. (2019). Losartan treatment augments chemotherapy efficacy and reduces ascites by normalizing the tumor stroma in ovarian cancer models. PNAS 116(6):2210-2219 [DOI:10.1073/pnas. 1818357116]
- Voutouri C., Kirkpatrick N.D., Chung E., Mpekris F., Baish J.W, Munn L.L, Fukumura D., Stylianopoulos T., Jain R.K. (2019). Dynamics of vessel cooption in brain tumors revealed by integrative experimental and mathematical modeling studies. PNAS 116(7):2662-2671 [DOI:10.1073/pnas. 1818322116]
- Mpekris F., Baish J.W., Stylianopoulos T., Jain R.K. (2017). Role of vascular normalization in benefit from metronomic chemotherapy. PNAS, 114(8):1994-1999 [DOI:10.1073/pnas.1700340114]

Per H. Nilsson

Ph.D.
Associate Senior Lecturer, Centre for Biomaterials Chemistry,
Linnaeus University, Kalmar Sweden;
Researcher, Department of Immunology,
University of Oslo, Oslo, Norway
Host Response to Biomaterials Laboratory (HoRB)

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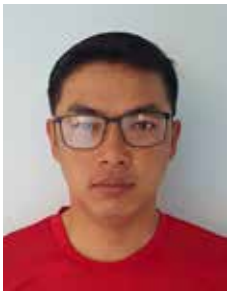
I am an associate senior lecturer and principal investigator of the research group "Host Response to Biomaterials" laboratory. I share my time equally between the Centre for Biomaterial Chemistry, Linnaeus University, Kalmar, Sweden, and the Department of Immunology, University of Oslo, Oslo, Norway. My time is allocated to research and teaching at approximately 75% and 25%, respectively. The focus of our research in the group of Host Response to Biomaterials Laboratory (HoRB) is to characterize how biomaterials perform in a biological system, with emphasis on the acute intravascular immune and thrombotic responses. We are a relatively junior research group with five people, including one post-doc, two Ph.D. students, and a technician. Our current main projects deal with i) the immunothrombotic response to nanoparticles in human whole blood, ii) the inflammatory and thrombotic *in vivo* response to implantation of a mechanical heart pump, so-called LVAD, and iii) the impact of the thrombotic response on inflammation. My research is mainly funded by grants from the Norwegian and Swedish research councils. I have my master's degree in Biomedicine from the University of Kalmar, Sweden, in 2006. I took my doctoral degree in Biomedical Sciences at Linnaeus University, Kalmar, Sweden, in 2012 with a compiled thesis with the title "Interactions between platelets and complement with implications for the regulation at surfaces", which included, e.g., two main authorships in Biomaterials. I continued as post-doc and later researcher in the group of Tom Eirik Mollnes at the University of Oslo. The overall focus of our research in Oslo was innate immune activation in sterile and septic inflammation. From that time, I have senior authorships in e.g., PNAS and the Journal of Infectious Diseases.
In miscellaneous information, I am a board member in the European Complement Network (http://www.ecomplement.org/) and a dedicated marathon runner with a fascination with ultra-long distances.

RECENT PUBLICATIONS:

- Thorgersen EB, Barratt-Due A, Haugaa H, Harboe M, Pischke SE, Nilsson PH, Mollnes TE: The role of complement in liver injury, regeneration and transplantation. Hepatology 2019.
- Wibroe PP, Anselmo AC, Nilsson PH, Sarode A, Gupta V, Urbanics R, Szebeni J, Hunter AC, Mitragotri S, Mollnes TE: Bypassing adverse injection reactions to nanoparticles through shape modification and attachment to erythrocytes. Nature Nanotechnology 2017, 12(6):589-594.
- Harboe M, Johnson C, Nymo S, Ekholt K, Schjalm C, Lindstad JK, Pharo A, Hellerud BC, Ekdahl KN, Mollnes TE, Nilsson PH: Properdin binding to complement activating surfaces depends on initial C3b deposition. Proceedings of the National Academy of Sciences 2017:201612385.
- Engberg AE, Nilsson PH, Huang S, Fromell K, Hamad OA, Mollnes TE, Rosengren-Holmberg JP, Sandholm K, Teramura Y, Nicholls IA, Nilsson B, Ekdahl KN: Prediction of inflammatory responses induced by biomaterials in contact with human blood using protein fingerprint from plasma. Biomaterials 2015, 36:55-65.
- Nilsson PH, Ekdahl KN, Magnusson PU, Qu H, Iwata H, Ricklin D, Hong J, Lambris JD, Nilsson B, Teramura Y: Autoregulation of thromboinflammation on biomaterial surfaces by a multicomponent therapeutic coating. Biomaterials 2013, 34(4):985-994.



Huy Quang Quach

Quang Huy Quach is a postdoctoral fellow at Department of Immunology, Oslo University Hospital. He is working within Norwegian Complement Research Group led by Dr. Tom Eirik Mollnes.
Currently, his research focuses on the development of ex vivo whole blood model for the evaluation of biocompatibility of biomaterials. This includes the interaction of biomaterials with different components of whole blood, such as the complement system, coagulation system, granulocytes, monocytes, platelets. The goal of this study is to fabricate an ex vivo whole blood model that better mimics human whole blood at its physiological conditions for the evaluation of biocompatibility of biomaterials.
Quang Huy holds a Bachelor degree in Biotechnology from Can Tho University, Vietnam; Master degree in Nanobiotechnology from Korea University of Science and Technology, Republic of Korea; and PhD degree in Biomedical Engineering from National University of Singapore, Singapore. His master thesis focused on the devel-

opment of fluorescent assays for the detection of cancer-related enzymes, including telomerase and DNA methyltransferase. During his doctoral research, Quang Huy worked on the interactions of selected nanomaterials with the complement system of innate immunity and the development of nanomaterial-based subunit vaccine against dengue virus.

RECENT PUBLICATIONS

- QH Quach, J Jung, H Kim, BH Chung, "A simple, fast and highly sensitive assay for the detection of telomerase activity", Chemical Communication, 2013.
- QH Quach, BH Chung, "A signal-on fluorescent assay for DNA methyltransferase activity using a methylation-resistant endonuclease", Analyst, 2014.
- QH Quach, JCY Kah, "Non-specific adsorption of complement proteins affects complement activation pathways of gold nanomaterials", Nanotoxicology, 2017.
- QH Quach, RLX Kong, JCY Kah, "Complement activation by PEGylated gold nanoparticles", Bioconjugate Chemistry, 2018.
- QH Quach, SK Ang, JJH Chu, JCY Kah, "Size-dependent protective activity of gold nanoparticle-based subunit vaccine against dengue virus", Acta Biomaterialia, 2018.

Mohammad Ramezani

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Mohammad Ramezani received his Pharma. D. degree from School of Pharmacy, Mashhad University of Medical Sciences, Mashhad, Iran in 1988. He granted a scholarship from the Ministry of Health and Medical Education of Iran to continue his education toward a PhD degree at the Department of Chemistry, Dalhousie University, Canada in 1996. After graduation, he continued his career as an Assistant Professor at the Department of Pharmaceutical Biotechnology, School of Pharmacy, Mashhad University of Medical Sciences. He was then promoted to position of Associate and full Professor in 2000 and 2004, respectively. Recently, he was promoted to the position of Distinguished Professor. The main focus of Dr. Ramezani's research is to develop functional nanomaterials for targeted gene and drug delivery purposes. He has published over 330 research papers in highly cited journals such as Advanced Functional Materials, Biomaterials, Journal of Controlled Release, Nanomedicine and International Journal of Pharmaceutics. He is also the Editor-in-Chief of the Nanomedicine Journal published by the Nanotechnology Research Center, Mashhad University of Medical Sciences. Dr. Ramezani has won the National Razi prize for best investigator in 2009 and 2018.

SELECTED PUBLICATIONS:

- F. Hosseini Shamili, M. Alibolandi, H. Rafatpanah, K. Abnous, M. Mahmoudi, M. Kalantari, S. M. Taghdisi, M. Ramezani* (2019). Immunomodulatory properties of MSC-derived exosomes armed with high affinity aptamer toward mylein as a platform for reducing multiple sclerosis clinical score. Journal of Controlled Release, 299, 149-164 (IF: 7.901).
- M. Shahriari, M. Zahiri, K. Abnous, S. M. Taghdisi, M. Ramezani*, M. Alibolandi (2019). Enzyme responsive drug delivery systems in cancer treatment. Journal of Controlled Release, 308, 172-189 (IF: 7.901).
- F. Charbgoo, M. Alibolandi, S. M. Taghdisi, K. Abnous, F. Soltani, M. Ramezani* (2018). MUC1 aptamer-targeted DNA micelles for dual tumor therapy using doxorubicin and KLA peptide. Nanomedicine: Nanotechnology, Biology, and Medicine, 14(3), 685-697 (IF: 5.570).
- M. Mohammadi, S. Taghavi, K. Abnous, S. M. Taghdisi, M. Ramezani*, M. Alibolandi (2018). Hybrid Vesicular Drug Delivery Systems for Cancer Therapeutics. Advanced Functional Materials, 28 (36), DOI: 10.1002/adfm.201802136, (IF: 15.621).
- S. M. Tghdisi, N. M. Danesh, M. Ramezani, R. Yazdian-Robati, K. Abnous (2018). A Novel AS1411 Aptamer-Based Three-Way Junction Pocket DNA Nanostructure Loaded with Doxorubicin for Targeting Cancer Cells in Vitro and in Vivo. Molecular Pharmaceutics, 15 (5), 1972-1978 (IF: 4.396).

Pouria Ramezani

Pouria Ramezani was born on September 1991 and after his graduation from high school in 2013, he was admitted to Pharm.D. program at School of Pharmacy, Mashhad University of Medical Sciences, Mashhad, Iran. He is currently senior Pharm. D. student having passed 213 credits successfully and will be defending his thesis soon. During his Pharm. D. curriculum, he has been involved in research activity focusing on Nanomedicine. The title of his thesis is "synthesis of targeted and controlled release hybrid polymersomes encapsulating SN38 *in vitro* and *in vivo*" which demonstrates preparation of MMP-2 responsive carrier being loaded with SN38 to deliver the desired drug molecule to colon adenocarcinoma. The goal of his study is to investigate how the intelligent carrier could increase therapeutic index of the encapsulated anti-cancer agents and secondly, to develop aptamer-targeted enzyme-responsive carrier which could act as a dually effective nanomedicine against cancer. Pouria has finished writing the manuscript from the results of his thesis project and it is under review by the Journal of Nanomedicine.
He has also been the co-author of four articles published in peer-reviewed journals. The total citations to his articles is 102 and his H-index is 4 according to Google Scholar. He has been awarded the "Researcher of The Year 2018" at the research week ceremony at Mashhad University of Medical Sciences, Mashhad, Iran as well as a 3rd place at the 22nd Iranian Pharmacy Students Seminar (IPSS). He also attended the well-recognized ISCOMS congress held by University of Groningen, Netherlands and was the session winner in Biomaterials category. He is very keen to continue his education towards a Ph.D. Degree in Nanomedicine.

Nicolas Ritt

PhD candidate
Nicolas started studying Biomedical Chemistry with a focus onOrganic Chemistry and Biochemsitry at Johannes Gutenberg-University in Mainz in 2011. During his studies he specialized on Macromolecular Chemistry and Biopolymers. He wrote his Bachelor thesis in the group of Professor Dr. Holger Frey and graduated in July 2014 as Bachelor of Science.
He subsequently started his master studies in Biomedical Chemistry and was accepted as exchange student at College of Engineering at the renowned Seoul National University in Seoul, South Korea. From August 2014 to March 2015 he worked and studied abroad in the group of Prof. Dr. Kookheon Char to further deepen his knowledge in Macromolecular Chemistry.
After he returned to Johannes Gutenberg University, he continued his master studies and in 2016 Nicolas wrote his master thesis with the title "Difunktionelle Triblock Copolymere als Transfektionsa-

gentien" at the group of Professor Dr. Rudolf Zentel and graduated as Master of Science in February 2017.
Following his graduation, Nicolas started his PhD studies at the group of Professor Zentel in May 2017. His research interests focus on the synthesis of multifunctional block-copolymers via RAFT-polymerization and the synthesis and characterization of well defined polyplexes as innovative transfection agents.
Currently he is associated in the CRC 1066: Nanodimensional polymer therapeutics for tumor therapy.

Silvia Lucia Rizzelli

PhD candidate
Silvia started studying Biomedical Chemistry with a focus on Organic Chemistry and Biochemsitry at the Johannes Gutenberg-University of Mainz in autumn 2011. During her studies she specialized on Analytical Chemistry and Macromolecular Chemistry. She wrote her Bachelor thesis in the group of Professor Dr. Rudolf Zentel in the field of Material Science. From October 2014 to March 2015 she spent a research semster at the University of Sheffield in England. There she worked in the Group of Prof. Dr. Steven P. Armes on the topic of non aqueous pickeling emulsions. After she returned to Mainz, she graduated in September 2015 as Bachelor of Science.
She subsequently started her master studies in Biomedical Chemistry specializing in Biophysical Chemistry and Bioanorganic Chemistry.
Silvia wrote her master thesis at the group of Professor Dr. Rudolf Zentel with the title "P(HPMA) based Transfection agents and graduated as Master of Science in September 2017.
Following her graduation Silvia started her PhD studies at the group of Professor Zentel in September 2017. Her research interests focuses on the synthesis of multifunctional block-copolymers via RAFT-polymerization and the synthesis and characterization of well defined nanoparticular structures for nanomedical application.
Currently she is associated in the CRC 1066: Nanodimensional polymer therapeutics for tumor therapy.

Mohammad Mahdi Sabahi

I am Mohammad Mahdi Sabahi, 6th year medical student at Hamedan University of Medical Sciences. I'm learning statistical analysis, research methodology, and scientific writing by taking a second degree as MPH. In the past several years, my research has largely remained focused on neurologic disorder, which yielded several publications.
I am determined to continue a residency in neurosurgery upon finishing my med school on 2021 and I have already started empowering myself by engaging in research and clinical training programs and I'll be doing my neurosurgery internship at Medical University of Vienna on March 2020.
As you see in my curriculum vitae and my experiences, I am committed to giving my best to any situation, and I'm extremely coordinated, goal-directed and I have excellent teamwork, team management and conflict resolution according to my colleagues. Also, I'm fluent in English and Persian, and I have a good knowledge of Arabic and Turkish, moderate knowledge of German and some knowledge of French.
In an attempt to develop skills in neurosurgery research, I have embarked my research journey by engaging in two original studies regarding immunotherapy of glioblastoma and spinal surgery in the Brain and Spinal Cord Injury Research Center. Research into Neurosurgery, Neurology and Neurosciences are of particular interest to me, as is my aspiration to work as a neurosurgeon in the future. I have often received positive feedback from my supervisors at different university hospitals of the Hamedan University of Medical Sciences on my clinical skills in patient assessment and I always look forward to receive constructive feedback on my progression. Following is my 5 recent publications:

RECENT PUBLICATIONS

- https://precisionnanomedicine.com/article/10592
- https://www.futuremedicine.com/doi/abs/10.2217/fvl-2019-0046
- https://www.ncbi.nlm.nih.gov/pubmed/31378723
- https://www.ncbi.nlm.nih.gov/pubmed/30447146
- https://www.ncbi.nlm.nih.gov/pubmed/31193574

Arunsajee Sae-be

Ph.D. student in Pharmaceutics (2nd year), Department of Pharmacy, Faculty of Pharmacy, Mahidol University, Bangkok, Thailand, 10400
T +66 98 015 4159 E-Mail: Arunsajee.s@gmail.com

Miss Arunsajee Sae-be is a Ph.D. student in Pharmaceutics (2nd year), Department of Pharmacy, Mahidol University. She is interested in nanomedicines, nanomaterials, cancer targeted drug delivery, cancer theranostics and tissue engineering. Her main research focuses on the development of 3D-liver spheroids and theranostics for liver cancer treatment.She studied in a pharmacy program and received the Bachelor degree in Pharmaceutical Science from Srinakharinwirot University, Thailand from 2010 to 2016. Her senior project associated with antibacterial and antifungal activities of essential oils which were entitled "Anti-Trichophyton mentagrophytes and anti-Staphylococcus aureus activities of essential oils from Zingiberaceae and Rutaceae families". After she graduated from Srinakharinwirot University, she served as a regulatory affairs pharmacist to prepare the registration dossier of new generic and generic drugs as well as inspected other activities to ensure that all activities were in compliance with applicable regulations at Siam Bheasach Co, Ltd from 2016-2018. Furthermore, she participated in the ASEAN regulation meeting.
In 2018, she has studied the Doctoral degree in Pharmaceutics at Mahidol University, Thailand and received the Royal Golden Jubilee Scholarship to research cancer theranostics. She started to fabricate 3D-liver spheroids. She participated in the International Conference and Exhibition on Pharmaceutical Sciences and Technology, Thailand 2019 (PST2019) as a poster presenter. The poster was entitled "The development of 3D-spheroid model for anticancer drug screening". She received the silver prize of the best poster award.

Amir Sh. Saljooghi

Amir Sh. Saljooghi was born and raised in Iran, Kerman. He received his B.Sc. in chemistry from Tehran University in 2003 and afterward subsequently completed his Ms.C. and Ph.D. degrees in Bio-Inorganic chemistry in 2010 with Prof. S. J. Fatemi. The thesis title was "Clinical evaluation of Deferasirox, Desferrioxamine and Deferiprone as single and combined for removal of Cd(II) and Tl(III) ions in rats as a biological model ".

Afterward he joined the Department of Chemistry at Ferdowsi University of Mashhad (FUM), Mashhad, Iran in 2010, where he is now Associate Professor of Bio-inorganic Chemistry, and Director of the Medicinal Inorganic Chemistry Group. His scientific interests are firmly based in the areas of medicinal inorganic chemistry and coordination chemistry; he has been involved over 10 years with nano bio-inorganic chemistry systems, metal ion decorporation, and the role of metal ions in chelation therapy, as well as chemotherapeutic metal complexes and ligands. Amir Sh. Saljooghi has published more than 40 research papers, and 6 books (in Persian) related to Inorganic, Bio-inorganic, and physical inorganic chemistry chemistry.
Recently, he and his research team focused on Theranostics platforms for cancer therapy, especially the role of Metal Organic Frameworks (MOFs), Mesoporous silica nanoparticles (MSNs), Polyoxometalates (POMs), and etc. in targeted drug delivery.

Maximilian Scherger

PhD
Maximilian Scherger studied biomedical chemistry at the Johannes Gutenberg University, Mainz (Germany), and received his master's degree in 2018. He is currently a PhD student in the group of Dr. Lutz Nuhn at the Max Planck Institute for Polymer Research and a member of the Integrated Research Training Group within the Collaborative Research Center 1066, Mainz (CRC 1066). He received a doctoral scholarship of Fonds der Chemischen Industrie and his PhD topic is about responsive carrier systems for the delivery of small molecular immunomodulators.

RECENT PUBLICATIONS

- "Multiarm Polycarbonate Star Polymers with a Hyperbranched Polyether Core from CO 2 and Common Epoxides" M. Scharfenberg, J. Seiwert, M. Scherger, J. Preis, M. Susewind, H. Frey, Macromolecules 2017, 50, 6577–6585.

Sascha Schmitt

Ph.D-Student
Sascha Schmitt studied chemistry at the Johannes-Gutenberg University of Mainz (Germany). He receives his master degree in 2018 concentrating on simultaneous dynamic and static light scattering on PEG based hydrogels. Currently he is working on his Ph.D. under the supervision of Prof. Dr. H.-J. Butt and Dr. Kaloian Koynov at the Max Planck Institute for Polymer Research. His research focuses on fluorescence correlation spectroscopy on polymer based systems for drug delivery.

Inbar Shreiber-Livne

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I am a Ph.D student in The Norman Seiden Multidisciplinary Graduate program from the Nanotechnology & Nanoscience institute, under the supervision of Ass. Prof. David Meiri (the Laboratory of Cancer Biology and Cannabinoid Research) and Prof. Alejandro Sosnik (Laboratory of Pharmaceutical Nanomaterial Science). My Research fields in this doctorate study include, chemistry, physics and engineering of polymers, nanoparticles, nanomedicine and cannnabinoids. My research topic is "Cannabinoid-Containing Polymers as Novel Nano Delivery Platforms".
In 2016 I completed a M.Sc. degree from the Materials Engineering department in the Technion, Israel, for which I received a full scholarship. This work involved several disciplines, including chemistry, physics and engineering of polymers, porous and responsive polymers, hydrogels and crosslinking mechanisms. The research title was: "Polymers: PolyHIPEs: Self-crosslinked, high porosity hydrogels through emulsion templating".
I completed in 2014 my B.Sc from the Chemical Engineering department in the Technion, Israel. In 2012 I received the Dean's excellence award for graduate students. My senior year research project was performed under the supervision of Prof. Moshe Narkis from the Chemical Engineering department in the Technion. This work dealt with the development of supercapacitors and for which I received the Seiden Prize for multidisciplinary undergraduate student's project (2013) in the area of Nano-electronics. Following completion of this research project I continued to work as a research assistant in Prof. Narkis' lab during the years 2013-2014.
I was a teaching assistant in the faculties of Biology and at the Department of Materials Science and Engineering. The courses included Biology 1, Introduction to biochemistry and enzymology and Introduction to Materials Engineering.

Jenny Schunke

PhD student
In 2017, I received my Bachelor's degree in molecular biology from the Johannes Gutenberg-University in Mainz. Afterwards, I completed my Master's degree in Biomedicine and focused on the investigation of therapy-resistance developing in chemotherapeutically treated penis carcinoma cell lines. Since October 2019, I am a PhD student of the AG Mailänder at the University Medical Center (Department of Dermatology) and the MPI for Polymer Research in Mainz. In my current PhD project, I am working on the targeting of dendritic cells and the antigen-specific activation of T cells using nanoparticles, including adjuvants and antigens, *in vitro* and *in vivo* in different melanoma models. The Research Training Group (CRC 1066), of which I have been a member since January 2020, connects young researchers working on nanodimensional polymeric therapeutics for tumor therapy and gives me the opportunity to exchange ideas and experiences in an interdisciplinary environment.

Yang Shi

Dr. Yang Shi works as a Group Leader at RWTH Aachen University Clinic in Germany since 2016. Prior to that, he was appointed Associate Professor at South China University of Technology in 2015 and obtained his PhD degree from Utrecht University, the Netherlands, with Prof. Wim Hennink in 2014. He has published >35 articles (total citations>1000, H index 19) in peer-reviewed journals including Chemical Society Reviews, Nature Nanotechnology, ACS Nano, Advanced Healthcare Materials, and Biomacromolecules. In 2016, he was recognized as "Rising Star" by the 4th Symposium on Innovative Polymers for Controlled Delivery. In 2019, he was awarded with the Europe Award by International Pharma Sciences Foundation/Rottendorf Stiftung. He is currently a Guest Editor for Theranostics and serves as a reviewer for journals including JACS, ACS Nano, Nano Letters, and Angewandte Chemie. His group focuses on nanomedicines and macroscale biomaterials for cancer chemotherapy and immunotherapy.

RECENT PUBLICATIONS

(*corresponding author, #shared first author, total 35 publications, H index=19)

- Sun Q, Barz M, De Geest BG, Diken M, Hennink WE, Kiessling F, Lammers T*, and Shi Y*. Nanomedicine and macroscale materials in immuno-oncology. Chemical Society Review. 2019, 48, 351–381.
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- Shi Y, van der Meel R, Theek B, Oude Blenke E, Pieters EH, Fens MH, Ehling J, Schiffelers RM, Storm G, van Nostrum CF, Lammers T, Hennink WE*. Complete regression of xenograft tumors upon targeted delivery of paclitaxel via Π-Π stacking stabilized polymeric micelles. ACS Nano, 2015, 9, 3740–3752.
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Sara Shokooh Saremi

Born in 1989, Sara received her Pharm.D Degree (Professional Doctorate in Pharmacy - in Iran is adapted from the US education system and is not equivalent to PhD) from Mashhad University of Medical Sciences (Mashhad, Iran) in 2015. Following that, she became a PhD candidate of pharmaceutical nanotechnology. During her PhD courses, she was the top student and she achieved the highest degree among her classmates in the board exam which is the qualification exam held by the university.

She is highly interested in drug delivery systems, especially liposome bilayers, and their utilization in overcoming problems such as active compound's poor absorption or their low bioavailability.

As her very first experience in the field of drug delivery, she started to work on encapsulating a hydrophilic antigen in liposomes; at the same time, setting up a method to isolate dendritic cells from mice bone marrow. She sat up the above-mentioned method for the first time in Iran, successfully.

After few years of experience, as her PhD thesis, she is now working on a novel liposome formulation for encapsulating a tyrosine kinase inhibitor named "Lapatinib" and therefore, increasing its delivery to tumor cells while decreasing the usual administered dose and its side effects. She also works on several other projects and helps other students to accomplish their work.

She would like to assess the effects of liposome formulation of the encapsulated drugs *in vitro* and *in vivo* and compare the results with the active compound itself. In addition, she would like working toward gaining improved knowledge about the tumor microenvironment in different types of cancers, especially breast cancer and utilizing the information in targeting to the tumor site and improving outcomes.

Andrei P. Sommer

Dr. Sommer is an independent researcher and consultant. He received his diploma in physics (1992) and his PhD in physical chemistry (1998) both Philipps University Marburg, Germany. He specialized in nanomedicine, biomedical engineering, photomedicine and materials science at Ulm University, Germany, where he worked until 2016 as senior scientist. He is a visiting professor at the faculty of science of ISRA University, Jordan.

MAJOR ACHIEVEMENS IN SCIENCE AND MEDICINE

- Discovery of the principle of skin aging and rejuvenation method via irradiation with 670 nm light.
- Discovery of the principle to reduce intra- and extracellular Amyloid-β via 670 nm light and EGCG.
- Discovery of the principle of a biological photo field-effect transistor operating in mitochondria.
- Discovery of the relationship between mitochondrial spectral sensitivity and the solar spectrum.
- Discovery of treatment parameters to prevent outbreak of herpes labialis using red laser light.
- Design of method to refill ATP reservoirs in oxidatively stressed cells and tissues via R-NIR light.
- Design of III. generation Petri dish based on nanodiamond for better cell performance/vitality.
- Design of method to remove particulate matter from air in polluted cities via plants and water.
- Design of nanodiamond-based biosensors for detection of organic compounds on Mars.
- Design of and antibacterial and biocompatible metallic alloys for extended space flights.

PUBLICATIONS

More than 100 peer reviewed papers (95% first author)

Monika Stahl

The presenting author studied pharmacy at the Friedrich-Alexander-University Erlangen-Nürnberg, Germany from 2012-2017. After practical training in pharmacy and industry, she joined the group of Achim Goepferich, Department of Pharmaceutical Technology, University Regensburg in 2018 as a Ph.D. student.

Judith Stickdorn

PhD student

Judith Stickdorn studied biomedical chemistry at the Johannes Gutenberg University, Mainz (Germany), and received her master's degree in 2018. During her studies she could gain first research experience by spending 7 months in the laboratories of Eduardo Fernández-Megía at the Centro Singular de Investigación en Química Biológica y Materiales Moleculares in Santiago de Compostela (Spain). She is currently a PhD student in the group of Dr. Lutz Nuhn at the Max Planck Institute for Polymer Research and a member of the Integrated Research Training Group within the Collaborative Research Center 1066, Mainz (CRC1066). Her PhD topic is about nanogel-based vaccines for cancer immunotherapy.

PUBLICATIONS

- J. Stickdorn, L. Nuhn, Reactive-ester derived polymer nanogels for cancer immunotherapy, Eur. Polym. J. 124 (2020) 109481.

Fabian H.L Starsich

Fabian Starsich (born Sep. 22, 1990, in Vienna, Austria) received his MSc. in Process Engineer-ing from ETH Zurich, Switzerland in 2014. He then joined the Particle Technology Laboratory at ETH and received his Ph.D. in 2018 under the supervision of Prof. Dr. Sotiris E. Pratsinis. The title of his thesis was "Multifunctional nanoparticles for targeted theranostics". He developed magnetic nanoparticles for thermal therapy1 and as contrast agents for magnetic resonance imaging[2,3], as well as fluorescent nanoparticles for imaging in the near-infrared[4].

In the following, he worked for 1.5 years for the multi-award spin-off Haelixa AG on nano-particle-based tracers for the food industry. He then found his way back to academia and joined the Nanoparticle Systems Engineering Laboratory of Prof. Dr. Inge Herrmann at ETH Zurich and Swiss Federal Laboratories for Materials Science and Technology as a Postdoctoral Research As-sociate. His research now focuses on the development of novel nanosystems for biomedicine for diagnostic and therapeutic clinical applications. Specifically, he tries to understand the im-portance of agglomeration and resulting nanoparticle interaction phenomena concerning applica-tion efficiencies (i.e. targeting, therapeutic effects, diagnostic capabilities).[5] To this end, he works in close collaboration with the University Hospital Zurich.

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Mohammad Taleb

Ph.D. student

Mohammad is a Ph.D student at National Center for Nanoscience and Technology, China. He received his B.S. degree from Shahid Beheshti University (Tehran, Iran) in 2015, followed by an M.S. degree from University of Chinese Academy of Sciences (NCNST) in 2017. He accepted in Ph.D qualification exam from NCNST, Chinese Academy of Sciences immedietly after his M.S. by CAS-TWAS felowship. His main interests are design of bio-inspired materials to overcome the barriers in tumor therapy and nanobiomedicine. He would like to working toward the controlling the properties of multi-functional nanoparticles in order to allow specific targeting and regulation of tumor cells and their microenvironment (vessel normalization, immune system regulation, ...). His research interests include targeting and regulation of tumors and their microenvironment mediated by intelligent functional nanomaterials for diagnostic and therapeutic applications, especially Breast cancers.

RECENT PUBLICATION:

- [co-author] Targeting delivery of platelets inhibitor to prevent tumor metastasis, 2019 Bioconjugate Chemistry. [IF 4.349]
- [first-author] Dopamine Delivery via pH-Sensitive Nanoparticles for Tumor Blood Vessel Normalization and An Improved Effect of Cancer Chemo-therapeutic Drugs, 2019 Advanced Healthcare Materials. [IF 6.27]
- [co-author] Sustained release of sodium deoxycholate from PLGA–PEG–PLGA thermosensitive polymer, 2018 Artificial Cells, Nanomedicine and Biotechnology. [IF 4.462]
- [co-author] Surface Functionalization of Polymeric Nanoparticles with Umbilical Cord-Derived Mesenchymal Stem Cell Membrane for Tumor-Targeted Therapy, 2018 ACS Applied Materials & Interfaces. [IF 8.456]
- [co-author] Delivery of small interfering RNA against Nogo-B receptor via tumor-acidity responsive nanoparticles for tumor vessel normalization and metastasis suppression, 2018 Biomaterials. [IF 10.273]

IN PREPARATION:

- [co-author] Platelets and their role in cancer metastasis, from a saint to a demon, 2019 Science China Chemistry. [IF 6.085]

Dennis Unthan

Doctoral candidate

I completed my a-levels in 2010. After doing community service in a psychiatric clinic I started studying chemisty at the Johannes Gutenberg University of Mainz in the winter semester 2011/2012. My bachelor's thesis was carried out at the Max Planck Institute for Polymer Research in the research group of Frederick Wurm in 2016. After doing an intership at the Institute of Molecular Sciences in Valencia I joined the group of Matthias Barz in 2018 for my Master's thesis. I graduated in March 2019 (Master of Science) and am a doctoral candidate since august 2019. My research focuses on the synthesis and characterization of novel polysarcosine-based lipopolymers, their formylation and application as siRNA-delivery systems.

RECENT PUBLICATIONS

- C.Muhl, D. Unthan, M. Conrad, M.Barz, Synthesis and characterization of bisalkylated polysarcosine-based lipopolymers, European Polymer Journal 2019, 120, 109223

Moritz Urschbach

PhD Student

Moritz took up his studies in Biomedical Chemistry at the Johannes Gutenberg-University Mainz in 2013 and obtained his bachelor degree in 2016. He continued his studies in Mainz to strengthen his knowledge at the interface of chemistry and biomedicine. During internships in the groups of Prof. H. Frey and Prof F. Rösch, Moritz gained insights into polymer chemistry and contrast agents for fMRT. Thereafter, he joined the group of Prof. Pol Besenius to work on his master thesis about the synthesis of TA-MUC1 functionalized peptide amphiphiles for the incorporation in supramolecular polymers. The thesis was awarded with the Adolf Todt-prize for excellent scientific work in 2018. He subsequently started his doctoral studies in Mainz under the supervision of Prof. Besenius where he is curretly working on the development of self-assembling, glycopeptide decorated structures to build up supramolecular antitumor vaccines. Moritz is also part of the research training group of the Collaborative Research Center "Nanodimensional polymer therapeutics for tumor therapy" (SFB 1066) which deals with the development of new multifunctional nanoparticle-systems for tumor immunotherapy.

Francisco Martin Vazquez-Meza

Francisco Martin Vazquez-Meza has a Bachelor's degree on clinical biologist chemist, he is currently doing his masters on "Health Sciences" in The University of Sonora (UNISON) in Mexico. He is developing the thesis titled "Characterization of MDA-MB-231 and Mexican Breast Cancer Cells by Raman Microespectroscopy and Atomic Force Microscopy", under the co-supervision of Dr. Aracely Angulo Molina & Dr. Monica A. Acosta Elías. His project is part of the collaboration between University of Sonora and University of Applied Sciences and Arts-HLS Northwestern Switzerland (FHNW). Dr Uwe Pieles from FHNW and Dr. Carlos Arturo Velazquez Contreras from UNISON also are participating as mentors.

RECENT PUBLICATIONS OF RESEARCH GROUP:

- Acosta-Elías, M. A., Burgara-Estrella, A. J., Sarabia-Sainz, J. A. I., Silva-Campa, E., Angulo-Molina, A., Melendrez R. & Pedroza-Montero, M. 2017. Nano alterations of membrane structure on both γ-irradiated and stored human erythrocytes. International Journal of Radiation Biology. p. 1306-1311 6 p.
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Chrysovalantis Voutouri

PhD

Dr. Voutouri earned a PhD degree in Mechanical and Biomedical Engineering from the University of Cyprus where he studied the mechanics of solid tumors and how mechanical forces affect the delivery and efficacy of chemotherapy and nanomedicine. Since March 2018, he is a Postdoctoral fellow at the Cancer Biophysics Laboratory at the University of Cyprus and an Instructor at the University of Cyprus and European University Cyprus.

Dr. Voutouri current research interests involve the modulation of the mechanical Tumor Microenvironment to improve tumor perfusion, the delivery of nanomedicines and immunostimulations. He also performs a mixture of preclinical studies and mathematical model simulations to identify optimal treatment strategies based on the combined use of nanomedicines and immunotherapeutic drugs.

Dr. Voutouri has participated in research projects funded by the Research Promotion Foundation of Cyprus, New Strategic Infrastructure Units – Young Scientists, (CancerNanoMED), European Research Council Proof of Concept (ERC-2018-PoC-838414 CancerFingerPrints), European Research Council 336839-ReEngineeringCancer and the Research Promotion Foundation of Cyprus. In the last six years, he has co-authored 21 scientific articles in peer-review journals (h-index=10, >337 citations), 7 of which in journals with impact factor >5. His publication record includes articles in PNAS, ACS Nano, Neoplasia,Theranostics and Journal of Control Release. He also has 5 papers and/or abstracts in referred conference proceedings and has given several podium presentations in International conferences. He is collaborating with Dr. Rakesh K. Jain, Professor at Harvard Medical School in Boston, MA, USA and Dr. James W. Baish, Professor of Biomedical Engineering at Bucknell University, USA.

RECENT PUBLICATIONS

- Panagi M., C. Voutouri, F. Mpekris, P. Papageorgis, M.R. Martin, J.D. Martin, C. Polydorou, M. Kojima, G. Ishii, K. Kataoka and H. Cabral and T. Stylianopoulos. TGF-β inhibition combined with Doxil normalizes the metastasis microenvironment towards antitumor immunity. Theranostics.
- Mpekris F., C. Voutouri, J.W. Baish, D.G. Duda, L.L. Munn, T. Stylianopoulos, R.K. Jain. Combining microenvironment normalization strategies to improve cancer immunotherapy. PNAS
- Voutouri C., N.D. Kirkpatrick, E. Chung, F. Mpekris, J.W. Baish, L.L. Munn, D. Fukumura, T. Stylianopoulos and R.K. Jain. Dynamics of vessel cooption in brain tumors revealed by integrative experimental and mathematical modeling studies. PNAS [DOI: 10.1073/pnas.1818322116].
- Martin J.D., M. Panagi, C. Wang, T.T. Khan, M.R. Martin, C. Voutouri, K. Toh, P. Papageorgis, F. Mpekris, C. Polydorou, G. Ishii, S. Takahashi, N. Gotohda, T. Suzuki, M.E. Wilhelm, V.A. Melo, S. Quader, J. Norimatsu, R.M. Lanning, M. Kojima, M.D. Stuber, T. Stylianopoulos, H. Cabral, and K. Kataoka and H. Cabra. The antiemetic dexamethasone increases nanocarrier delivery by normalizing the tumor microenvironment. ACS Nano
- Voutouri C. and T. Stylianopoulos. Accumulation of mechanical forces in tumors is related to hyaluronan content and tissue stiffness. PLoS One 13(3): e0193801 [DOI: 10.1371/journal.pone.0193801].

Adam Alexander Walters

PhD
Research Fellow, School of Pharmaceutical and Cancer Sciences, Kings College London.
Adam presently works as a Maplethorpe research fellow under the mentorship of Professor Khuloud Al-Jamal's at King's College London. In this position his work is focussed on the development of rationally designed nano formulations for the delivery of immunologically active small molecules and biologicals. He has an interest in the use of nucleic acids for either gene delivery, gene knock down or immuno-stimulation, primarily in cancer models. A key component of Adam's work is the identification of synergistic modalities for co formulation in nano systems.
Prior to this engagement Adam had a 5-year stint as a Research Associate at the Jenner Institute, University of Oxford, working with Dr Anita Milicic and Prof. Adrian Hill in investigating the controlled delivery of antigen for induction of spatially and temporally restricted immune responses for malaria vaccination.
Throughout his career, though disease models and platforms have changed, the consistent theme of Adam's research has been the development of novel formulation with special focus on the interaction of biomaterials with the immune system.

RECENT PUBLICATIONS:

- Hassan, H., Diebold, S. Smyth, L., Walters, A., Lombardi, G. and Al-Jamal K., T. 'Application of carbon nanotubes in cancer vaccines: achievements, challenges and chances' 2019 Journal of Controlled Release
- Faruqu, F., N., Tzu-Wen Wang, J., Xu, L., McNickle, L., Ming-Yiu Chong, E., Walters, A., Gurney, M., Clayton, A., Smyth, L., A., Hider, R., Sosabowski, J. and Al-Jamal K., T. 'Membrane radiolabelling of exosomes for comparative biodistribution analysis in immunocompetent and immunodeficient mice – a novel and universal approach' 2018 Theranostics
- Mei, K-C., Ghazaryan, A., Teoh, E., Z., Summers, H., D., Li, Y., Ballesteros, B., Piasecka, J., Walters, A., Hider, R., C., Mailänder, V. and Al-Jamal, K., T. 'Protein-Corona-by-Design in 2D: A Reliable Platform to Decode Bio–Nano Interactions for the Next-Generation Quality-by-Design Nanomedicines' 2018 Advanced Materials
- Thompson, C., Lourenco, J., Obolski, U., Walters, A., Edmans, M., Palmer, D., Kooblall, K., Carnell, G., O'Connor, D., Bowden, T., Pybus, O., Pollard, A., Temperton, N., Lambe, T., Gilbert, S., and Gupta, S. 'A naturally protective epitope of limited variability as influenza vaccine target' 2018 Nature Communications
- Gola, A., Silman, D., Walters, A., Sridhar, S., Uderhardt, S., Salman, A., M., Halbroth, B., R., Bellamy, D., Bowyer, G., Powlson, J., Baker, M., Venkatraman, N., Poulton, I., Berrie, E., Roberts, R., Lawrie, A., M., Angus, B., Khan, S., M., Janse, C., J., Ewer, K., J., Germain, R., N., Spencer, A., J. and Hill, A., V., S. 'Prime and Target Immunization Protects Against Liver-Stage Malaria in Mice' 2018 Science Translational Medicine

Alina Zenych

Alina Zenych received her B.Sc. and M.Sc. degrees from the National Technical University of Ukraine "Kyiv Polytechnic Institute" in Medical Instruments and Systems. During 2013 - 2014, she performed a one-year Erasmus Mundus Action 2 exchange program at the University of Groningen in the Netherlands. In 2016, she obtained an M.Sc. from Paris Descartes University in France in Biomedical Engineering (Molecular and Cellular Biotherapies). Currently, as a recipient of the INSPIRE project fellowship within Marie Skłodowska-Curie grant, Alina is a Ph.D. candidate in nanomedicine and works on molecular diagnostics and targeted therapy of thrombotic diseases using polysaccharide-based nanocarriers in the Laboratory for Vascular Translational Science - INSERM U1148, Sorbonne Paris Nord University. She published as a co-author a research article in a peer-reviewed journal Biomaterials (10.1016/j.biomaterials.2018.12.023) and submitted a review article to Biomaterials (under revision), both dedicated to nanomedicine in thrombotic pathologies.

Hana Zivotska

Hana Zivotska got her Bachelor and a Master's degree in Chemistry and Food Technology at Mendel University in Brno, Czech Republic. The aim of these works was to evaluate changes in the content of milk during the year and its impact on chosen technological properties and effect of season on chosen quality parameters of milk via statistical evaluation of data.
Hana is currently in the second year of her PhD studies in the Research Group for Molecular Biology and Nanomedicine at the Department of Chemistry and Biochemistry, Mendel University in Brno, Czech Republic. Her main focus is on biomimetic peptide-based ligands for active targeting of cancer cells. The main goal of this research is to find suitable ligands with a high-targeting efficiency that lead to increased efficiency of anti-cancer treatment. During her study, she also spent one week at University College Dublin, Ireland by joining the Flow Cytometry Summer School program. She also attended conferences in Czech republic (MendelNet2019 in Brno and BOD 2019 in Brno).

CLINAM

European Foundation for Clinical Nanomedicine

12/2020

Basel, October 26–28

ABSTRACTS SPEAKERS

EXTRACELLULAR VESICLES INCREASE THE ENZYMATIC ACTIVITY AND IMPROVE THE EFFICACY OF ENZYME REPLACEMENT THERAPY IN LYSOSOMAL STORAGE DISORDERS

IBANE ABASOLO[1,2,3], Joaquin Seras-Franzoso[1,2], Zamira V. Díaz-Riascos[1,2,3], José Luis Corchero[2,4], Patricia González[1,2], Natalia García-Aranda[1,2,3], Mònica Mandaña[1,2,3], Roger Riera[5] , Ana Boullosa[1,2,3], Sandra Mancilla[1,2,3], Alba Grayston[6], Marc Moltó-Abad[1,7], Elena Garcia-Fruitós[2,4], Rosa Mendoza[2,4], Guillem Pintos-Morell[1,7], Lorenzo Albertazzi[5], Anna Rosell[6], Josefina Casas[8,9], Antonio Villaverde[2,4], Simó Schwartz Jr[1,2]

[1] Drug Delivery & Targeting, CIBBIM-Nanomedicine, Vall d'Hebron Institut de Recerca (VHIR), Universitat Autònoma de Barcelona (UAB), 08035 Barcelona, Spain.

[2] Networking Research Center on Bioengineering, Biomaterials and Nanomedicine (CIBER-BBN), 08035 Barcelona, Spain

[3] Functional Validation & Preclinical Research (FVPR), CIBBIM-Nanomedicine, Vall d'Hebron Institut de Recerca (VHIR), Universitat Autònoma de Barcelona (UAB), 08035 Barcelona, Spain

[4] Institut de Biotecnologia i de Biomedicina (IBB), Universitat Autònoma de Barcelona (UAB), Bellaterra, 08193 Barcelona, Spain

[5] Nanoscopy for Nanomedicine Group, Institute for Bioengineering of Catalonia (IBEC), C/Baldiri Reixac 15-21, Helix Building, 08028 Barcelona, Spain

[6] Neurovascular Research Laboratory, Vall d'Hebron Institut de Recerca (VHIR), Universitat Autònoma de Barcelona (UAB), 08035 Barcelona, Spain.

[7] Rare Diseases Unit, Reference Center for Inherited Metabolic Disorders, Vall d'Hebron University Hospital, 08035 Barcelona.

[8] RUBAM, Biological Chemistry, Institute of Advanced Chemistry of Catalonia (IQAC-CSIC), C/Jordi Girona 18-26, 08034 Barcelona, Spain

[9] Networking Research Center on Hepatic and Digestive Diseases (CIBEREHD), 08034 Barcelona, Spain

Extracellular Vesicles (EVs), comprising microvesicles, exosomes and other secreted membrane vesicles, are naturally occurring delivery systems released by most cell types [1]. Given their ability to transfer bioactive components and overcome biological barriers, EVs are being increasingly explored as vehicles for therapeutic agents. In this work, we propose the use of EVs as direct delivery platforms for α-galactosidase (GLA) and sulfoglucosamine sulfohydrolase (SGSH) proteins, defective in Fabry disease and Sanfilippo A syndrome (MPSIIIA). LSDs are congenital rare diseases caused by the lack or malfunction of a lysosomal protein, mainly enzymes. Noteworthy, most LSDs are currently treated with enzyme replacement therapy (ERT). This approach is based in periodic intravenous infusions of human recombinant enzymes. Nevertheless, as in other protein therapeutics, ERT has important drawbacks, including poor biodistribution, low enzyme half-life, inability to cross the blood brain barrier and high immunogenicity, which often limits it efficacy [2].EVs were isolated from mammalian CHO DG4 and HEK293 cells stably or transiently overexpressing GLA and SGSH enzymes, respectively [3]. Direct purification of EVs from cell supernatants was found to be a simple and efficient method to obtain highly active GLA and SGSH proteins. In fact, EV-derived proteins display 10-fold higher specific enzymatic activity than their soluble counterparts. In Fabry disease *in vitro* models, EVs carrying GLA (EV-GLA) were rapidly uptaken and driven directly to the lysosomes, restoring enzyme functionality much more efficiently than clinical reference agalsidase-alfa. In mice, EVs from CHO cells were well tolerated and distributed among all main organs after intravenous (i.v.) or intraaterial (i.a.) administrations. Moreover, in GLA knockout mice, repeated i.v. administrations of EV-GLA outstanded free enzyme reducing globotriaosylceramide (Gb3) and lysoGb3 levels in different tissues, including the brain. Overall, our results demonstrate that EVs obtained from mammalian cell lines overexpressing lysosomal enzymes work as natural drug delivery systems, improving the activity and the efficacy of the recombinant enzymes and facilitating the surmount of the blood brain barrier.

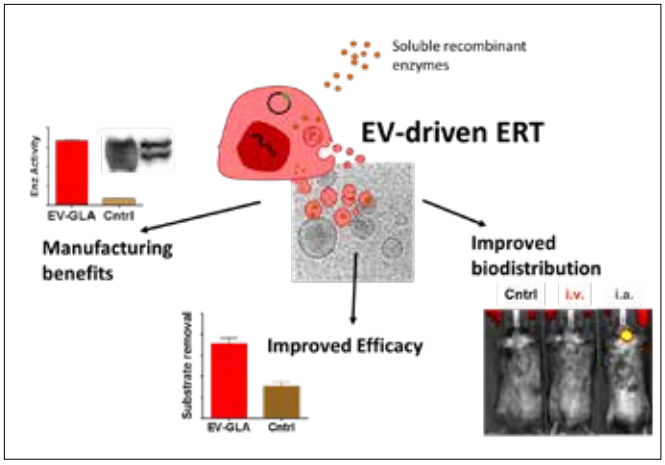

Figure 1. *EVs directly purified from cells transfected with plasmid coding for lysosoma enzymes could be used to improve the Enzyme Replacement Therapy (ERT) in Lysosomal Storage Disordes (LSD). In this work, EVs encapsulating the defective enzyme in Fabry disease (EV-GLA) have shown to be more active than the free enzyme (Cntrl) in vitro and in vivo.*

ACKNOWLEDGEMENTS

This study has been supported by ISCIII (PI18_00871 co-founded by Fondo Europeo de Desarrollo Regional (FEDER)), and CIBER-BBN (EXPLORE) granted to IA and SGR/2017-2019 granted to SS. Culture of CHO cells and soluble protein purification was partially performed at the Protein Production Platform of CIBER-BBN/UAB and the ICTS Nanobiosis. In vivo assays were performed also at the ICTS "NANBIOSIS", specifically by U20/FVPR of CIBER-BBN/VHIR. JSF was supported by an AECC post-doctoral fellowship and NG-A by a PERIS grant from the Catalan Government (SLT006/17/270). We are thankful Alexandre Garcia for his assistance in the Gb3 and LysoGb3 determinations and and to the "Servei de Microscòpia"-UAB for technical assistance in CryoTEM imaging.

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[2] "New strategies for the treatment of lysosomal storage diseases" Parenti G, Pignata C, Vajro P, Salerno M. Int J Mol Med. **31(1)**:11-20 (2013)

[3] "Method for producing enzymes". Schwartz S Jr, Abasolo I, Seras-Franzoso J, Corchero JL. Patent P201930056.

DEVELOPMENT OF A CELL THERAPY WITH GENE-MODIFIED PRIMARY CELLS

KARIN ABITORABI, MSC

Today's Cell Therapies are mainly focused on autologous transplantation of human primary cells with and without gene-modification. There are multiple ways to modify human primary cells. In this presentation we will focus on how to develop a Cell Therapy from research to the clinic and which gene-modification techniques are currently being utilized in the field. An outlook into fully automated technologies and their use in cell therapies from isolation of cells of interest to final formulation of the drug product will be discussed.

MOBILE NETWORK FOR ENVIRONMENTAL AND HEALTH SENSING

GABRIEL AEPPLI, ETHZ, EPFL AND PSI

We describe an approach to personal health and environmental monitoring based on networked smartphones with compact plug-in sensing modules. The modules take advantage of modern nanotechnology, optics and synthetic biology to deliver, in concert with the processing power and pre-exisiting sensors embedded in the smart phones, unique signatures of disease markers as well environmental toxins dissolved in a variety of fluids. The network allows data, associated with particular locations owing to GPS/GIS, from many smartphones to be aggregated and analyzed, and correlated with other geographically-based data. This will provide unique insights into the prevalence and spreading of toxins in the case of environmental sensors, and, analogously, the epidemiology of medical conditions associated with particular biomarkers. In addition, the plug-in sensors will greatly enhance the personalized health monitoring platform already present on smartphones. We illustrate the concepts with first stage trials (conducted by the spin-out company Aquaffirm) of an electrochemical test for arsenic contamination of water in Bangladesh, and an optical assay for fluoride contamination in Mexico. The arsenic test takes advantage of the high specificity of enzymes produced using synthetic biology.

REFERENCE

People Fixing the World “Detecting a lethal poison in drinking water”
https://www.bbc.co.uk/programmes/p07mf3fr

IMMUNOMODULATION WITH NUCLEIC ACID NANOPARTICLES

KIRIL AFONIN

The integration of nanotechnology into modern therapeutics provides additional control of unique physicochemical properties including size, surface charge, hydrophobicity, and the addition of moieties for biomedical applications. The ability to fine-tune these parameters subsequently allows for the improved efficacy of therapeutic treatments and has implications for the future of personalized medicine. The functionally-versatile molecule, RNA, plays an essential role in living systems and the new discipline of RNA nanotechnology investigates how this intriguing biopolymer can be programmed to assemble into defined shapes and sizes with specified biological activities. Multifunctional nucleic acid-based nanoparticles (NANPs) hold tremendous potential in biomedical applications because of their programmability, biocompatibility, and precise control over their formulation. However, the immunotoxicity and immunomodulatory effects of NANPs are largely unknown and must be defined to permit the successful translation of this technology into the clinic. The understanding of how particular NANPs can trigger the immune response may also open the possibilities to a new field where NANPs are used not only for drug delivery but also as vaccine adjuvants. To address such fundamental problems in a timely fashion, we initiated the very first systematic investigation of NANP recognition by human immune cells. Despite the anticipations, we did not find a strong, uniform immune response for all NANPs. Instead, the tests found varying and specific responses from different immune cells, depending on each NANP’s shape and formulation. Our results strongly suggest that NANPs can be used not only as nanoscaffolds for controlled drug delivery but also as a tool for communication with the immune system. The ability to control the amounts of cytokines that immune cells produce in response to various NANPs can be used synergistically as an additional therapeutic modality together with the therapeutic agents embedded into the NANPs’ structures.

SELECTIVE ENTRY OF LIPID VESICLES INTO THE BRAIN POST INTRACEREBRAL HAEMORRHAGE OFFERS NOVEL THERAPEUTIC OPPORTUNITIES

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The integrity of the blood brain barrier (BBB) is compromised after intracerebral haemorrhage (ICH), but this has not been utilized to enhance drug delivery into the brain. Previously we showed that we can target ischemic-stroke lesions by selective translocation of liposomes through the site of BBB disruption. Here, we hypothesised that similar selective targeting is possible in ICH, with loss of BBB integrity being the primary mechanism by which entrance of liposomes is permitted.

ICH was induced in mice (C57BL/6) by intra-striatal collagenase injection. Liposomes were injected intravenously at 3h, 24h & 48h post ICH and accumulation in the brain studied using in-vivo optical imaging and histology. BBB integrity, brain water-content, and iron accumulation were characterised using dynamic contrast-enhanced MRI and quantitative relaxometry (T1 and R2* mapping). Optical imaging data show a biphasic liposome uptake into the haematoma; an early phase (3-24h) followed by a second phase (48-72h) post ICH. MRI measurement of BBB integrity showed a similar biphasic pattern, suggesting a common transport mechanism. Both the water content and the iron content were highest at 3h after ICH and reduced gradually over time.

Our findings suggest that selective liposomal accumulation into the haematoma is linked to biphasic BBB hyper-permeability after ICH, offering a novel route for drug delivery into the haemorrhaged brain.

EXOSOME-MEDIATED RNAI OF PAK4 PROLONGS SURVIVAL OF PANCREATIC CANCER MOUSE MODEL AFTER LOCO-REGIONAL TREATMENT

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Pancreatic cancer (PC) remains one of the most aggressive and devastating malignancies, predominantly due to the absence of a valid biomarker for diagnosis and limited therapeutic options for advanced disease. Exosomes (Exo) as cell-derived vesicles, are widely used as natural nanocarriers for drug delivery. P21-activated kinase 4 (PAK4) is oncogenic when overexpressed, promoting cell survival, migration and anchorage-independent growth. In this study, we validate PAK4 as a therapeutic target in an *in vivo* PC tumour mouse model using Exo nanocarriers following intra-tumoural administration.

METHODS

PC derived Exo were firstly isolated by ultracentrifugation on sucrose cushion and characterised for their surface marker expression, size, number, purity and shape. siRNA was encapsulated into

Exo via electroporation and dual uptake of Exo and siRNA was investigated by flow cytometry and confocal microscopy. *In vitro* siPAK4 silencing in PC cells was assessed by western blotting, flow cytometry, and *in vitro* scratch assay. *In vivo* efficacy (tumour growth delay and mouse survival) of siPAK4 was evaluated in PC bearing NSG mouse model. Ex vivo tumours were examined using Haematoxylin and eosin (H&E) staining and immunohistochemistry.

RESULTS

High quality PC derived PANC-1 Exo were obtained. siRNA was incorporated in Exo with 16.5% loading efficiency. Exo and siRNA co-localisation in cells was confirmed by *in vitro* imaging. PAK4 knockdown was successful at 30 nM Exo-siPAK4 at 24 h post incubation *in vitro*. Intra-tumoural administration of Exo-siPAK4 (1 µg siPAK4 and 7.7 × 1011 Exo, each dose, two doses) reduced PC tumour growth and enhanced mice survival ($p < 0.001$), with minimal toxicity observed compared to polyethylenimine (PEI) used as a commercial transfection reagent. H&E staining of tumours showed significant tissue apoptosis in siPAK4 treated groups.

CONCLUSIONS

PAK4 interference prolongs survival of PC bearing mice suggesting its candidacy as a new therapeutic target in PC. PANC-1 Exo demonstrated comparable efficacy but safer profile than PEI as *in vivo* RNAi transfection reagent.

ACKNOWLEDGEMENT

Funding is provided from the Marie Skłodowska-Curie actions, "Horizon 2020", European Union.

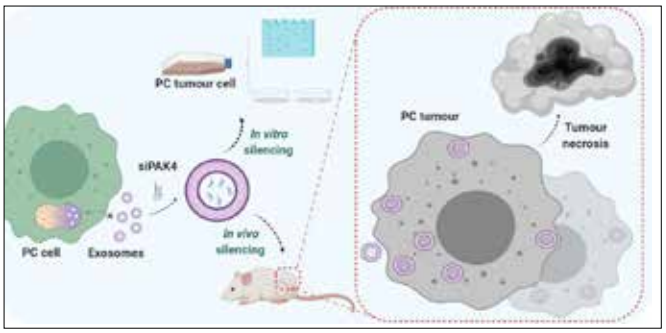

Figure 1. *Designing PANC-1 exosome carrying siPAK4 for pancreatic cancer therapy in mouse model.*

TARGETING METASTATIC PROSTATE CANCER VIA ANTI-PSMA EXOSOME-MIMETICS

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Introduction: Prostate cancer (PC) is the second cause of death in men worldwide. A range of anti-cancer drugs have been used to treat metastatic PC. However, they have shown limited efficacy in prolonging overall patients' survival, due to drug resistance and limited drug accumulation at disseminated tumors, primarily to the bone. Therefore, there is an unmet need to develop novel strategies to target metastatic PC tissues efficiently. Exosomes are nano-sized, cell-derived vesicles have shown an intrinsic homing ability to a wide range of cells. In the present work, we report efficient metastatic PC targeting using our novel prostate-specific membrane antigen (PSMA)-targeted exosome-mimetics (EMs).
Material & Methods: A stably transfected PSMA-peptide expressing monocytes U937 cell line was established. PSMA-targeted EMs were prepared by serial extrusion of the transfected U937 monocytes. The PSMA-targeted EMs were characterized by nanoparticle tracking analysis (NTA), transmission electron microscopy (TEM), and western blotting. The binding of the PSMA-targeted EMs to recombinant human PSMA protein was confirmed by ELISA. Also, cellular uptake studies were performed using flow cytometry and confocal laser scanning microscopy. Their doxorubicin loading capability was assessed. Finally, *in vivo* biodistribution, tumor targeting, and safety studies of targeted EMs were carried out in C4-2B (PSMA +ve) and PC3 (PSMA -ve)- tumor-bearing mice.
Results: The engineered EMs exhibited high protein yield, good drug loading, and exosome markers expression. The expression of PSMA targeting peptide, and its binding to PSMA receptors was confirmed *in vitro*. Finally, successful solid and metastatic tumor accumulation was achieved *in vivo* with PSMA-targeted EMs, in the absence of *in vivo* toxicity.
Conclusion: Our engineered PSMA-targeted EMs, could offer a promising drug delivery system for metastatic PC, based on its drug loading capacity, tumor targeting, and safety *in vivo*.

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MODIFYING INTRACELLULAR FATE THROUGH NANOTECHNOLOGY TO WIDEN THE THERAPEUTIC INDEX

CAMERON ALEXANDER
(University of Nottingham)

The efficacy of many drugs is dependent not just on their mode of action, but also the efficiency by which they are delivered to tissue, cellular and sub-cellular targets. This is especially the case for many new biological therapeutics, such as peptides, proteins and nucleic acids, but it is also true for many conventional small molecule drugs. As a consequence, there has been an intense focus on developing carriers and delivery systems for active pharmaceutical ingredients, with many new advances possible through applications of nanotechnology.
In this presentation the use of synthetic polymers as carriers for a variety of drugs will be explored. Recent data have shown that the potency of methotrexate is altered by the route through which it enters cells, with some polymer carriers enhancing the activity of the drug by avoiding the normal receptor-mediated uptake pathway. These polymers can be 'tuned' for active or passive uptake via modulation of their surface functionality and architecture. Similarly, polymers for delivery of DNA and RNA can exhibit different intracellular trafficking dependent on the structures they form when in complexes with the nucleic acids.

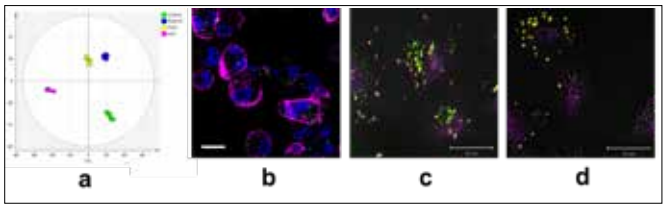

Figure 1. In (a) is shown a Principal Components Analysis (PCA) clustering of the metabolic pathways altered in THP-1 macrophages when treated with methotrexate (MTX), PLGA, and PLGA containing MTX, while in (b) confocal microscopy images of the same cells with PLGA-MTX are presented (nuclei = blue, cell membranes = purple, polymers = green). Images (c) and (d) show A549 cells with branched vs hyperbranched polycations-DNA complexes, respectively (purple = nuclei, yellow = polymer, red = DNA).

The presentation will consider how variations in the chemistry, structure and architecture of new synthetic polymers can be exploited to increase the efficacy of drug delivery, and some of the challenges in taking these materials beyond the research laboratory and into the clinic.

PRECISION NANOMEDICINE USING IRON OXIDE NANOPARTICLES AND ROBOTICS

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Among the wide variety of nanosystems studied for the purpose of medical applications, superparamagnetic iron oxide nanoparticles (SPONs) represent a versatile platform that can be potentially utilised both for disease diagnostics and for therapy. This dual functionality allows their use as a precision medicine tool to improve the assessment of individual disease burden and to adapt the therapeutic regimens to patients' needs.

Diagnostic potential of SPIONs lies in their strong T2 and T2* effects in magnetic resonance imaging (MRI), allowing an increase of the technique sensitivity almost to the cellular level. Consequently, SPION-enhanced MRI has been clinically applied for detection of tumor sites and lymph node metastases, but also for improved characterization of atherosclerotic plaques, myocardial infarcts and thrombosis. In terms of therapeutic benefits, magnetic nanoparticles offer a possibility of highly precise targeting, which allows the adaptation of the dose and a local drug enrichment in the target tissue. This strategy of drug delivery, which additionally reduces systemic toxicity, is called Magnetic Drug Targeting (MDT). In this approach, SPIONs conjugated with appropriate drugs are directed to the diseased tissues or vasculature regions by an external magnetic field. The efficacy of MDT had been previously demonstrated by our group in cancer and was recently confirmed in the settings of atherosclerosis. One of the important therapeutic aims of nanomedicine is to move from systemic thrombolytic drug medication towards the targeted therapeutics that minimise side effects and improve treatment efficacy in stroke. MDT finds the application also in this field. SPIONs can further be used for multiple regenerative approaches, including magnetically-assisted tumor immunotherapy based on SPION-labelled T-cells, stent and graft endothelialisation using SPION-labelled endothelial cells, as well as Magnetic Tissue Engineering (MTE), which allows patient-specific layer-by-layer formation of hierarchical tissue constructs.

The potential of SPIONs for precision medicine can be further enhanced by the combination of Nanomedicine with robotics. The steering system for robotized magnet operation recently implemented at SEON is expected to refine the targeting precision and efficacy. Moreover, in combination with currently developed image-assisted magnet positioning, it can contribute to the improved clinical utility and speed up the translation of MDT to the future bedside applications.

Figure 1. *Robot-operated magnet for precise accumulation of drug-loaded SPIONs.*

PRECISION NANOMEDICINE AND BEHIND THE CONTROVERSIAL DIRECTIONS OF SCIENCE PUBLICATIONS

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Society needs reliable science now more than ever, but progress cannot be made without scientists sharing reliable information. A publisher has to run a sustainable business but needs the oversight of scientists to ensure that the primary interests of all parties are equally served. Scientists provide content, supply quality control, improve scientific merit, and as users they read, share, judge, and utilize content.

Science publishing has many problems today. Some of them are of external origin, created by short-sighted politics (denial of science by authorial politicians to justify short-term revenues as opposed to long term needs), and others are internal due to the rapid expansion of science itself (into the unknown), and the imbalance between the interests of science, authors, and the publishing business.[1]

Forced to chase originality and novelty, many investigators now pursue only novel materials and complicated approaches to fulfill the criteria to make their manuscripts acceptable. [2] Originality and novelty are important, but without in-depth and reproducible studies and R&D knowledge, it is impossible to develop practical (nano)medicines for everyday use.

After many years of evolution, nanomedicine is merging into medicine. There are many great achievements and even more promises on this field. However, in addition to great promises, there are also many challenges, especially in the areas of communication and of commercialization. We need not only revolutionary discoveries, but affordable and economically competitive drugs, devices and clinical applications. Thus, nanomedicine has to be fueled by successful business and it must be accepted by the medical community.

We, a group of professionals and lead scientists, have decided to create our own non-profit publishing company (https://andoverhouse.org) and launch Precision Nanomedicine, he official journal of CLINAM and the International Society for Nanomedicine. It is a gold open access journal that promotes all progressive and rational aspects of nanomedicine and publishes articles and news covering topics from basic science through commercialization. Authors retain the ownership of their copyrighted material, which can be freely downloaded for non-commercial use.

The speaker will describe this new endeavor and discuss them with the audience.

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BIO CONVERGENCE IN DRUG DELIVERY – FROM THE BENCH TO THE PATIENTS

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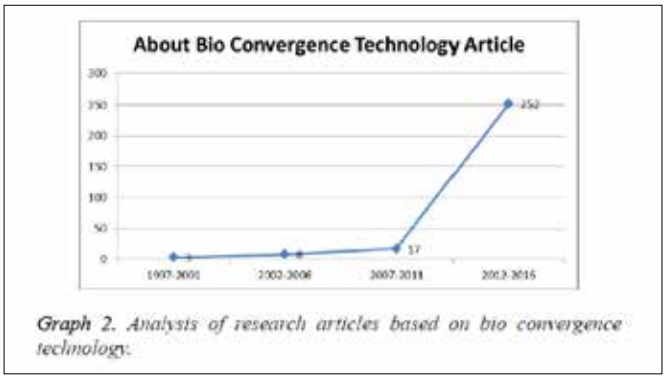
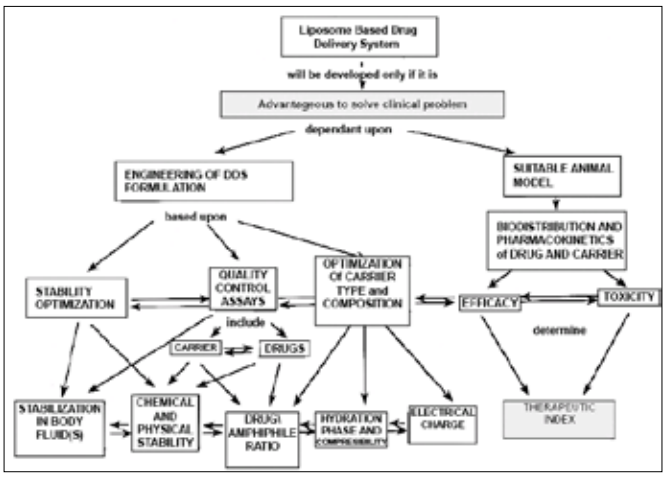
Bio-Convergence is a term in increase use by industry academia and many other fields that require innovative product development. It requires merging of previously distinct technologies into a new form which may involves new practices. Bio-convergence became a prerequisite for the development of complex drug – products.



Graph 2. Analysis of research articles based on bio convergence technology.

Biomedical Research (2017) Health Science and Bio Convergence Technology: Special Issue: S407-S409 Edition-II
About bio convergence technology, bio technological medicine and drugs: Analysis of literature.Kurtulus Ongel1, Utku Eser2, Murat Altuntas3 and Dilek Aksay4*

In my presentation I will describe the use of Bio-convergence for designing and producing liposomal nano-drugs with the focus on two liposomal nano-drugs the anticancer nano-drug Doxil® and the antibiotic novel drug nano-mupirocin. The development of liposomes-based nanodrugs is described by the "Concept-Map" shown below.



Concept map describing development of liposomal formulations. Critical points are marked by gray background.

The presentation will discuss how the merging of few disciplines including: computation, machine learning and AI as theoretical tools, chemistry, physics, physical-chemistry, nano-technology, pharmacy biology, toxicology and medicine as experimental tools interact in order to enable the development of these two highly complex super-complex drugs. The clinical success of Doxil with which more than 700,000 patients were treated world-wide and the preclinical success in the treatment of infectious diseases caused by bacteria resistant to major antibiotics are a good proof to the power of using Bio-convergence for developing complex nano-drugs for the treatment of major diseases

POLYPEPT(O)IDES FOR THE THERAPY OF INFECTIOUS DISEASES

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The enormous potential of polymeric nanomedicines arises from the possibility to combine desirable material properties with compartmentalized functionalities in one distinct nanoparticle,[1] to encapsulate and deliver drugs more specifically to the desired site of action and/or maintain sustained release over elongated time frames. The current treatment of tuberculosis (TB) requires administration of four drugs over six months and the patients are likely to suffer from side effects such as liver toxicity. This leads to poor patient compliance and thus promotes the development of multi-drug-resistant and extensively drug-resistant strains of Mtb. Nano-sized drug delivery systems may address these current limitations. Griffiths and co-workers have recently demonstrated that even i.v. injected PeptoMicelles (micelles based on polypept(o)ides)[2-4] can accumulate passively at granulomas in zebrafish embryo and mouse models, which provides a novel approach for tuberculosis therapy.[5] The accumulation process, however, requires stealth-like nanoparticles with enhanced serum stability.[6]
With respect to these needs we established polypept(o)ide-based micelles, which are either stabilized by π-π interactions or bio-reversible covalent core-crosslinking. The size and morphology of these micelles can be adjusted from spherical (dh=30-100 nm) to worm-like (dh=60-200 nm, aspect ration up to 6) shapes. In the core of micelles various anti-TB drugs can encapsulated by either hydrophobic interactions, π-π stacking or covalent attachment, while the corona depending on the application route, which can be intranasal liquid infusion, powder or aerosol inhalation or intravenous injection.
The use of tailor made polymeric micelles of adjustable size, shape and functionality enables us to enhance the efficiency of tuberculosis therapy in the zebra fish embryo model using various drugs, which suffer from limited bioavailability. Further studies in rodent models are ongoing.

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RNANTIBODY® AS AN THERAPEUTIC OPTION

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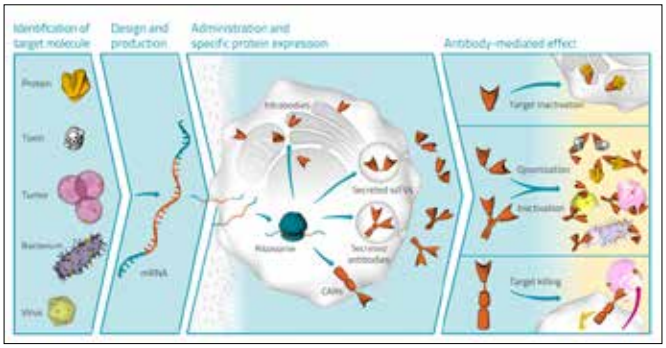
Key Words: mRNA, lipid nanoparticle, Antibodies

The direct delivery of mRNA has emerged as a promising alternative to protein therapeutics, overcoming fundamental flaws associated with the latter technology. One important application of therapeutic proteins is the use of antibodies for passive immunization against infectious diseases. Recently, we demonstrated that a single intravenous injection of antibody-encoding mRNA rapidly generates neutralizing antibody titers, providing protection against lethal rabies infection *in vivo*. Expanding the scope of our mRNA technology, several different applications of RNAntibody are shown in the field of infectious disease but also oncology.

Figure 1 – *RNAntibody® - the body makes its own functional antibodies without vaccination*



CLINICAL CASE STUDY: LIPOSOMAL METHYL PREDNISOLONE, HOW PRECLINICAL STUDIES HELPED THE TRANSLATION

YAELLE BAVLI

PEGylated nanomedicines are known to induce infusion reactions, a type of hypersensitive reactions, in a non-negligible percentage of population. These reactions can be mild and transient but in some rare cases they can be life-threatening. For this reason, increasing efforts are put into the early detection of drugs causing such adverse effects. Infusion reactions have been linked to activation of the complement system but also in many cases to the presence in the blood of anti-PEG antibodies, especially IgG and IgM, either pre-existing or elicited after exposure to the PEGylated drug. In addition, these anti-PEG antibodies can cause Accelerated Blood Clearance (ABC), a phenomenon where the PEGylated nanodrug is cleared very rapidly from the blood upon repeated injections, decreasing the drug's therapeutic efficacy. Unfortunately, the *in vitro* tests currently available can give an estimation of the immunogenic potential of a drug but do not reflect the incidence or the magnitude of the infusion reactions they can trigger in patients. In order to find the relevant biomarkers for a good *in vitro-in vivo* correlation, extensive tests must be conducted.

We got the opportunity to conduct such tests when a formulation of PEGylated nano-liposomes previously developed in our lab was used under a compassionate approval to treat a 21-year-old patient suffering from a rare and life threatening intracardiac manifestation of IgG4-related disease (IgG4 RSD). The nanoliposomal formulation, NSSL-MPS, consists of PEGylated nano-liposomes of approximately 80nm diameter remotely loaded using a trans-membrane calcium acetate gradient with the weak acid steroid prodrug methylprednisolone-hemisuccinate. We showed in previous studies that NSSL-MPS were highly efficacious in numerous animal models of inflammatory diseases such as multiple sclerosis, rheumatoid arthritis, Duchenne muscular dystrophy, local inflammation, cerebral malaria and lupus (Turjeman K. and Barenholz Y., Journal of drug targeting, 2016). IgG4 RSD is a rare disease characterized by infiltration of "masses" composed mostly of lymphocytes and fibroblasts in different organs that can result in multi-organ dysfunction and even mortality. The treatment of reference is usually the administration of glucocorticoids but the patient was already treated with high doses of steroid and started to show severe steroid-related toxicity.

Before the "first-in-human" administration, the NSSL-MPS formulation was fully characterized at the physicochemical level and tested *in vitro* for signs of complement activation (quantification of iC3b). In addition, the hematocompatibility profile of NSSL-MPS was assessed with tests that included cytokines and chemokines release, hemolysis effect, leukocyte procoagulant activity, platelet aggregation, collagen-induced platelet aggregation and plasma coagulation time.

The treatments consisted of 8 weekly injections in two courses of treatment (5- and 3-weeks long, set 6.5 months apart) with increasing doses of NSSL-MPS ranging from 50 mg to 300 mg MPS. Due to ethical requirements, the patient received premedication before each infusion that included corticosteroids, acetaminophen and H2 blockers to reduce the risks of infusion reactions. Before, during and after each treatment, plasma and serum samples were collected. In vivo tests included the quantification of different markers of complement activation during the infusion (iC3b, Bb, SC5b-9 and thromboxane B2), but also the detection of anti-PEG IgG and IgM antibodies in the serum, either naturally present before the treatment or elicited by the injection of NSSL-MPS. In addition, we performed a pharmacokinetic study to measure the occurrence of accelerated blood clearance and monitored the patient for the detection of clinical signs of infusion reactions.

The results of *in vitro* tests showed that at clinically relevant concentration, NSSL-MPS did not induce complement activation (Figure 1) and did not induce a positive response in the remaining tests of our *in vitro* panel detailed above. Of the 4 test markers of complement activation (Figure 2), only Bb showed an increase during the second course of treatments (injections 4-6) but since there was no clinical manifestations of infusion reactions, it was defined as subclinical complement activation.

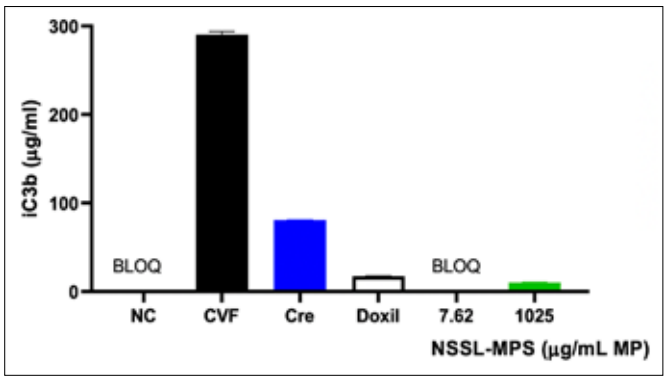


Figure 1 *Evaluation of in vitro activation of the complement system. Different concentrations of NSSL-MPS were incubated in human plasma for 30 min and the increase in iC3b was measured and compared to iC3b activation by Cobra Venom Factor (CVF).*

BLOQ, below limit of quantitation; Cre, Cremophor-EL; CVF, Cobra Venom Factor; NC, Negative Control. BLOQ is <0.13 µg/ml.

Before the first injection of NSSL-MPS, the patient had no detectable levels of anti-PEG antibodies and the treatment with NSLL-MPS did not elicit the production of such antibodies. This is in good correlation with the results of the pharmacokinetic study (Figure 3) that show a similar long circulating time of the liposomal drug after repeated injections, and therefore an absence of accelerated blood clearance. In addition, the NSSL-MPS provided good protection from the adverse effects inherent to glucocorticoids treatment.

Prior to initiating this compassionate use protocol, the patient had progressed under several lines of treatment. But since the treatment with NSSL-MPS, her disease stabilized with normalized levels of IgG4 and no growth of the mediastinal mass on MRI follow ups (performed twice a year). It is very likely that the NSSL-MPS treatment was efficacious in this outcome and contributed to the stabilization of the disease.

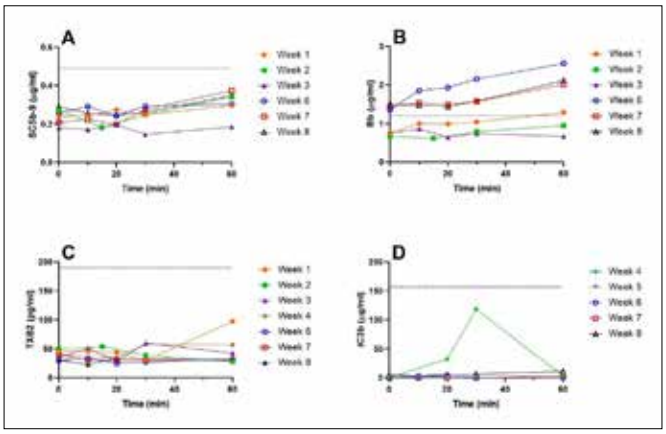


Figure 2 *Markers of in vivo complement activation in plasma. Immediately before the beginning of NSSL-MPS infusion (0) and 10, 20, 30 and 60 minutes after the beginning of the infusion, the following markers of complement activation were quantified in the plasma of the patient: SC5b-9 (A), Bb (B), TXB2 (C) and iC3b (D). The dash line indicates the upper limit of normal values. TXB, Thromboxane.*

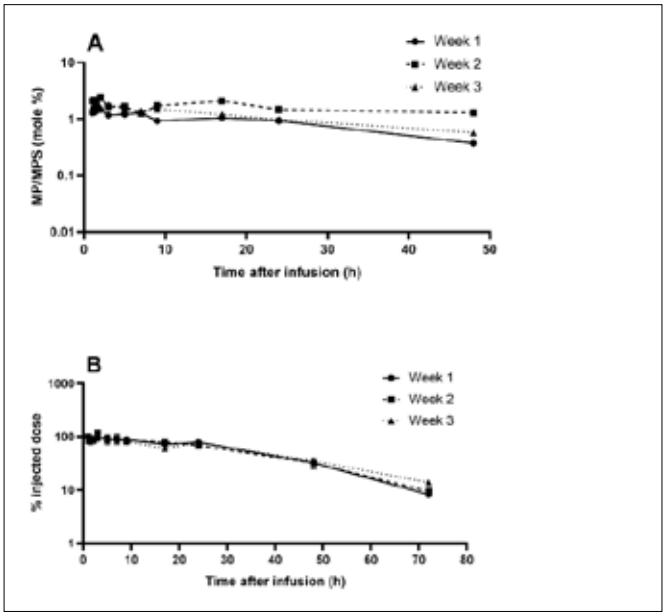


Figure 3 *Levels of methylprednisolone hemisuccinate (MPS) and its active metabolite methylprednisolone (MP) in the patient's serum for treatments 1-3. (A) MP to MPS ratio (mole %) following 1-hour slow infusion of NSSL-MPS for treatment 1, 2 and 3. (B) Serum levels (as percent of injected dose) of MPS following slow infusion of NSSL-MPS for treatment 1, 2 and 3 (treatment with 50, 100 and 150 mg liposomal MPS respectively).*

In conclusion, this first in human administration of NSSL-MPS provided us the opportunity to try to understand if a panel of *in vitro* tests performed before the treatment can correlate with the *in vivo* changes in biomarkers levels during and following NSSL-MPS drug-product infusion, concomitantly with the presence of clinical signs of infusion reactions. This approach can and should be used during the development of new formulations of PEGylated nanodrugs in order to attempt to detect as early as possible the potential immunogenicity of the drugs.

BIOCHEMICAL FUNCTIONALITY OF MAGNETIC PARTICLES AS NANOSENSORS: HOW FAR AWAY ARE WE TO IMPLEMENT THEM INTO CLINICAL PRACTICE?

BEATRICE BECK SCHIMMER

In the past, many different magnetic nanosensors were developed and are currently under evaluation for the diagnosis, but also the treatment of various diseases. While some of the nanosensors are designed for an intracorporeal application such as drug carriers, others are foreseen to purify blood in an extracorporeal approach. Some promising technologies in the field of nanosensors will be presented, highlighting the use of such materials including own data. At the same time risk aspects are discussed. A special emphasis is put on the importance of interdisciplinary approaches during the development of such materials for medical use as well as the regulatory challenges.

THERANOSTIC NANOPHYSICS FOR THE DECIPHERING, PREVENTION AND THERAPY OF BRAIN DISEASES

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Brain diseases realize the first medico-economic cost in Europe triggering handicap, death and chronicle cognitive impairment. Targeted molecular therapy and precision medicine has been successfully developed in other organ locations but failed inside the brain. Brain inaccessibility and functionality is the first bottleneck explaining our failure to detect early and cure many brain diseases from brain cancer to neurodegenerative diseases. Moreover, each disease involves connected neurological and molecular networks, that explain adaptation and relapse from most targeted therapies. We developed a new nanomedicine brain precision approach implementing locally a non-lesional miniaturized high-resolution deciphering of the brain pathologies. Chemically and micro-nano-structured silicon integrated to functional brain neurosurgery devices was developed. This provide a unique non-lesional bioharvesting strategy to explore the brain pathology locally with high resolution. Safety was demonstrated and regulatory industrialization was implemented providing the opportunity for a pilot first in human trial. This trial that is now completed fully demonstrating safety, clinical operability and compatibility with last generation omics technologies. Using this knowledge, a connected theranostic approach is now developed targeting multiple connected pathways using repositioned drugs to neutralize molecular adaptation and relapse. Another modality to neutralize molecular adaptation is to move from drug to physical neuromodulation using innovative nanoparticles delivered locally in connection with physical intervention such as magnetic vibration or electric stimulation. The success of deep brain stimulation in movement disorders is a perfect demonstration of the potential therapeutic impact of physics for brain pathologies. Two examples will be developed. The first is the use of graphene to inhibit glioblastoma invasion inside the brain. The second is the use of spintronics nanoparticles activated to vibrate using magnetic fields. Both demonstrated an efficient therapeutic activity to neutralize glioblastoma invasion in preclinical models.
Regulatory issues as well as the need for rigorous preclinical, biocompatibility, ethical and industrial prerequisites validation will be discussed as a mandatory step to translate at the bedside.
Our failure to cure brain diseases could be also explained by an epistemological and societal omission of the inaugural cause of brain pathologies. Strong evidences clearly demonstrate the impact of environmental factors such as pesticides, ferromagnetic nanoparticles or nanoplastic. We must be alerted about the con-

nected irreversible alteration of human intelligence. Nanotechnologies are a pivotal etiology but also crucial tools to prevent this biomedical emergency. The new project EcoBrainPrint will use our Brain Nanoimprint technologies to make possible the inaccessible exploration of environmental body contamination and impact. All the nanomedicine community should join to the emergency objective aiming to characterize, understand and neutralize the environmental human body impairment.

MULTICOMPONENT SUPRAMOLECULAR POLYMERS AS A PLATFORM FOR THE DESIGN OF GLYCOCONJUGATE VACCINES

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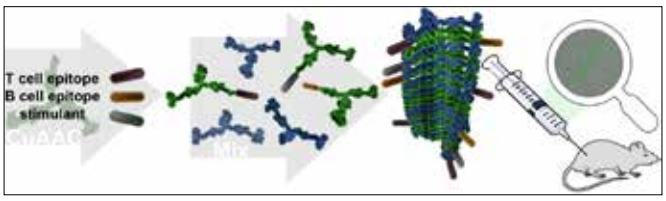
Peptide secondary structures can be harnessed to design monomers capable of self-assembling into nano-scaled supramolecular structures in aqueous media.[1,2] Decorating the surface with immunogenic molecular patterns results in pathogen-mimicking entities and potential vaccine candidates.[3] In the context of antitumor vaccines, the challenge is to overcome self-tolerance mechanisms to enforce an immune response against endogenous, tumor-associated glycopeptide motifs.[4] To this end, a co-stimulation of B cells with Th cells is mandatory, which we aim to achieve using a co-presentation of different epitopes and immunostimulating agents at the surface of multicomponent supramolecular polymers.
Mucin 1 (MUC1) is well-known for undergoing alterations in O-glycosylation during tumorigenesis, and is thus an excellent tumor-associated target structure for immunotherapy. In this contribution I discuss the use a fully synthetic glycopeptide from the MUC1 tandem repeat sequence, which consists of 22 amino acids bearing the Tn and 2,3-Sialyl T tumor associated antigens. As T cell epitope we chose a small fragment from highly immunogenic tetanus toxin (p30). Additionally, an imidazoquinoline as potent TLR7/8 agonist,[5] was synthesized. These epitopes were conjugated to supramolecular monomers and mixed in aqueous solution to yield a polymeric vaccine formulation. The vaccines were administered intraperitoneally to C57BL/6 mice and the antisera were collected after three further boosts. High antibody titers of the IgG type were observed in all mice. Furthermore, FACS analysis confirmed the high binding affinity of the generated antibodies to T47D tumor cells. These results support the potential of this modular supramolecular platform approach for the development of antitumor vaccines.



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MODULATION OF MACROPHAGE POLARIZATION IN THE TUMOR MICRO-ENVIRONMENT BY NANOPARTICLES

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The vertebrate immune system has evolved to a specialized network of different cell types protecting the organism against foreign invading pathogens while at the same time ensuring tolerance to self-tissues. In addition, the immune system is believed to keep self-tissues under surveillance with the aim to identify and eliminate malignant cells. Yet, tumors can develop in the presence of a functioning immune system, demonstrating the imminent medical need to generate novel immunotherapies for the treatment of cancer. A main obstacle for such an endeavor is the fact that tumor cells have developed sophisticated immune evasion strategies leading to inefficient anti-tumor immune responses. Here, we have explored a molecular mechanism of metabolic communication deployed by highly glycolytic tumors for immunoevasion. Here, high glycolytic activity of the tumor resulted in high acidification of the tumor microenvironment that can be sensed by tumor-associated macrophages (TAM) in a G protein–coupled receptor– cAMP-dependent manner. Sensing of acidification resulted in expression of the transcriptional repressor ICER in tumor-associated macrophages that led to their functional polarization toward a non-inflammatory phenotype and promoted tumor growth. Collectively, our findings identify a molecular mechanism of metabolic communication that was exploited by high-glycolytic-rate tumors for evasion of the immune system. Therefore, sophisticated therapeutic strategies that ensure targeted drug delivery to TAM are required to maintain self-tissue tolerance while at the same time strengthening anti-tumor immune responses. In this presentation I will highlight the molecular mechanisms underlying acidification-based tumor immune evasion and how these mechanisms can be targeted by nanoparticle-based drug delivery to enforce efficient anti-tumor immunity.

NANOPARTICLE DESIGN STRATEGIES FOR EFFECTIVE LIVER CANCER IMMUNOTHERAPY

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Liver cancer has a very poor prognosis, being the second most common cause of death from cancer worldwide. Despite the increasing knowledge on the molecular mechanisms underlying hepatic carcinogenesis, effective therapeutic strategies are still an unmet clinical need. To maximize site-selectivity and therapeutic efficacy reducing systemic side effects, drug delivery systems (beads) may be applied locally through transarterial chemoembolization (TACE), and may be loaded with chemotherapeutic drugs or immunostimulants. In our case, the cyclic dinucleotide (CDN) 2`3`cGAMP was used as a potent immune modulator. CDNs activate the stimulator of interferon genes (STING) pathway that plays an important role in the activation of type I interferons in antigen presenting cells (APCs) in response to cytosolic nucleic acid ligands.

PLATFORMABILITY OF RNA DRUG DELIVERY; THE CASE OF EXPERT, BRINGING IMMUNE ACTIVATING MRNA TO THE CLINIC

SVEN EVEN BORGOS[1] AND RAYMOND SCHIFFELERS[2] ON BEHALF OF THE EXPERT CONSORTIUM

[1]SINTEF Industry, Dept. of Biotechnology and Nanomedicine;
[2]University Medical Center Utrecht, Dept. of Clinical Chemistry and Haematology

The H2020 EXPERT project (EXpanding Platforms for Efficacious mRNA Therapeutics; https://www.expert-project.eu/), which started end of 2019, aims at developing a new off-the-shelf delivery system for RNA-based nanomedicines. Specifically, one of our target indications in the project is triple negative breast cancer. We will address this cancer by intratumoral injection of the immunostimulatory mRNA TriMix®, which is exclusively licensed to one of the project partners (eTheRNA Immunotherapies Nv). We will explore different nanomedicine-based delivery solutions, spanning the spectrum from established (LNPs) via emerging (cell-penetrating peptides) to exploratory (exosomes). The LNP formulation, once optimized early in the project, will go through quality-by-design production optimization, comprehensive quality and safety testing according to EUNCL protocols, GMP scale-up, and finally first-in-man clinical testing in cancer patients.
We will give an update on progress in the EXPERT project, and address in a broader sense i) why RNA drugs, and mRNA in particular, is ideally suitable for nanomedicine based platform creation; and ii) the particular challenges met in formulation and characterization of these drugs. Importantly, this applies not only to therapeutic formulations addressing an existing pathology, but equally to the burgeoning field of synthetic mRNA vaccines that hold the potential to transform vaccinology.

EC: TOWARDS SCIENCE-BASED REGULATIONS FOR NBCDS, THE INTERNATIONAL PERSPECTIVE

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Fig.1: *The main categories of regulatory challenges*

The Horizon 2020 project "REFINE" aims to advance the regulatory science in the field of nanotechnology based health products. As a first step, we have reviewed existing guidance documents, scientific articles and communications released by the regulatory community. Six major challenges associated with the regulation of nanotechnology-enabled health products were identified, summarized and explained in REFINE's white paper[1] (Fig.1). However, the white paper is only the starting point to launch a public debate and the scientific community is now invited to provide their feedback on how those challenges can be addressed. Our EU-survey can be found on http://refine-nanomed.com/news/. During the Global Summit on Regulatory Science, which was held in Ispra (Italy) in September 2019, highlights of the REFINE white paper were presented ahead of publication and regulatory scientists from academia, industry and competent authorities confirmed the need for further research in the presented fields.

In particular, the lack of methodologies was highlighted as an area where (pre)-standardisation activities should be initiated and intensified. The experimental work packages of the REFINE project have taken up this task and are currently developing and optimising methods that are urgently needed in order to i) assess similarity of follow-on products, ii) address safety requirements of emerging nanotechnology enabled health products and iii) strengthen the implementation of the quality/safety-by-design approach. In order to support the laboratory work with the prioritisation and selection of methods for further optimisation, we have started to map the regulatory information needs in five areas against methods that are already widely used and well-developed in the research environment and as such might qualify to enter into the standardisation process.

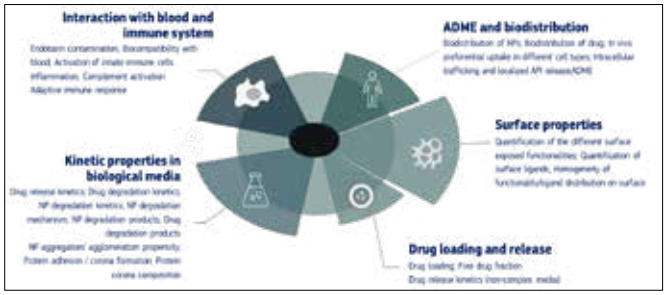

Fig.2: *Areas of methodological gaps that require test development, optimisation and standardisation*

Acknowledgement: We would like to thank the regulatory community for their continous feedback and their responses to our surveys. We are also grateful to be a partner in the REFINE consortium that is always willing to discuss and to provide constructive feedback on our work. This project has received funding from the European Union's Horizon 2020 research and innovation programme under Grant Agreement No 761104.

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DISSEMINATION FOR LAY PUBLIC IN NANO-MEDICINE : A GAME FOR NANOMEDICINE IN ATHEROSCLEROSIS

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Novel technologies are usually developed far from public scrutiny, but it is generally assumed that positive uptake by society will follow. Genetically modified food broke the pattern when strong public opposition developed in 1999, first in the UK and then Europe. This forced a change in how biotechnology was governed, focused on the need to engage with publics, rather than educate them to accept the technology. As the next new technology, nanotechnologies became subject to widespread public consultation, with the aim of avoiding 'another GM situation'. The 'nano' word was unfamiliar to many lay people, and risks of strange particles were thought to be a major potential stumbling block?

Unlike GM, however, nanotechnologies are not a single scientific concept, but a multiplicity of applications as diverse as medicine and paint, loosely linked by the nano scale. Publics found it hard to engage and consultations did not achieve much impact. Nonetheless, the concept of Responsible Research and Innovation (RRI) emerged in within the EU, as an umbrella approach to taking account of wider societal and ethical issues in the development of new and emerging technologies. It stresses the need to engage with a range of stakeholders and publics, with the aim that the applications of the technology are broadly coherent with societal values and wishes. RRI looks for an orderly approach with time for technologists and society to reflect. CRISPR genome editing research has gone so rapidly and in so many different directions, that it resembles an avalanche which is overwhelming the more reflective development that RRI advocates. With the requirement for clinical trials nanomedicine provides a much better scope for RRI.

Its underlying assumption is that nanomedicine will help treat sick or injured people that we struggle to treat at present, or can forewarn us of a medical condition in time to intervene, maybe even prevent it. We assume that most people would agree that it's a good thing, and will welcome the drugs, clinical procedures, devices and tests that nanomedicine is enabling. But acceptance may not happen automatically.

CRISPR and GM

Genome editing is revolutionising the field of genetics, for example in novel modifications of food animals, but it is happening in a way that presents a significant challenge to the RRI concept.

Clinam brings together scientists and clinicians applying nanotechnology to clinical medicine. Its underlying assumption is that nanomedicine will help treat sick or injured people that we struggle to treat at present, or can forewarn us of a medical condition in time to intervene, maybe even prevent it. We assume that most people would agree that it's a good thing, and will welcome the drugs, clinical procedures, devices and tests that nanomedicine is enabling. But acceptance may not happen automatically. The 'nano' word is still unfamiliar to many lay people: isn't it something to do with fancy high tech materials, and strange particles? That sounds OK for paints, solar panels and tennis rackets, but it could be a bit scary if it comes close to my body. As nanomedicine becomes more established in clinical use, it is important that we build bridges to the general public and patients.

In the recently completed EC FP7 NanoAthero project, to demonstrate the clinical feasibility of nanosystems for imaging and treating atherosclerotic plaques, we sought to address this is to engage with people interactively in the form of a Democs* card game. The Democs concept is a group activity for 6-8 people on a new technology, using cards from which people learn, discuss together and come to their own views. It has been widely for over 15 years, on different subjects as diverse as climate change and tuberculosis. We created a game to explore nanomedicine in general and its application to atherosclerosis. In this talk we will present the concept, some of the first results from playing the game, and explore its potential for wider use.

The value of Democs is that is a grassroots method, without needing experts or any prior technical knowledge - the cards are the 'expert'. It can be played by groups of people anywhere, something to do with your friends one evening. It uses three sets of cards: case studies and factual cards to introduce the scientific topic in ways understandable to lay people, and then 'issue cards' to present ethical and social implications for discussion. The group context is of great value in helping people to learn together, benefit from each other's viewpoints, and to seek to come to both individual and collective understandings. At the end, participants are invited to give written statements and opinions, and these qualitative outputs can then be analysed.

* Democs (also known as Decide) was invented in 2001 by Perry Walker, then of the New Economics Foundation. The NanoAthero game is available via www.nanoathero.eu and www.edinethics.co.uk/democs/nanoathero

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POLYMERIC MICELLES IN CANCER THERAPY

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Targeting tumors with drug-loaded nano-scaled carriers is a promising strategy for systemic cancer treatment. Polymeric micelles have demonstrated high potential as nanocarriers capable of effectively overcoming biological barriers for controlling the distribution and function of bioactive agents in the body. Here, I introduce our recent achievements on developing polymeric micelles assembled through multimolecular interactions between block copolymers and the loaded drugs as translationable nanomedicines. Compared with free small therapeutic agents, polymeric micelles can selectively accumulate in solid tumors through the increased permeability of tumor tissues by controlling various physicochemical aspects, such as size and charge, as well as by modifying the surface of the micelles with ligands recognizing the tumor vasculature, such as glucose, peptides or antibody fragments, for promoting selective tumor extravasation. Moreover, because the effective delivery of nanomedicines to solid tumors is hindered by abnormalities in the tumor vasculature and stroma, which can drastically reduce tumor perfusion and the delivery of blood-borne drugs and cells, strategies capable of normalizing the tumor interstitial space and the vasculature would further improve the tumor targeting ability. In particular, we found that dexamethasone, which is a glucocorticoid steroid that is given to cancer patients to manage chemotherapy-induced toxicities in various clinical trials using polymeric micelles, repairs vessel leakiness and normalizes the mechanical tumor microenvironment towards increasing vessel permeability and improving tumor targeting. In addition, polymeric micelles can be applied for boosting the efficacy of immunotherapies by delivering bioactive payloads promoting antitumor immunogenic signals, such as chemotherapy promoting antigenicity and adjuvanticity, as well as decreasing immunosuppression in tumors. In this way, by using polymeric micelles delivering epirubicin, which are under phase I/II clinical evaluation, we were able to improve the efficacy of immune checkpoint inhibitors (ICI), namely, anti-PD1 antibodies, to achieve curative responses even in ICI-resistant tumor models, while circumventing immunosuppressive side effects.

FROM SOPS TO STANDARDS FOR NANOMEDICINE

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The accurate measurement of the important properties of nanoparticles enabled pharma-ceuticals (NEP) is one of the key factors for the characterization of NEPs and for their translation from laboratory research to approved medicinal products. There is a strong request from regulators (FDA and EMA) to develop internationally recognized standards for the accurate measurements of quality attributes, that are currently lacking.
The European Union Nanomedicine Characterisation Laboratory (EUNCL), in addition to providing acces to its characterization infrastructure, has developed standard operating procedures (SOPs) for NEP assessment. These SOPs and protocols are open access and available to all.
In this presentation I will briefly illustrate the different SOPs and protocols developed by EUNCL (in collaboration with NCI-NCL), the process for their internal quality control, and the steps that we are taking towards more formal standards.
The most pressing standards are being developed in the EU-US Collaboratory on Nano-medicine, an initiative lead by JRC and NIST and involving regulatory and non regulatory agencies, scientists from profit and non-profit research institutions. In particular, I will illustrate the first methods for the measurement of chemical composition and particle size distribution of liposomal drug products.

ORALLY ADMINISTERED, POLYMERIC BILE ACID NANOPARTICLES TO RESTORE GLUCOSE CONTROL AND INDUCE IMMUNE TOLERANCE IN EARLY AND ADVANCE DIABETES

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Type 1 diabetes (T1D) is an autoimmune disease resulting from the attack of immune cells to pancreatic insulin-secreting β-cells. The actual triggers of T1D are not fully understood however, it is recognized that both, genetic and environmental factors contribute to its initiation and progression. Nowadays, it is generally accepted that the disorder represents a continuum from asymptomatic manifestations towards lifelong insulin dependence, and that the process encompasses well defined disease stages (1). Treatment options for T1D rely almost exclusively on the administration of exogenous insulin, which can be delivered as self-injections or with the help of insulin pumps. Unfortunately, the century old insulin replacement therapy does not tackle the root cause of the disease, it is not perfect controlling glucose homeostasis and it is associated to multiple complications that diminish the patients' quality of life.
Immunotherapy represents a hope for the treatment of T1D and many other autoimmune diseases. However, until now all tested immune interventions have been fundamentally based on immunosuppressive mode of actions, and although some of them displayed benefits, they increased the risk of infections. Thus, there is an urgent medical need to develop new therapies aiming to restore immune tolerance, without impairing general immune function. These new products should be amenable for patients in all stages of the disease and, ideally, they should use a convenient route of administration that is non-invasive and facilitates patient compliance.
Here we report the development of a nanocarrier platform based on polymerized ursodeoxycholic acid (pUDCA) that delivers biologic and/or chemical cargo using the oral route. Ingested pUDCA nanoparticles are stable and protected from degradation through the intestinal tract and, when they reach the ileum, they are efficiently uptaken and incorporated into the enterohepatic circulation. The absorption process is further enhanced by monocyte/macrophage internalization that is mediated by binding of pUDCA to the specific bile acid receptor, G Protein-Coupled Bile Acid Receptor 1 (GPBAR1/TGR5), expressed on the surface of these cells. Agonism of GPBAR1 on antigen presenting cells has been reported to facilitate M2 phenotype differentiation, to suppress IL-12 and IL-23 and to stabilize and/or enhance IL-10 production (2). GPBAR1 is also expressed on intestinal L cells and pancreatic a-cells. In these cases, pUDCA binding to GPBAR1 induces the production of the incretin, Glucagon-Like Peptide 1 (GLP-1), and consequently triggers endogenous insulin production (3). Because pUDCA nanoparticles accumulate in the pancreas environment, they carry the intrinsic potential to reduce inflammation and promote insulin production, two major hallmarks required for effective glucose control.
The efficacy of our pUDCA platform was first tested in a model of cyclophosphamide (CY) induced diabetes. Administration of CY to the T1D-prone, non-obese diabetes (NOD) mouse strain leads to a rapid diabetes development with almost 100% incidence within 10 days of treatment. Two consecutive oral doses of pUDCA nanoparticles, but not other monomeric or polymerized bile acid nanocarriers, delivered to NOD mice at days 1 and 2 following CY administration, resulted in 50% protection from hyperglycemia. Disease prevention was maintained for up to 30 days in the absence of any other treatment. pUDCA nanoparticles were loaded with the mTOR inhibitor rapamycin ($pUDCA_{RAPA}$) to further explore prevention with a complementary immunomodulator. Under the same experimental conditions, pUDCARAPA nanoparticles stabilized normal blood glucose levels in a dose dependent manner, reaching almost complete control in the group treated with pUDCARAPA nanoparticles that incorporated the highest concentration of rapamycin. Of note, rapamycin alone, or encapsulated into poly-[lactic-co-glycolic acid] (PLGA) nanoparticles, showed little effects and only in a subset of treated mice. Interestingly, normoglycemia in the pUDCARAPA treated animals correlated with a decrease of CD8+ effector T (Teff) cells and with a concomitant increase of CD4+, CD25+, Foxp3+ regulatory T (Treg) cells in the pancreas draining lymph nodes. All together, these results indicate that PUDCA nanoparticles possess an intrinsic immune regulatory potential, that is enhanced by rapamycin, and that prevents disease progression in the early stages, i.e., those currently referred as stage 1 or 2 [(1)]. Stage 1 and 2 T1D patients display ≥2 autoantibodies to pancreatic antigens, some signs of dysglycemia (in the case of stage 2) and are suffering a silent destruction of pancreatic b-cell mass, despite they do not have yet the typical clinical symptomatology of T1D, i.e., polyuria, polydipsia, weight loss, fatigue, diabetic ketoacidosis, etc. The mechanisms of CY-induced T1D in NOD mice is associated with a reduction in the number and function of Treg cells, which it is believe to tilt the immune balance towards an aggressive autoimmune response against pancreatic β-cells [(4)]. This process involves tissue damage and the release of autoantigens in a microenvironment that promotes epitope spreading and disease progression. In such environment, we anticipate that antigen presenting cells will incorporate rapamycin from $pUDCA_{RAPA}$ nanoparticles and pancreatic antigens from the local environment and use such combination to instruct cognate T cells to differentiate into bonafide pancreatic-antigen specific Treg cells [(5)]. The implication of this finding is that pUDCARAPA nanoparticles have the potential to prevent disease progression in late stage 2 as well as early onset stage 3 T1D patients.
Restoration of normoglycemia in established T1D (stages 3 and beyond) is a more challenging task, since the general belief is that these patients have reached a point of no return that made them life-long dependent to exogenous insulin. To this end, we orally treated NOD mice that already developed spontaneous disease (glucose levels ≥ 200 mg/dL), with pUDCA nanoparticles encapsulating insulin (pUDCAINS). Our aim was to combine the direct effects of exogenous insulin as antigen and for short term glucose regulation, with the intrinsic incretin and immunoregulatory effects of pUDCA. A single seven days course of oral pUDCAINS nanoparticles to diabetic NOD mice was sufficient to restore nor-

moglycemia for 40 days in all treated mice and to extend this benefit to 40% of them for additional 50 days. These effects could not be achieved with soluble insulin, independently of whether the administration followed the oral, subcutaneous or intraperitoneal route. The NOD mouse model represents an excellent tool for the *in vivo* study of T1D and for predicting the efficacy of novel interventions in human disease. However, many protocols successfully working in NOD mice have not translated to the human situation, often due to differences in dosing, timing and/or interpretation of subtle immune differences among the two species (6). For this reason, we decided to further evaluate the efficacy of pUDCAINS nanoparticles in a large animal model, namely the alloxan-induced Ossabaw swine diabetes model [7]. Like in NOD mice, diabetic swine that were daily gavaged with pUDCAINS nanoparticles displayed a progressive restoration of blood glucose levels, reaching normoglycemia by treatment day 7. These results suggest that pUDCAINS is an excellent drug to maintain glucose homeostasis in T1D patients with advance disease and perhaps also in individuals suffering the insulin resistance form of diabetes (type 2 diabetes, T2D) that have advanced into b-cell failure. Due to the intrinsic anti-inflammatory and incretin effects of the pUDCA, we believe these patients will also have a local reduction in their ongoing inflammatory processes (i.e., in the absence of general immunosuppression) and a chance for a recovery of b-cell function once all the inflammatory autoimmune stress is released. This concept is supported by studies from the Network for Pancreatic Organ Donors with Diabetes (nPOD) showing that pancreata from T1D patients with long-standing disease retain insulin production capacity [8].

In summary, we are presenting the development of an oral nanocarrier platform based on pUDCA that is capable of delivering diverse immunoregulatory cargo, while simultaneously displaying intrinsic therapeutic properties. These facts, together with the exquisite preference to accumulate in the pancreas environment, enable pUDCA nanoparticles with the transformative potential to intervene in all stages of T1D, which is a step forward towards the eradication of this disease.

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TOWARDS A UNIVERSAL INFLUENZA THERAPY

MARK CHIU

There is a need to develop an effective “broadly-reactive” or “universal” influenza therapies capable of treating both seasonal influenza strains. Although there are a number of novel influenza vaccine approaches are currently under evaluation, there is still a paucity of specific treatments for those patients with weakened immune systems like elderly and young patients. One approach is the generate multiple mechanisms to target influenza via the “Achille’s heel” of the virus, i.e. conserved viral proteins or protein regions shared amongst seasonal and prepandemic strains. The approach towards eliciting a broader immune response capable of conferring protection against the diversity of currently circulating seasonal influenza strains. I review a universal biologics strategy with an emphasis on targeting the HA glycoprotein.

THREE-DIMENSIONAL GRAPH CONVOLUTIONAL NETWORK (3DGCN) FOR DEEP-LEARNING PREDICTION OF DRUG-TARGET INTERACTIONS

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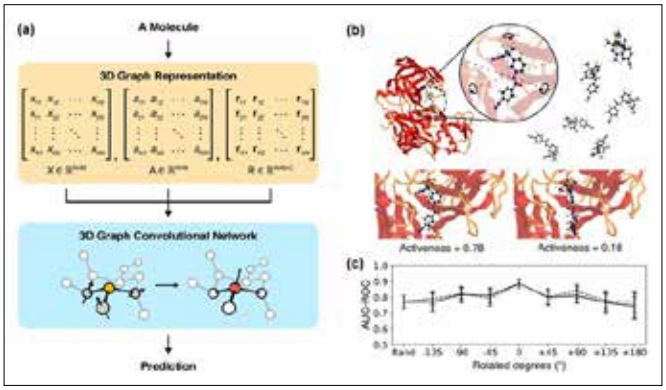
The game-changing wave of deep learning (DL) touched the chemistry community and has revolutionized the way problems in chemistry are being solved. Theoretical approaches to the calculation of physicochemical properties of molecules have been challenged by DL-based methods. Reaction prediction and retrosynthetic analysis also have been tackled with great promise by various DL strategies, such as convolutional neural network, recurrent neural network, and neural-symbolic network approaches. Several years of research have shown that deep neural network and other DL models generally outperform the conventional machine-learning models or physical calculations used in chemistry.

The early models in DL chemistry used traditional chemical representations, such as fragment-type fingerprints or other molecular descriptors that had been developed for chemoinformatics. Considering that DL models are believed to “learn” the representations, the paradigm of molecular input has shifted from molecular descriptors to representation learning, which directly interprets the molecular structures. On the other hand, graph convolutional network (GCN), which handles graph-structured data and which is specialized in network problems, has recently been adapted in DL chemistry. GCN takes a molecular graph, through direct substitution of the molecular structure with a connected simple graph, as the input and applies the convolution on each node and its neighborhoods. Given the molecular graph, the GCN uses recursive updates of nearest-neighbor features for possible refinement of the molecular structure into an intended property. However, the

molecular graph, widely used in chemistry, is an inherently two-dimensional (2D) representation, composed of vertices and edges, which lacks the spatial topology of the atoms and bonds in 3D space. Several attempts, including the algorithms that introduced the multiple distance-dependent weights simulating the decay of atomic influences over space, used by the interatomic Euclidean distances, have been made to provide and interpret the spatial information on molecules, but these reported methods do not take the bond directions into account, and therefore do not represent the 3D molecular structures fully for DL prediction. Particularly, the information on 3D structures of molecules including chirality is important for interpretation and prediction of drug-target interactions (DTIs). In this work, we propose an advanced DL algorithm, incorporating the 3D bond features of molecules into a vanilla GCN, which would efficiently predict the chemical tasks related to the molecular topology, such as DTI prediction (Figure 1).

Figure 1. (a) Schematic for prediction processes in the 3DGCN. (b) Illustration and example of rotation-dependent activeness of ligands to b-secretase 1 for the BACE dataset. The ligands are rotated, gradually or randomly, and provided to the previously trained 3DGCN model for activeness prediction. b) Performance-evaluation results upon ligand rotations. A trained model is provided with ligand molecules rotated along the x (solid), y (dashed), or z (dotted) axis, and the performance is measured.



WHERE TO GO WITH NANO?

DAAN J.A. CROMMELIN

The rapid rise in interest in 'nanomedicines' in the academic world over the last twenty years and the claims of success led to calls for reflection. The main focus of this presentation will be on answering the question: 'where to go with nanomedicines'? Guiding principles for future research will be outlined based on learnings with the most successful nanomedicines family within the broad field of nanomedicine so far: liposomes. An analysis of currently clinically tested, approved and marketed liposome-drug combinations provides these insights.

Reference: D.J.A. Crommelin, P. Van Hoogevest, G. Storm. The role of liposomes in clinical nanomedicine development. What now? Now what? J. Controlled Release. 318, 2020, 256-263.

SPLENIC UPTAKE OF GANGLIOSIDE-CONTAINING LIPOSOMES IS MEDIATED BY CD169+ MACROPHAGES AND STIMULATES STRONG IMMUNE RESPONSES.

JOKE M.M. DEN HAAN[1],

Joanna Grabowska[1], Alsya J. Affandi[1], Katarzyna Olesek[1], Hakan Kalay[1], Martino Ambrosini[1], Paul Crocker[2], Yvette van Kooyk[1], Gert Storm [3]

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CD169-expressing macrophages are present in spleen and lymph nodes to capture pathogens. CD169, also known as Siglec-1, is a sialic-acid binding lectin that binds to several endogenously expressed gangliosides. Here we tested anionic ganglioside-containing liposomes for their binding and uptake by murine and human CD169+ antigen presenting cells and their capacity to induce immune responses.

Both human and murine splenic CD169+ antigen presenting cells specifically bound and took up ganglioside-containing liposomes. In vitro studies with human CD169+ monocyte-derived dendritic cells demonstrated antigen presentation to CD8+ T cells after uptake of ganglioside/antigen-containing liposomes. Intravenous immunization of mice with ganglioside/ovalbumin-containing liposomes showed binding to CD169+ macrophages and the induction of ovalbumin-specific CD8+ and CD4+ T cell and B cell responses. T cell, but not B cell, responses were dependent on CD169+ macrophages as well as Batf3-dependent cross-presenting dendritic cells.

In conclusion, anionic ganglioside-containing liposomes efficiently target antigen to CD169+ antigen presenting cells, demonstrate a strong capacity to stimulate immune responses, and should be further explored as a cancer vaccination strategy.

RESULTS FROM A REGISTRATIONAL TRIAL OF NANOPARTICLE ALBUMIN BOUND SIROLIMUS (ABI-009) IN ADVANCED MALIGNANT PECOMA

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ABI-009 (nab-sirolimus) is an albumin-bound nanoparticle version of sirolimus (an mTOR inhibitor) with a naoparticle size of approximately 100 nm, that can target various tissues based on mechanisms of albumin transport. Advanced Malignant PEComa is an extremely rare, aggressive sarcoma and relevant mutations in the mTOR pathway causing mTOR activation are considered as drivers for this disease. ABI-009 has significantly higher intratumoral drug levels, mTOR target suppression, and anti-tumor activity compared to other mTOR inhibitors in preclinical models. The AMPECT trial measured the effects of ABI-009 and is the first prospective study in advanced malignant PEComa.

Patients (N=34) received ABI-009 (100mg/m2 IV, weekly, 2/3 weeks) until progression or unacceptable toxicity. Primary endpoint: overall response rate (ORR) by independent radiology review (IRR). Key secondary endpoints included duration of response, DoR, progression-free-survival (PFS), PFS rate at 6 months (PFS6), overall survival, and safety. Exploratory endpoints included correlation of mutational status tumor genotype and outcome. Efficacy and safety results of this registrational trial will be presented.

ABI-009 is also being studied in several different diseases both in oncology and non-oncology applications. Results in these ongoing studies will also be presented

SAFETY TESTING OF IRON OXIDE CONTAINING NANOPARTICLES FOR LATER CLINICAL USE

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Superparamagnetic iron oxide nanoparticles (SPIONs) consist of iron oxide cores and various coatings. SPIONs have been used in drug delivery, hyperthermia, and enhanced resolution magnetic resonance imaging (MRI) techniques. A possible problem with the clinical application of these nanoparticles is their capability to cause hypersensitivity reactions (HSRs), which are also called complement (C) activation-related pseudoallergy (CARPA)[1]. A few types of SPIONs (e.g. ferucarbotran (Resovist®, Bayer Healthcare), ferumoxtran-10 (Sinerem®, Guerbet) and NC100150) had been designed and clinically tested as MRI contrast agents. In addition, ferumoxytol (Feraheme®, AMAG Pharmaceuticals, or Rienso®, Takeda Ltd.) has been approved for the treatment of iron deficiency in adult chronic kidney disease patients, and also used as MRI contrast agent[2]. However, both Resovist and Rienso were (regionally or globally) withdrawn from the market, the latter because of hypersensitivity reactions, some of which were fatal. In the case of Rienso, 35 deaths were reported worldwide.

For these reasons, there is an increasing interest in the prediction and prevention of CARPA caused by iron oxide-based nanoparticles, using laboratory assays and animal models. In our laboratory, the porcine CARPA model has been established. The major advantage of this model is its high sensitivity and human predictability. The mechanism of CARPA is rather complex. In addition to C activation, formation of anaphylatoxins can induce mast cells and macrophages to release vasoactive agents and inflammatory mediators. The symptoms of CARPA include hemodynamic and cardiopulmonary changes, among which pulmonary arterial pressure (PAP) elevation is most characteristic in pigs. In addition, hematological changes like leukopenia or leukocytosis, thrombocytopenia, skin reactions (flush and rash), and elevated TXB2 levels are also important markers of CARPA.

MATERIALS AND METHODS

Pigs: Domestic pigs (20-25 kg) were sedated with ketamine/xylazine (10 and 2 mg/kg, respectively) and anesthetized by isoflurane (2-3%) in O_2 flow. In spontaneously ventilating animals, the PAP was measured using a Swan-Ganz catheter introduced into the pulmonary artery via the right external jugular vein, while systemic arterial pressure (SAP) and heart rate (HR) were measured in the femoral artery. The left femoral vein was cannulated for blood sampling. Test agents were injected in bolus (~30 sec) via the left external jugular vein. Hemodynamic changes were continuously monitored using an AD Instruments (ADI) PowerLab System. Mean PAP, SAP and HR data were evaluated by the ADI LabChart software.

Blood sampling: Blood samples of 2 ml, each were collected from the pigs before (time 0), and at pre-determined time points (1-3-5-10-30 min) after the injection. Samples were collected into K_3-EDTA blood tubes, of which samples for TXB2 analysis were containing indomethacin. Aliquots of 100 µl blood were drawn into tubes with K_3-EDTA for haematological analysis. Blood was centrifuged at 1500 rpm for 10 min at 4 °C, and plasma was stored at -80 °C until analysis.

Thromboxane B2 levels: Plasma TXB2 (the stable metabolite of plasma TXA2) levels were measured with an ELISA kit (Cayman Chemicals).

Test items: To induce CARPA, zymosan was utilized as direct complement activator. Feraheme and Resovist served as positive control for SPIONs. SEON Dex-USPIO (30 nm)[3], a dextran-coated SPION - provided by the Section of Experimental Oncology and Nanomedicine (SEON), University Hospital Erlangen - was used as test agent.

RESULTS

Cardiovascular effects of an i.v. bolus injection of Feraheme (5 mg/kg) is shown in Fig. 1. Feraheme induced a 3-fold, prolonged increase in PAP with a transient increase in SAP and a compensatory decrease in HR, showing typical symptoms of severe CARPA. At the end of the experiment i.v. zymosan (0.1 mg/kg) exhibited similar, but somewhat milder CARPA compared to the effects of Feraheme.

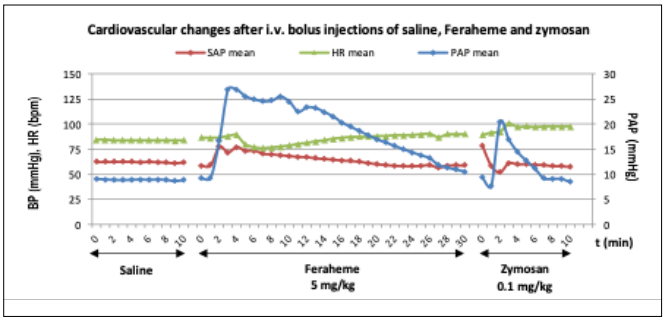

Figure 1. *Time course of cardiovascular (PAP, SAP and HR) changes by Feraheme injection in a pig. Doses are given in mg iron/kg. Curves were constructed pre-injection and from the 0 to 30 min readings after injection, evaluated at 1 min intervals.*

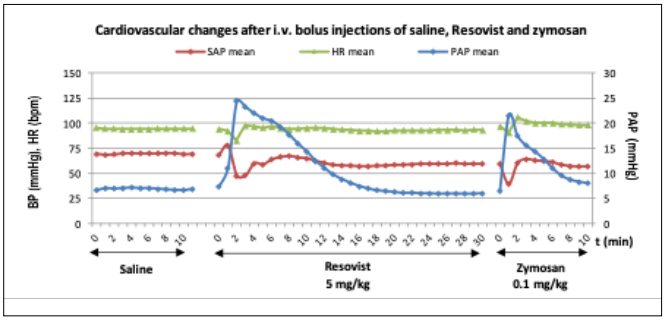

Figure 2. *Time course of cardiovascular (PAP, SAP and HR) changes by Resovist injection in a pig. Doses are given in mg iron/kg. Curves were constructed pre-injection and from the 0 to 30 min readings after injection, evaluated at 1 min intervals.*

In another experiment cardiovascular effects of an i.v. bolus injection of Resovist (5 mg/kg) was studied (Fig. 2). Resovist also elicited severe CARPA, with a 3.5-fold increase in PAP but its duration was shorter. SAP and HR showed transient changes only. Upon i.v. administration of zymosan (0.1 mg/kg) a similar CARPA to the previous study could be seen.

Cardiovascular effects of consecutive bolus administrations of 0.5 mg/kg and 5 mg/kg i.v. dextran-coated SPIONs with 30 nm diameter (SEON Dex-USPIO) are shown in Fig. 3. None of the boluses, low or high dose, led to changes in any parameters (PAP, SAP or HR). At the end of the experiment 0.1 mg/kg i.v. zymosan induced moderate-to-severe CARPA.

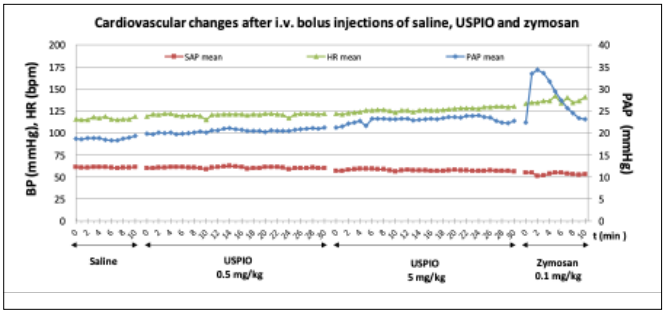

Figure 3. *Time course of cardiovascular (PAP, SAP and HR) changes caused by consecutive SEON Dex-USPIO injections of increasing doses in pigs. Doses are given in mg iron/kg. Mean values of 2 animals*

are averaged. Curves were constructed pre-injection and from the 0 to 30 min readings after injection, evaluated at 1 min intervals.

CONCLUSIONS

This study investigated the immune reactive properties of commercial, as well as newly formulated SPIONs in a highly sensitive model of CARPA in pigs. Our previous experience with other SPIONs - similar to literature data of HSRs in humans – and our current results with Feraheme and Resovist indicated the possible induction of massive CARPA by such nanoparticles. However, applying special synthesis procedures and surface coating by dextran, the way it is done in SEON Dex-USPIO, revealed reaction-free nanoparticles. This implies a significant advancement on this field, as well as the potential safety for later clinical use of these SPIONs.

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A NOVEL PEPTIDE BASED NANOPARTICLE-FOR MRNA AND GENE EDITING THERAPEUTICS:

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The recent discovery of CRISPR (clustered regularly interspaced short palindromic repeats)-Cas9 (CRISPR-associated protein 9) has provided a highly efficient tool for site-directed genome engineering in eukaryotic cells, opening new perspective for innovative gene therapy. However, *in vivo* administration of the RNA-guided nuclease CAS9 and of the associated guide RNA (gRNA) remains a limitation to the overall potency and for the therapeutic application of the CRISPR-CAS9 system.
We have developed a new delivery platform that can potently protect and deliver active CAS9 mRNA/gRNA complexes in a variety mammalian cell lines, including primary and T cells. ADGN-technology is based on short amphipathic peptides that form stable neutral nanoparticles with nucleic acids through non-covalent electrostatic and hydrophobic interactions. We demonstrated that ADGN-nanoparticle promotes *in vivo* delivery CRISPR/Cas, leading to a robust editing of a selected target gene in specific organs or in tumors.
Activating mutations in KRAS play potent roles in cancer initiation, propagation, and maintenance. Although they represent important therapeutic targets, no effective treatment for direct inhibition of KRASG12V and KRASG12D mutants have been reported. ADGN-CRISPR technology was successfully applied to impair cancer cell proliferation by directly targeting G12V and G12D mutations of the KRAS oncogene.
We demonstrated that combining highly specific gRNAs targeting KRASG12V and KRASG12D mutants with ADGN-nanoparticles, efficiently and selectively silenced KRASG12V and KRASG12D in colorectal and pancreatic cancer cells. ADGN-nanoparticles containing CRISPR/gRNA targeting KRASG12D abolished PANC1 tumor growth and induced permanent KRASG12D gene editing in the tumor with an in indel frequencies of 76%. ADGN-nanoparticles containing CRISPR/gRNA targeting KRASG12V abolished SW403 tumor growth and induces KRASG12V gene editing in the tumor with an in indel frequencies of 82%. In contrast, no effect on tumor growth were observed with ADGN-nanoparticles containing none specific gRNA or gRNA targeting other KRAS mutations. ADGN/mRNACas9/gRNA abolishes colorectal and pancreatic tumor growth *in vivo*, without inducing any toxicity, emergence of off target or other KRAS mutation.
ADGN-nanoparticle containing mRNACas9/gRNA were effective in targeting selectively mutated KRAS both *in vitro* and *in vivo*. Our study provides a proof-of-concept that ADGN/CRISPR can be applied to target driver mutations of cancers *in vivo* and permanent disrupt the oncogenic alleles, leading to inhibition of tumor growth.

EMERGING BIOMARKERS OF NANOPARTICLE IMMUNOTOXICITY: AN OUTLOOK IN FUTURE

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Despite the sophistication and many therapeutic advantages, the clinical translation of nanotechnology-formulated drug products is often complicated by the immune-mediated toxicities. Cytokine storm, fever-like reactions, and complement activation are among the most common and best-studied acute dose-limiting toxicities. Immunotoxicity due to the alteration in the immune system's function, including but not limited to the immunosuppression and autoimmunity, take longer to develop and are less understood both in terms of the nanoparticle structure-activity relationship and methodologies appropriate for monitoring these toxicities. This presentation will review existing and emerging biomarkers of nanoparticle immunotoxicity, and propose experimental strategies for improving our understanding of the immunological safety of nanomedicines. Case studies discussing the role of nanoparticle physicochemical properties and their contribution to the immunotoxicity will be used to support the proposed strategy. Recommendations for future research directions will be offered.

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NANOPARTICLE-LOADED MICROARRAY PATCHES TO EXTEND PHARMACOKINETIC EXPOSURE

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Queen's University Belfast

Anti-HIV therapy has generated excellent clinical results, leading to a decline in mortality and morbidity for HIV positive patients globally. Antiretrovirals (ARVs) can also be used to prevent HIV transmission and pre-exposure prophylaxis (PrEP) strategies have been developed for subjects at high risk of exposure. Currently available formulations necessitate lifelong, daily dosing and suboptimal adherence can increase the risk of treatment and PrEP failure. Recently, the development of novel ARV long acting injectable formulations to support administration once a month has been greeted with great excitement within the scientific, clinical and patient communities. Current obstacles to effective roll-out and clinical implementation of the LA strategy are: a) only two ARVs with compatible formulations b) intramuscular injections typically require a skilled healthcare worker and can lead to problems with needlestick injuries, inappropriate reuse and needle phobia all important concerns c) multiple large volume injections are necessary d) formulations can require a cold-chain.
Clearly, there is an urgent need for complementary delivery systems for LA ARVs that can be self-applied with increased adherence, facilitating the combination of multiple potent drugs, while

avoiding complexity related to intramuscular injection and reducing overall treatment costs. We have developed a minimally-invasive approach using dissolving microarray patches (MAPs) containing LA drug particles. Upon skin insertion, the MAPs rapidly dissolve in interstitial fluid, depositing the controlled release drug particles in the viable skin layers, forming individual nano-implants that slowly release their drug payload for absorption, supporting long acting pharmacokinetics.

Our novel MAP approach has multiple advantages over conventional HIV treatment and PrEP strategies. Firstly, it resolves the stigma and "pill fatigue" associated with long term daily oral administration. MAPs can enhance adherence and avoid needle-stick injuries and large volume injections with respect to injectable LA formulations and there is no need for administration by skilled healthcare workers. Administration strategies based on MAPs will reduce treatment costs, support the discrete self-application by patients and the co-administration of multiple drugs

Our multidisciplinary research is based on several integrated activities. Firstly, we use computational modelling to select optimal drug and formulation candidates and to identify suitable dosing strategies. Based on the computational simulations, we then choose appropriate drug particle systems to then be embedded in MAPs for administration. Drug particles and MAPs are comprehensively tested through *in vitro* and *in vivo* approaches and pharmacokinetic modelling used to further rationalise strategies for bridging to humans, supporting identification of the most promising drug combinations. We aim to ultimately generate an innovative weekly or monthly administration strategy for HIV treatment and prevention in adults with potential applications in special populations, such as children, combining safe and potent ARVs in a user-friendly, discrete and innovative technological platform.

ACKNOWLEDGEMENTS: Funded by USAID, though the generosity of the American people.

ADDRESSING THE REGULATORY BOTTLENECKS OF NANOMEDICINES DURING PRIMARY RESEARCH PLANNING

ROSY FAVICCHIO

Translational nanomedicines can exploit newly discovered strategies to target specific cells or tissues, and can do so reducing systemic side effects. A large number of targeted and activatable therapeutic nanoparticles have been developed and published, yet the pathway to their testing in a clinical setting and their approval for use in patients remain ridden with hurdles. Journals active in the translational medicine space may help address some of these hurdles earlier on in the bench to bedside pipeline. Although only few nanoformulations have been approved for human use, a large number of ongoing clinical trials suggests that the upcoming years will be critical to the field, possibly leading to a demonstration of clinical benefit for specific nanopharmaceuticals. Improved delivery, increased therapeutic effects and limited off-target effects are often claimed in original research articles, nevertheless the design of experimental preclinical research, often assessed in rodent models of disease, doesn't directly evaluate the translational potential of new and improved formulations. Improvements in the design of preclinical research settings, including the use of advanced disease models and comparisons to the clinically recommended therapies that are directly relevant to the intended clinical application, can anticipate the requirements of regulatory and funding agencies and will help improve the development of translationally viable nanomedicinal propositions.

NANOCARRIERS TARGETED TO THE MOSQUITO STAGES OF MALARIA: CURING THE INSECT HAS ITS ADVANTAGES

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[4]University of Twente, The Netherlands

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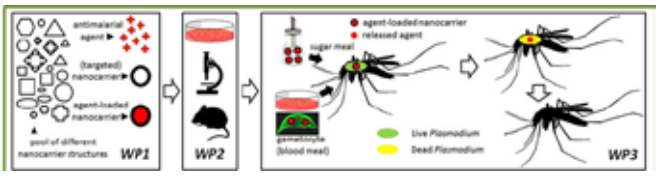
Whereas nanomedical approaches to cure diseases are prevalent in the developed world, there is an astonishing lack of nanomedicines being developed to treat the main causes of death in the developing world: infectious diseases, among which malaria is prominent. The unmet medical and patient need of malaria eradication will not be achieved unless the targeted delivery of new drugs is vastly improved. Encapsulation of drugs in targeted nanovectors is a rapidly growing area with a clear applicability to infectious disease treatment, and pharmaceutical nanotechnology has been identified as a potentially essential tool in the future fight against malaria. Polymers offer virtually unlimited diversity in chemistry, dimensions and topology, which renders them a class of materials that is particularly suitable for applications in nanoscale drug delivery strategies. The objective of the NANOpheles project is to design polymeric nanovectors for the delivery of antimalarial agents to *Plasmodium* stages in the mosquito, and to characterise the efficacy of nanovectors and antimalarial agents to reduce mosquito infectiousness. This objective will be achieved through (i) **synthesis** of nanocarriers capable of encapsulating antimalarials (currently used drugs and future antimicrobial peptides, antibodies, and dsRNA) and of preventing their degradation in storage conditions, (ii) **engineering** of targeted nanovectors capable of delivering their antimalarial contents to *Plasmodium* stages in the *Anopheles* mosquito, and (iii) **evaluation** of the effect of selected nanovectors (loaded with antimalarial agents) on the mosquito stages of *Plasmodium* and their transmission capacity in a murine model of malaria. NANOpheles unites groups which are leading laboratories in nanoparticle synthesis, targeted drug delivery to *Plasmodium*-infected cells, molecular and cell biology of malaria, mouse models and mosquito vectors of malaria, and clinical aspects of malaria.



Acknowledgments: (1) ERA-NET Cofund EURONANOMED III. European Innovative Research & Technological Development Projects in Nanomedicine. Project title: **"NANOpheles. Development of nanovectors for the targeted delivery in *Anopheles* mosquitoes of agents blocking transmission of *Plasmodium* parasites."** (EURONANOMED2017-178). (2) *Acciones de Programación Conjunta Internacional*, Ministry of Economy, Industry and Competitivity, Spain. Project title: **"NANOpheles. Development of nanovectors for the targeted delivery in *Anopheles* mosquitoes of agents blocking transmission of *Plasmodium* parasites."** (PCIN-2017-100).

METATHERAPEUTICS: A NEW GENERATION OF TREATMENTS?

MAURO FERRARI, Affiliate Professor of Pharmaceutics, University of Washington, Seattle (USA)

Chemotherapy, biologically target therapy, radiotherapy, nanotherapy, and immunotherapy are honored, and extremely valuable approaches to the treatment of cancer and other diseases, and will continue to be for the foreseeable future, each with their strengths, limitations, and appropriate combinations of patient and indication. They will also be an integral part of the future in a different manner: The expectations and dreams of precision medicine cannot be met by a single disciplinary approach, and will require a true convergence of the the various "-therapies" with novel approached, from the digital world, math, physics, an dother disciplines. MetaTherapeutics are a first emergent new class of innovatively executed precision medicines, expanding on current paradigms, and bringing new hope against current untreatable diseases.

REGULATION OF NANOMATERIALS BY THE THERAPEUTIC GOODS ADMINISTRATION

ANNE FIELD

The Therapeutic Goods Administration (TGA) is part of the Australian Government Department of Health, and is responsible for regulating medicines and medical devices. The TGA administers the Therapeutic Goods Act 1989 and associated Regulations, applying a risk management approach designed to ensure that therapeutic goods supplied in Australia meet acceptable standards of quality, safety and (as appropriate) efficacy. The TGA regulates therapeutic goods through a combination of pre-market assessment and post-market monitoring and enforcement of standards. In addition, the TGA is responsible for the licensing of Australian manufacturers and for verifying overseas manufacturers' compliance with the same standards as their Australian counterparts. The TGA approves and regulates products through established principles of risk/benefit analysis. The TGA's approach to risk management involves identifying, assessing and evaluating the risks posed by therapeutic products, and applying any measures necessary for treating the risks posed.

In 2009 the TGA participated in a whole of government program known as the National Nanotechnology Strategy (NNS; subsequently known as the National Enabling Technologies Strategy or NETS). Under this program, the TGA reviewed the capacity of existing regulatory arrangements to assess and manage issues arising from the use of nanotechnology in the therapeutic arena. This review included the establishment of databases for both existing and in-line therapeutic products. In addition, the TGA reviewed the scientific literature related primarily to therapeutic goods that incorporate nanomaterials to identify any regulatory gaps or concerns, and a regulator's training program in nanotechnology was held.

Examples of therapeutic products incorporating nanotechnologies approved by the TGA and included in the Australian Register of Therapeutic Goods (ARTG include medicines composed of nanosized particles, specialised drug delivery systems (including liposomal and polymeric substances), metals and metal oxides used as diagnostic agents as well as sunscreens and excipients used in therapeutic products.

The TGA has access to excellent nanometrology standards and services provided by the Nanometrology Section of the National Measurement Institute (NMI), which is part of the Australian Government's Department of Industry, Science, Energy and Resources. The NMI develops, maintains and delivers measurement infrastructure, expertise, standards and services, including measurement standards and services for nanotechnology to assist Australian researchers and industries to capitalise on growth and commercialisation opportunities, and contribute to effective health, safety and environmental regulation for nanotechnologies. In addition the NMI actively participates in the development of documentary standards for nanotechnologies at both the national and international level.

The TGA recognizes the need to maintain capacity building and engage internationally to ensure development of appropriate guidelines. Currently, the TGA is co-chair of the International Pharmaceutical Regulators Nanomedicine Working Group. Consultation is underway on a proposal to update the Australian Essential Principles for Medical Devices regulation to align with the EU General Safety and Performance Requirements for Medical Devices Essential Principles and Labelling documents. As part of this consultation, the TGA is examining issues relating to the regulation of medical devices incorporating nanotechnology.

FROM MANUFACTURING TO BED: SPECIAL CONSIDERATION FOR THE HANDLING OF NANOMEDICINES

BEAT FLÜHMANN, Global Lead Nanomedicines, Vifor Pharma Ltd

Colloidal solutions of iron carbohydrates are widely used for the intravenous treatment of iron deficiency and iron deficiency anemia. For intravenous application as infusion the drug is diluted in saline solution. In observational studies an increase in adverse events was observed depending on the dilution volume. This phenomenon can be explained by the physico-chemical properties of nanoparticles which are affected by the nature of the diluent and the concentration. Comparison of different iron sucrose preparations diluted in saline solution for infusion revealed in some preparations a change in particle size over time pointing for some of the preparations to quality and stability problems. This findings are good examples that special precautions are needed in a clinic for the selection, storage and handling of nanomedicines and established practice known from small molecules needs to be adapted.

DESIGNING NOVEL NANOSYSTEMS CONSISTING ON COMBINED GENE AND IMMUNE THERAPIES FOR NON-SMALL CELL LUNG CANCER

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Lung cancer is one of the leading causes of death nowadays, representing a high social and economic cost for most countries. Non-small cell lung cancer (NSCLC) is the most common type of lung cancer, developed not only by genetic predisposition, but also by toxic habits of the population. In addition, the fact that current therapies are not efficient enough to stop tumor growing makes the problem worst. Current standard of care for NSCLC patients consist on the administration of chemotherapeutic drugs. However, these therapies showed a limited efficiency in killing tumor cells, and, at the same time, they produce high toxic side effect due to its systemic distribution (Zappa and Mousa, 2016; Karachaliou *et al.*, 2018).

In the last years, immunotherapeutic alternatives, mainly based on checkpoint inhibitors, have demonstrated a superior performance for some type of cancers, mainly for melanoma, but this is not the case of NSCLC patients, where tumors are usually highly aggressive, not localized and difficult to access by treatments (Moya-Horno *et al.*, 2018; Oliveres *et al.*, 2018). To make the problem worse, the variability between patients represents a bottleneck step, which is believed that may be faced by the use of combined therapies, to attack cancer cells using different approximations, thus empowering therapies to decrease cancer cells resistance.

However, immunotherapies are gaining importance and it is be-

lieved nowadays that combination therapies, that attack tumors from different mechanisms of action, will have the better outcomes in the near future. Consequently, novel combination treatments are required.

Following this trend, we **aim** to develop a combined gene and immunotherapy against NSCLC based on the use of poly(beta aminoester) (PBAE) nanoparticles encapsulating different kind of nucleic acids to suppress tumor cells growing by altering different cellular mechanism.

PBAE are biocompatible and biodegradable polymers that can be synthesized including cationic end-oligopeptides to promote the complexation of anionic nucleic acids by electrostatic interactions thus facilitating the formation of small polymeric nanoparticles (***Figure 1***) (Fornaguera *et al.*, 2018; Fornaguera, Castells-sala, *et al.*, 2019; Fornaguera, Guerra-Rebollo, *et al.*, 2019). Their backbone structure, including aminoester groups, is able to be protonated when the pH decreases, thus enhancing the endosomal scape to achieve the expression of the encapsulated gene material. In addition, their lateral chains can be modified by the addition of targeting moieties, to achieve a selective cell type transfection after their systemic administration. Moreover, the possibility to lyophilize them approaches this strategy to clinical use. There are many studies demonstrating the suitability of these polymers for various gene delivery applications.

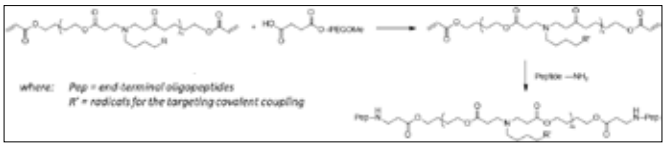


Figure 1: *Synthesis of oligopeptide-modified PBAEs.*

In the current work, we will use them as gene carriers in the two parts of the combined therapy.

On the one hand, we designed an antisense therapy, by the encapsulation of a small interfering RNA (siRNA) that encodes for mTOR, an overexpressed gene in NSCLC cell that avoids cell apoptosis (Gandhi *et al.*, 2018; Mossmann, Park and Hall, 2018)"id":"ITEM-1","issue":"10","issued":{"date-parts":[["2018"]]},"publisher":"Pharmaceutical Research","title":"Bioreducible Poly(Amino Ethers. In this case, we have used a polymer that includes a specific targeting for tumor cells. We have formulated nanoparticles with sizes lower than 200 nm (by DLS and TEM), with a selective cytotoxicity only for tumor cell models (by MTT *in vitro* tests), while being safe (non-toxic) for healthy model cells. This toxic effect only on cancer cells was demonstrated to be caused by knocking-down mTOR gene as a model tumor gene (by western blot and RT-qPCR analysis) (***Figure 2***) (publication pending).

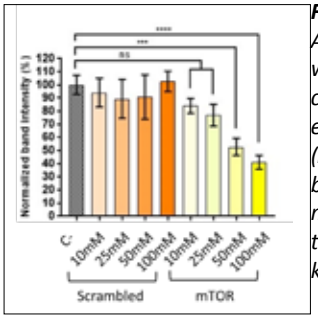
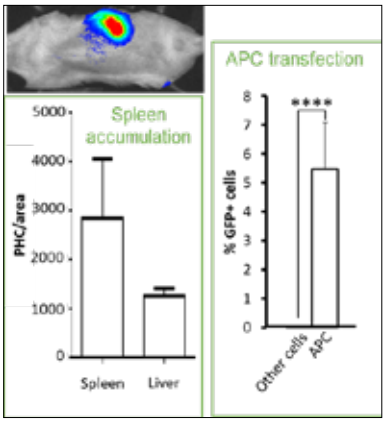


Figure 2: *Expression of mTOR in A549 model lung cancer cells by western blot analysis, after their incubation with PBAE nanoparticles encapsulating either the functional (mTOR) or the non-coding (scrambled) siRNAs. As it can be observed, nanoparticles promote cell transfection and a selective dose-dependent knock down of mTOR gene.*

On the other hand, PBAE nanoparticles have been used as cancer vaccines, with the purpose of re(awakening) the tumor immune response. In this case, nanoparticles were loaded with messenger RNA (mRNAs) encoding for tumor associated antigens. We demonstrated that they were specifically directed to dendritic cells, as professional antigen presenting cells (APCs) (by flow cytometry of disaggregated cells after intravenous administration) and thus appropriate to promote tumor immune killing (***Figure 3***) (Fornaguera *et al.*, 2018).

Figure 3: *Summary of the biodistribution study in healthy mice: Nanoparticles are accumulated in the spleen, as observed by luminescence studies using an mRNA that encodes for Fluc; where they preferentially transfected APCs, as demonstrated by flow cytometry studies using mRNA encoding for GFP and colocalizing this signal with that of cell surface markers.*



Summarizing, we have designed a novel combined therapeutic approach for NSCLC patients based on polymeric nanoparticles encapsulating siRNA targeting oncogenes added to cancer vaccines that showed promising results in first proof-of-concept studies.

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A COMPUTATIONAL PROTOCOL FOR THE IN SILICO MATURATION OF ANTIBODY FRAGMENTS

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[1] BINDesignER can be downloadex here: https://github.com/migsoler/BINDesignER

An ideal binder should be capable of capturing with high affinity, sensitivity, and specificity a target molecule, such as an organic molecule or a biomolecule. Examples include antibodies and nanobodies typically optimised *in vivo*, as well as DNAs and RNAs based aptamers evolved *in vitro*, and peptides optimised *in silico*. This latter opportunity offers invaluable advantages as computational design allows designing binders capable of capturing its target through a chosen binding site.
In particular an algorithm first proposed by Laio and co-workers, was able to optimize simultaneously the sequence and conformation of small peptides in order to reach a high binding affinity to a target organic molecule [1]. The same algorithm was then adapted for the generation of peptides for protein recognition. Computationally-generated peptides have been shown to pinpoint the binding site they have been designed for, being that a pocket or a surface exposed binding site, with uM affinity [2,3]. The same algorithm has now evolved into BINDesignER[1]: a computational protocol for the optimisation of peptides and for the *in silico* maturation of antibody fragments.
Our most recent achievement is the design of binders for biomarker recognition or, more precisely, the *in silico* maturation of small antibody fragments (also known as VHH or nanobodies) capable of recognizing chosen epitopes of Her2 (Figure 1a), a biomarker associated with breast cancer recurrence [4]. The method iteratively attempts point mutations in the VHH hypervariable loops and evaluates the resulting binding affinity towards the target (Figure 1b). The optimization brings to a gradual decrease of all the scoring functions with which the VHH/Her2 binding affinity was evaluated (Figure 1c). The optimised VHH mutant showed significantly enhanced experimental affinity with respect to the original VHH it matured from (Figure 1d).

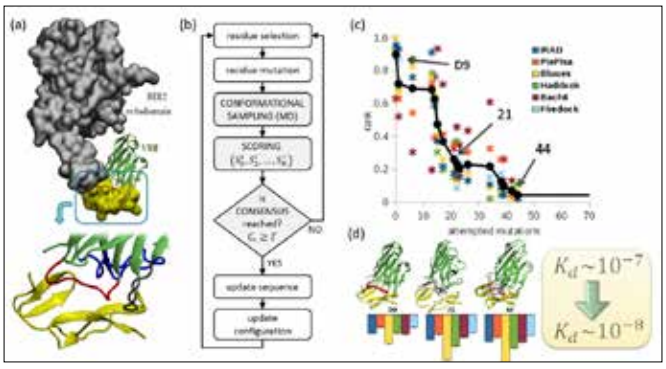

Figure 1 (a) Starting binding conformation between D9 (green) and HER2 ectodomain (yellow/gray), and close-up on the HER2 the domain employed in the design simulations with highlighted CDR1 (red, to be optimized), CDR2 (gray), CDR3 (blue); (b) Algorithm diagram; (c) Global (black circles and line) and scoring-function specific (star) ranking scores of the bindings between VHH mutants and HER2 as obtained along the optimization where three selected mutants are indicated by arrows; (d) simulation snapshots of the three selected mutants and binding scores averaged over the last 100 ns of 250ns long molecular dynamic simulations (scoring functions have been rescaled in order to make the relative variations visible).

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PHARMACOKINETIC AND CLINICAL CORRELATIONS OF PEGYLATED LIPOSOMAL MITOMYCIN-C PRODRUG (PROMITIL) IN COLORECTAL CANCER PATIENTS

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Background: Pegylated liposomal (PL) mitomycin-c lipidic prodrug (MLP) may be a useful agent in patients with metastatic colo- rectal carcinoma (CRC). We examined the pharmacokinetics and clinical observations in a phase 1A/B study with PL-MLP.
Methods: Plasma levels of MLP were examined in 53 CRC patients, who received PL-MLP either as single agent or in combi- nation with capecitabine and/or bevacizumab. MLP was determined by an HPLC-UV assay, and its pharmacokinetics was analyzed by noncompartmental methods. The correlation between clinical and pharmacokinetic parameters was statistically analyzed.
Results: PL-MLP was well tolerated with a good safety profile as previously reported. Stable Disease was reported in 15/36 (42%) of efficacy-evaluable patients. Median survival of stable disease patients (14.4 months) was significantly longer than of progressive disease patients (6.5 months) and non-evaluable patients (2.3 months). MLP pharmacokinetics was stealth- like with long T½ (~1 day), slow clearance, and small volume of distribution (Vd). The addition of capecitabine and/or bevacizumab did not have any apparent effect on the pharmacokinetics of MLP and clinical outcome. High baseline neutrophil count and CEA level were correlated with faster clearance, and larger Vd. Stable disease patients had longer T½ and slower clearance than other patients. T½ and clearance were significantly correlated with survival.
Conclusions: PL-MLP treatment results in a substantial rate of disease stabilization in metastatic CRC, and prolonged survival in patients achieving stable disease. The correlation of neutrophil count and CEA level with pharmacokinetic parameters of MLP is an unexpected finding that needs further investigation. The association of long T½ of MLP with stable disease and longer survival is consistent with an improved probability of disease control resulting from enhanced tumor localization of long-circulating liposomes and underscores the relevance of personalized pharmacokinetic evaluation in the use of nanomedicines.
Ref: Gabizon A, Tahover E, Golan T, Geva T, Perets R, Amitay Y, Shmeeda H, and Ohana P, **Investigational New Drugs**, Epub Jan 18, 2020.

GRANAGARD: A NANOFORMULATION OF POMEGRANATE SEED OIL FOR THE PREVENTION OF NEURODEGENERATIVE DISEASES

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Department of Neurology, Hadassah University Hospital and Granalix Biothechnologies, Jerusalem Israel

Neurodegenerative diseases, such as Alzheimer's (AD), Creutzfeldt-Jacob (CJD), Parkinson and others, are late onset fatal brain disorders that affect large numbers of individuals in our society. Since diagnosis usually is performed after considerable death of brain cells over long periods of time, there is a clear unmet need for the prevention/delay of disease onset in subjects at risk, which constitute most of the population. Prevention of these diseases requires administration of candidate compounds for many years to healthy individuals, implying such reagents must to be safe and compatible with "maintenance" drugs. To this effect, and since oxidative stress, aggregation of individual key disease proteins and mitochondrial dysfunction are the common denominators in the pathogenesis of all neurodegenerative conditions, we developed GranaGard a nanoformulation of pomegranate seed oil (PSO), which main component is Punicic Acid (PA), one of the strongest natural antioxidants. While PA, as well as its main metabolite, Conjugated Linoleic acid (CLA) cannot reach the brain following administration of natural PSO, GranaGard targets CLA to the brain in significant levels. CLA, in addition to its antioxidant nature, is a calpain inhibitor, an innovative target in the search for neurodegenerative disease treatment. Long term administration of GranaGard to transgenic models of CJD and AD demonstrate a strong neuro protective effect on neurological, cognitive and pathological markers of these diseases. This includes reduced neuronal death, delay of disease onset, reduction of key disease protein accumulation and restoration of mitochondrial activity. No side effects in humans or mice have been reported so far. We conclude that long term administration of GranaGard is safe and may be effective for the prevention/delay of neurodegenerative diseases. Clinical trials to this effect are in progress.

REDEFINING CANCER IMMUNOTHERAPIES FOR PATIENTS

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Chief of laboratory of Integrative Cancer Immunology, Cordeliers Research Center, Paris, France.

To-date, the evaluation of the prognosis of cancer patients relied on the anatomic extent of tumor (TNM-classification). However, this classification provides limited prognostic information and does not predict response to therapy. We redefined cancer by integrating the immune system to transfer cutting-edge medicine to the patients. We have previously shown that tumors from human colorectal cancer with a high-density of infiltrating memory and effector-memory T-cells are less likely to disseminate to lympho-vascular and perineural structures and to regional lymph-nodes. We also demonstrated the critical tumor-microenvironment parameters determining the dissemination to distant metastasis. We found that the combination of immune parameters associating the nature, the density, the functional immune orientation and the location of immune cells within the tumor was essential to accurately define the impact of the local host-immune reaction on patients' prognosis. We defined these parameters as the "immune contexture". We characterized the immune landscape within human tumors, and showed the importance of several adaptive immune cells. We described the immunophenotype and antigenome associated with immune escape mechanisms and demonstrated mechanisms associated with pre-existing and proliferating intratumoral T-cells.
Based on the immune contexture, a standardized, simple and powerful digital-pathology-based immune stratification-system, termed "Immunoscore", was delineated having a prognostic power superior to that of the currently used cancer staging-system. Tumor invasion parameters were statistically dependent on the host-immune reaction. A worldwide consortium validated the prognostic value of the consensus Immunoscore, using a standardized-assay.
We have demonstrated the significant role of Immunoscore and immunoediting in affecting metastatic dissemination in space and time. We hence proposed a "parallel immune selection model" of tumor evolution incorporating the effects of the immune system in shaping and driving metastatic spread. Further analyses revealed a large inter- and intra-metastatic immune heterogeneity. Immunoscore and T and B cell score (TB score) from the least-infiltrated metastasis are the most associated with survival. Thus, tumor progression, invasion and recurrence are dependent on pre-existing immunity and on Immunoscore. Finally, novel concepts underpinning tumor evolution at the pre-cancerous stages, and predictive and mechanistic immune signatures of response to cancer therapies will be discussed.

RISK MANAGEMENT FOR NANOMEDICAL PRODUCTS

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In general, regulatory requirements as well as regulatory/scientific guidance on nanotechnologies and nanomaterials applied in medicinal products as well as medical devices are emerging. Common requirement for both of these types of medical products is the need to apply basic principles of risk management throughout the lifecycle of the products. This way, inherent safety and quality by design can be achieved. A new regulatory framework for medical devices was recently published in Europe. The new regulation contains specific requirements on the application of risk management, and also several provisions for nanomaterials. Apart from a definition, specific attention for safety with regard to the use of nanomaterials is required. Moreover, a specific classification rule leads to different routes for conformity assessment depending on the potential internal exposure to nanomaterials. For the implementation of these provisions, work is currently ongoing in European Commission Working Groups to develop guidance. For nanomedicinal products, a specific risk to be managed is related to immunotoxicity responses. Recently, we proposed a strategy for nonclinical regulatory immunotoxicity testing of nanomedicinal products. Standard development organisations like ISO, CEN and ASTM are working on standards that can help implementation of proper risk management and emerging regulatory requirements for nanomedicinal products and medical devices. Furthermore, work done by the International Pharmaceutical Regulators Programme, as well as in European projects such as REFINE (Regulatory Science Framework for Nano(bio)material-based Medicinal Products and Medical Devices) will provide important contributions for this purpose.

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THE NANOPRIMER: A NANOPARTICLE DESIGNED TO TRANSIENTLY OCCUPY THE MONONUCLEAR PHAGOCYTIC SYSTEM IN ORDER TO INCREASE NANOMEDICINE-BASED TREATMENTS EFFICACY

MATTHIEU GERMAIN

Most nanomedicines are limited in their efficacy due to a sub-optimal biodistribution/accumulation in the target tissues. A large part of the administered dose remains useless due to the high rate of clearance by the mononuclear phagocytic system (mainly by Kupffer cells in liver). We designed a new approach based on the use of an engineered, biocompatible nanoparticle—the Nanoprimer—that is administered before the nanomedicine in order to transiently and specifically occupy the liver clearance pathways responsible for sub-optimal therapeutic bioavailability. The Nanoprimer's effect is only based on its specific physico-chemical properties, there is no active principal ingredient encapsulated in it. Here we demonstrate the ability of the Nanoprimer to redefine the bioavailability and efficacy of nanomedicines loaded with small molecules or nucleic acid. We evaluated the biodistribution and the safety of the Nanoprimer alone and of an irinotecan loaded liposome combined with the Nanoprimer.

The Nanoprimer presents a rapid accumulation within liver and spleen and was well tolerated with no sign of toxicity observed using therapeutic dosage. The administration of the Nanoprimer increases systemic bioavailability of different type of nanomedicines and enhances their accumulation in target tissues. This improved bioavailability is correlated with an increased secretion of hEPO by 30%, for mRNA-loaded nanomedicines, an increased silencing of FVII by 50%, for siRNA-loaded nanomedicines, and an increased anti-tumor efficacy by 50%, for irinotecan-loaded nanomedicines.

Here, we showed the safe profile of the Nanoprimer and its ability to increase the treatment outcomes for different nature of nanomedicines. The separation of the functions ensuring the efficacy of a treatment into two distinct objects opens perspectives for designing future nanomedicines and a shift in the therapeutic paradigm.

NANOTECHNOLOGY AT THE US NATIONAL CANCER INSTITUTE

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Nanotechnology has been providing novel, paradigm shifting solutions to medical problems and to cancer, in particular. In order to further these research goals, NCI formed a program called Alliance for Nanotechnology in Cancer which was initiated in 2004. The program funds Centers of Cancer Nanotechnology Excellence (CCNEs) – translational arm of the effort, smaller grants focused on fundamental mechanisms of delivery and device characterization, and cancer nanotechnology training programs. An intramural arm of the Alliance - Nanotechnology Characterization Laboratory provides a characterization support to evaluate clinically promising nanomaterials and establish their physical, pharmacological and toxicological characteristics.

CCNEs, which had a tremendous impact, both scientifically and commercially, on cancer nanotechnology community will be closing in summer 2020 after 15 years of successful operation and $330M investment from NCI. They contributed to a significant increase in overall interest in nanotechnology for cancer and NCI growing its investment into nanotechnology from $100M per year in 2008 to over $200M in 2018. CCNEs produced over 3400 papers, but more importantly, the technologies that the CCNEs were developing resulted in the formation of over 100 start-up companies, which entered over 30 clinical trials (Phase I and Phase II) to-date. These accomplishments went well beyond what NCI funding could support and were possible due to significant leveraging of NCI investment with additional funds from the government, philanthropy, and corporate investment. Nanotechnology has integrated well into NCI funding portfolio and is being practiced through multiple funding opportunities of the institute. In this presentation I will discuss a current status of cancer nanotechnology efforts funded by NCI and also describe future opportunities and strategies in this growing field. Further progress is likely to follow two parallel tracks. First one will be associated with on-going translation to the clinical environment; while the second with the development of new tools and techniques in research arena. It is expected that nanomedicine will be moving beyond the delivery of small molecule drugs and will be exploring new exciting opportunities in immunotherapy, gene therapy, combination therapies, and intra-operative imaging.

VALIDATION AND PRECLINICAL TRANSLATION OF DENDRITIC POLYSULFATES AS INTRINSIC TUMOR TARGETED DRUG COMPLEXES

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Today, the state-of-the-art targeted tumor therapy is frequently based on poorly water-soluble inhibitors of tumor cell signaling and growth. While the tumor signaling inhibitors selectively target crucial and indispensable pathways of tumor cell growth and survival, they widely distribute in the healthy tissue and do not accumulate in tumors and metastasis at high concentrations due to their physicochemical properties. We discovered the strong tumor and inflammation targeting property of the biocompatible polymer dendritic polyglycerolsulfate (dPGS).[1] Based on its strong tumor targeting property, dPGS copolymers were synthesized for targeted drug delivery and biologically evaluated. Dendritic dPGS-based polymer micelle and the dPGS dendritic copolymer are highly potent candidates for targeted delivery of poorly water-soluble drugs.[2,3] For this purpose the hydrophobic anticancer drug doxorubicin was encapsulated within both the cleavable and non-cleavable dPGSPCL copolymer micelle. We showed *in vitro* growth inhibition of human MCF-7 mammary carcinoma cells with both formulations. Further proof of concepts is provided by the treatment of established human mammary MCF-7 xenografts in nude mice. We demonstrated that both cleavable and non-cleavable dPGS copolymer micellar formulations of doxorubicin may increase the survival of tumor bearing mice compared to vehicle-treated controls or doxorubicin. However, stable long-term survival 100% for 80 days of implanted tumors was only achieved by repeated treatment with cleavable doxorubicin-loaded dPGS copolymer micelles (Figure 1).[3]

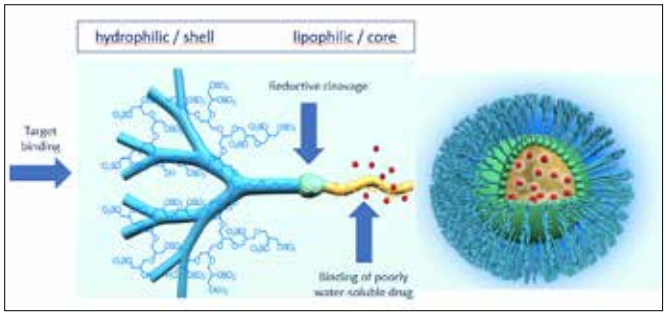

Fig. 1: *Multifunctional dPGS copolymer micelles for encapsulation of hydrophobic drugs.*

These results show that the combination of dendritic polyglycerol sulfate and poly(caprolactone) can lead to promising candidates for drug delivery systems, as they demonstrate high anti-inflammatory potential, biodegradability and superior tumor targetability. These new drug carrier systems can promote a controlled and more efficient treatment with chemotherapeutics, *in vivo* since the drug distribution can be located at its side of action.

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APPROACHES FOR NEXT GENERATION LIPID NANOPARTICLE TECHNOLOGIES FOR RNA DELIVERY

HEINRICH HAAS

Lipid nanoparticles comprising ionizable lipids are versatile delivery systems for therapeutic application of RNA. Although having been already successfully translated into clinical practice, there is need for further development of this technology in order to allow its application in a wider range of therapeutic settings.

Here we present approaches for engineering m-RNA LNPs with improved biological activity and safety. Physicochemical particle characteristics as a function of composition and process conditions were thoroughly investigated and correlated with biological activity. Small angle X-ray scattering (SAXS) measurements were applied to reveal insight into the internal organization of the particles. We used polysarcosine (pSar), a polymer made of repetitive units of the endogenous amino acid sarcosine (N-methylated glycine) instead PEGylated lipids as a tool for particle (size) engineering in order to avoid disadvantages connected with PEGylated particles. In combination with novel ionizable lipids, and depending on the particle characteristics, potent spleen or liver signals were obtained. The systems may be promising for development of next generation potent and safe RNA therapeutics for clinical translation.

TALIDOX, THE WORLD'S SMALLEST LIPOSOMAL DOXORUBICIN: INSIGHTS FROM FIRST APPLICATIONS IN PATIENTS AND IMMUNO-ONCOLOGICAL POTENTIAL

STEFAN HALBHERR, Founder and CEO, InnoMedica

InnoMedica has established an economically efficient method for GMP compliant industry-scale manufacturing of ultra-small liposomes down to about 30nm in diameter. The small particle size, along with other physico-chemical features such as lipid composition and surface design, give rise to previously unseen pharmaceutical effects and favorably affect drug biodistributions. In essence, InnoMedica is exploiting the liposome-derived biodistributions to address a broad spectrum of unmet medical need, predominantly in oncology and neurology.

Talidox (Targeted Liposomal Doxorubicin) is InnoMedica's most advanced liposome product, making use of ultra-small liposomes, drug-loading and release mechanisms, as well as surface design parameters, all acting in synergy. The unique particle architecture of Talidox gives rise to unprecedented serum-half life, a key parameter when trying to hit solid tumors with nanoparticles, as only the availability of the particle in the blood stream for a long period of time will enable specific interaction of the engineered nanosurface with cancer tissue. Particle size in conjunction with serum half-life can positively affect the degree of drug accumulation in tumors, as extravasation from the blood stream towards tumor tissue is more likely to happen with smaller particles but demands the availability of the particle in the first place. When actually reaching tumors, smaller particles do have advantages such as increased cellular uptake and faster drug release, but also a deeper and more thorough perfusion of the tumor due to facilitated diffusion, ultimately leading to stronger antineoplastic effects. Importantly, the architecture of Talidox does not lead to aggravation of any side-effects but instead led to the widening of the therapeutic window in the upper end enabling higher dose intensities. With sight on immuno-suppression that typically coincides with often-observed hemotoxic effects of chemo drugs Talidox proves well tolerated with only very mild forms of neutropenia observed after a number of treatment cycles. Strong induction of immunogenic cell death (ICD) is another key feature of doxorubicin, which is focused towards the inside of the tumor over a longer period of time through the Talidox carrier system. In sum, Talidox has the potential to become a valuable formulation of doxorubicin that can be used to better treat a broad panel of neoplasms either in mono- or combo-therapy settings. A number of common side-effects are heavily reduced, such as myocardial damage, myelosuppression, infections, hair loss, nausea, vomiting, and more synergies with the body-own immune system or also exogenous immunotherapies such as monoclonal antibodies or CAR-T cells can be harnessed. Last, through image guided decision making, quantification of Talidox accumulation in tumors (Tumor Accumulation Score, TAS) can be used to predict therapeutic efficacy and identify high-response patients in a rational and clinically feasible way, taking into account "tumor drug uptake" as a predictive biomarker approach. This will substantiate an important step away from empiricism towards personalized treatments without the need of biopsies, DNA sequencing, or receptor expression quantification, but instead will take intra- and inter-tumor variability of one patient at the very same time into the decision making process. Along with procedures to increase tumor uptake such as targeted radiation or heating, therapeutic efficacy can be further elevated to new heights.

MAKING MEDICINES WITH NEW BEHAVIORS

STEFAN HALBHERR, Country Manager/CEO, InnoMedica

Nature is inarguably full of nanotechnology. One important aspect of the natural design of biological systems seems to be the element of compartmentalization, as evidenced by the important roles that cell membranes, nuclei, mitochondria, virus envelopes, etc. fulfill.

Here we report our advancements in the understanding of how lipid nanoparticle design was inspired by nature and how it can be put to use to profoundly impact tissue distribution patterns. We show how this principle ultimately generates new, biologically compliant medicines with new behaviors that address unmet medical needs.

While nanoparticle size might be the most obvious critical quality attribute (CQA), there seems to be much more to the picture. Particle net charge is important to steer nanoparticle accumulation between the liver, spleen and lungs. On the same turn the phospholipid composition of bilayer plays a crucial role to navigate *in vivo* biodistributions, enabling highly diverse distribution patterns that also include drug delivery to the brain. Furthermore, we found that surface PEGylation can further influence *in vivo* behaviors to some extent but biodistribution-effects from underlying phospholipid compositions remain, although with a delay.

Applications in oncology are most advanced and enable unique induction patterns of immunogenic cell death (HMGB1, Calreticulin, secreted ATP). Treatments with cytotoxics that normally would cause a significant number of adverse events are greatly enhanced in terms of safety and tolerability.

We are further expanding the use of natural compartmentalization to treat currently un- or poorly treatable neurological disorders

such as Parkinson's disease with therapeutic nanolipid rafts and are moving the frontier of RNA-based therapeutic interventions to enable precise immune stimulation.

THE EFFECT OF HEPARIN ON THE CELLULAR UPTAKE OF NANOCARRIERS

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Few studies have considered the interaction of nanocarriers with drugs and the implications for their individual efficiency. Here, we demonstrate that heparin, a common anticoagulant, interacts with nanocarriers.Hence, nanocarriers, pre-coated with heparin and plasma in different conditions (Figure 1, A), were incubated with cancer cells, immune cell lines as well as primary cells from human blood and tested for cellular uptake by flow cytometry.
We found that HeLa cells, monocytes and macrophages reacted differently to the presence of heparin: the uptake of the pre-coated nanocarriers (NC) decreased for HeLa and primary monocytes, while it increased for macrophages **[1]**. Further, the timing of the heparin's addition to the nanocarrier had an impact on uptake. Proteomics revealed that heparin induced no major changes in the protein corona composition. Thus, we suggest that heparin itself, through its adsorption on the nanocarriers, was responsible for the change in cellular uptake.
Heparin turns out to be an uptake-modulator for immune cells, which interacts specifically on cell types. Therefore, on one hand we investigated, if co-administration of heparin can modify the undesired uptake of nanocarriers by macrophages. For this question we examined clinically relevant liposomal formulations. On the other hand, we tried to modulate the uptake of a nanovaccine into dendritic cells by co-treatment with heparin.
In a first *in vitro* setup (Figure 1, A), the tested nanocarrier system was incubated with murine spleenocytes. The culture medium therefore was supplemented with varying concentrations of heparin before the nanocarrier was added. Multicolor flow cytometry was applied to investigate distinct macrophage populations.
In the following *in vivo* setup (Figure 1, B), mice were heparinized intravenously to a final concentration of 4 U/ml blood before nanocarriers were injected. The cellular uptake into macrophages and antigen-presenting cells of spleen and liver was screened by multicolor flow cytometry.

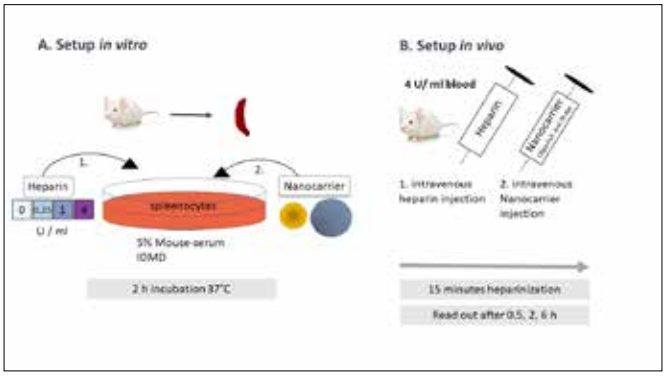

Figure 1. Illustration of the experimental setup to investigate the effect of co-treatment by heparin. *A) in vitro setup: A mixture of spleen cells, together with various concentrations of heparin (0-4 U/ml) in cell-culture medium supplemented with mouse serum was incubated with fluorescently labelled nanocarriers for 2h. B) in vivo setup: Mice were heparinized to a final blood concentration of 4 U/ml heparin for 15 minutes before the fluorescently labelled nanocarriers were injected. For both setups, the cells have been classified into distinct subpopulations by multicolor flow cytometry in order to additionally measure the uptake into macrophage subpopulations by fluorescence.*

In vitro, we showed, that heparin reduces the uptake of protein-capsules significantly stronger into the macrophage populations than into dendritic cells. *In vivo* a co-application of heparin did not cause this target-oriented drive towards dendritic cells for protein capsules. This result of uptake-modulation by heparin *in vitro* may favor the effect of the here applied nanovaccine. The investigated type of protein-capsule harbors an antigen-shell (ovalbumine) and is supposed to evoke a specific immune response by the uptake into antigen-presenting cells.
For the doxil-like liposomes, there was a tendency of uptake-reduction by increasing heparin concentrations observed. *In vivo,* no effect by heparin could be detected.
In short, heparin induced no major changes in the protein corona composition. Thus, we suggest that heparin itself, through its adsorption on the nanocarriers, was responsible for the change in cellular uptake. This study highlights the importance of carefully timing the injection of multiple drugs. To some degree, we show the ability of anticoagulants to modify the efficacy of a nanotherapy.

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RATIONALIZING NANOPARTICLE DESIGN: FROM ARCHITECTURE TO FUNCTION

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The vast complexity of biological systems make the engineering of nanoparticulate delivery vehicles ex-tremely challenging. Successful translation of nanomedical approaches from concept to clinics are critically dependent on the robustness of the therapeutic ap-proach. In my presentation, I will discuss a holistic ap-proach based on the combination of experimental and simulation work aiding the design of next genera-tion nanomaterials. I will present the rationalized design of a nanoparticle-based surgical glue, which ac-tively supports the different phases of wound healing in a temporally orchestrated manner. Additionally, multiscale multimodal imaging of the nanoparticle fate *in vivo* will be presented.

MINI-NANO DELIVERY SYSTEM FOR DRUGS AND CHECK POINT INHIBITORS FOR BLOCKING GROWTH OF HER2+ BREAST CANCER

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Despite the progress in immunotherapy of tumors, such as melanoma, lung, and prostate cancers, surprisingly little is known about the role of the immune system in human HER2+ breast cancer (BC) development. The microenvironmental immune system of BC is deregulated with the capability of cancer cells to evade immune surveillance by inactivating cytotoxic T lymphocytes (CTL) and mac-

rophages (MF). We developed the treatment of primary and brain metastatic HER2+ BC using combined delivery of checkpoint inhibitors (anti-CTLA-4 and/or anti-PD-1 mAbs) to the tumor site (primary and brain metastatic, as brain is an immune privileged site) with parallel inhibition of c-Myc syntheses, the master signaling regulator, cancer stem cell marker and immune modulators of BC cells.
Methods: Mini-nano drugs blocking HER2+ positive breast cancer were peptide-targeted polymer-drug conjugates for successfully arresting growth of breast cancers1, Figure 1.

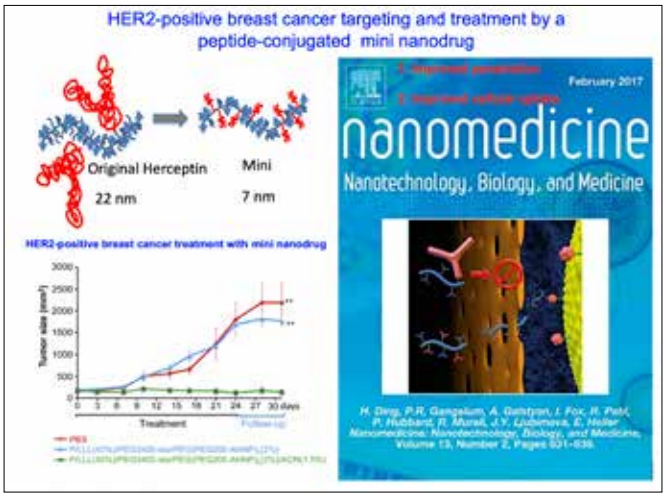

Figure 1. *Growth of HER2-positive BT474 human breast cancer tumors in mice that received IV tail injections of either PBS (red line), the mini nanodrug (green line; 2.4 mg/kg AON, 1.5 mg/kg AHNP) or the "empty" drug carrier (blue line; 0 mg/kg AON, 1.5 mg/kg AHNP). Injections were given twice per week, for a total of 8 injections, and were followed by one week of observation. Statistical significance was calculated with reference to the PBS group (p<0.001 and marked as **). Abbreviations: PBS, phosphate-buffered saline; P, poly(β-L-malic acid); LLL, leucyl-N-leucyl-N-leucine; PEG200 or PEG3400, short- or long-sized polyethylene glycol; starPEG, 8-arm PEG carboxylic acid (-COOH); AHNP, anti-HER2/neu peptide; AON, antisense oligonucleotide against HER2 synthesis.*

New small-size nano scale immunoconjugates *(NIC)* based on natural polymer, poly (β-L-malic acid) (P), were synthesized and physico-chemically characterized. The lead NIC P/PEG/LLL(40%)/AON(c-Myc)(2.0%)/AP-2 (2.0%) with antisense (AON) against c-Myc and HER2, trileucine peptide, endosome escape unit (LLL) [2-5] and AP-2[3] peptides for BBB crossing were characterized for toxicity and biodistribution.
NICs were designed for efficient delivery of immune stimulators and anti-cancer agents through biological barriers to the tumor site/cells (primary BC and brain cancer brain metastases (BCBM), with *novel tumor-targeted and blood brain barrier (BBB)-passing peptides replacing previously used mAbs*. Peptides are conjugated through starPEG to increase the number of peptides per polymalic acid (P) polymer for efficient delivery and/or therapy.For the treatment experiments, mice were used bearing subcutaneous (s.c.) or intracranial inoculated D2F2/E2 HER2+ cells expressing BC.

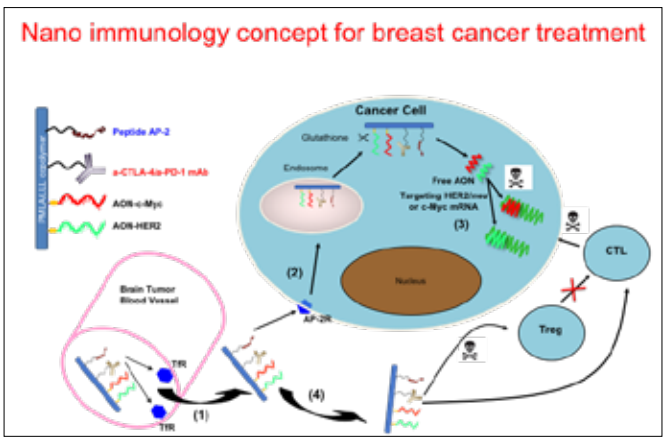

Figure 2. *Structure and mechanisms of action of nano immunoconjugates (NIC) to treat primary and brain metastatic BC. Upper left, the general structure of NIC that can be used alone or in combination with other NICs. <u>The simplest version (route 1,2) is proposed to be used for treatment.</u> It has a PMLA backbone, an endosomal escape unit (LLL), AP-2 or iron mimic peptide for BBB and breast tumor targeting, AON against synthesis of tumor molecular markers, c-Myc and/or HER2 to induce tumor cytotoxicity. In the advanced version (route 1,4), NIC also contains the checkpoint inhibitor mAb αPD-1 or αCTLA-4. Route 1,2: NIC action in primary HER2+ tumors and their brain metastases. <u>The proposed mechanisms of action:</u> After intravenous administration, NIC reaches the tumor or metastasis through AP-2 ligand or TfR and by the same ligands is tumor cell-internalized by LRP-1- or TfR-mediated endocytosis. LLL allows endosomal escape, and AON to c-Myc and/or HER2 is cleaved from the NCI by cytoplasmic glutathione and free to block tumor target mRNA. Route 1,4: The attached mAbs neutralizing checkpoint inhibitors suppress Treg locally at the tumor site and also in peripheral blood stream (dual anti-cancer and immune effect), removing Treg "brake" from CTL and natural killer cells (NK), thus maximizing their tumor killing potential.*

Results: Flow cytometry, immunohistochemistry and western blot analysis methods revealed that BBB-passing NIC are bearing CTL and anti-tumor MF -activating aPD-1 or aCTLA-4 Abs to boost both systemic and also local anti-tumor immune response, increasing the efficacy of immune stimulation in particular against BC brain metastases.
Detailed data are reported for breast and metastatic tumors: A, Serum cytokine expression in mice bearing s.c. D2F2/E2 BC. Compared to free aPD-1 (Group 1), treatment with P/AP-2 /aPD-1 (Group 2), and P/AP-2/aPD-1/AON c-Myc (Group 3) significantly increased serum expression of IL-10 and especially of IL-12 p70. B, similar results were obtained for intracranial tumors. C, Tumor size of s.c. D2F2/E2 breast cancer is significantly reduced after treatment with NIC in Groups 2 and 3 vs. PBS or Group 1. D, Survival in mice bearing intracranial D2F2/E2 breast cancer is significantly increased after treatment with NIC in Groups 2 and 3 vs. PBS or Group 1. Kaplan-Meier curves (log-rank test) are shown. *, p<0.05, **, p<0.01, ***, p<0.001, by one-way ANOVA and Bonferroni's multiple comparisons test. These data suggest that successful delivery of aPD-1or aCTLA-4 to the tumor across BBB elicited significantly stronger immune response compared to free mAb. This was accompanied by an increase in CD8+ and activated CD8+CD69+ CTL.
A novel therapeutic approach was used consisting of premedication after the intravenous I.V. administration of NIC using antihistamine triprolidine and platelet activating factor antagonist CV-6209. This important approach allowed us to greatly reduce toxicity of checkpoint inhibitor mAbs and use I.V. route closely mimicking the clinical approach, as opposed to preclinical use of intraperitoneal injections[3].
Conclusions: Our data show that nano scale immunoconjugates blocking Treg cells and c-Myc can inhibit growth of aggressive HER2+ BC and their brain metastases, directly eliminating cancer cells and eliciting a local and systemic long-term broad-spectrum immune response, to prolong animal survival.
Support: NIH grants U01 CA151815, R01 CA188743, R01 CA206220, R01 CA209921.

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GRAPHENE QUANTUM DOTS FOR PARKINSON'S AND ALZHEIMER'S DISEASES

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Emerging evidence indicates that the pathogenesis of Parkinson's disease (PD) is strongly correlated to the accumulation of alpha-synuclein (α-syn) aggregates. While anti-aggregation therapy for α-syn remains an attractive strategy for novel therapeutic intervention in PD, there has been no clinical success in anti-aggregation agents for the disease to date. Graphene quantum dots (GQDs) are of burgeoning interest in biomedical sciences due to their outstanding chemical and optical properties as well as their biocompatibility. Here we show that GQDs exhibit anti-amyloid activity through binding to α-syn fibrils. Employing biophysical, biochemical, and cell-based assays, we find that GQDs exhibit notable potency in not only inhibiting fibrillization of α-syn but also disaggregating preformed α-syn fibrils in a time-dependent manner. Remarkably, GQDs rescue neuronal death and synaptic loss, reduce Lewy body (LB)/Lewy neurite (LN) formation and prevent neuron-to-neuron transmission of α-syn pathology, as well as ameliorate mitochondrial dysfunctions induced by α-syn preformed fibrils (PFFs) in neurons. In addition, administration of GQDs protects α-syn PFFs-induced loss of dopamine neurons, LB/LN pathology, and behavioural deficits *in vivo*. The finding that GQDs function as an anti-aggregation agent provides a great potential novel therapeutic target for the treatment of PD and related α-synucleinopathies. The similar mode of action can be applied to Alzheimer's diseases (AD) as we recently confirmed *in vivo* that the amyloid-β aggregates are substantially removed by injecting GQDs.

THE IMPACT OF SURFACE CHEMISTRY ON THE PERFORMANCE OF PAPER-BASED IMMUNOASSAYS

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Clinical diagnostic needs today are rapidly changing. Fast global passenger and cargo communication, changing climate patterns and pollution result in an increased probability of epidemics as is apparent with the coronavirus outbreak in China in early 2020. A fast and, importantly, appropriate response is crucial to contain outbreaks and improve patient outcomes. Therefore the development of rapid, affordable and preferably point-of-care diagnostics for a wide array of diseases is paramount. Paper-based immunoassays fulfil much of the above needs and additionally are cheap and easy to produce, and relatively easy to dispose of. Their simplicity allows for a broad range of designs from a straight forward single use device to a more complex LEGO-like customizable test, to an integrated device which can leverage mobile phone cameras and GPS tracking for advanced data logging and mapping thus building live networks and real time epidemic monitoring. There is a large range of labels which may be applied to indicate the presence of a specific target molecule in paper-based biosensors. Nanoparticles decorated with antibodies, commonly referred to as immunoprobes, are attractive because they can be seen by eye without further instrumentation or labelling steps thereby broadening applicability. The result readout of such tests may be as simple as "one line negative, two lines positive" written on the casing itself (Figure 1a).

Though promising there are several challenges which delay widespread application of paper-based assays, such as their low sensitivity and selectivity when compared to laboratory methods (e.g. PCR). Usability of such tests may be further limited by inconclusive or confusing results. For example this may mean the appearance of one line, but in the wrong place, a very broad line or just high background. Often these problems are addressed during test development and optimization, in some cases by the application of phones or other devices to improve signal analysis. While this solution works well, it adds time and cost to test development while the use of external devices may not be feasible in some cases as hardware specifications and availability varies and is outside of the developer's control.

In our work we approach this problem from an immunoprobe design perspective. We monitor how applying a progressively more controlled surface chemistry impacts the performance of model tests then expand our findings to more realistic systems. Surface functionalization used for immunoprobe synthesis were PEG backfill, NHS and hydrazide chemical grafting. We monitor the change of immunoprobe size and distribution with immunoprobe synthesis (Figure 1b), their signal distribution on the test (Figure 1c and d), and binding (K_D^{eff}) and limit of detection (LOD) to gauge test performance (Figure 1d). Our results suggest that the link between surface chemistry and test performance is not straightforward. While better for colloidal stability, chemical grafting on PEGylated surfaces yields worse test performance and suboptimal immunoprobe distribution on the paper assay. On the other hand PEG backfilled particles offer better distribution and performance, however, test results are more variable, its selectivity is lower may be more confusing. This may be attributable to number of accessible grafted antibodies on the particle surface, as opposed to total number of grafted antibodies. While we have only observed these principles for a number of antibody-antigen binding couples, we expect them to be widely applicable. If true there are several implications for test and devise design. PEG backfill immunoprobes may be required if the target molecule is available at low concentrations in the medium, though an interpretational aid, such as a mobile phone app, may be necessary. In cases where the target antigen has a high enough concentration in the test medium, chemical grafting may be more appropriate which would result in a more straightforward interpretation and improved reproducibility.

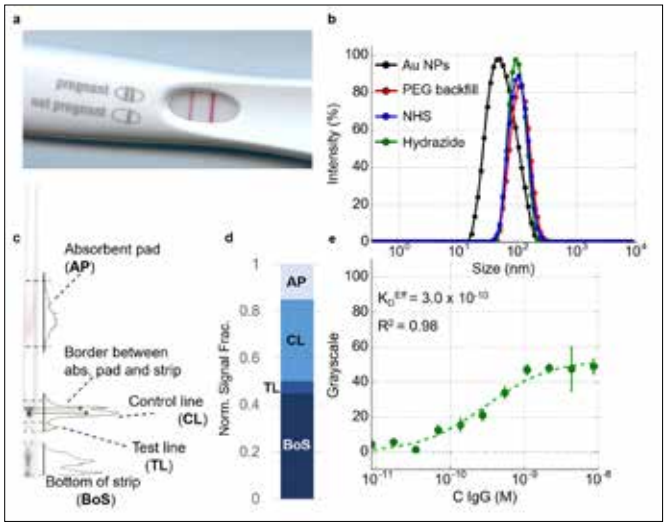

Figure 1. *(a) Example paper-based lateral flow assay available on the market. (b) Change in particle diameter with antibody grafting. Example immunoprobe distribution on a dipstick assay as seen on (c) an image and (d) after analysis. (e) Test performance measured via K_D^{eff} and LOD obtained from an antigen titration.*

CHALLENGES IN CAR-T CELL THERAPY STANDARDIZATION

ALEXANDER HUBER

The therapy Kymriah uses patients T-cells to be equipped with a chimeric receptor that recognizes CD19+ cells. These reprogrammed T-cells recognize B-cell malignancies to treat cancer indications like pediatric and adult ALL, diffuse large B cell lymphoma (DLBCL) or non-Hodgkin's lymphoma (NHL). Kymriah is a personalized therapy: One patient, one batch and this requires the implementation of a very complex supply chain for a large number of materials. Some of the critical materials are tailor-made and single sourced for this therapy such as T-cell stimulating paramagnetic beads coated with anti-CD3/CD28 and lentiviral vectors for T-cell transduction. In addition, the manufacturing process needed to be adapted from an academic setting into a fully approved GMP process that is able to supply the global demand for the indications mentioned. The described challenges require efforts for standardization not only in the manufacturing process but also in the supply chain for CAR-T cell therapies.

NANOMEDICINE AND CONVERGING TECHNOLOGIES: TOWARDS GLOBAL HEALTH

PATRICK HUNZIKER

CLINAM is about advancing health by suited leading edge science, in particular nanomedicine and other "converging" technologies.
The scientific introduction contrasts the large number of annual scientific publications in emerging technologies with the comparatively limited impact of technological innovations seen in practical application in healthcare in developed countries and the huge unmet needs in the significant proportion of people suffering from poverty diseases.
It explores stumbling blocks in realizing real-world benefit from fascinating scientific insights and lays the ground for the interdisciplinary setting that makes CLINAM a unique opportunity to go beyond mere science by developing projects and establishing consortia focussed on translating scientific progress to the benefit of individuals and society.

DEPOLARIZED DYNAMIC LIGHT SCATTERING (DDLS) CHARACTERIZATION OF ANISOTROPIC NANOPARTICLES FOR BIOMEDICAL APPLICATIONS

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The recent emergence and increasing interest in anisotropic nanoparticles opens up various applications in nanomedicine and in the biomedical area. For example, several teams highlighted the interest of shaping magnetic nanoparticles with anisotropic geometries, in order to apply mechanical forces within biological cells [1, 2]. Anisotropic gold nanoparticles also, are showing great potentialities in biomedical applications because of the possibility to tune and customize some of their properties like the localized surface plasmon resonance (LSPR) toward near-infrared region [2] for bio imaging or *in vivo* photothermal applications. Characterizing the size and shape of these nanoparticles is thus crucial to better control their synthesis and their final properties.
Dynamic Light Scattering (DLS) is a well-known and very efficient technique to characterize the size of nanoparticle in suspension. DLS technique is based on the Stokes-Einstein equation which assumes that the analyzed particles are spherical, thus providing an equivalent "hydrodynamic sphere" diameter. For that reason, DLS is usually limited to isotropic objects and cannot describe accurately anisotropic particles. Here we report the implementation of an innovative DDLS setup with multi-angle measurement features and simultaneous measurements on the two-polarization axis (Fig 1a) enabling fast and accurate characterization of anisotropic nanoparticles. Using an appropriate calculation model, we show that this new setup not only detects the presence of anisotropic particles but also get access to their aspect ratio, after careful calibration of the method with model rod-like nanoparticles. As a demonstration of the capabilities of this new DDLS system, examples of achieved measurements on Gold nanorods and magnetic filament are presented.

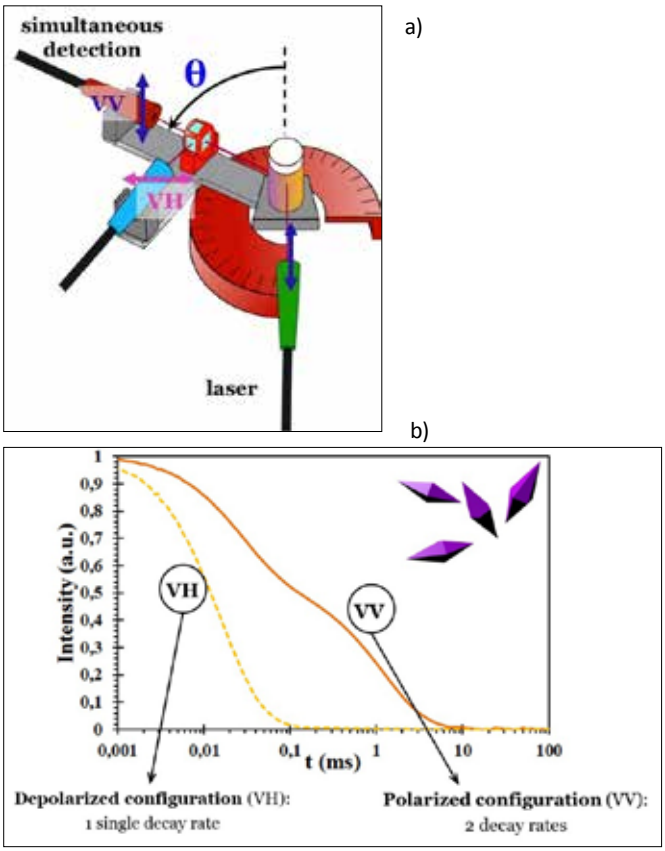

Fig1. *a) Sketch of DDLS setup with varying scattering angle Θ (goniometer) and ''a polarization dichroic prism enabling the simultaneous collection of both parallel (IVV) and perpendicular (IVH) polarizations of the scattered intensity. b) Correlograms of IVV and IVH obtained on Bi-pyramid Gold nanoparticles. The VV correlograms presents two relaxation modes,[7] related to translation (transl) and rotational (rot) diffusions respectively in accordance with the particle elongated shape.*

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THE MECHANOBIOLOGY OF MACROPHAGE INFLAMMATION: A NOVEL WAY TO STUDY, DIAGNOSE, AND TREAT AGE-RELATED INFLAMMATION

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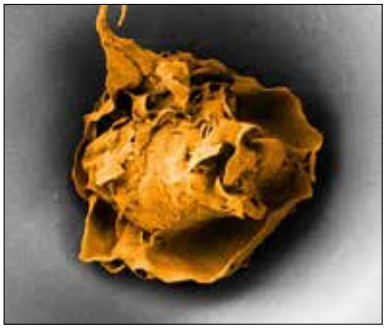

Despite the fundamental physiological role of inflammation as a defence mechanism against infections or tissue injury, when inflammation becomes sustained and prolonged it is detrimental to health and leads to tissue degeneration. This phenomenon when it occurs in older people is called 'inflammaging and is attributed in part to chronic macrophage inflammatory activation. The field of biogerontology has paid little attention to the emergent field of mechanobiology – how changes in the biophysical properties of the microenvironment can affect macrophage phenotype and function during ageing and macrophage induced inflammaging. In our recently published work, we have shown that externally applied physical forces can regulate macrophage activation via changes in gene expression, which are coupled with altered nuclear morphology and temporally regulated by mechanomodulating chromatin compaction (HDAC3 dependent), epigenetic alterations (H3K36me2) and transcription factor complex activity (MRTF-A-SRF). This field is now at the stage where we can apply this new knowledge to understanding the mechanobiology of macrophage activation during ageing and identify novel therapeutic targets to reduce inflammaging. Identifying the mechanobiology based novel mechanisms underlying inflammaging would have enormous socioeconomic benefits because if the current demographic trends continues, the global population aged 60 years or over is projected to reach nearly 2.1 billion by 2050 of which the majority will exhibit symptoms of inflammaging.

Insight into the pathological mechanisms underlying inflammaging lies in the observation that macrophage hyperactivation is associated with altered expression of nuclear envelope (NE) proteins, with potential mechanical effects on the nucleus that then influence its pro-inflammatory state. By combining state-of-the-art genomics and epigenomics techniques complemented with novel bioengineering platforms, we have uncovered the potential role played by the mechanobiology of NE proteins as part of the underlying molecular mechanisms involved during macrophage activation. Using mice knock-out for different NE genes and a combination of RNA-Seq, ChIP-Seq, and ATAC-Seq experiments, we have uncovered: a) the effect of NE protein on pro-inflammatory gene expression programs, b) critical histone modifications involved during macrophage activation and their regulation by NE protein, and c) NE protein dependent changes in chromatin accessibility during macrophage activation, respectively. Further, using high-resolution imaging, novel bio-informatics pipelines, and confirmatory western blotting experiments along with using pharmacological inhibitors, we have also uncovered the class of NE dependent transcription factors, which are necessary to drive pro-inflammatory gene expression, and their potential to be used as therapeutic targets for reducing inflammaging. Finally, we have provided new category of nuclear mechanics based biophysical biomarkers and novel bioengineering based diagnostic platforms, which will provide an accurate, rapid and inexpensive diagnosis of macrophage inflammation during inflammaging and sepsis because the current gold standard methods are time-consuming, costly and require a lot of starting patient material (blood or DNA).

Altogether, our current study will provide new multidisciplinary strategies to conduct advanced research into macrophage activation, human ageing and inflammaging, based on the emerging field of mechanobiology.

ACCELERATING THE DEVELOPMENT OF TRANSFORMATIVE NANOMEDICINES WITH NXGEN MICROFLUIDICS TECHNOLOGY

LLOYD JEEFFS

Nanomedicines are transforming the treatment of serious diseases with unmet medical needs. Oncology, rare diseases and infectious diseases are three areas where advances in target discovery, nanoparticle drug delivery technologies and nanoparticle manufacturing are converging to drive innovation. Precision NanoSystem's NanoAssemblr® platform accelerates nanomedicine development and manufacturing using microfluidics technology.

The preparation of nanoparticles using microfluidic mixing has been widely used as a dependable and predictable research tool for developing a wide range of novel formulations containing a broad spectrum of active pharmaceutical ingredients. To date, much of this work has been performed on previously published microfluidic structures such as flow focusing and staggered herringbone mixers. We have developed a novel, next-generation mixing structure (NxGen) that overcomes the challenges of scaling up complex nanoparticle formulations. Specifically, this NxGen mixer preserves time invariant mixing conditions over a wide range of flow rates (0.2 to 20 L/h), thus enabling the predictable development of many classes of nanoparticles including liposomes, PLGA nanoparticles and nucleic acid lipid nanoparticles. This high capacity NxGen mixer technology has been integrated with a GMP system to manufacture large scale nanoparticle batches to support IND enabling studies and clinical trials.

NANOMEDICINE IN DRUG DEVELOPMENT: AMBISOME AND THE NEXSTAR/GILEAD LIPOSOME PARADIGM

GERARD M JENSEN

AmBisome (liposomal amphotericin B) is among the earliest approved liposomal therapeutics, and has been in commercial use since the early 1990s. The session will show recent trials and elucidate the pharmacokinetics. The speech report results from other liposomal and lipid complex applications from Gilead including an update on work with the anti-viral Remdesivir.

COMPLEX GENERICS CONTAINING NANOMATERIALS

WENLEI JIANG

Per the GDUFA II commitment letter, complex drug products generally include products with 1) complex active pharmaceutical ingredients (APIs); 2) complex formulations; 3) complex routes of delivery; 4) complex dosage forms; 5) complex drug-device combination, or 6) other products where there is complexity or uncertainty concerning the approval pathway or possible alternative approach would benefit from early scientific engagement. In this presentation, Specific criteria on how to determine complex API, complex routes of delivery, complex dosage forms and formulations, complex drug and device combinations to support complex drug product classification will be introduced. Regulatory research, guidance development, and approval of complex products containing nanomaterials will be focused.

DEVELOPMENT OF NANOMEDICINE REFERENCE MATERIALS FOR PROTEIN NANOPARTICLES

MICHAEL J.W. JOHNSTON
Biologic and Radiopharmaceutical Drugs Directorate
Health Products and Food Branch, Health Canada

The development of reference standards for the assessment of nanoparticle facilitates cross-laboratory studies and the effective transfer of particle characterization methodology. Suitable reference standards should be stabile during stable long-term storage. We examine the behaviour of albumin nanoparticles reconstituted from a lyophilized state and demonstrate that albumin nanoparticles from fatted protein (dodecanoic acid) show improved performance when reconstituted from a lyophilized state versus nanoparticles fabricated with defatted protein. We also demonstrate the fatted albumin nanoparticles are stable for at least 6 months when stored at -80ºC and that the reconstitution methodology is readily transferable. Furthering our work, we adapt a microfluidic manufacturing process to facilitate production of larger quantities of nanoparticles.

NANOCRYSTAL – POLYMER PARTICLES FOR A SUSTAINED TREATMENT OF OSTEOARTHRITIS

OLIVIER JORDAN

Drug nanocrystals have attracted much attention due to their unique properties, and some of them made their way to the market. Further combination of this nanotechnology with polymer particles may be used to treat slowly evolving diseases such as osteoarthritis, a highly prevalent disease and unmet need. Examples of particles to treat inflammation or stimulate chondrogenesis on the long term will be detailed. Using nanocrystal-polymer particles, the bioactivity of kartogenin, a stem cell chondrogenic promotor, was maintained over 2 months (reduced VEGF and Adamts5 expression). The cartilage was protected from degradation. The approach provides a proof-of-concept of an extended drug delivery system with the potential to treat human OA.

NEXT-GENERATION GENE EDITING TECHNOLOGY FOR ALLOGENEIC IMMUNE CELL THERAPEUTICS

STEVE KANNER

Gene editing is a promising technology with applications in a variety of fields, including human therapeutics. One of the challenges presented by gene editing is the potential for undesired off-target edits. In order to successfully deploy genome editing for clinical applications, it is critical to design strategies that maximize on-target activity while minimizing off-target editing. Caribou's next-generation gene editing technology offers improved specificity over the first generation through the use of novel guides that contain both DNA and RNA, called CRISPR hybrid RNA-DNA (chRDNA). With this platform, Caribou is advancing a therapeutic product pipeline that includes allogeneic CAR-T cell therapies for the treatment of cancer. The lead products are being developed to treat selected hematological malignancies.

EVALUATION OF BOVINE MILK EXOSOMES AS NANO-MEDICINAL DELIVERY VEHICLE FOR LOCKED NUCLEIC ACID ANTISENSE OLIGONUCLEOTIDES (LNA-ASO)

MICHAEL KELLER

"Parenteral or intrathecal administration of antisense oligonucleotides (ASO) have enabled treatment of liver and CNS based diseases, respectively, thanks to the inherent high exposure of the ASO in these tissues. To extend the possible scope of indications to treat with ASO, we looked at feasibility concepts to deliver ASO LNA into tissues where exposure with unformulated ASO LNA generally is insufficient for PD effect. To this end, we investigated the bovine milk derived extracellular vesicles as a potential drug delivery vehicle for ASO LNA. Improved isolation and purification procedure, biophysical characterization, *in vitro* and finally *in vivo* evaluation are presented."

A CANCER SURVIVOR'S JOURNEY; A PATIENT'S PERSPECTIVE

LORA KELLY, MSN, BSN, BSIE, RN

Lora Kelly is a seven year pancreatic cancer survivor enduring chemotherapy for the third time. Lora is a Master's prepared Licensed Nurse and an Industrial Engineer who continues to work full time as the Director of Clinical Nursing Education (DCNE) at Harrisburg Area Community College (HACC). Lora teaches fundamental and medical surgical nursing skills at a college level as well as Directs the Clinical Education for the school's Nursing Program. Lora works closely with pancreatic cancer patients and their caregivers via a pancreatic cancer support group which she has facilitated for several years. Lora also founded and Chairs the Board for the Central Pennsylvania Chapter of the National Pancreas Foundation (NPF) which focuses on pancreatic cancer education and fundraising for research. The Controlled Release Society (CRS) featured Lora's journey in an article published in a special edition of their journal, May 2019, A Cancer Survivor's Journey.
https://www.sciencedirect.com/science/article/pii/S0168365919300884?via%3Dihub

DEVELOPMENT OF TACLANTIS AND OTHER NOVEL DERIVED FORMULATIONS

AJAY KHOPADE

Poor aqueous solubility is the most trivial looking problem in the pharmaceutical world. The sheer number of technologies reported in literature is mind boggling. The space has become increasingly crowded with hundreds of buzz words. The challenge is to navigate through those complexities and constraints to derive a new idea that translates to a product. Another challenge is also to protect the scope of the idea i.e. creating reasonable space within the narrow space. The presentation will cover a case study of TACLANTIS which has successfully navigated a path from ideation to the market passing through stringent or moderately stringent toxicology and clinical tests. Technology is quite different (IP protected) yet based on common knowledge of physical chemistry driving the self-assembly phenomenon. It solves solubility problem yet is distinct in its application and approach to solve patient compliance problem.

- Self-assembly strategies for insoluble drug delivery
- Fast disintegrating particles in bio-systems
- Uncompromised pharmacokinetics, safety and efficacy
- Data package bridge enabling approval
- IP protection

Take Home Message: Critical role of drug delivery technology in differentiated products

SAFETY ASSESSMENT OF SARAH NANO-TECHNOLOGY IN SWINE MODELS

SARAH KRAUS[1], Raz Khandadash[1], Ekaterina Sigalov[1], Doron Suchi[1], Moshe Eltanani[1], Pazit Rukenstein[1], Ofra Rivlin[1], Cheri Tal[1], Abraham Nyska[2], and Ofer Shalev[1]

[1] New Phase Ltd., Petah Tikva, Israel;

[2] Consultant in Toxicologic Pathology, Timrat, and Sackler School of Medicine, Tel Aviv University, Israel.

:

The Sarah Nanotechnology (New Phase Ltd., Israel) is a medical device that comprises Sarah Nanoparticles (SaNPs) and an Electromagnetic Induction System (EIS). Sarah Nanotechnology strategy is aimed primarily for the treatment of stage IV small cell lung cancer (SCLC).

SaNPs are multicore NPs containing encapsulated iron oxide, administered intravenously (IV) to the patient and become localized via the Enhanced Permeability and Retention (EPR) effect into the tumor vicinity. Following delivery and accumulation in the malignant tissue, the patient undergoes a partial-body electromagnetic field (EMF) application with the EIS at 290 kHz ± 20%. The SaNPs absorb the electromagnetic energy and convert it to thermal energy, reaching a pre-determined temperature (50±3°C), thereby heating the malignant cells and causing hyperthermic cell death at sub-ablative temperatures.

For its therapeutic effect, the SaNPs need to accumulate in the tumor. However, accumulation in healthy organs is an important risk consideration and therefore, potential adverse effects and biodistribution were assessed in swine. Previous studies conducted by New Phase Ltd. have shown that SaNPs are stable, safe, and biocompatible *in vivo* in small animal studies (mice, Guinea pigs, and rabbits).

Pigs are considered to be one of the major animal species used in preclinical toxicology sharing similar anatomic and physiologic characteristics with human, particularly those related to thermophysiology, such as comparable thermal mass, surface area, water loss through skin, metabolic energy per unit surface area, cardiac output, thermo-regulatory mechanisms, and electromagnetic and thermal properties, making them clinically relevant.

Aim: Toxicity and biodistribution studies were performed in order to evaluate the effects of SaNPs with or without EMF application in healthy swine models.

Methods: All protocols were reviewed and approved by an Institutional Animal Care and Use Committee (IACUC) and followed officially approved procedures for the care and use of laboratory animals.

The following studies were conducted:

1. Sub-acute systemic toxicity and biodistribution of increasing SaNP doses of 60%, 80%, and 100% (without EMF) were examined in Sinclair minipigs for a follow up period of 30 days.
2. Sub-acute systemic toxicity and biodistribution were examined in Göttingen minipigs treated with 60% and 100% single doses of SaNPs followed by EMF application with the EIS (30 min. of continuous radiation at 30 mT) (e.g. full treatment) and a follow up period of 30 days.
3. Chronic systemic toxicity and biodistribution of repeated SaNP doses of 100% (without EMF) were evaluated in a Sinclair minipig that received 3 IV infusions at an interval of one month between each dosing session. The animal was followed up for 93 days to assess any potential safety issues.

Blood and tissue samples were collected for biodistribution analysis using particle electron paramagnetic resonance (pEPR) which is based on a low-field and low-frequency electron paramagnetic resonance enabling the quantitation of superparamagnetic iron oxide NPs. The main advantage of the pEPR method relies on its ability to distinguish between endogenous and exogenous iron sources, showing greater sensitivity for exogenous magnetic NPs.

Results: Doses of 60%, 80% and 100% of SaNP alone, and 60% and 100% doses of SaNP with EMF were well tolerated with no adverse reactions in any of the pigs up till 30 days.

All clinical parameters (blood pressure, heart rate, O_2, CO_2, and body temperature) were stable throughout the administration of SaNP doses.

There were no significant changes in the clinical pathology parameters (Hematology & Chemistry) and most changes observed were within normal ranges.

Histopathology analysis demonstrated no treatment-related toxicity in any of the organs examined (liver, lungs, kidney, spleen, brain, heart, lymph nodes) in all animals.

The presence of iron oxide containing NPs was identified by Prussian blue (PB) staining. Minimal multifocal PB stained macrophages were noted in the lungs but were not associated with any inflammation and therefore considered as not adverse. No such PB positive granules were noted in any of the other organs examined.

Analysis of iron content by pEPR showed that SaNPs accumulated primarily in the lungs, liver, and spleen. Excretion of SaNP from pigs that received 60%, 80%, and 100% doses (without EMF) was 83%, 45%, and 39%, respectively, after 30 days. For the pigs that received 60% and 100% doses (with EMF), the excretion of the SaNP was 75% and 59%, respectively, after 30 days. In the animal that received 3 repeated doses, the total SaNP percentage that accumulated in vital organs was 52% which corresponds to 48% SaNP that was cleared from the animal's body after 93 days.

Conclusions: The main findings of the studies show that single doses as well as 3 repeated doses of SaNP without EMF application were well tolerated in swine models *in vivo*.

In the animals that received a full treatment, a dose level of 60% and 100% SaNP followed by EMF application was well tolerated. There were no SaNP-related or EMF-related clinical observations noted during the dosing, exposure, or observation periods.

The absence of inflammation, necrosis and adverse reactions suggest that the SaNPs remain intact in the body and do not degrade or cause toxicity or thermal damage, even after 3 repeated doses of SaNPs.

These safety assessments open new avenues for generating SaNPs and EMF application as a potential novel therapeutic modality for cancer patients.

COMPLEX 3D CELL STRUCTURES ON INORGANIC SURFACES

SILKE KROL, Laboratory for personalized medicine, National Institute of Gastroenterology, "S. de Bellis" Research, Hospital, Castellana Grotte, Bari (I)/Encytos B.V.

Contact: silke.krol@aol.com

We will present a novel platform for 3D cell growth. A patent application is in preparation and therefore details of the study as well as the cell growth platform will be presented in the oral presentation.

NANOMEDICINE AND THERANOSTICS IN THE ERA OF IMMUNOTHERAPY

TWAN LAMMERS

Nanomedicines are 1-100(0) nm-sized carrier materials designed to improve the biodistribution and target site accumulation of systemically administered (chemo-) therapeutic drugs. By delivering drug molecules more efficiently to pathological sites, and by preventing them from accumulating in healthy tissues, nanomedicines are able to improve the balance between efficacy and toxicity. Nanomedicines rely on the Enhanced Permeability and Retention (EPR) effect for efficient target site accumulation, which is notoriously known to be highly variable, both in animal models and in patients. To tackle this high heterogeneity in EPR, and to improve the therapeutic performance and clinical translation of cancer nanomedicines, we are working on "smart" systems and strategies to modulate and monitor tumor-targeted drug delivery. In addition, by inducing immunogenic cell death, modulating the tumor immune microenvironment and targeting antigen-expressing cells in the spleen and

in lymph nodes, nanomedicine can help to improve the efficacy of cancer immunotherapy. In the present lecture, several of the above strategies will be highlighted, including pharmacological and physical modulation of tumor blood vessels and the microenvironment, theranostic concepts for individualized and improved nanomedicine treatment, and induction of immunogenic cell death and immunotherapy responses using novel nanomedicine formulations.

BRAIN THERANOSTICS WITH EXTRACELLUAR VESICLES: GLYMPHATIC/LYMPHATIC DISPOSAL

DONG SOO LEE, Yoori Choi
Seoul National University, Seoul Korea

Brain theranostics combining therapy with imaging diagnostics will herald the realization of therapeutic success in neurodegenerative diseases (Lee, Nucl Med Mol Imaging, 2019). In vivo companion diagnostics of brain theranostics is done using radionuclide-labeled therapeutics. Extracellular vesicles have been proposed as candidates of therapeutics neurodegenerative diseases (Lee, PRNano, 2019), though exact roles are not known of extracellular vesicles in the brain and cerebrospinal fluid.
Many clinical trials failed to treat Alzheimer's disease (AD), except for the recent news of aducanumab trial. Therapy with Aducanumab or the similar should have been based upon the better understanding of the 'waste disposal' of the culprits of AD from brain parenchyme, which included 1) degradation on site, 2) cell-mediated clearance, 3) back-transfer through blood-brain-barrier (BBB), and 4) glymphatic-lymphatics drainage (Xiao, Preprint, 2020 Lee, bioRxiv 2020).
Pathophysiologic roles of amyloid, tau and microglia and other cells are recently figured out, as solute amyloid evoke hyperexcitability of neurons and solute/exosome-contained tau suppress/soothe neurons (Busche, Nat Neurosci, 2019). If this fails, amyloid make plaques and tau is hyperphosphorylated by NLRP3 of microglia (Ising, Nature, 2019) and released to the insterstitum which is taken up by astrocytes and other neurons. Solute amyloid built up in the brain parenchyma after lymphatic ligation of deep cervical lymph nodes and were washed out by repeated focused ultrasound (FUS) to increase in the CSF. Obviously, brain solute amyloid immunohistochemistry normalized with improvement of mouse memory-test results in 5xFAD mouse AD model (Lee, Choi, Park, 2020, bioRxiv).
We propose that extracellular vesicles with its contents of subset of amyloid or filamentous/fibrillar forms of tau are cleared by the repeated FUS and also that whereabouts of extracellular vesicles can be traced using Tc-99m-labeled extracelluar vesicles injected to the intrathecal space of model mice (Sarker, Suh, Choi, Hwang, Lee, work in progress). Extracelluar vesicles participate in the facilitated clearance of toxic solutes from brain interstitium via glymphatic and finally lymphatics to deep cervical lymph nodes, during deep sleep in physiologic states, which is impaired during aging. FUS or other methods of neuromodulation can help dispose wastes from brain in the early neurodegenerative diseases, by stimulating astrocytis AQP4 and/or enhancing immunity (Choi, Bae, Suh, Choi, Lee, work in progress) or simulating non-REM sleep in sleep deprivation (Liu, 2017, Neurosci Meth).

THE DIGITAL TWIN, AN ESSENTIAL TOOL IN PERSONALIZING THERAPY AND PREVENTION

HANS LEHRACH, Director em., Max Planck Institute for Molecular Genetics, Berlin (D)

We are all different. We have different genomes, different environments, different behavior, different disease histories and often similar, but molecularly different diseases. It is therefore not surprising that we often react very differently to therapies, and particularly to drugs, affecting the complex (and also different) molecular networks in each of us. This can delay effective treatment, cause significant and sometimes lethal side effects (every year close to 200,000 Europeans die of 'adverse drug reactions', and many more suffer some form of medical consequences). This contributes significantly to the very high (and still increasing) cost of healthcare in many parts of the world (4.5 billion € per day in Europe).
To identify the optimal drug or preventive measure for every patient, we can use a strategy that has proven highly successful in many other areas: when faced with complex problems, we cannot avoid making mistakes, but we can make them safely, cheaply and quickly on computer models of the situation, rather than in reality. We therefore design new cars or planes first as computer models and test them in virtual rather than real crash tests. We train pilots on flight simulators rather than on real planes full of passengers, and we predict the weather using computer models rather than getting surprised by life threatening storms. To be able to choose the right drug for every patient, we will similarly need 'digital twins', which can be 'treated' systematically with all available therapies or therapy combinations, allowing us to select the one that has performed best on the digital twin, which, in contrast to the real patient, is never harmed by any drug.
To be able to construct such digital twins of each patient, we have to understand and model the relevant molecular (and other) processes that determine a patient's response to different therapies, we have to carry out a detailed (mostly molecular) analysis of the patient to be able to individualise and initialise these models, and we have to determine the parameters to solve the large systems of differential equations which are used to predict the response of these models (and therefore ultimately that of the patient to the therapy), still a major bottleneck in completing this process.
Digital twins will become an essential part of our health care system, reducing otherwise unavoidable mistakes and unnecessary costs, accompanying us through our lives to help us to deal intelligently with our own health and well-being.

ARE WE BUILDING ON THE SHOULDERS OF GIANTS OR ON A NANOBUBBLE?

RAPHAËL LÉVY, University of Liverpool, Liverpool, UK; rapha@liverpool.ac.uk; @raphavisses

The excitement for nanoscience and nanomedicine started in the late 1990s and early 2000s with a combination of scientific (e.g. new syntheses of well-defined nanomaterials) and political developments (e.g. the launch of large scale funding initiatives in the USA and Europe). Twenty years later, the field continues to mature and develop but concerns have also emerged about reproducibility and the poor rate of translation into clinical applications. In this context, it is highly instructive to look back at the ideas and early work that shaped the current research landscape. In an attempt to answer the question asked in the title, I will review the history of the concept of nanoparticles crossing biological barriers, and I will present a post-publication peer review experiment where 20 highly cited articles (published before 2008, over 40,000 citations in total) which relate directly or indirectly to the interactions of nanoparticles and cells were critically reviewed using Twitter and PubPeer to share findings.

ORGANELLE-SPECIFIC TARGETING OF POLYMERSOMES INTO THE CELL NUCLEUS

LIM, RODERICK[1], Christina Zelmer[1,2], Ludovit P. Zweifel[1], Larisa E. Kapinos[1], Ioana Craciun[2], Zekiye P. Güven[3], Cornelia G. Palivan[2]
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[2] Department of Chemistry, University of Basel, Switzerland

[3] Supramolecular NanoMaterials and Interfaces Laboratory, Ecole Polytechnique Fédérale de Lausanne, Switzerland

Organelle-specific nanocarriers are being sought after for delivering therapeutic agents into the cell nucleus. This necessitates nucleocytoplasmic transport (NCT) but little is known as to how large objects infiltrate the intracellular barrier posed by nuclear pore complexes (NPCs). Here, we have constructed ~60 nm-diameter, nuclear localization signal (NLS)-conjugated polymersome nanocarriers (NLS-NCs) and studied the NCT mechanism underlying their selective nuclear uptake. Detailed chemical, biophysical, and cellular analyses show that nuclear transport receptors are required to authenticate, bind, and escort NLS-NCs through NPCs while Ran guanosine triphosphate (RanGTP) promotes their release from NPCs into the nuclear interior. Ultrastructural analysis by transmission electron microscopy further resolves NLS-NCs on transit in NPCs and inside the nucleus but not the majority of control blank NCs. Our findings demonstrate the efficacy for polymersomes to deliver encapsulated payloads directly into cell nuclei.

REFERENCE:

Zelmer et al. (2020) Organelle-specific targeting of polymersomes into the cell nucleus. Proc. Natl. Acad. Sci. USA, 117(6) 2770-2778.

CONCENTRATION-DEPENDENT VERSUS CONCENTRATION INDEPENDENT SAFETY AND BIOCOMPATIBILITY: CONSIDERATIONS FOR NANOMEDICINES

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The evaluation of the toxicity, and compatibility, is an important step in the development of new therapeutics and is part of regulatory compliance. The field of nanomedicine is no different however, there is the possibility that, given their unique properties, additional factors should be taken into consideration when the compatibility of nanoparticles, and nano formulations, is being assessed. Concentration dependant toxicity is related to the amount of drug or nanoparticle that accumulates within a cell or tissue. However, the mechanisms of intracellular accumulation differ between drug molecules and nanoparticles, mainly due to differences in size. Nanoparticle accumulate within cells via endocytosis and phagocytosis whereas drug molecule accumulation is, primarily, via uptake transporters. This differential route of uptake may impact on the subcellular distribution of the materials and thereby affect concentration dependant effects. In this presentation, we will discuss our work on nanoparticle compatibility and how we have observed concentration independent effects on the biocompatibility of antiretroviral formulations and representative nanoparticle systems. We suggest that these issues should be taken into account when determining exposure-response relationships for nanomedicines and other delivery systems.

POLYMERIC NANOCONJUGATES FOR MRI BRAIN TUMOR DIFFERENTIAL IMAGING AND TREATMENT

VLADIMIR A. LJUBIMOV, Eggehard Holler, Liron Israel, Zachary Grodzinski, Julia Y. Ljubimova, Rameshwar Patil, Keith L. Black

Nanomedicine Research Center, Departments of Neurosurgery, and Biomedical Sciences, Cedars-Sinai Medical Center, Los Angeles, California, USA

Figure 1

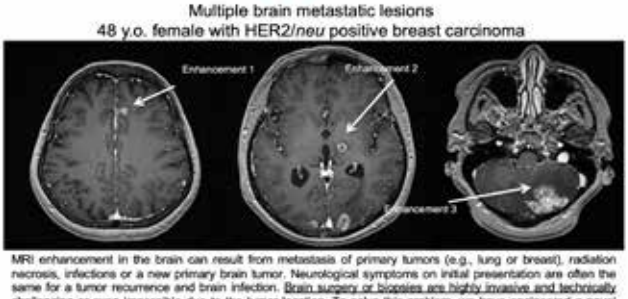


Figure 2

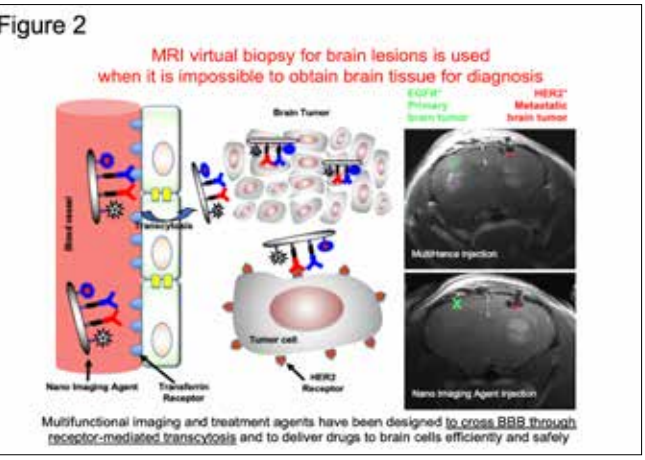
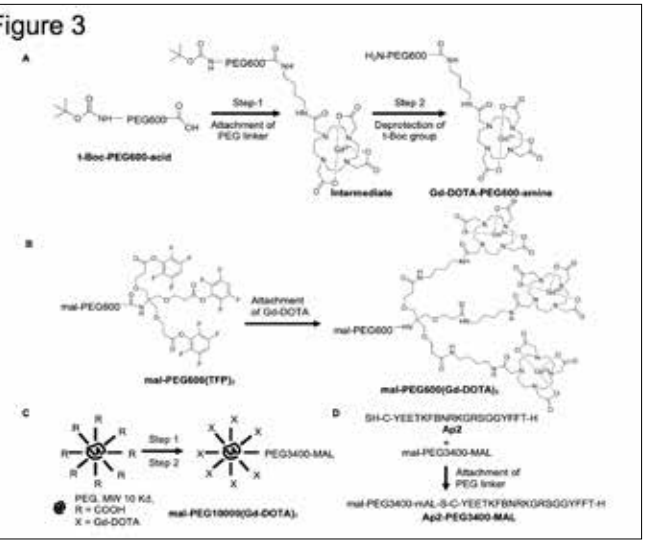


Figure 3

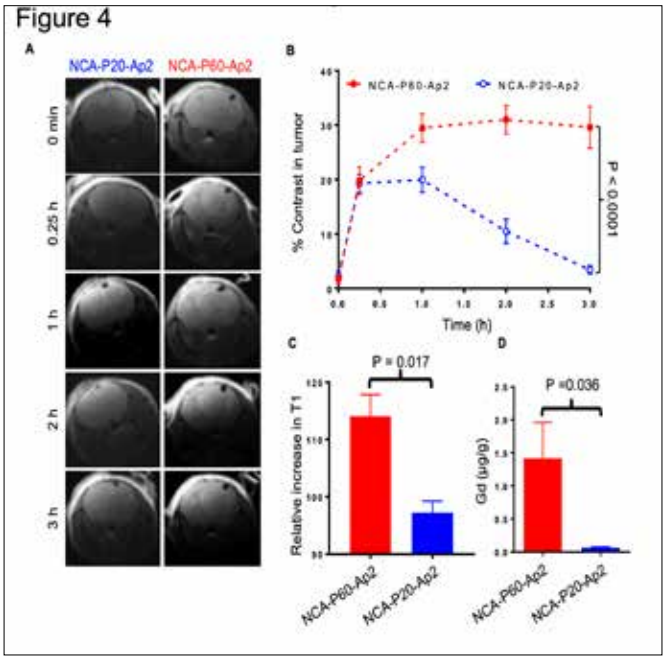


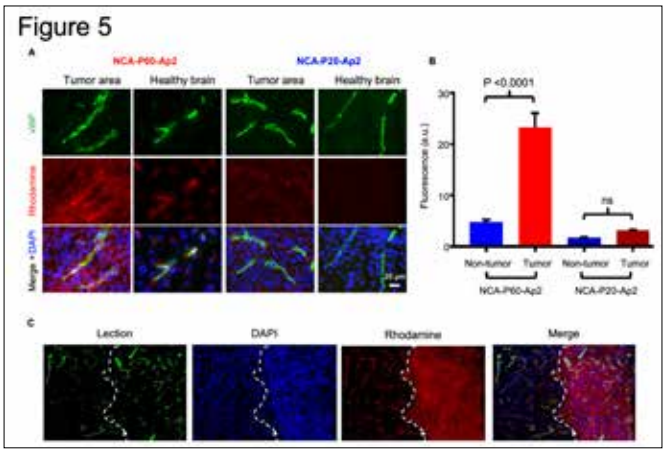
A natural nanobiopolymer, polymalic acid (PMLA), was used here as a nanoplatform for the family of PolycefinTM drugs for imaging and treatment of primary and metastatic brain tumors[1]. Pathological MRI enhancement in the brain may result from primary or recurrent brain tumor, radiation necrosis, metastasis from primary cancer (e.g., lung, breast), stroke, inflammation or infection (Fig. 1). Biopsies of the brain have risk for serious complications including stroke and death. The purpose of the study was to establish the validity of a polymalic acid (PMLA)-based natural nanobiopolymer for the differential tumor-type imaging and for treatment. Our new model of double human brain tumors in mice mimicking brain metastasis (BM) allowed us to develop noninvasive differential tumor diagnostics based on MRI (Fig. 2) and a subsequent efficient BM treatment with nanodrugs.

By virtue of anti-transferrin receptor (TfR) mAb attached to nano contrast agent (NCA) and ensuring transcytosis through blood-brain barrier (BBB) and mAbs targeting tumor cell markers for specific cancer cell uptake (anti-HER2 or anti-EGFR), the NCAs with

Gd-DOTA tracer accumulated in BMs and distinguished between different tumor types on MRI[2].

It should be noted that we used the "classical" approach for BBB drug delivery with anti-TfR antibody. We further optimized this approach for peptide-based BBB delivery[3,4]. In general, our technology is based on biodegradable and nontoxic PMLA nano platform that can be applied to any molecules crossing BBB in order to deliver specific agents able to recognize various brain pathological conditions by "MRI virtual biopsy".





Methods: Antibody and peptide targeted NCAs were synthesized using polymalic acid platforms of different sizes. NCAs contained covalently attached moieties for tumor-specific targeting (antibodies or peptides for BBB delivery by transcytosis, antibodies to differentiate EGFR or HER2/neu positive brain tumors), tumor cell elimination (antisense oligos to EGFR or HER2), a unit for endosome disruption and drug release into the tumor cell cytoplasm (trileucine), and imaging (MRI tracer Gadolinium, Gd-DOTA).

Mice had human EGFR+ (MDA-MB-468 breast or A549 lung cancer, or human U87 primary glioma cells) and HER2+ (MDA-MB-474 breast cancer) tumor cells stereotactically implanted. NCA was injected into the tail vein for imaging.

Gd-DOTA intermediates with varied chain length and branches (4- and 8-arm PEG) and AP-2 peptide (mini-NCAs) were prepared (Figs. 3B and C) and evaluated. Signals in healthy brain and tumor were quantified over time using Bruker BioSpec 94/20USR 9.4-Tesla MRI system. Delivery of contrast agents across BBB was studied by fluorescent microscopy.

Results: Imaging. After reaching a maximum in the first hour post injection, high signal values prevailed for 3 hours for Gd-DOTA-NCA, but declined rapidly for clinical Gd. By differential MRI with anti-HER2 (Trastuzumab) or anti-EFGR (Cetuximab) antibody attached to NCA, it was possible to reliably differentiate HER2+ from EGFR+ metastatic brain tumors by non-invasive imaging (Fig. 2). Treatment. Single polymer-attached drugs were successfully used to treat brain metastases that are not curable by the drugs commonly used to treat corresponding primary cancers. Animal survival after PolycefinTM treatment was significantly higher than in control animals: 65% increase for lung cancer, 47% for HER2-positive breast cancer, and 97% for triple-negative breast cancer.

In our work described above we have shown that certain antibodies were required to target PMLA conjugates across BBB and deliver antisense and contrast agents to glioma cells. Recently, we studied the efficiency of certain peptides,2 which allowed mini-NCA to cross BBB. The Ap-2 peptide (TFFYGGSRGKRNNFKTEEY) that demonstrated strong BBB crossing ability was selected for further study (Figs. 3 and 4).

When NCAs were fluorescence-labeled with rhodamine for microscopic analysis, high fluorescence intensity outside the blood vessels (green) was observed for NCA-P60-Ap2 (Fig. 5).

In healthy brain most of the intensity was visually noticed for mini-NCA (NCA-P60-Ap2) only in vessels, but upon microscopic optical quantification, fluorescence in the tumor outside vessels was also detected. However, fluorescence intensity was significantly higher for NCA-P60-Ap2 than for NCA-P20-Ap2 ($P<0.0001$, Fig. 5). This suggested that Ap-2 targeting provided high BBB penetration activity and is useful for glioma targeting with MRI contrast agents.

Conclusions: 1. The MRI "virtual biopsy" approach may assist in choosing the right treatment regimen for primary and metastatic brain cancers. The method of targeted dynamic MRI contrast enhancement was applicable to single and multiple brain tumor lesions differentially highlighted based on specific cell surface markers, e.g., HER2 or EGFR. A single MRI scan appears to be sufficient for diagnosis, optimally around 2-3 hrs after I.V. injection of a NCA. Therefore, a patient could leave MRI facility with a noninvasively typed BM within 3 hrs. If one NCA cannot give a clear diagnosis, a NCA with another targeting specificity could be administered in 12-24 hrs. Importantly, these NCAs can be easily modified with other antibodies to target tumors with different markers.

2. We later optimized the dynamic contrast-enhanced MRI agents for brain tumor targeted imaging using PMLA-based NCAs. We also reasoned that the use of specific antibodies afforded circumstantial chemical synthesis, costs, instabilities, storage and volume preparation for clinical imaging. We resolved this issue by replacing antibodies using affine peptides, which recognized tumor markers and were successfully tested in novel polymer conjugates for imaging and drug delivery.

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NANO IMMUNOTHERAPEUTICS FOR DELIVERY OF CHECKPOINT INHIBITORS TO ACTIVATE GLIOBLASTOMA LOCAL IMMUNE SYSTEM

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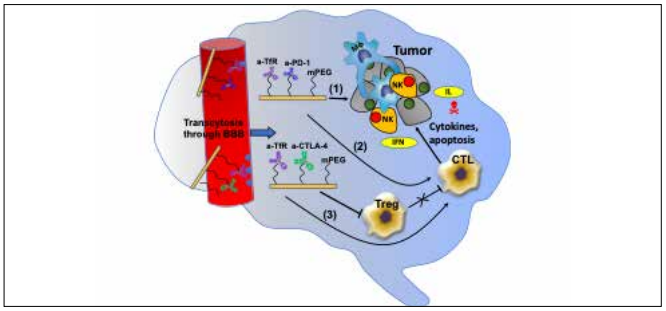

Fig. 1. *Proposed mechanism of glioma treatment with delivery of checkpoint inhibitors anti-CTLA-4 and anti-PD-1 mAbs crossing BBB as part of NICs: (1) anti-PD-1 initiates central pathway inhibition with cancer cell attack; (2) anti-PD-1 provides local Treg inhibition, (3) anti-CTLA-4 provides local inhibition of Treg. IL, interleukin. mPEG, polyethylene glycol. From Galstyan et al, Nat Commun. 2019.*

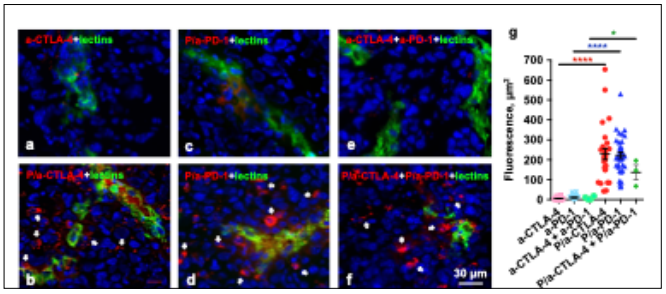

Fig. 2. *Drug distribution on brain tumor sections. Blood vessels were stained with lectins (green). Free rhodamine-labeled mAbs (a-CTLA-4 or a-PD-1 or their combination) are virtually absent outside of the blood vessels (a, c, e). NICs with a-CTLA-4 and/or a-PD1 or their combination (red, arrows) are distributed mostly outside the blood vessels in the tumor parenchyma, significantly more than free mAbs (b, d, f). g, three to seven images of different brain sections for each animal were quantified. Red fluorescence outside the vessels was expressed as positive area in μm². * = p<0.05; **** = p<0.0001 (ANOVA + Sidak's posttest). From Galstyan et al, Nat Commun. 2019.*

Introduction. Glioblastoma has a very poor prognosis that has not improved in the past 30 years. Tumor microenvironment is important for malignant growth, invasion, and escape from the immune surveillance. It also serves as a niche for cancer stem cells that are responsible for tumor resistance to therapy and development of recurrence. Tumor microenvironment comprises acellular and cellular elements.

Immune cells (cytotoxic and regulatory T lymphocytes, natural killers, macrophages) are an important part of the cellular component of the tumor microenvironment and influence the course of cancer growth and spread. Blockade of cytotoxic T-lymphocyte-associated antigen 4 (CTLA-4) using the antagonistic monoclonal antibody (mAb) ipilimumab was the first strategy to achieve a significant clinical benefit for melanoma patients. Systemic administration of "checkpoint inhibitors", anti-CTLA-4 and/or anti-PD-1, and anti-programmed cell death ligand 1 (PD-L1) mAbs can suppress some tumors, but has low efficacy against brain tumors due to antibody inability to cross BBB after IV injection. We designed nanoimmunoconjugates (NICs) based on poly(β-L-malic acid) platform that delivered checkpoint inhibitor mAbs by BBB transcytosis to the syngeneic intracranial glioma in order to boost local brain immune system for glioblastoma therapy (Fig. 1).

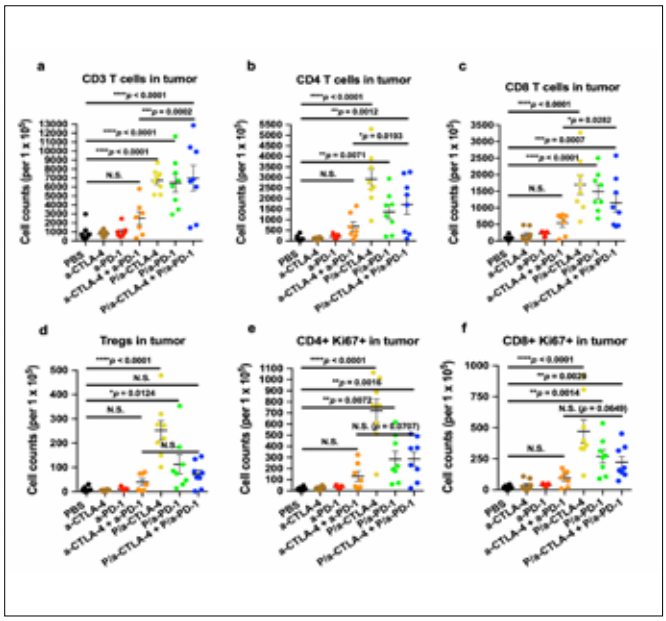

Fig. 3. *Increase of tumor infiltrating T cells revealed by flow cytometry. GL261 brain tumors were analyzed by the spectral flow cytometry (SONY Biotechnology) and tumor-associated T cells are shown as cell counts in each treatment group. a, CD3+ T cells; b, CD3+CD4+ T helpers; c, CD3+CD8+ T effectors; d, CD3+CD4+Foxp3+ Tregs; e, Ki67+ proliferating CD4+ cells; f, Ki67+ proliferating CD8+ cells. About 100,000 events/sample were recorded and analyzed by the SA3800 software. Data are mean ±SEM. * = p<0.05;. ** = p<0.01; *** p<0.001; **** = p<0.0001; N.S. = non-significant (one-way ANOVA with Sidak's posttest). N=6 per group. From Galstyan et al, Nat Commun. 2019.*

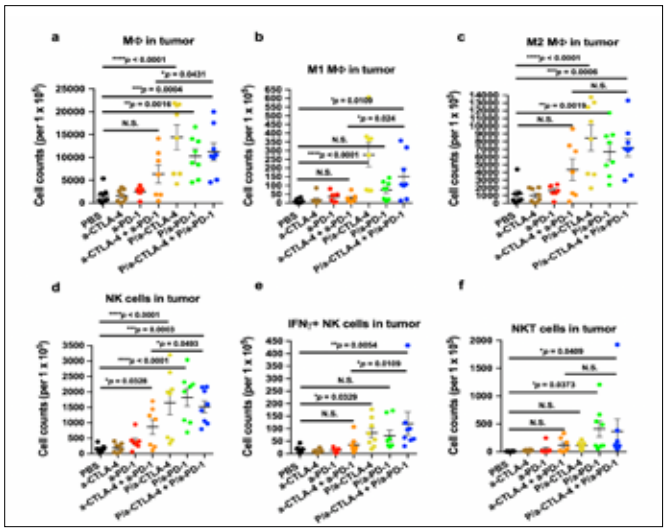

Fig. 4. *Increase of tumor macrophages, NK and NKT cells by flow cytometry. GL261 brain tumors were analyzed by the spectral flow cytometry and various types of tumor-associated immune cells are shown as cell counts in each treatment group. a, Macrophages (MΦ): CD3-F4/80+; b, M1 type MΦ: CD3-F4/80+iNOS+CD206-; c, M2 type MΦ: CD3-F4/80+CD206+iNOS-; d, Natural killer (NK) cells: CD3-NK1.1+; e, Interferonγ+ NK cells; f, NKT cells: CD3+NK1.1+. Approximately 100,000 events/sample are recorded and analyzed by the SA3800 software (SONY Biotechnology). Data are mean ±SEM. *,= p<0.05;. ** = p<0.01; *** p<0.001; **** = p<0.0001; N.S., non-signif-*

icant. N=7 per group. From Galstyan et al, Nat Commun. 2019.

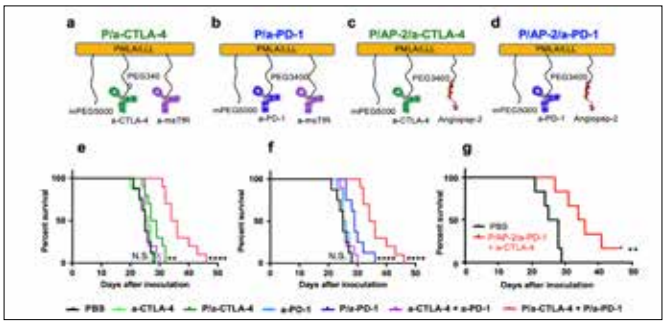

Fig. 5. *Increased GL-261-bearing mouse survival after treatment with NICs. a-d, Structures of NICs containing PMLA/LLL backbone, 2% mPEG5000, a-msTfR (or AP-2) and a-CTLA-4 or a-PD-1. Kaplan-Meier plot of animal survival after treatment with e, PBS, a-CTLA-4, P/a-CTLA-4, a-CTLA-4 + a-PD-1, and P/a-CTLA-4 + P/a-PD-1. Treatment with NICs significantly increased survival compared with free mAbs, their combination and PBS. f, treatment with PBS, a-PD-1, a-CTLA-4 + a-PD-1, P/a-PD-1, and P/a-CTLA-4 + P/a-PD-1. Both NIC treatments significantly improved survival compared to free mAbs, their combination and PBS. g, treatment with PBS, and P/a-CTLA-4+P/a-PD-1. This experiment was performed with AP-2 peptide to cross BBB as an alternative to a-TfR mAb. NIC treatments significantly improved survival compared to free mAbs and PBS. Data are mean ± SEM, N=6 per group. P-values were obtained using a log-rank test, **, p < 0.01; ****, p < 0.0001. PMLA, polymalic acid. From Galstyan et al, Nat Commun. 2019.*

Materials and Methods: Synthesis of nano immunotherapeutics. Nano immunoconjugates (NIC) crossing BBB were synthesized on poly(β-L-malic acid) (P) platform: P/mPEG5000(2%)/LLL(40%)/ms a-TfR(0.2%)/a-CTLA-4(0.2%) (P/a-CTLA-4) and P/mPEG5000(2%)/ LLL(40%)/a-msTfR(0.2%)/a-PD-1(0.2%) (P/a-PD-1) and with peptide Ap-2 for BBB delivery P/PEG/ mPEG5000(2%)/LLL(40%)/AP-2 (2.0%) a-CTLA-4 and P/PEG5000(2%)/LLL(40%)AP-2 (2.0%)/a-PD-1. Physico-chemical, pharmaceutical, and toxicological parameters of NICs were determined using pull-down ELISA, FTIR analysis and *in vitro* and *in vivo* PK and toxicity tests. Brain tumor treatment. Syngeneic GL261 murine glioma cells were intracranially inoculated into C57/ BL mice. Treatment groups were injected IV with either PBS, a-PD-1 and a-CTLA-4 as a control, or polymer-conjugated a-PD-1(P/a-PD-1), a-CTLA-4 (P/a-CTLA-4) or a combination of polymers with antibodies/peptides, (P/a-CTLA-4 + P/a-PD-1).

Flow cytometry analysis. GL261 mouse brain tumors were processed to obtain single cell suspensions and stained for CD3, CD4, CD8, CD69, IFNγ, FOXP3 (Treg), CD68 & CD80 (macrophages), CD25 and NKp46 (NK cells), and Tmem119 (microglia). Fluorescence-activated cell sorting (FACS) analysis was performed on SONY SA3800 analyzer (SONY Biotechnology). Various types of tumor-associated immune cells were presented as the cell counts in each treatment group. Approximately 100,000 events/sample were recorded and analyzed by the SA3800 software (SONY Biotechnology). One-way ANOVA and pairwise two-tailed t-test were performed to determine statistical significance between treatment groups. Data were quantified using flow cytometry software and FlowJo software (Tree Star). Cytokines were measured in serum using cytokine multiplex assay (Bio-Rad) on a Bio-Rad Bio-Plex 200 instrument with Bio-Plex Manager Software: IL-1β, IL-2, IL-4, IL-5, IL-6, IL-10, IL-12(p70), IFNγ, and TNFα levels. Statistical analysis was performed using one-way ANOVA and Tukey's posttest.

Immunohistochemistry. Cryostat brain tissue sections were blocked in 5% BSA and 0.1% Triton X-100 in PBS, fixed with 1% paraformaldehyde, immunostained and mounted with ProLongGold Antifade (Thermo Fisher) medium containing 4',6-diamidino-2-phenylindole (DAPI) to counterstain cell nuclei. Images were captured using a Leica DM6000 B microscope (Leica). Blood vessels were detected using DyLight 488-labeled tomato lectin (0.6 μg/μL) and FITC-labeled RCA120 lectin at (2 μg/μL), injected as a 120 μL bolus, 15 minutes prior to euthanasia. Optical imaging data were analyzed using ImageJ Fiji software.

Results: Activation of glioblastoma local immune environment. A versatile drug carrier based on polymalic acid was used to deliver covalently conjugated anti-CTLA-4 and anti-PD-1 mAbs to brain tumor cells using IV injections, which was never attempted before due to mAb toxicity after multiple IV injections in mice. Since the animals were for the first time preconditioned by treatment with anti-histamine Triprolidine and platelet activating factor antagonist CV6209, this toxicity leading to anaphylaxis was avoided. BBB crossing and tumor accumulation of NICs (in contrast to free checkpoint inhibitor mAbs) was documented (Fig. 2).

NICs stimulate T cell and macrophage response in tumors. NIC treatment of mice bearing intracranial GL261 GBM resulted in local brain immune system activation. Flow cytometry analysis showed a significant increase of the CD3+, CD4+ and CD8+ T cell populations in the tumor tissue after treatment with NICs, especially in animals treated with P/a-CTLA4, P/a-PD-1, and their combination (co-injection), compared to PBS and free mAbs or their combination (Fig. 3). IFNλ+ NK and NKT cells and anti-tumor M1 macrophages were also significantly increased in treated tumors. The most pronounced effect was observed when two NICs with checkpoint inhibitors were combined. Similar results were obtained using immuinohistochemistry. Fig. 4 shows a significant increase of anti-tumor M1 macrophages in NIC-treated tumors as compared with a combination of free checkpoint inhibitor mAbs.

NIC treatment improves animal survival. We next tested whether NICs with conjugated BBB-crossing antibody to
transferrin receptor (TfR) could increase glioblastoma-bearing animal survival after 5 IV injections. As shown in Fig. 5, NICs P/CTLA-4 and P/PD-1 significantly improved survival of primary brain tumor-bearing mice compared to free anti-CTLA-4 and anti-PD-1 ($p<0.04$ and $p<0.004$, respectively). The combination P/CTLA-4 + P/PD-1 showed the highest survival efficacy compared with CTLA-4, PD-1, and PBS groups ($p<0.001$, $p<0.04$, and $p<0.0001$, respectively). We also used an alternative mechanism of nanodrug delivery through BBB with polymer-conjugated Angiopep-2 (AP-2) peptide, which is a synthetic low-density lipoprotein receptor (LRP-1) ligand. Animal experiments showed that both NIC variants significantly prolonged survival with maximum effect combining both checkpoint inhibitor mAbs (Fig. 5g).

Conclusions: We combined nanotechnology and immunotherapy advances to deliver nanoscale immunoconjugates (NIC) drugs across the BBB and treat GBM. This study resulted in successful use of polymer-based carriers with covalently attached immunotherapeutics to activate brain local immune response and treat brain tumors. Because of intravenous administration of drugs, some general immune system activation also occurred resulting in increased serum cytokines such as IL-1β, IL-2, IL-12(p70), IFNλ, and TNFα. The combined treatment with nano immunodrugs and other modulators of tumor microenvironment may significantly increase the efficacy of therapy of brain gliomas independent of pronounced genetic heterogeneity of these tumors.

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MANAGEMENT SCIENCE FROM CLINAM PERSPECTIVE

BEAT LÖFFLER

Management Science (MS) is the interdisciplinary study of problem solving and decision making in organizations, with strong links to management, economics, business, engineering, management consulting, and other fields. This nice definition does omit the first issue of all matters which is those potentially using this at later stage. Let's look at the step before: The argument known as "Meno's Paradox" is as follows: "If you know what you're looking for, inquiry is unnecessary. If you don't know what you're looking for, inquiry is impossible. Therefore, inquiry is either unnecessary or impossible. Instead of that Debate is the best tool to make keen projects, cooperation and successful progresses. CLINAM creates a neutral platform for debate of those who know what they are look-

ing for and to learn what others are looking for. CLINAM widens the horizon of knowledge by direct multi-dialogues. Inquiry here becomes a valuable mix of curiosity and imparting knowledge.

TRANSLATION TO THE CLINIC OF AN ULTRASMALL GADOLINIUM BASED NANOPARTICLE: AGUIX

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The talk will describe the translation to the clinic of AGuIX nanoparticles (NPs) that are actually in phase 2 clinical trial with a focus on Phase Ib clinical trial: Nano-Rad (NCT02820454) for the treatment of brain metastases by whole brain radiation therapy in combination with AGuIX nanoparticles.

AGuIX NPs are ultrasmall nanoparticles (~5 nm) made of polysiloxane matrix and surrounded by gadolinium chelates covalently grafted on the inorganic matrix [1] (Figure 1.A). These NPs have shown preclinically important radiosensitizing properties *in vitro* and *in vivo*, effective targeting of tumors by enhanced permeability and retention effect, high MRI positive contrast and elimination by the renal way after intravenous administration [2]. After scale-up and cGMP production, regulatory toxicity tests on monkeys and rodents have shown no sign of toxicity [3]. Based on these positive preclinical results, a first-in-man phase 1 clinical trial with intravenous administration of AGuIX NPs has been filed in 2016 and completed in 2018 on 15 patients suffering from multiple brain metastases from four types of primary tumors (melanoma, lung, colon and breast) [4]. During this clinical trial, one administration of AGuIX NPs was performed before radiotherapy irradiation of 30 Gys in two weeks (Figure 1.B). After intravenous administration, accumulation of the nanoparticles in the tumors is observed by MRI (Figure 1.C and D) and signal is still observed 7 days after administration of the nanoparticles proving retention of the NPs in the tumors (Figure 1.E). Due to very encouraging radiotherapy results, ANSM (French regulatory office) has authorized in 2019 two phase 2 clinical trials on the same indication (Nano-Rad 2, NCT03818386 and Nano-Stereo, NCT04094077).

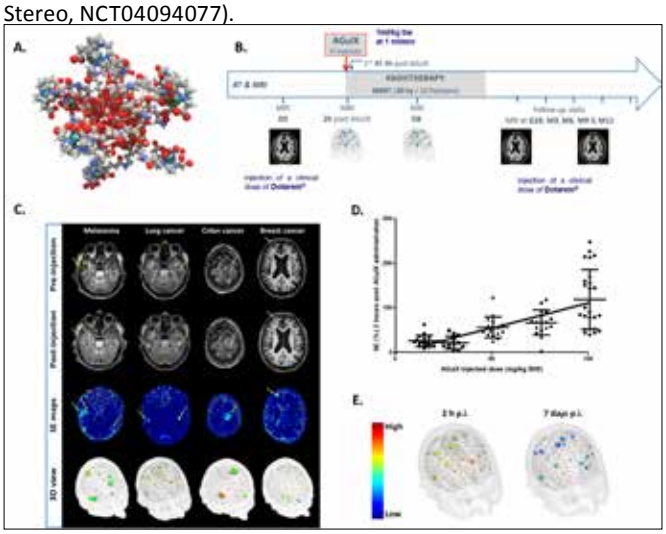

Figure 1. *A. Representation of AGuIX nanoparticles. B. Protocol of Phase Ib NanoRad clinical trial. C. MRI signal of brain metastases issued from 4 different types of primary cancers two hours after administration of AGuIX Nps. D. Signal enhancement in tumors depending of the injected dose two hours after intravenous administration. E. MRI signal 2 hours and 7 days after intravenous administration.*

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ROLE OF THE PROTEIN CORONA IN NANOPARTICLE UPTAKE BY IMMUNE CELLS

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Immune cells are specifically prone to be targeted by nanocarriers. Especially phagocytotic cells like macrophages or dendritic cells take up objects in the nanometer range naturally. This offers a wide field of opportunities for applications of nanocarriers to target these cell populations. Dendritic cells are the central “conductors” of the immune systems. They orchestrate the other cells to react to antigens or get tolarized against specific proteins and other molecules. Therefore they are central in every field of vaccination, cancer immunotherapy, allergy induction and treatment as well as autoimmune disease. Macrophages on the other hand are either part of the tumor environment as tolerizing and immunosuppressive cells or at least cells where a lot of nanocarriers end up unintentionally when applied as drug carriers. Therefore targeting the specific cell type of dendritic and/or macrophagocytotic cells as well as avoiding the huge compartment of unspecific and unwanted uptake is central to the development of nanoparticles as drug delivery devices. We have investigated and elucidated some of these mechanisms and show how to exploit these further for *in vivo* studies. To promote drug delivery to exact sites and cell types, the surface of nanocarriers are functionalized with targeting antibodies or ligands, typically coupled by covalent chemistry. We also show that we can use a pre-adsorption process to intentionally convey targeting antibodies to the surface of the nanocarrier. Pre-adsorbed antibodies or also ligands like interleukin-2 (IL-2) remain functional and are not completely exchanged or covered up by the biomolecular corona, whereas coupled antibodies are more affected by this shielding. But also the other proteins which are adsorbed to nanocarriers will remodel the targeting and the fate of the nanocarriers in the organism. For this we demonstrate how the biomolecular corona can contribute to a mistargeting and how we can one day may be able to predict the effect of the mistargeting in the end.

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NEW APPROACHES TO THERAPEUTIC MODIFICATION OF IMMUNE-MEDIATED INFLAMMATION IN VULNERABLE ATHEROSCLEROTIC PLAQUES

HARALD MANGGE, Dr. Gunter Almer
Clinical Institute of Medical and Chemical Laboratory Diagnostics, Medical University of Graz

Atherosclerosis (AS) leads to myocardial infarction and stroke, and remains worldwide the main cause for mortality. Vulnerable atherosclerotic (AS) plaques are responsible for these life-threatening clinical endpoints. Atherosclerosis is a chronic, complex inflammatory disease with interactions between metabolic dysfunctions, lipids, vascular, and immune cells. Although, macrophages and T cells have been in the focus of research since the last ten years, B cells producing antibodies and regulating T and natural killer (NKT) cell activation are more important than former thought. New results showed that the B cells exert a prominent role with proatherogenic and protective facets mediated by distinct B cell subsets and different immunoglobulin isotypes. These new insights come amongst others from observations of the effects of innovative B cell targeted therapies in autoimmune diseases like systemic lupus erythematosus (SLE) and rheumatoid arthritis (RA). These diseases associate with AS, and beneficial side effects of B cell subset depleting (modifying) therapies on atherosclerotic concomitant disease has been observed. This presentation will reflect the significance of B cell activation in AS with emphasis on vulnerability of AS plaques. It will also discuss the clinical therapeutic potency of B-cell modulations on the process of AS. The CANTOS study (NCT01327846) demonstrated that immune-mediated inflammation is a realistic target for improved treatment of atherosclerotic disease.

SINGLE PARTICLE MEASUREMENT OF NUMBER, SIZE AND CHARGE IS REQUIRED FOR CONFIDENCE IN NANOMEDICINE ENGINEERING AND DEVELOPMENT

MARIANNE MARCHIONI

In nanomedicine development size matters, the number of particles matters and the particle surface properties matter. As nanomedicine products evolve out of university research groups to detailed development, clinical testing and clinical use, the quality and discipline around measurement and analysis becomes increasingly critical. Measurement technology has developed to meet this need and extremely accurate measurements can now be done quickly and easily.
One important aspect is the required resolution of measurement. It has been clear for some time that DLS for instance, lacks the resolution necessary to make nanoparticle measurements in the real world. It is expected that regulators will soon require a minimum level of resolution for nanoparticle measurement, which will likely rule out DLS data. That would be a welcome and profound change for the nanomedicine field.
Repeatability of the measurements by third parties is also an essential component. That logically means that the measurements need to provide the real dimensions and not artificial numbers. Precise single particle measurements are required for this to occur.
TRPS offers the level of detail and certainty that the medical world requires. The importance of showing size and number is now well understood. Current developments are aimed at increasing the accuracy and resolution of TRPS single particle charge measurements by a factor of 10.
The extracellular vesicle research community is beginning to make advances using extracellular vesicles, mainly exosomes as a new class of therapeutics. There a number of different configurations including gene therapy, drug delivery and stem cell derived exosomes. These are biological particles, typically more heterogeneous than synthetic particles and typically more adapted for survival in the body than synthetics such as liposomes. The need for precise isolation and measurement of these nano-bio structures provides some additional challenges which TRPS is being developed to meet.

BRAIN CLINICAL INFORMATICS – PROMISE AND BARRIERS TOWARDS PRECISE MEDICINE

MIRA MARCUS-KALISH

These last months forced us to face new challenges, looking at the broad band picture of the human being functioning in his surroundings as one complex system. That involves both micro (in-body, physical, mental, etc.) and macro (environmental, cultural, economics, etc.) features. The collection of relevant accurate data, knowledge and technologies, created the need to bridge barriers of data curation and to apply sophisticated analysis tools to meet the great promise of personalized, precise and reliable medicinal treatment.
Thus, realizing the unity of nature, science and technology from the nanoscale towards converging knowledge and tools at a confluence of disciplines - is a major goal, as was envisioned by the NSF in 2001 (NBIC) and further at the joint EU-US WTEC effort “Converging of Knowledge, Technology, Society” (2013). The COVID-19 outbreak has quickly become a global health emergency regarding physical health while facing major mental threat and stress. People are exposed to unexpected deaths and threats following social isolation and physical discomfort, as well as cultural, behavioral and environmental impact. More specifically, as an example, there is a strong association between stress-related disorders, such as Post Traumatic Stress Disorder (PTSD), and the development of neurodegenerative diseases, which increase the need for early diagnosis and treatment. These understandings reemphasized the need to collect all available data in a broad band variety, across countries and continents, and to combine, verify and embed all knowledge, tools and technologies in the various disciplines, while bridging all curation and adaption barriers, preserving privacy and ensuring reliable, advanced analysis tools. The working hypothesis is that the comprehensive systemic analysis will allow better distinction between pathological and physiological in both PTSD and neurodegeneration, serve as a clinical predictor for individuals at risk and help clinicians to provide the most suitable therapeutic treatment. Furthermore, the large-scale availability of the metabolic profiles will allow us to identify key metabolic biomarkers of the underlying disease that might shed light on the following progression of PTSD to neurodegeneration. However, to better understand the underlying neurobiology of PTSD pathogenesis and resilience, these trajectories must be linked with co-occurring developments in objectively measured domains (e.g., cognitive, physiologic, psychophysical, neuroimaging, genomic).
The Brain Medical Informatics Platform (MIP), developed by the EU Human Brain Flagship Project, as part of the EBRAINS platform, is one feasibility study along these lines. It involves broad clinical data collection from 30 hospitals, converging all available knowledge and data, embedding new technologies for data privacy, preservation and curation as well as sophisticated analysis tools. Our lab has contributed valuable components, such as the 3C analysis, advanced tools for validation, and methods for efficient interaction with the simulation environment on EBRAINS. The MIP and EBRAINS offer a framework that can help to meet the great promise of personalized, precise and reliable medical treatment.

As one case study, a PTSD prospectively tracked cohort, containing 180 individuals at initial high risk for PTSD, at three time points (1, 6, and 14-months following a traumatic event), was analyzed at TAU in collaboration with the Tel Aviv Medical Center, including concurrently documenting brain structure and function, cognitive functioning, and co-occurring clinical symptoms. The aim was to uncover neurocognitive moderators underlying PTSD symptom trajectories, by prospectively documenting multi-modal dimensions of the evolving psychopathological consequences of traumatic events. Advanced computational methodology was utilized combining both theory- and data-driven approaches, to find potential biomarkers and mechanism-related classification of recent trauma survivors. We found both a cognitive construct which emerged as a significant predictor of PTSD (i.e., cognitive flexibility), as well as neuroanatomical risk factors for PTSD severity (i.e., hippocampus and CSP volumes) both of which could guide early management and objective long-term monitoring.

WORLDWIDE DEVELOPMENT AND CHARACTERIZATION OF NANOMATERIALS / NANOMEDICINES

SCOTT MCNEIL, Professor of Nanopharmaceutical and Regulatory Sciences, Dept. of Pharmaceutical Sciences, University of Basel, Switzerland

Thorough characterization is foundational in the development and scale-up of nanomedicines, and is required step for regulatory evaluation. Characterization involves physicochemical characterization, safety and efficacy testing, and comparisons with current standards of care. This talk will provide an overview of those techniques, and serve as an introduction to the worldwide efforts covered in this session.

FROM PRECISION TO POPULATION: HARNESSING NEW KNOWLEDGE TO REDUCE GLOBAL HEALTH BURDEN AT SCALE

CLIVE MEANWELL

The health care investment sector has performed creditably over the last 20-years – lagging only consumer discretionary spending and, more recently, information technology. Within the healthcare sector there have been pockets of exceptional opportunity: Investing in medical products for infrequent cancer subsets, inflammatory diseases and rare diseases has fared well. Large pharmaceutical firms have favored such capital deployment, in tandem with dividends and share buy-backs, to create shareholder value. The biotechnology subsector has also focused most of its investments in this direction. By contrast, investments in technologies which address the drivers of prevalent conditions which constitute most current and future healthcare burden have been held back. Perceived high technical risk of systems biology, human behavior, and environment; high costs for R&D, and for marketing and sales; and constrained, slow revenue growth consequent to payers' management of budgets, and eroding margins due to intermediary-market participants in some countries such as the US, have each contributed. Yet prevalent diseases present the greatest burden of disease worldwide for the foreseeable future, wielding enormous negative economic and social impact (accentuated during pandemics). For example, hypertension alone, afflicts 3.5 billion people, brings $370 billion in direct medical costs (10% of all), $3.6 trillion indirect costs, and more than 200 million disability-adjusted life years, half of them in China, India, Russia, Indonesia, and the United States, each year. Today hypertension is treated with variably effective life-style modification, and drugs which were invented 20-60 years ago.

Given the perennially dismal economic consequences and social burden of poor underlying health – accentuated recently by COVID-19 – technological and business model innovations are needed, including massive improvement in R&D efficiency; earlier engagement of payer-provider systems and a thoughtful mix of medicinal and digital therapeutics.

HYPHENATION OF ELECTRICAL ASYMMETRICAL FIELD-FLOW FRACTIONATION WITH MULTI-ANGLE LIGHT SCATTERING AND NANOPARTICLE TRACKING ANALYSIS FOR MULTI-DIMENSIONAL CHARACTERIZATION OF LIPOSOMES AND EXOSOMES IN COMPLEX BIOLOGICAL MEDIA

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The quest for innovative and groundbreaking drug candidates is continuing at high pace, especially in the field of bio- and nanomedicine. A lot of effort is currently being put into the rational design of such candidates in order to improve their quality and efficacy. However, the increasing complexity of bio- and nanomedical drug candidates also demands for more sophisticated analytical tools that enable a better understanding of their physico-chemical properties, especially in complex human and biological media, thereby allowing a deeper insight into their behavior in more realistic real-world application scenarios. Key parameters that need to be addressed here are e.g. the concentration and surface charge as well as the particle size and number concentration. Both Field-Flow Fractionation (FFF) [1] and Nanoparticle Tracking Analysis (NTA) [2] are well-established and powerful analytical techniques that provide this crucial information thus being valuable tools to enhance the quality and efficacy of innovative bio- and nanomedical drugs. The concept of coupling of AF4 to NTA was already previously investigated for the characterization of nanomaterials [3,4]. We here present a true online coupling of FFF with NanoSight NTA. The overall analytical setup comprised an Electrical Asymmetrical Flow FFF (EAF4) channel that was connected in-line to a UV- and Multi-Angle Light Scattering detector. Hyphenation to the NS300 NTA system was achieved by taking advantage of the slot outlet option of the EAF4 channel with a T-piece in order to further reduce the detector flow to an NTA-compatible flow rate of 12 µl/min. The hyphenated setup is displayed in Figure 1.

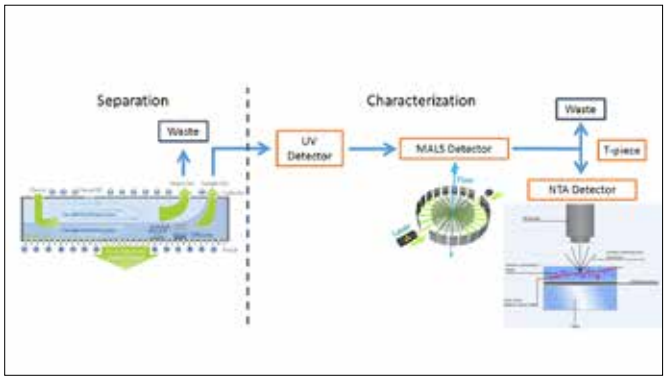

Figure 1: *Schematic of the online coupling of Electrical Asymmetrical Flow Field-Flow Fractionation with UV-, Multi-Angle Light Scattering- and NanoSight Nanoparticle Tracking Analysis detection (EAF4-UV-MALS-NTA).*

The established analytical setup was initially tested against 100 nm polystyrene beads (PS100) prior to use with biologically relevant particles like liposomes and exosomes, both prepared in ultrapure water and biological media such as Dulbecco's Modified Eagle's Medium (DMEM) to simulate a complex biological environment. While EAF4-UV-MALS was able to provide the radius of gyration

R_g and electrophoretic mobility (surface Zeta potential) (**Figure 2**), coupling with NTA additionally enabled access to the hydrodynamic diameter D_h as well as the particle number concentration of samples in both media, as exemplarily displayed for liposomal Doxorubicin HCl in DMEM (**Figure 3**).

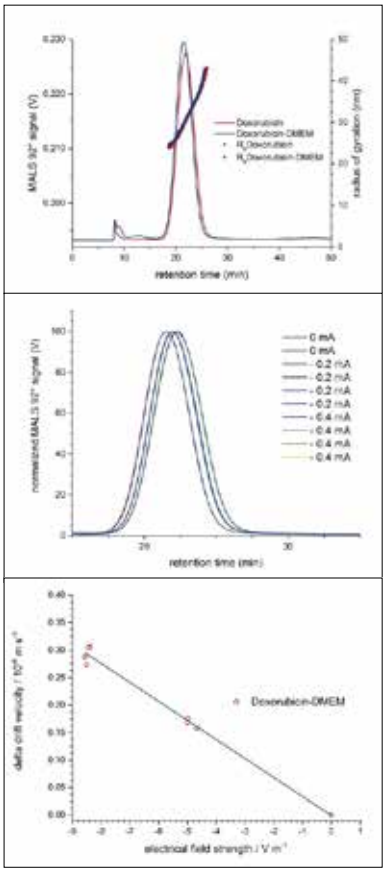

Figure 2: *Left: EAF4-MALS fractograms of liposomal Doxorubicin HCl both in 0.5 mM sodium chloride solution (red trace) and DMEM (blue trace) in cluding respective radii of gyration (blue and red dots). Middle: Observed retention time shifts of liposomal Doxorubicin HCl in DMEM induced by the application of an electric field during EAF4-MALS separation. Right: Drift velocity plotted against the applied electric field to derive the electrophoretic mobility of liposomal Doxorubicin HCl in DMEM.*

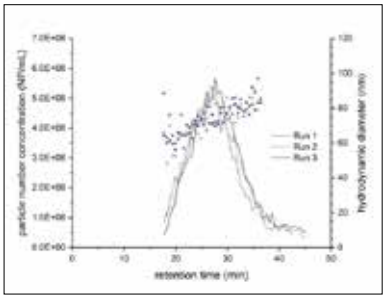

Figure 3: *Particle number distribution overlaid with the hydrodynamic diameter of liposomal Doxorubicin HCl in DMEM obtained from three consecutive EAF4-MALS-NTA measurements.*

The obtained results of the EAF4-MALS-NTA analysis of liposomal Doxorubicin HCl are summarized in **Table 1** clearly highlighting that there is no size alteration observable when the sample is suspended in DMEM versus 0.5 mM sodium chloride solution. However, at the same time, the increase in Zeta potential may indicate a change in the composition of the liposome surface, which might be attributed to the adsorption of DMEM constituents and the formation of a protein corona, which does not significantly alter the size of the liposome itself though.

Table 1: *EAF4-MALS-NTA results for liposomal Doxorubicin HCl and Doxorubicin-DMEM samples. Online hyphenation of EAF4-MALS and NTA is a powerful analytical setup for the characterization of nanomedical particle systems as the presented approach provides both effective separation and high resolution particle characterization in both simple and complex environments prior to more costly in vivo studies supporting the drug development process.*

	MALS results		NTA results			EAF4 results
Sample / Measurand	D_g at peak maximum (nm)	D_g range (nm)	D_h at peak maximum (nm)	D_h range	particle concentration at peak max (NP mL^{-1})	Zeta potential (mV)
Liposomal Doxorubicin HCl	62	48 - 82	80	65 - 95	7.5 E^{+08}	-34.6 ± 1.5
Doxorubicin-DMEM	62	48 - 82	75	60 - 95	5.4 E^{+08}	-45.2 ± 1.5

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CLINICAL EXPERIENCE WITH IV LIPOSOMAL PREDNISOLONE TARGETING INFLAMMATION: RESULTS FROM A PHASE III RANDOMIZED ACTIVE-CONTROLLED TRIAL

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Glucocorticoids (GC) are potent anti-inflammatory drugs but their systemic (parenteral/oral) use in inflammatory disorders such as rheumatoid arthritis and inflammatory bowel disease is limited by poor target localization and toxic effects in healthy organs. Targeted delivery of GCs to the site of inflammation with long-circulating liposomes can improve the therapeutic index. This approach has proven successful in our rat and murine arthritis studies and in several other preclinical inflammatory disease models.

Over the past years we evaluated this novel treatment concept in a series of smaller and larger trials in patients with a variety of diseases of inflammatory origin. Doses of 37.5 up to 300 mg prednisolone in long-circulating liposomes (Nanocort) have been studied in patients with rheumatoid arthritis (RA), kidney disease, colitis ulcerosa (UC), and patients with Graves' orbitopathy.

Recently we completed a Phase III randomized, double-blind, active controlled, multi-center study in which Nanocort was compared with IM injection of equipotent dose methylprednisolone acetate (Depo-Medrol®) in patients experiencing a flare from RA.

172 patients with active RA were enrolled and 150 patients were randomized into one of three groups: Nanocort 75 mg IV infusion and IM saline injection, Nanocort 150 mg IV infusion and IM saline injection or Depo-Medrol® 120 mg IM injection and IV saline infusion. Dosing in each group occurred at Baseline and Week 2. Study visits occurred at Week 1, 2, 3, 4, 6, 8 and Week 12 to assess efficacy and safety evaluations. Primary endpoint was EULAR responder rate at Week 1. Safety was determined by the occurrence of adverse events (AE) during treatment and 12 week follow-up.

The results show that IV Nanocort at both dose levels was clearly superior to IM Depo-Medrol in terms of EULAR Response (Good/Moderate) at Week 1, with p-values of 0.007 and 0.018, respectively. Treatments were well tolerated with a comparable pattern of AEs across the treatment groups, though the Nanocort groups had a higher incidence of hypersensitivity reactions during infusions.

We conclude that liposomal GC targeting is a safe and efficacious novel treatment strategy for several diseases with an inflammatory component.

PERSONALISED MEDICINE FOR RENAL DYSFUNCTION USING AGULX NAOPARTICLES

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Population ageing, increased obesity and cardiovascular diseases are predominant factors leading to chronic renal diseases. Renal dysfunctions lead to fibrosis which then result into chronic renal failure, which incidence is 130 to 150 millions/year in Europe. At this stage, patients are treated by dialysis or transplantation. Based on this socio-economic impact, diagnosis and treatment of renal failure have become a major public health issue. Today, diagnosis of renal dysfunction is indirectly evaluated via plasmatic creatinine measurements or albuminuria. However, these values are not sensitive and present high variability between individuals. After suspicion of renal impairment, imaging modalities are used such as MRI. As of today, small molecules such as DOTAREM are mainly used with rather limited applications. Therefore, agents providing more information on the molecular levels using the current clinical techniques used could improve the patient care. The agent proposed is AGUIX, which are small nanoparticles of 3 nm currently in phase II clinical trial as radiosensitizer in glioblastoma, and that we evaluated in this study as a marker of the tubular function.

MATERIALS AND METHODS

AGulX nanoparticles[1], consisting of a functionalized polyorganosiloxane matrix with Gd DOTA chelators and chromophore Cy5.5. Multi-imaging modalities were used to show the potential interest of using AGuix in two models of renal dysfunctions, ureteral artery obstruction and folate. The multiparametric MRI (T2, T1 weighted and ADC maps) method was adapted to the imaging probe size. The imaging method was based on a dynamic acquisition with high and low temporal resolution, providing signal enhancement curves and numerical analysis with 3TP 3 Time Point type method. Results were compared with Mann-Whitney *U* test. A histological study was performed for data interpretation.

RESULTS

Significant signal decrease was observed between the UUO pathological mice kidneys as compared to the contralateral kidney or sham. The dynamic AguIX MRI data computed with our developed 3TP method were compared to DCE profiles with classical commercial Gd complex contrast agent DOTAREM. Significant differences of 3TP parameters were measured in the cortex between sham and UUO DCE 3TP profiles (respectively from p= 0.0048 to p=0.008, and from p=0.0009 to 0.0012). Similar dynamic profiles were obtained by optical imaging. Histological study confirmed and rationalized the *in vivo* results related to the internalization of the probes into the tubules, which were specifically affected by the pathology.

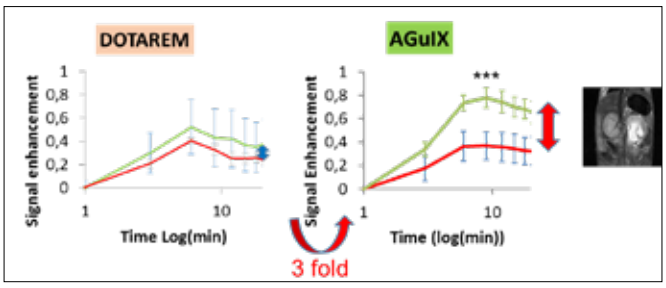

Figure 1 *Dynamic profile of the DCE MRI images of the controlateral (green) /UUO (red) cortex kidney with DOTAREM and AguIX contrast agent.*

CONCLUSION

We showed that AGUIX could be advantageously used to determine renal pathologies related to renal tubular dysfunction. This was evidenced on two pathological mice model by DCE-MRI and optical imaging, and is being currently conducted on other relevant models of renal pathologies together with PET imaging.

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ASSESSING NANOMEDICINE SAFETY THROUGH REAL-TIME MITOCHONDRIAL RESPIRATION PROFILING AND METABOLOMICS

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Mitochondria are the most efficient provider of intracellular energy. The four-mitochondrial inner membrane protein complexes are responsible for generating the mitochondrial membrane potential ($\Delta\psi_m$). Complexes I, III and IV are the proton exchangers that maintain $\Delta\psi_m$ by generating a net outward proton transfer across the inner membrane. Accordingly, $\Delta\psi_m$ is negative inside and 180–200 mV. This makes mitochondria a highly attractive target for polyfectamines and lipofectamines, which are commonly used in nucleic acid transfer protocols. Consequently, this induces mitochondrial impairment (and particularly through the inhibition of cytochrome *c* oxidase) in conjunction with polycation-mediated perturbation of plasma and other organelle membranes (e.g., early endosomes, endoplasmic reticulum) [1]. Our efforts have shown that polycation structure (linear versus branched) and size impact cellular bioenergetics differently [2,3]. Furthermore, through a pan-integrated real-time medium-throughput bioenergetics (phosphorylation control protocol and respiratory flux ratios), metabolomics and biomembrane profiling we have unravelled integrated dynamics and compartmental mechanisms pertaining polycation cytotoxicity [2,3]. Through these approaches we have provided a comprehensive cell-dependent 'structure-safety' correlation mapping for rapid selection of safe macromolecules, peptides and vectors for cellular targeting and transfection [4–7]. One recent example is a multifunctional brain-specific phage mimetic for rapid and safe nucleic acid delivery to cerebral endothelial cells, neurons and microglial cells on intravenous injection [8].

Finally, through respiratory mapping we have been able to select suitable polycations to prevent vemurafenib resistance in melanoma cells [9]. Indeed, acquired resistance to the oncogenic $BRAF^{E600}$ inhibitor vemurafenib is a major clinical challenge in the treatment of melanoma. Thus, combined treatment of selected polycations and vemurafenib diminishes the metabolic flexibility of melanoma cells, making them unable to shift between glycolysis and mitochondrial oxidative phosphorylation according to energy demands.

Polycations exert considerable detrimental effects on melanoma cells at concentrations better tolerated by epidermal melanocytes and act synergistically with vemurafenib in effectuating bioenergetic crisis, DNA damage and cell death selectively in melanoma cells [9]. Therefore, comprehensive screenings of pre-existing libraries of structurally different and chemically modified polycations could potentially identify effective macromolecules for cancer therapy in combination with small molecule drugs. Within the context of cancer therapy, these developments may be expanded for construction of functional programmable nanoparticles from polycations (e.g., through layer-by-layer assembly and environmentally controlled dis-assembly) as well as polycation-drug conjugates.

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COMPLEMENT MODULATION OF NANOMEDICINE PERFORMANCE IN HEALTH AND DISEASE

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The complement system is an integral constituent of the innate immune system orchestrating various protective, regenerative and inflammatory responses [1]. Nanomedicines can potentially trigger complement activation and as such the role of complement system in nanomedicine integrity performance and safety is multifaceted [1]. Thus, complement plays important roles in regulation and synchronization of nanomedicine opsonisation and phagocyte clearance. Our recent studies have shown that C3 opsonisation is continuous and variable *in vivo* and demonstrated a vital role for non-specific blood proteins in complement activation [2,3]. Indeed, some of these proteins generate necessary antigenic epitopes for docking of a few natural antibodies on some, but not all nanoparticles, which subsequently serve as target for nascent C3b attack and formation of C3bBb-properdin convertases. Dynamics of non-specific blood protein binding might therefore explain interindividual disparities in complement activation kinetics and opsonisation efficacy.

Nanoparticles are widely tested in vaccination strategies both as an antigen depot systems and adjuvants. Part of nanoparticle adjuvanticity may be related to their complement activation properties, since upon C3 cleavage, liberated C3d (in fluid phase and surface bound) can induce B lymphocyte activation for maintenance of long-term B cell memory [1]. Indeed, B cell complement receptors (CD21 and CD35) cooperate with B-cell antigen receptor to efficiently recognize C3dg-tagged nanoparticles/antigens and signal augmentation is achieved through CD19 recruitment. Complement also plays a role in negative selection of B lymphocytes, but the role of complement in tolerance to self-antigens are apparently restricted to those self-antigens that are evolutionary conserved.

Uncontrolled complement activation is deleterious to the host, causing cellular injury and contributing to the development of disease. For instance, we have shown that intratumoural complement activation by nanomedicines can promote tumour growth [4]. This is partly due to C5a liberation resulting in influx of immunosuppressive Treg cells. C5a also facilitate neutrophil recruitment to tumour microenvironment, which further help with cancer progression. C5a also stimulates the production of functionally active tissue factor in peripheral blood neutrophils, which results in tumour growth and metastases. Among its other roles, C5a can inhibit the production of IL-12 in macrophages and induce macrophage polarization by activation of the nuclear factor-κB. C1q is also capable of inducing macrophage polarization and suppressing macrophage NLRP3 inflammasome activation. C1q polarized macrophages express elevated levels of programmed death-ligand 1 and -2 and promote T_{reg} proliferation. In addition to these, the possible role of nanoparticle-mediated intracellular complement (complosome) activation is poorly explored, but this may modulate cell physiology and metabolism in tumour microenvironment (as well as in adjacent health tissues) and initiate resistance to immunotherapies. On the other hand, controlled nanomedicine-directed complement activation could be beneficial in selected pathological conditions that will benefit from influx of immunosuppressive cells.

There are reports claiming a causal role for complement anaphylatoxins in adverse reactions to nanomedicines [reviewed in 5]. These reports have correlated nanoparticle-mediated complement activation in human serum to cardiopulmonary responses in pigs, and suggested the utility of the porcine model in nanomedicine safety assessment. Contrary to these, compelling evidence indicate a direct role for some macrophage sub-populations (and other immune cells) in infusion-related reactions independent of complement activation [1,5,6]. Moreover, our recent results with isolated porcine pulmonary intravascular macrophages excludes the role of complement activation in bioactive mediator release on nanomedicine challenge, and therefore questions the scientific validity of the porcine model (and other cloven-hoof animals) in nanomedicine safety assessment, which is irrelevant to human cases [1,5]. Accordingly, we call for development of more accurate biomarker screening and diagnostic imaging to predict infusion reactions to nanomedicines in humans.

Finally, considering multifaceted roles of the complement system in modulating nanomedicine performance, development of species- and pathway-specific complement and complement-receptor inhibitors could open up new avenues for assessing the role of complement and complosome in nanomedicine safety [7]. Nevertheless, through concerted "nanomedicine structure-complement function" mapping we have been able to engineer a panel of complement-safe nanomaterials and nanopharmaceuticals [1,8].

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"RECENT ADVANCES IN ANTISENSE TECHNOLOGY AT IONIS PHARMACEUTICALS"

BRETT MONIA

The development of antisense technology as a novel drug discovery platform to treat a broad range of rare and common diseases is now validated. Antisense medicines offer tremendous hope for patients afflicted with serious diseases that cannot be addressed with traditional drug discovery platforms. With numerous antisense medicines recently receiving market authorization along with a large and diverse pipeline of medicines on the horizon, antisense is poised to revolutionize the practice of medicine for generations.

A NANOTECHNOLOGY PLATFORM FOR TYMPANIC MEMBRANE REGENERATION

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Figure 1 – *Approach developed for the manufacturing of TM regenerative scaffolds with four nano features: nanofibers, nanofibrils, nanoparticles and nanovibration*

The restoration of a damaged tympanic membrane (TM) can only be accomplish by mimicking the peculiar anatomy and function of the several nanoscale features. The optimal selection of these nano features can be decisive for an optimal regeneration of a contaminated and chronically inflamed microenvironment as a consequence of recurrent infections such as chronic otitis media (COM). Auto/allografts from other tissues sources are still the clinical gold standard but with suboptimal outcomes. The 4NanoEARDRM project (EuroNanoMed III) aims to synergize 4 different nanotechnologies in a biofabrication process for an optimal TM restoration, including acoustic, regenerative and therapeutic cues, to achieve an alternative therapeutic approach for COM (Figure 1).

METHODS

To achieve a nano-featured temporary TM implant for a suitable regeneration, properties such as anti-infective, immunomodulatory, otocompatible and acousto-mechanic properties are needed. These properties were developed as follows: a) An anisotropic TM scaffold based on a nanocomposite of poly(ethylene oxide terephthalate)/poly(butylene terephthalate)/chitin nanofibril (PEOT/PBT/CN), fabricated via electrospinning (ES) and additive manufacturing (AM) with radial and circular key anatomical features of the human TM [1]; b) CNs were chosen to sustain an anti-inflammatory microenvironment by virtue of their immunomodulatory properties; c) Antibiotic-loaded polymer nanoparticles (NPs) were prepared via nanoprecipitation and water-in-oil-in-water emulsion; d) Nanoscale acousto-mechanical response was investigated via Laser Doppler Vibrometry (LDV) and Optical Coherence Tomography (OCT).

RESULTS

The biofabrication process has been optimized to allow the production of complex TM-like geometries within 80 µm of thickness and 400 nm ES fibers. *In vitro* studies with human mesenchymal stromal cells (hMSCs) demonstrated good cytocompatibility, showing hMSC alignment along the AM and ES fibres. Otocompatibility of the PEOT/PBT/CN composite at different weight ratios was assessed using OC-k3 inner ear cells. A strong immunomodulatory activity was observed for the prepared scaffolds decreasing pro-inflammatory interleukins (ILs) IL-1α, IL-1β, IL-6 and IL-8 in human keratinocytes. PEOT/PBT/CN (CN:PEG 50:50 w/w) stimulated human β-defensin 2 expression. NPs loaded with ciprofloxacin were successfully prepared with two different manufacturing strategies and also incorporated into electrospun fibers. NPs resulted more effective against *S. aureus* and *P. aeruginosa* than free drug [2]. Acousto-mechanical performance of TM scaffolds evaluated by LDV and OCT were comparable to the *in silico* models developed showing the strong influence of the geometries manufactured.

DISCUSSION & CONCLUSIONS

Our biofabrication strategies allowed the improvement of the geometrical limitations previously reported. This was achieved by the reduction of the AM fiber features and the reduction of the nanosized ES fibers. Nanocomposite fibers manufactured showed enhanced bioactive (anti-inflammatory and indirect antibacterial) properties needed to promote TM healing. Antibiotic-loaded NPs embedded in TM scaffolds could deliver locally an optimal therapy. TM scaffold geometry was tuned to accomplish effective sound transmission.

ACKNOWLEDGEMENTS

4NanoEARDRM project (EuroNanoMed III).

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IMPLICATIONS OF CARBON NANOPARTICLES FOR POTENTIAL BIOSENSORS AND THERAPEUTIC APPLICATIONS

DEBABRATA MUKHOPADHYAY

There are several different carbon nanoparticles including single-walled carbon nanotube (SWNT), Nano-Diamond (ND), which have the promise to be employed for different clinical implications with proper modification. Herein, we will discuss our recent works related to two different kind of carbon nanoparticles for future clinical use. Previously, we showed the utilization of a near-infrared fluorescent SWNT sensor array to measure the single-molecule efflux of NO and H2O2 from human umbilical vein endothelial cells (HUVEC) and cancer cells in response to angiogenic stimulation or chemotherapeutic drugs. In addition, we described that the SWNT platform can be employed for detection and therapeutic outcome of chemotherapeutic drugs in real-time. Recently, we have shown that Nano-Diamond (ND) could have a great promise in future clinical use as drug delivery system due to its unique bio-compatible character. This topic will be discussed in details as well.

THERAPEUTIC TARGETING OF TRAINED IMMUNITY WITH NANOBIOLOGIS

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Immunotherapy is revolutionizing the treatment of diseases. Most of the immunotherapy strategies currently being developed engage the adaptive immune system. In recent years, emerging evidence has shown that the innate immune system displays long-term changes in its functional program through metabolic and epigenetic programming of myeloid cells (monocytes, macrophages, dendritic cells). Therefore, targeting myeloid cells and their progenitors is a powerful 'therapeutic framework' to regulate the delicate balance of immune homeostasis, priming/training and tolerance. This Presentation will showcase how nanobiologic-based immunotherapies can be applied to induce trained immunity. Through extensive screening, we identified a nanobiologic lead candidate. We found that this nanobiologic's anti-tumor effects result from trained immunity-induced myelopoiesis caused by the activation of hematopoietic stem cells and epigenetic rewiring of multipotent progenitors. We determined that the induction of trained immunity overcame the immunosuppressive tumor microenvironment and potentiated concurrent immune checkpoint inhibition in this melanoma model refractory to anti-PD-1 and anti-CTLA-4 therapy. In conclusion, we show that rationally designed nanobiologics can promote trained immunity and elicit a durable anti-tumor response either as a monotherapy or in combination with checkpoint inhibitor drugs.

NANOMATERIALS IN MEDICINE

BERT MÜLLER, Biomaterials Science Center, Department of Biomedical Engineering, Medical Faculty, University of Basel, Switzerland

Nanomaterials are characterized by extensions from 1 to 100 nm at least in one of the three orthogonal directions. Therefore, they exhibit properties that differ from the well-known bulk properties. A characteristic example of our everyday life is the sun crème. The titania nanoparticles protect the skin from the ultraviolet radiation much better than any previously used product.

Consequently, it is not surprising that our understanding of the human anatomy, physiology and biomechanics is entering a new era involving the true nanometer scale. The medicine-oriented presentation includes selected examples on (i) the three-dimensional nanoimaging of human teeth in health and disease [1] as well as of selected brain tissues [2], (ii) the mechanically responsive, non-spherical liposomes for the targeted release of vasodilators in constricted blood vessels [3], and (iii) the nanotechnology-based polymeric medical implants with unique mechanical and electrical properties [4] for a variety of applications ranging from artificial muscles via sensor-enhanced devices for the oral cavity to neural interfaces for stimulation. Since the establishment of the Biomaterials Science Center in 2007, the interdisciplinary research team has published more than 300 papers – most of them with clinical relevance. Founded in 2019, the two startup companies Bottmedical AG and Acthera Therapeutics AG originated from the nanoscience activities.

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NANO-ENABLED IMMUNOTHERAPY FOR CANCER AND THE TREATMENT OF ALLERGIC AND AUTOIMMUNE DISEASE

ANDRÉ NEL, Distinguished Prof. of Medicine at UCLA, Associate Director California Nano Systems Institute

Nanoparticles have made a big impact on the development of immunotherapy for cancer and serious allergic disorders, demonstrating the ability to develop new therapeutics that are capable of boosting immunogenic effects in the setting of "cold" tumor microenvironments in solid cancers, as well as the ability to induce tolerogenic effects that suppress antigen-specific immune hyperreactivity in the setting of asthma or autoimmune disease. I will delineate cancer immunotherapy from the perspective of inducing immunogenic cell death (ICD) by silicasome carriers and liposomes that provide an endogenous vaccination approach through the delivery of chemotherapeutic agents in combination with other active pharmaceutical ingredients. Immunogenic cell death leads to the activation of cytotoxic T-cells by the generation of "eat-me" and immunological danger signals, which can be further propagated at the TME delivery site by additional interference in checkpoint and immune metabolic pathways. This intervention can increase the number of immunotherapy responders to checkpoint inhibitors, in addition to inducing immune memory that can eradicate tumor metastases. The second part of my talk will focus on the induction of antigen-specific immune tolerance by liver-targeting tolerogenic nanoparticles, which leads to the generation of antigen-specific regulatory T cells and that can suppress allergic inflammation in the lung and autoimmune disease processes. Moreover, both treatment modalities, i.e., ICD-inducing nanocarriers and liver-targeting tolerogenic nanoparticles, can be used on the translational side to generate new therapeutics that can be implemented to treat two major disease processes by using contrasting design features.

WELL-DEFINED POLYMER-PRODRUG CONJUGATES FOR CANCER THERAPY

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SUMMARY

We report on the design of a new class of polymer prodrug nanocarriers by using the "*drug-initiated*" method (Figure 1),[1] which consists in the controlled growth of vinyl polymers from anticancer drug-bearing initiators to prepare well-defined and high drug content polymer prodrug nanoparticles with *in vitro* and *in vivo* anticancer activity.[2] This method is robust and versatile as was applied to different drugs including Gemcitabine, Cladribine and Paclitaxel, to different polymers and to different drug/polymer linkers to adjust the drug release kinetics and thus the cytotoxicity.[3,4] Fluores-

cent polymer prodrug nanocarriers can also be produced in a similar fashion from a fluorescent dye-bearing initiator.[5] This approach was further developed to yield heterotelechelic polymer prodrugs for drug delivery, imaging and combination therapy.[6]

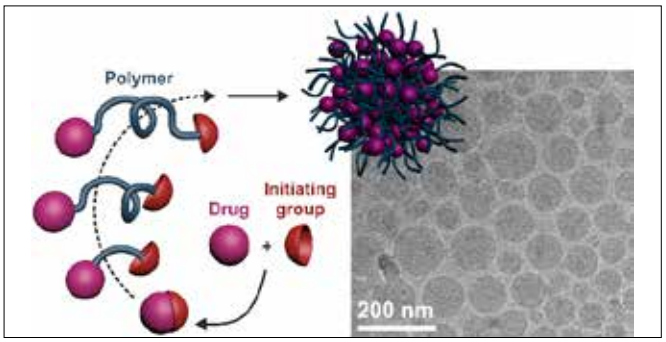

Figure 1. *Drug-initiated synthesis of polymer prodrug nanoparticles.*

We also present our recent achievements to confer degradability to vinyl polymers[7] by using controlled radical ring-opening polymerization (rROP) using cyclic ketene acetal (CKA) monomers. In particular, 2-methylene-4-phenyl-1,3-dioxolane (MPDL) was copolymerized with oligo(ethylene glycol) methyl ether methacrylate (OEGMA) or methyl methacrylate (MMA) by NMP to produce well-defined, non-cytotoxic and degradable copolymers.[8] This approach was eventually applied to the "*drug-initiated*" synthesis of degradable polymer prodrug nanocarriers with tuneable cytotoxicity towards cancer cells.[9]

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INTEGRATING EXPERIMENTAL AND COMPUTATIONAL PHARMACOLOGY FOR INTELLIGENT DRUG DESIGN

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G protein-coupled receptors (GPCRs) regulate many physiological and pathophysiological processes and are a major drug target – covering ~30% of FDA-approved drugs on the market. Designing drugs that control the GPCR-driven cellular responses requires understanding of how these receptors encode and transfer information. Both, i) receptor conformational changes and ii) activation dynamics of GPCRs can affect the downstream signaling events. A thorough understanding of how ligands activate and regulate these processes is thus crucial for drug development and optimization.
At InterAx we use mathematical modeling to integrate theoretical knowledge with experimental data of cellular GPCR signaling events. As a result, deeper mechanistic insights into the dynamic cellular signaling systems activated by these receptors are achievable.

As a showcase of the InterAx platform, an ordinary differential equations (ODE) model describing the trafficking and signaling of the beta-2 adrenergic receptor (B2AR) in response to various agonists (asthma drugs) was developed. Time-resolved data of i) receptor internalization and recycling and ii) cyclic AMP messenger accumulation were used to calibrate the model. The model allows to predict experimental outcomes, derive previously inaccessible parameters of B2AR signaling and delivers insights into drug actions on the B2AR. Overall the approach opens a way for more rational, intelligent, design of drugs with desired system-level response.

POLYMERIC NANOFORMULATIONS TO PROMOTE IMMUNOTHERAPY RESPONSES

LUTZ NUHN, Max-Planck-Institute for Polymer Research, Mainz (Germany)

Over the past three decades, synthetic polymeric nanoformulations have been carefully investigated for use as chemotherapeutics to improve cancer therapies. Alternatively, ongoing oncology research has identified the immune system as promising target to re-install the body's own self-healing capacities and to sustainably eliminate abnormal malignancies. Throughout the last decade, many highly potent immune modulatory small molecules could be identified to address these promising targets, yet, unfavorable pharmacokinetics causing systemic inflammatory side-effects limits their clinical translatability.[1]
Due to their immediate affinity, recognition and processing by immune cells, macromolecular carriers provide unique opportunities to improve the delivery of such small molecules and enhance their efficacy in cancer immunotherapy. However, facile and straightforward access to multifunctional as well as biodegradable polymeric nanoformulations remains a key challenge.

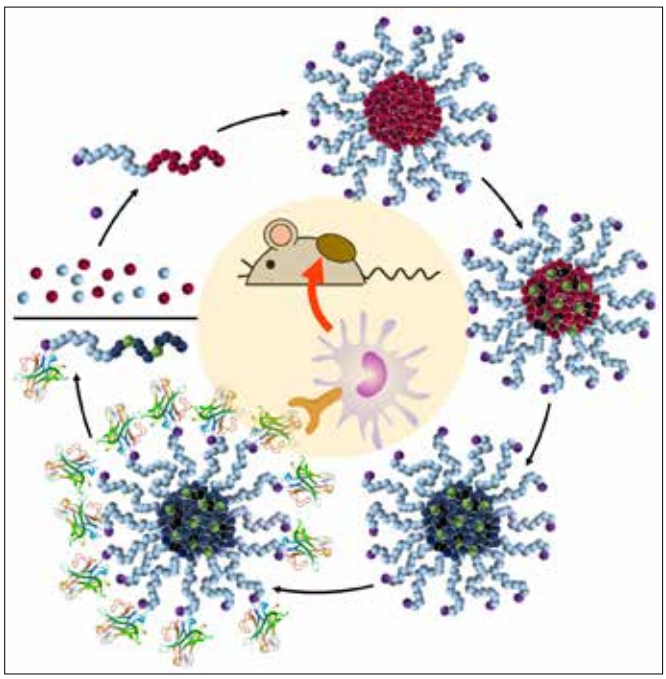

Figure 1: *Fabrication process of multifunctional nanogels derived from reactive precursor block copolymers for targeted delivery of immune modulatory small molecules.*[2]

RESULTS AND DISCUSSION

Spontaneous self-assembly of amphiphilic block copolymers is a straightforward method to access well-defined supramolecular topographies in the nanometer regime. However, unless stabilized by additional physicochemical crosslinks, such polymeric architectures are highly responsive towards disintegration upon exposure to complex biological media. Therefore, controlled stabilization of

polymeric nanoformulations is a key requirement to avoid rapid disassembly and premature drug release under such conditions.
To address such requirements, we are developing reactive amphiphilic polymers forming polymeric precursor micelles that can be further derivatized and stabilized inside their cores and, thus, transformed into hydrophilic nanogels.[2,3]
During this procedure, incorporation of hydrophilic cross-links, fluorescent dyes or drugs can be achieved affording therapeutically active nanogels. Ketal-crosslinks inside the micelle cores exhibit stimuli-responsive particles that unfold into single polymer chains upon exposure to endosomal pH.[4] Immune modulating molecules attached to the core enable localized immune responses and, thus, trigger targeted immunotherapy against e.g. liver fibrosis or cancer.[5]
Additionally, nanogels can be fabricated by precursor polymers with orthogonal reactive groups that are exposed to the carrier surface.[6] These entities are used to decorate the particle surface with targeting units or tumor-specific antigens for eliciting cancer immunity after intravenous injection.
Overall, such polymer-based nanogels can be considered as versatile platform for delivering immune modulating cues into the immune system and thus, provide opportunities to resolve further needs in cancer immunopharmacology.

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INNOVATIVE CELL AND GENE THERAPIES FOR NON-ONCOLOGY INDICATIONS

MAGDALENA OBARZANEK-FOJT, Fellow, Drug Product Lead, GDD TRD Biologics & CGT, Novartis (CH)

Advanced Therapy Medicinal Products (ATMPs) are a new class of medicines for human treatment that in last two decades revolutionized our approach in fighting a disease. To date, multiple ATMPs reached the market and are successfully used in oncological or non-oncological indications, such as Kymriah (CAR-T), Zolgensma (AAV9) or Luxturna (AAV2). The technical development process for ATMPs may be distinctive from standard biologics, being very specific depending on the modality, route of administration and indication. As an example, cell-based gene therapy would undergo different development paths determined by the type of gene editing technology used, i.e. using viral vectors or CRISPR technology, while vector-based gene therapies would require more standardized approach, focusing on a yield increase and purity to increase the number of doses per batch. In any case, regulatory and GMP requirements are an indispensable part of ATMPs development to be taken into account for the product design. In this talk, two examples of Novartis drug product development for ATMPs will be discussed, (1) a cell-based personalized therapy to treat hematological disorder and (2) an adeno-associated vector (AAV)-based therapy for ophthalmic application. This talk will focus on challenges and opportunities related to ATMPs development strategies.

GENE THERAPY MANUFACTURE

MAGDALENA OBARZANEK-FOJT, Fellow, Drug Product Lead, GDD TRD Biologics & CGT, Novartis (CH)

Gene Therapy is a subclass of the ATMPs (Advanced Therapy Medicinal Products) which aims at delivering genes with a therapeutic function either directly into a cell of human body or via ex-vivo genome-modified human cells. Lentiviral vectors are commonly used to deliver genetic information into a human cells ex-vivo, thanks to their potency to carry a relatively large "cargo". Although in this context the lentiviral vector is not a drug product per se, the manufacturing process needs to follow requirements typical for standard biopharmaceuticals, such as plasmids and master cell bank generation, harvest, purification, buffer exchange, formulation and fill and finish. The production process involves also challenges and a need of innovative solutions to overcome difficulties related to peculiarities of this type modality, such as robustness of plasmid transfection, low in process stability or recovery or poor yield. In addition, it requires fine alignments with gene therapy development and manufacturing to assure timely delivery of therapy to a patient. This talk will focus on lentiviral vector product development and manufacturing.

INTEGRATED ASSESSMENT OF PHARMACOKINETICS FOR NANOMEDICINE DEVELOPMENT

ANDREW OWEN

A robust understanding of pharmacokinetics underpins successful medicine development across the pipeline from drug discovery, preclinical and clinical development, regulatory approval and post marketing. In many cases, nanomedicine development seeks to augment therapies through modification of pharmacokinetics by improving bioavailability, extending plasma half-life or tuning distribution so as to specifically target diseased cells or tissues. Accordingly, the pharmacodynamics of nanotechnology-enabled products, in terms of efficacy and safety, is underpinned by a robust understanding of the pharmacokinetics, and different mechanisms to those with small molecules sometimes apply. Therefore, different mechanisms must be considered for effective nanomedicine development, and this requires a holistic approach to understanding the nanotechnology-components as well as the therapeutic payload itself. The importance of pharmacokinetics from early *in vitro* transporter and enzyme metabolism studies through first-in-human dose prediction and rationalising the exposure-response relationship will be discussed in this introduction to the session.

RAPID DEPLOYMENT OF AN MRNA VACCINE FOR PANDEMIC RESPONSE

DON PARSONS

The SARS-CoV-2 pandemic has highlighted the need for the rapid development of vaccines to counter emerging viral threats. A vaccine based on mRNA offers important advantages in manufacturability and scalability in comparison to traditional vaccine platforms in such a scenario. The mRNA-1273 vaccine candidate was deployed to Phase I human trials with unprecedented speed; and the vaccine is currently in Phase III. Moderna leveraged their personalized vaccine manufacturing infrastructure, developed for production of single-patient neoantigen oncolytic vaccines, to enable supply of Phase I studies. A rapid and extremely substantial scaleup of this technology was required to supply Phase III studies and ultimately, should those trials prove successful, to produce 100's of millions of doses. This talk will address some of the challenges associated with rapid development and scaleup of a nanotechnological drug delivery system.

THE ROAD AHEAD FOR MRNA NANO-FORMULATIONS

DON PARSONS

Nanoformulations for the delivery of mRNA have achieved a significant point of divergence in their history. With the development and deployment of mRNA vaccines against SAR-CoV-2, these formulations will be manufactured at large commercial scale and distributed globally. Pharmaceutical considerations such as cold chain management have emerged as a critical priority. At the same, the potential for these formulations beyond vaccines remains a largely unexplored frontier from a clinical perspective. Use of these formulations for systemic delivery of protein therapeutics has shown promise preclinically; and initial clinical data have been promising. This area shows significant promise for future development.

NANOMEDICINES TO DELIVER DUAL-TARGETING DUAL-ACTION PT(IV) CHEMOTHERAPEUTIC COMPLEXES FOR ENHANCED ANTICANCER ACTIVITY AND REDUCED NEPHROTOXICITY

GIORGIA PASTORIN [a][e], Maria V. Babak, [a][b] Yang Zhi, [a] Bertrand Czarny, [c] JIONG WEI WANG, [d] GERT STORM, [d] MATTHIAS WACKER, [a] Wee Han Ang, [b][e] Dan Gibson, [f]

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Introduction: The discovery of cisplatin as a (Pt)-based anti-cancer drug in 1965 was an important milestone. Nowadays, cisplatin and its analogues, all acting at the nuclear DNA as their biological target, are still used in 40% of all chemotherapy treatments. However, these drugs have toxic side effects (e.g. kidney dysfunction).

Hence, we have conceived a novel dual-targeting platform to ensure strategic delivery of dual-action Pt prodrugs. The dual action stems from Pt-induced DNA damage in combination with "mitochondrial reprogramming" of cancer cells by dichloroacetate (DCA). Interestingly, while combination treatments of Pt anticancer drugs with mitochondrial sensitizers like DCA were reported to be more effective than respective monotherapies, DCA is unable to permeate cells. So, if released outside mitochondria, this small molecule competes with pyruvate for the mitochondrial entry via mitochondrial pyruvate transporter, thereby abrogating its anticancer effect. Therefore, its conjugation to Pt(II) pharmacophores yields Pt(IV) pro-drugs that are more effectively uptaken inside cells due to their increased lipophilicity.

Materials & methods: The first targeting was established by liposomal nanoencapsulation of Pt complexes (including cisplatin and mitochondrial sensitizers) to attain high accumulation [1] at the tumor site. After the release of the Pt prodrug inside cancer cells, a second stage of targeting directed the Pt prodrugs to the mitochondria [2]. Toward that purpose, we prepared two series of non-symmetric cis,cis,trans-Pt(IV) complexes, each containing an axial TPP mitochondria targeting ligand with either cisplatin (CDP) or oxaliplatin (OXP) as pharmacophores, differing only on the nature of the other axial ligand. Upon intracellular reduction, these Pt prodrugs released two bioactive molecules [3], acting on the mitochondrial and on the nuclear DNA [4] (Figure 1, taken from reference 4).

Results: Our Pt system showed low micromolar cytotoxicity in both sensitive and resistant A2780 human ovarian cancer cell line. The incorporation of DCA into a Pt(IV) scaffold improved cytotoxicity up to 5-fold, indicating the beneficial effects of mitochondrial sensitizers on the overall activity of Pt(IV) complexes. Concurrently, all our compounds did not cause ROS induction even at longer time points and were able to induce p53-mediated mitochondrial caspase 3-dependent apoptosis of cancer cells.

The most potent complexes also displayed excellent activity in a colon CT26 adenocarcinoma tumor model in Balb/C mice, characterized by complete tumor remission, accompanied by reduced kidney toxicity [3].

Conclusion: The research findings provide insights into the potential of nanomedicine to reach the diseased area, protect the incorporated bioactive molecules from premature deactivation and enhance delivery of drugs at the mitochondria while decreasing their side effects.

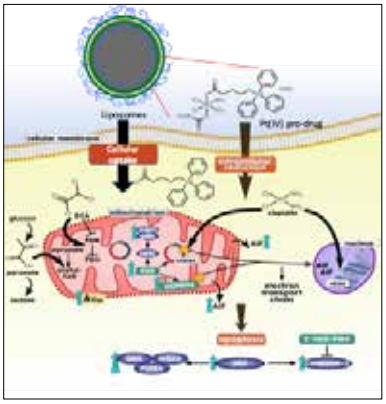

Figure: *Proposed mechanism of action of our Pt(IV) complex.*

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NANOTECHNOLOGY: OVER A DECADE OF PROGRESS IN RESEARCH, REGULATION AND POLICY AT U.S. FDA

ANIL PATRI, Chair, Nanotechnology Task Force and Director, Nanocore, U.S. Food and Drug Administration (FDA), National Center for Toxicological Research (NCTR), Jefferson, AR

The U.S. FDA has made significant progress in learning about nanomaterial and nanotechnology through internal research, collaborations and engagement with other stakeholders. Since the release of the Nanotechnology Task Force report in 2007, FDA has established infrastructure facilities, provided guidance documents to support industry, reviewed and approved many products that utilize nanotechnology, developed policy, facilitated consensus standards development and collaborated with domestic and international regulatory agencies and stakeholders to address knowledge and policy gaps. A summary of these activities is published in a report by the FDA in July 2020 and will be presented.
Disclaimer: The views expressed in this presentation do not necessarily represent those of the U.S. Food and Drug Administration

MANIPULATING CELLS' FUNCTION WITH NOVEL LIPID NANOPARTICLES: FROM RNA THERAPEUTICS TO GENOME EDITING

DAN PEER

Accumulating work points out relevant genes and signaling pathways hampered in human disorders as potential candidates for therapeutics. Developing nucleic acid-based tools to manipulate gene expression, such as siRNAs, mRNA and genome editing strategies, open up opportunities for personalized medicine. Yet, although major progress was achieved in developing RNA targeted delivery carriers, mainly by utilizing monoclonal antibodies (mAbs) for targeting, their clinical translation has not occurred. In part because of massive development and production requirements and high batch-to-batch variability of current technologies, which relies on chemical conjugation. Here I will present a self-assembled modular platform that enables to construct theoretically unlimited repertoire of RNA targeted carriers. The platform self-assembly is based on a membrane-anchored lipoprotein, incorporated into RNA-loaded novel lipid nanoparticles that interact with the antibody Fc domain. We show that a simple switch of 8 different mAbs, redirects specific uptake of siRNAs by diverse leukocyte subsets *in vivo*. The platform therapeutic potential is demonstrated in an inflammatory bowel disease model, by targeting colon macrophages to reduce inflammatory symptoms, and in Mantle Cell Lymphoma xenograft model, by targeting cancer cells to induce cell death and improve survival. In addition, I will discuss novel approach for delivering modified mRNA to specific cell types *in vivo* utilizing this platform and Cas9 mRNA combined with sgRNA and will show *in vivo* highly efficient Genome Editing with therapeutic benefit in cancer rodent models. This modular delivery platform can serve as a milestone in turning precision medicine feasible.

TALINEUREN: A REGENERATIVE NANODRUG AGAINST NEURODEGENERATION

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Talineuren (TArgeted LIposomal NEUro REgenerati**o**N) is an innovative liposomal formulation of the glycosphingolipid GM1 ganglioside. A number of highly desirable pharmacological effects have already been described for GM1, such as the general promotion of neuronal survival, dendritogenesis, axon sprouting, interaction and stimulation of neurological growth factors, immuno-modulation, and potentially disease-modifying effects in Parkinson's disease. Treatment with GM1 has been documented to be very well tolerated and effective. However therapeutic breakthrough has been hampered by the necessity of high-frequency repeated injections and the limited availability of the molecule at the required sites in the body, e.g. the brain.
Talineuren has been designed to address the issues of repeated drug administration and GM1 biodistribution. A couple of key parameters of the liposome act in synergy leading to altered behaviour of the liposomal formulation compared to the free compound. In one peculiarity, the phospholipids used in Talineuren produce a natural long-circulation behaviour, omitting the need of surface PEGylation, and simultaneously favouring uptake by the CNS. Mechanisms causing this uptake are subject of ongoing investigation. Talineuren's small diameter is 36 nm and may positively influence the endocytotic uptake in CNS, but might also uptake into other tissues and clearance organs. In stark contrast to the current theory of ligand-targeted nanomedicines, Talineuren's biodistribution seems to be only marginally affected by the addition of GM1, even though the zeta potential drops to-42mV after insertion of GM1 and despite the fact that the surface exposed glycan-moiety of GM1 has plenty of possible, highly abundant binding partner receptors in the body such as galectin-1, galectin-3, factor H, NGF, TrkA, or also alpha-synuclein and amyloid-beta.
To date Talineuren has been investigated in a number of *in vitro* and *in vivo* studies. In cell culture, Talineuren leads to enhanced neuronal survival. This effect can be amplified by the addition of neuronal growth factors. These findings are in line with previously published effects of non-liposomal GM1. In vivo, Talineuren leads to elevated dopamine levels in the brains of MPTP-treated mice, a model of Parkinson's disease. It is particularly noteworthy that the dose level of GM1 could be reduced by a factor of 4 in Talineuren to 7.5 mg/kg compared to injected forms of free GM1 dosed at 30 mg/kg and that therapeutic efficacy was still superior in the Talineuren group compared to free GM1 even at reduced doses. Moreover, it was possible to administer Talineuren via the oral route, bypassing the need for subcutaneous or intravenous injections. Clinical translation of Talineuren to treat Parkinson patients is ongoing and expected to start in 2021. Unlike the majority of therapeutic approaches in Parkinson, Talineuren does not address a specific mechanism of disease but instead aims for multipronged stimulation of neuronal survival. Trials of *in vivo* efficacy of Talineuren in Huntington's disease are ongoing. Biodistribution studies with fluorescently labelled Talineuren liposomes show an intriguing case where unprecedented amounts of liposomes reach the CNS and distribute to different areas of the brain, including but not limited to the substantia nigra that is particularly affected by Parkinson's disease. If clinical trials are successful, Talineuren could become a dogma changing medicine and make disease-modifying therapy in neurodegenerative conditions like Parkinson possible.

NITRIC OXIDE-DEPENDENT BIODEGRADATION OF GRAPHENE OXIDE REDUCES INFLAMMATION

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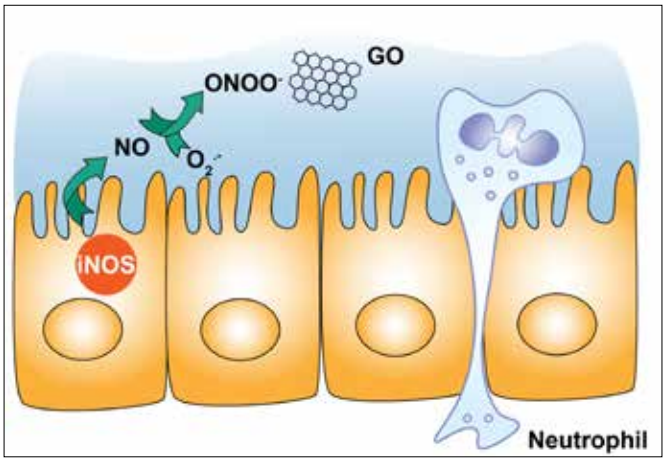
Understanding the biological fate of graphene-based materials is crucial to assess their potential risk for humans following unintended exposure [Fadeel et al. ACS-Nano. 2018;12(11):10582-620]. Here, we explored the biodegradation of graphene oxide (GO) following oral exposure in zebra fish. The lateral dimensions of GO were less than 20 µm and the thickness around 5 nm, as determined by transmission electron microscopy (TEM) and atomic force microscopy (AFM), respectively. Using Raman confocal microscopy, we were able to provide evidence of biodegradation of GO in the gastrointestinal tract 24 h after oral exposure of zebra fish embryos (5 dpf). The *in vivo* biodegradation was blocked upon downregulation of nos2a encoding the inducible nitric oxide synthase (iNOS) by using morpholino oligonucleotides, or by pharmacological inhibition of iNOS using L-NAME, suggesting that the biodegradation was nitric oxide (NO)-dependent. Furthermore, NO and peroxynitrite generation in the gut were observed using DAF-FM-DA and DAX-J2 PON fluorescent probes, respectively. We then confirmed peroxynitrite-dependent degradation of GO *in vitro* by combining a superoxide-generating system, xanthine oxidase/xanthine (XO/X), with a NO donor (PAPA-NONOate), or by simultaneously producing superoxide and NO by decomposition of SIN-1. Degradation of GO was evidenced by TEM, AFM, and Raman spectroscopy. Finally, by using transgenic zebrafish Tg(mpx:eGFP), we could show that inhibition of biodegradation of GO induced more neutrophil infiltration into the gut, indicating that biodegradation served to reduce the inflammatory response triggered by GO. In sum, our findings suggest that GO is biodegradable both *in vitro* and *in vivo* through a nitric oxide-dependent pathway.



Nitric oxide (NO)-dependent degradation of GO. This schematic shows iNOS-dependent generation of NO leading to the generation of peroxynitrite and, subsequently, to biodegradation of GO.

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NOVEL STRATEGIES TO MODULATE TUMOR STROMA TO ENHANCE TUMOR PENETRATION

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Most solid tumors encompass abundant amount of tumor stroma which consists of cancer associated fibroblasts (CAFs), extracellular matrix (ECM) and various immune cells. The tumor stroma is known not only promote the tumor growth and metastasis but also for acting as a barrier to the penetration of chemotherapeutic agents [(1)]. In certain tumor types such as pancreatic ductal adenocarcinoma (PDAC), tumor stroma can compose up to 90% of the whole tumor mass. The deposition of extensive ECM within the tumor stroma compresses tumor vasculature thereby reducing the tumor perfusion as well as itself acts as a barrier for penetration. CAFs are the main producer of ECM. In recent years, we have designed several strategies to tackle the tumor stroma by targeting CAFs and thereby induced the penetration of chemotherapeutic agents.
Recently, we have identified a novel integrin target (ITGA5) in CAFs in pancreatic patient tumors, which was found to be linked to the poor overall survival of patients with PDAC (2). By knocking down ITGA5 in CAFs, we showed that CAFs lost their capacity to produce ECM and contract. Furthermore, co-injection of tumor cells (PANC-1) and CAFs (ITGA5 knockdown) showed reduced tumor growth compared to PANC-1 and CAFs in SCID mice. Thereafter, we designed an ITGA5 antagonizing peptide AV3 which deactivated patient-derived CAFs by inhibiting reducing α-SMA, ECM production (collagen, fibronectin) and contraction.
In vivo, we examined the effect of AV3 in two different co-injection tumor models (PANC-1 + pancreatic stellate cells (PSCs)) and (MIAPaCa + PSCs) as well as patient-derived xenograft (PDX) tumor models. Furthermore, we found that combination of AV3 with gemcitabine enhanced the anti-tumor effect of gemcitabine leading to reduction of 80% tumor growth. To investigate the mechanism of enhanced effect, we examine the effect of AV3 on tumor stroma and tumor accumulation. We found that treatment with AV3 significantly reduced the collagen deposition in all models. Also, we examined the accumulation of indocyanine dye (ICG) in mice after the treated with AV3 compared to control mice. We found that there was an enhanced accumulation of ICG in tumors at 24h in the AV3 treated mice which was visible in all tumor models. These data indicated that AV3 can reduce tumor stroma and thereby enhance the tumor perfusion of gemcitabine and enhanced the therapeutic efficacy [(2)].
In other studies, we have targeted human relaxin (RLN2) and FGF2 using superparamagnetic nanoparticles (SPION) to tumor stroma [(3, 4)]. Both RLN2 and FGF2 are endogenous biologicals and have been shown to elicit anti-fibrotic effects. We conjugated these biologicals to SPION using covalent conjugation. RLN2-/FGF2-SPION showed higher anti-stromal effects *in vitro* in TGF-β activated patient-derived PSCs representing CAFs and enhanced the effect of gemcitabine either in 3D tumor heterospheroid models (FGF2-SPION) or *in vivo* co-injection models (RLN2-SPION).
In conclusion, we have demonstrated that modulation of tumor stroma by inhibiting CAFs leads to reduced ECM deposition and contraction, thereby enhance the effect of chemotherapy in pancreatic tumor models.

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TRANSPARENCY, REPRODUCIBILITY AND TRANSLATION OF NANOMEDICINE! TACKLING THE COMPLEXITY

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The measurement of physical-chemical properties of complex biological samples is critical to assess their characteristics and product qualities at pre-clinical and clinical level, respectively.
The scientific and regulatory communities have been active in developing and promoting combined multiple orthogonal approaches to perform robust characterisation of complex biological samples.
In this work complementary approaches and insight on the challenges and limitations of the different techniques are presented.
Consideration on the increasing need to measure size and concentration in physiologically relevant media and the importance of standards, as suitable reference materials are also emphasized since these are critical for the translation and evaluation of the next nanomedicines clinical products.

AN RNA VACCINE DRIVES EXPANSION AND EFFICACY OF CLAUDIN-CAR-T CELLS AGAINST SOLID TUMORS.

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Chimeric antigen receptor (CAR)–T cells have shown efficacy in patients with B cell malignancies. Yet, their application for solid tumors has challenges that include limited cancer-specific targets and nonpersistence of adoptively transferred CAR-T cells. Here, we introduce the developmentally regulated tight junction protein claudin 6 (CLDN6) as a CAR target in solid tumors and a strategy to overcome inefficient CAR-T cell stimulation *in vivo*. We demonstrate that a nanoparticulate RNA vaccine, designed for body-wide delivery of the CAR antigen into lymphoid compartments, stimulates adoptively transferred CAR-T cells. Presentation of the natively folded target on resident antigen-presenting cells promotes cognate and selective expansion of CAR-T cells. Improved engraftment of CAR-T cells and regression of large tumors in difficult-to-treat mouse models was achieved at subtherapeutic CAR-T cell doses.

RESULTS FROM CLINICAL PHASE 1 AND 2 EVALUATIONS OF CPC634

CRISTIANNE RIJCKEN

Cristal Therapeutics is a clinical stage company developing innovative products for improved treatments of cancer patients or other diseases with a high medical need. Cristal Therapeutics' proprietary platform, CriPec, address the current lack of insufficient target efficacy by enabling selective tissue targeting of therapeutic agents upon intravenous administration via prolonged pharmacokinetics and improved biodistribution.
In cancer treatment, CriPec nanomedicines improve the pharmacokinetic profile and enhance tolerability via focusing the drug on the solid tumour, and enabling longer exposure over current treatment options available in the clinic. The release profiles are fully tuneable, whilst application via various routes of administration for a range of indications are possible. The use of targeting ligands on the CriPec® surface further increases selectivity for target cells. The versatile nanoparticulate platform can be combined with small molecules (including traditional chemotherapeutics), peptides and oligonucleotides. The manufacturing of CriPec® nanomedicines is a straightforward chemical process, including low cost of goods, large (GMP) scale production procedures already in place and option for freeze-drying.
The lead product, CPC634 (a CriPec® entrapped version of the widely used chemotherapeutic docetaxel), is in clinical phase 2 studies for the treatment of solid tumours. The clinical data so far have convincingly demonstrated the prolonged systemic circulation, the improved safety profile and the significantly enhanced tumour uptake as compared to the conventional docetaxel. Based on this clinical validation, Cristal Therapeutics is co-developing innovative nanomedicines in combination with biotech and pharma partners.
Cristal has in depth expertise on the rational design of nanomedicines, the early stage chemistry, the actual nanoparticle formulation, and in-depth analytical characterisation of the starting materials, intermediates, and final drug products. In addition, there is extensive *in vitro* and *in vivo* evaluation expertise, namely, to monitor either pharmacokinetic/biodistribution profiles, evaluate the safety and assess the efficacy profile, of nanomedicine compositions versus native drugs and other relevant controls. We have in depth expertise into GMP upscaling and manufacturing, a manufacturing license to release material for clinical trial use, experience with regulatory guidelines incl. consultation with competent authorities, clinical CRO selection and a Q system installed, to control all essential elements of drug development into the clinical space.
In addition, Cristal Therapeutics has developed a proprietary CliCr® as a superior click chemistry reagent with advantageous physicochemical properties providing fast access to desired stable conjugated products with high conversion rates. The technology allows conjugation of molecules to our CriPec® nanoparticles. The unique versatility of CliCr® enables its use in a wider range of areas spanning from therapeutics, nanomedicine, biomaterials all the way to diagnostics. CliCr® has as novel click chemistry reagent significant advantages over other marketed reagents in relation to conversion kinetics, stability, and applicability.
In the current corona pandemic, CriVac® allows to generate almost perfect non-viral mimics of the SARS-CoV2 virus particles and offers excellent possibilities for optimization of the immunogenic response. CriVac® is a unique antigen carrier platform based on CriPec® nanoparticle (which evidently lacks infectious ability), a size resembling a virus and whereby the desired numbers of antigen displayed is controlled via CliCr®. CriVac® particles have been shown to efficiently drain and accumulate to the lymph nodes upon subcutaneous injection and are taken up and processed by relevant immune cells there. CriVac® mimicks features of a live virus in a tailored manner to induce immunity safely and efficiently, offering a prophylactic vaccination strategy which will be readily adaptable to different pathogenic treats.
The main clients from Cristal Therapeutics comprise global biotech/pharma companies working in oncology, chronic inflammatory diseases, infectious diseases and other medical needs. Cristal Therapeutics is actively looking for collaborations to combine their

APIs with CriPec®, CliCr® and/or CriVac®, and to co-develop innovative pharmaceutical products towards clinical translation.
Cristal Therapeutics has raised more than € 20 million in private equity and more than € 10 million non-dilutive funding. The company is led by a management team of entrepreneurial life science executives and supported by an industry-experienced Supervisory Board and internationally renowned Scientific Board.

DETERMINING WHAT REALLY COUNTS: MODELING AND MEASURING NANOPARTICLE NUMBER CONCENTRATIONS (PNC)

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The issue concerning which dose metric to use in nanotoxicological studies (i.e., whether to use the mass, particle number, or surface area concentration to assess the response of cells or organisms to NP exposure) has been a topic of debate since nearly the beginning of the nanotoxicology field. While measuring a mass concentration for dissolved organic and inorganic substances is linearly related to their number concentration, the situation is more complex for NPs. Unlike dissolved chemicals, NPs have a distribution of sizes and shapes; they may undergo changes in test media such as dissolution or agglomeration; and the conversion from a mass- or surface area-based concentration to a number-based concentration requires a more complex formula than a simple linear correlation. Although the mass concentration is the most widely reported metric for the exposure concentration in nanoecotoxicological research, some studies have suggested that alternative dose metrics such as the surface area-based or a particle number concentration (PNC)-based metric may more accurately reflect the toxicological response observed. In addition, recent research efforts have also been made to evaluate the size distribution of NPs associated with test organisms after exposure and the PNC of the organisms' body burden after extraction from the tissue and resuspension into a liquid media.
In addition to their importance in nanotoxicology, PNC measurements also have regulatory importance. For example, the use of a PNC as the metric in some geographical locations such as the European Union (e.g., 50 % of the particles between 1 nm and 100 nm) has been proposed for determining if a substance is labelled as containing NPs.39 In addition, one key consideration for the use of OECD ecotoxicology test guidelines with NPs is what dose metric to use when evaluating if the change in the exposure concentration has exceeded the limit of ± 20 %.

One of the principle challenges in determining the PNC of NPs suspended in aqueous media using size distribution measurements is that different analytical procedures can give varying results. This stems partly from the potential for even a small number (1 %) of NP agglomerates to shift the whole size distribution to larger particle sizes for some techniques such as nanoparticle tracking analysis (NTA) and dynamic light scattering (DLS), given that these techniques are much more sensitive to larger particles. Other techniques such as single particle inductively coupled plasma-mass spectrometry (spICP-MS) would count agglomerates as part of the tail toward larger particles, but this would be unlikely to shift the full size distribution. In addition, NP size measurement techniques also measure slightly different properties of the NPs with some measuring only the NP core diameter (e.g., scanning electron microscopy (SEM) and spICP-MS), while other techniques (e.g., DLS and NTA) also measure the hydrodynamic diameter, which includes the NP surface coating (if the NP is being stabilized) and hydrated water ions. Therefore, previous studies typically show that DLS and NTA results yield larger diameters for NPs than results from other techniques that only measure the NP core. When converting from the measured size to the NP number concentration, it is unclear to what degree these size differences would impact the PNC. For some techniques that directly measure the NP core, there are also limitations such as low throughput analysis and challenges with sample deposition for SEM analysis. Some techniques also directly measure the NP number concentration such as spICP-MS, NTA, and potentially differential mobility analysis (DMA). However, it is unclear to what degree these direct PNC measurements agree among techniques or with PNC values derived using NP size measurements.

There have been several studies that have compared PNC measurements across laboratories for the same initial NPs using a single technique. Among studies utilizing spICP-MS, one study was performed by a post hoc analysis of previously published spICP-MS data for the National Institute of Standards and Technology (NIST) reference material (RM) 30 nm and 60 nm gold nanoparticles (AuNPs), and two other studies assessed silver nanoparticles (AgNPs) in food simulants or after addition to chicken meat. An interlaboratory comparison has also been conducted on polystyrene NPs and 30 nm AuNPs using NTA. In the spICP-MS interlaboratory comparison of AuNP results, the PNC recoveries for the 60 nm AuNP ranged between 63.9% and 99.95%, while the PNC recoveries for the 30 nm AuNP ranged between 14.8% and 102.2%, suggesting that larger NPs may yield better recoveries. Results for AgNPs yielded an even broader range with the average recovery (after removal of outliers) ranging between 0.6% and 39% compared to the expected values from the manufacturer. This result that could stem from numerous factors including particle dissolution, losses from adsorption to the containers, and how the transport efficiency was calculated. However, there has not yet been a comparison among techniques for measuring PNCs.

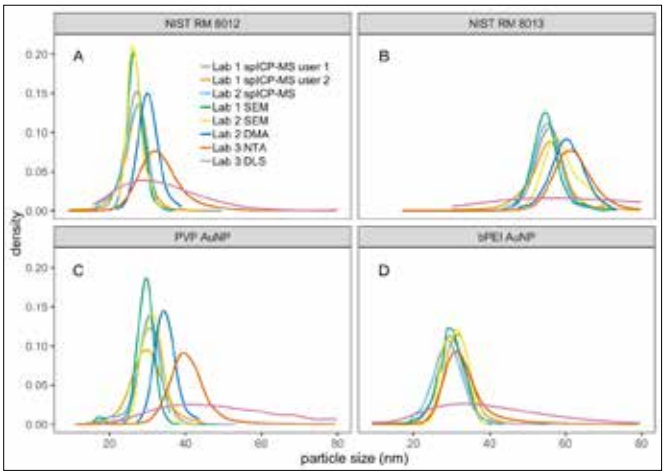

Figure 1 – *Kernel density plots for NIST RM 8012 (A), NIST RM 8013 (B), PVP AuNP (C), and bPEI AuNP (in plastic vials) samples (D). Values are provided for spICP-MS, DMA, NTA, SEM, and DLS. Results are not reported for the DMA analysis of the bPEI sample because of challenges with analyzing this sample.*

In this study, we conducted a multi-technique and multi-laboratory study to investigate the comparability of PNC results for four AuNPs. To minimize variability that could result from NP dissolution, matrix effects from complex aqueous matrices, or agglomera-

tion as a result of a high ionic strength media, a simple scenario was evaluated, namely AuNPs in water. Four monodisperse AuNPs were tested: two NIST RMs (8012 and 8013) and two commercially available AuNPs with different surface coatings which impacted the surface charge (positively-charged branched polyethyleneimine (bPEI) and negatively-charged polyvinylpyrrolidone (PVP)). Three samples were negatively charged: citrate-stabilized, AuNPs NIST RM 8012 and RM 8013 with nominal diameters of 30 nm and 60 nm, respectively, and the PVP AuNPs, while the bPEI AuNPs were positively charged. Because the measured mean values and shape of the size distributions were found to vary among techniques, statistical analysis was performed to understand the impact of variations in these and other parameters on the derived PNC results. The size distributions measured by the different techniques were also used to model the AuNP concentration that would reach the cells in an *in vitro* toxicity experiment, and approach that has been used to evaluate the toxicological effects of NPs on, for example, human macrophage and alveolar epithelial cells.

E. J. Petersen, A. R. Montoro Bustos, B. Toman, M. E. Johnson, M. Ellefson, G. C. Caceres, A. L. Neuer, Q. Chan, J. W. Kemling, B. Mader, K. Murphyb and M. Roesslein; Determining what really counts: modeling and measuring nanoparticle number concentrations, Environ. Sci.: Nano (2019) DOI: 10.1039/c9en00462a

WHAT CELLS CAN DO WITH NANOMATERIALS

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Engineered nanomaterials have unique properties for many applications and a better understanding of cellular effects upon direct exposure of (human) cells to these materials is prerequisite for their safe-by-design and successful use in any applications, including biomedicine, where targeting efficacy and low side effects are relevant. It is well described in the literature that the physico-chemical properties of nanomaterials such as size, shape, material and surface functionalization, strongly influence cellular uptake (Kinnear et al., 2017). However, their subsequent release, intracellular degradation of the materials, transfer to other cells, or translocation across tissue barriers are still poorly understood. The involvement of these cellular clearance mechanisms strongly influences the long-term fate of the nanomaterials used, especially when repeated exposures are taken into account (Bourquin et al., 2018). The aim of this presentation is to give an overview of uptake mechanisms, intracellular fate and the possible mode of clearances and to address the critical points that need to be considered for future research.

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[1]ENTRY OF NANOPARTICLES INTO CELLS: MECHANISMS AND CONSEQUENCES

KIRSTEN SANDVIG

Nanoparticles can be used to deliver drugs or other substances both *in vivo* and *in vitro* [2-4]. To enter cells the particles exploit the endocytic machinery, and they have been demonstrated to induce changes in cellular uptake and intracellular transport [5,6]. In the case of nondegradable particles the size may be of importance for blocking membrane traffic to different parts of the cell, and for degradable particles the degradation products may stress the cell and cause secondary changes in cell physiology. Crosslinking of cell surface molecules may cause signaling in cells [7], and nanoparticles have been found to induce macropinocytosis that facilitates uptake of particles. In several instances this process has been shown to be dependent on the large GTP-binding protein dynamin. To optimize nanoparticle delivery into cells one needs to understand the cellular mechanisms involved in their uptake. Such information may help in deciding the type of particle to use, the size of the particle as well as which components to include at the particle surface. Today we know that cells have different types of endocytic mechanisms [8], some giving rise to small vesicles (60-200 nm diameter), whereas other mechanisms such as macropinocytosis are required for uptake of larger particles. One should be aware of that cells growing in a polarized manner are likely to have endocytic mechanisms which are under differential influence of signaling substances at the two poles [9], and studies of nanoparticle uptake in nonpolarized cells may not give the same results as if uptake in polarized cells is investigated. It may be an advantage to study different cell types as their response to a given nanoparticle may vary, and one should be aware of that even apparently small differences in particle composition may contribute to different types of cell death [10]. Furthermore, increased cell density may induce changes in membrane lipids and intracellular transport [11], and modification of membrane lipids may change the mechanisms of uptake [12]. Clearly, well controlled conditions for the cell experiments performed and correct interpretation of the results obtained from cellular studies are essential. For instance, cholesterol is often mistaken for only being important for caveolar uptake, but is involved in several endocytic processes including micropinocytosis [3,8]. Also, robust methods to determine whether a particle is internalized or only at the cell surface are important to provide the investigator with correct data about uptake efficiency, and a challenge is that the drug in the nanoparticle often has to reach the cytosol or the nucleus to exert its action.

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TUMOR EXOSOME-BASED NANOPARTICLES AND ARTIFICIALLY CLOAKED VIRAL NANOVACCINES FOR CHEMO-IMMUNOTHERAPY APPLICATIONS

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The recent cutting-edge advances on nanomaterials is anticipated to overcome some of the therapeutic window and clinical applicability of many drug/peptide molecules and can also act as innovative theranostic platform and tool for the clinic in the future [1-4]. In the last decade, research on cancer immunotherapy resulted in a new set of potential treatments with promising results in the clinics [5-8]. Among these, immune checkpoint inhibitors are one of the few immunotherapies that have been clinically validated, yet with variable results, ranging from complete responses to hyperprogression. Amongst the different experimental treatments, active cancer immunotherapy hold great promises for the future. In this work, prominent nanosystems, such as biohybrid nanocomposites made of different nanoparticles (porous silicon and oncolytic virus) and cancer cell-based membrane materials are presented and discussed as potential platforms for the individualization of medical intervention and cancer immunotherapy applications. Examples on how these biohybrid nanomaterials can be prepared and scaled-up, as well as how they can be used to enhance the drug's targetability, intracellular drug delivery for both cancer chemo- and immunetherapy applications, will be highlighted and discussed. Overall, our results suggest that biohybrid nanomaterials are a versatile and advanced platform for cancer treatment with an interesting potential for present and future clinical impact given its easy tailorability to each patient, choosing a suitable inorganic or virus and obtaining cancer cells from biopsy.

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MOLECULAR BIOENGINEERED NANOMEDICINES FOR TARGETED DELIVERY OF PEPTIDES IN MODULATION OF DIABETES

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Diabetes mellitus is a metabolic and chronic disease in which the pancreas does not produce enough insulin, or the body cannot effectively use the insulin it produces. Most current treatments for type 1 diabetes mellitus, predominant in childhood, are invasive and painful and suffer from poor patient compliance, which contributes to a leading cause of mortality and morbidity. To maintain patient compliance, oral administration is the preferred drug delivery route. Yet, many drugs, including biopharmaceuticals do not endure the harsh environment of the gastrointestinal tract and cross epithelial barriers inefficiently. Functionalization of nanoparticles with ligands that can bind specifically to surface receptors may be one strategy to overcome these obstacles and to improve the delivery of biopharmaceuticals through biological barriers, as the intestine.
We have been explored mucodiffusive functional, multistage nanoparticles, engineered with innovative ligands to binding specific receptors, with potential to overcome the physical mucus barrier and mediating the transport across polarized epithelial cells.
Different monodisperse nanomedicines of 200 nm in size range, loaded with insulin, GLP-1 or GLP-1 analogues, have been developed by our group. Those nanoparticles have been modified to address specific targets, and pre-validated in well-establish *in vitro* cell-based models. When orally administered to diabetic animal models, a reduction of glycemia has been measured as a function of receptor targeting, with pharmacodynamics dependent on release kinetics.
In this talk, it will be discussed our recent advances in decorated nanoparticles for improved oral bioavailability of antidiabetic peptides.

STUDYING SMALL RNA TRANSFER WITH EXTRACELLULAR VESICLES

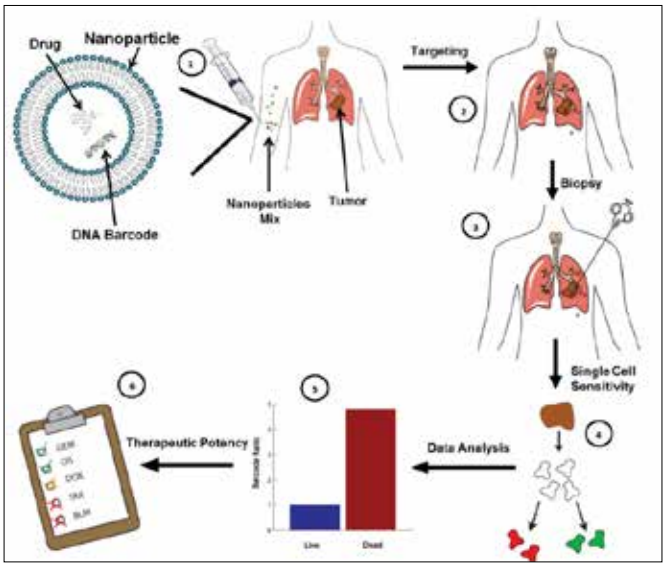
RAYMOND M. SCHIFFELERS, Olivier G. de Jong, Daniel E. Murphy, Imre Mäger, Eduard Willms, Antonio Garcia-Guerra, Jerney J. Gitz-Francois, Juliet Lefferts, Dhanu Gupta, Sander C. Steenbeek, Jacco van Rheenen, Samir El Andaloussi, Matthew J. A. Wood & Pieter Vader

Extracellular vesicles can transfer biological cargo between cells, including RNA. Up to now it has been difficult to study the biological processes that are involved in the delivery of small RNAs. I will discuss a highly-sensitive CRISPR-Cas9-based reporter system. Using this CRISPR operated stoplight system for functional intercellular RNA exchange (CROSS-FIRE), functional delivery of an sgRNA results in a color change of cells. This enables direct visualization of EV-mediated transfer of small non-coding RNA molecules at single-cell resolution.

BARCODED NANOPARTICLES FOR PRECISION CANCER MEDICINE: TARGETING PANCREATIC TUMORS WITH NANOTECHNOLOGY

AVI SCHROEDER, Associate Professor of Chemical Engineering, Technion – Israel Institute of Technology, Haifa 32000, ISRAEL; avids@technion.ac.il

Medicine is taking its first steps towards patient-specific cancer care. Nanoparticles have many potential benefits for treating cancer, including the ability to transport complex molecular cargoes including siRNA and protein, as well as targeting to specific cell populations.
The talk will discuss 'barcoded nanoparticles' that target sites of cancer where they perform a programmed therapeutic task. Specifically, liposomes that diagnose the tumor and metastasis for their sensitivity to different medications, providing patient-specific drug activity information that can be used to improve the medication choice.
The talk will also describe how the liposomal lipid composition can be used as multi-functional systems for degrading the pancreatic stroma to allow subsequent drug penetration into pancreatic adenocarcinoma, and how the nanoparticle configuration can be leveraged to induce an anti-tumor immune response.
The evolution of drug delivery systems into *synthetic cells*, programmed nanoparticles that have an autonomous capacity to synthesize diagnostic and therapeutic proteins inside the body, and their promise for treating cancer and immunotherapy, will be discussed.



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ADVANTAGES AND LIMITS OF ARTIFICIAL INTELLIGENCE (AI)

STEFAN SCHULZ

Today, AI mostly means machine learning . Can it learn from "real-world data" for the benefit of health care and medical research? AI has proved useful in image diagnosis and the processing of clinical language. Intelligent systems predict future events and support clinical decisions. AI can also improve the interface between clinicians and computers.
Yet there are limitations: large amounts of training data are needed, but shared data are difficult to construct due to patient privacy. Clinicians want to understand the rationale behind AI-based recommendations - they don't want black boxes. Systems are often not portable: AI trained in one hospital gets in trouble in a place with different data infrastructures and workflows. AI is often expected to revolutionize research: real-world data analytics is valuable for generating scientific hypotheses, but AI cannot substitute prospective studies .
Not to forget that traditional AI has provided logics-based standards like SNOMED CT. They allow, e.g., retrieving a patient with follicular lymphoma treated with Rituximab, when searching for NHL patients treated with monoclonal antibodies. Clinical data enhanced by standards are interoperable, reusable and thus of increased value also for training AI systems.

DELIVERY OF ANTI-CANCER STEM CELL DRUGS IN COLORECTAL METASTATIC CANCER

SIMO SCHWARTZ

Colorectal cancer (CRC) is a highly prevalent disease worldwide. Patient survival is hampered by tumor relapse and the appearance of drug-resistant metastases, which are sustained by the presence of cancer stem cells (CSC). Specific delivery of anti-CSC chemotherapeutic drugs to tumors by using targeted drug delivery systems that can also target CSC sub-population might substantially improve current clinical outcomes. CD44v6 is a robust biomarker for advanced CRC and CSC, due to its functional role in tumorigenesis and cancer initiation process. Here, we show that CD44v6-targeted polymeric micelles (PM) loaded with niclosamide (NCS), an anti-CSC drug, is a good therapeutic strategy against colorectal CSC and circulating tumor cells *in vivo*. HCT116 cells were sorted according to their CD44v6 receptor expression into CD44v6+ (high) and CDv44v6– (low) subpopulations. Accordingly, CD44v6+ cells presented stemness properties, such as overexpression of defined stemness markers (ALDH, CD44c3 and CXCR4) and high capacity to form colonspheres in low attachment conditions. NCS-loaded PM functionalized with an antibody fragment against CD44v6 (Fab-CD44v6) presented adequate size, charge, and encapsulation efficiency. In addition, Fab-CD44v6 significantly increased PM internalization in CD44v6+ cells. Further, encapsulation of NCS improved its effectiveness *in vitro*, particularly against colonspheres, and allowed to increase its intravenous dosage *in vivo*. Remarkably, functionalized PM accumulate in tumors and significantly reduce circulating tumor cells *in vivo*. In conclusion, CD44v6 targeted PM meet the essential conditions to become an efficient anti-CSC therapy.

USE OF CLICK CHEMISTRY TO PREPARE ORIENTATED DISPLAY OF ANTIBODIES ON NANO-MEDICINES

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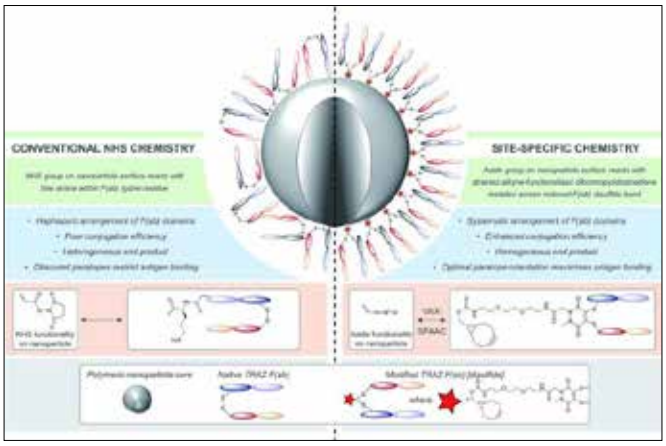
2 Department of Chemistry, University College London.

The successful development of targeted nanotherapeutics is contingent upon the conjugation of therapeutic nanoparticles to target-specific ligands, with particular emphasis being placed on antibody-based ligands. Thus, new methods that enable the covalent and precise installation of targeting antibodies to nanoparticle surfaces are greatly desired, especially those which do not rely on costly and time-consuming antibody engineering techniques. In this presentation we will discuss approaches for the highly controlled and oriented covalent conjugation of antibodies to nanoparticles using disulfide-selective pyridazinedione linkers and strain-promoted alkyne–azide click chemistry. This new approach paves the

way for the development of antibody-targeted nanomedicines with improved paratope availability, reproducibility and uniformity to enhance both biological activity and ease of manufacture.



COMPUTERIZED MEDICAL SYSTEMS, PREDICTIVE ANALYTICS AND BIG-DATA INFORMATICS FOR PRECISION MEDICINE

VARDA SHALEV is the managing director of the KSM research and Innovation Institute at Maccabi healthcare Services.

Maccabi is one of the world's largest healthcare providers with 2.5 million patients, representing 25% of the Israeli market with a nationwide representative distribution. Maccabi has long been recognized, both in Israel and abroad, as a unique and innovative healthcare system.

Since 1993, Maccabi has implemented electronic medical records (EMRs), documenting complete data on each patient in a central database. This comprehensive, systematic, high-quality database of a stable population of patients across their lifetime (less than 1% rate of attrition), is one of KSM's key assets.

The Ksm research and innovation institute is one of the world's finest medical research institutes. The institute's team of senior scientists, availing themselves of Maccabi's extensive pool of high quality data, work round the clock to offer knowledge-based solutions for caregivers, physicians, insurers, research centers, pharmaceutical companies, and health organizations worldwide. Their insights and revelations enable many of the dramatic leaps and bounds seen in today's real-world medicine and patient care.

This one-stop-shop research facility answers client needs in myriad areas, through advances in epidemiological research, clinical trials, and medical technology innovation.

In her speak, Prof. Shalev will tell the stories of the establishment of the institute and many of the companies we cooperate with is medical fields ranging from symptom checkers through medical imaging and even voice biomarkers- all based on the KSM research activities and Maccabi healthcare services unique data assets.

EFFECTIVE STRATEGIES FOR TISSUE SPECIFIC CRISPR/CAS GENE EDITING USING SYNTHETIC NANOPARTICLES

DANIEL J SIEGWART

CRISPR/Cas (clustered regularly interspaced short palindromic repeat / CRISPR-associated protein (Cas)) is a revolutionary gene editing technology with wide-ranging utility. The safe, non-viral delivery of gene editing components would greatly improve future therapeutic utility, including for correction of mutations that cause genetic diseases. I will present and report the development of synthetic nanoparticles that are able to achieve high levels of gene editing in targeted organs and cells. The chemical foundation underlying non-viral carriers will be presented, including materials such as zwitterionic amino lipid (ZAL)-based lipid nanoparticles (ZNPs),[1] degradable dendrimer-based lipid nanoparticles (DLNPs),[2-5] and Selective ORgan Targeting (SORT) nanoparticles.6 These nanoparticles are uniquely able to deliver long nucleic acids (Cas9 mRNA, targeted sgRNA, donor DNA) or Cas9 ribonucleoprotein (RNP) complexes with sgRNA (+/- donor DNA) to enable gene knockout and correction.[1, 6] Delivery of low sgRNA doses (15 nM) can reduce protein expression by >95% in cells. In contrast to transient therapies (e.g. RNAi-mediated mRNA degradation),[2, 7-9] we show that LNP delivery results in permanent DNA modification, where the >95% decrease in protein expression is sustained indefinitely even after multiple rounds of cellular division *in vitro* and *in vivo*. In mice, intravenous co-delivery of Cas9 mRNA and sgTom1 permanently induced expression of floxed tdTomato in the liver, kidneys, spleen, and lungs of genetically engineered mice with precise organ selectivity. Correction of a mutation causing Duchenne muscular dystrophy (DMD) via an exon skipping approach will be highlighted as a functional application of CRISPR/Cas in muscle. We have also been able to create organ-specific tumor models in the lungs and livers of mice. In addition, I will show how co-delivery of Cas9 mRNA and sgPCSK9 by liver SORT LNPs enabled complete ~100% knockout of serum and protein levels of PCSK9, a therapeutically attractive target for treatment of cardiovascular disease. Successful correction of mutations by Homology Directed Repair (HDR) will also be discussed if time allows. The effectiveness of ZNPs, DLNPs, and SORT LNPs for delivery of long RNAs provides a chemical guide for the rational design of future synthetic carriers. Such insights have recently enabled applications in mRNA replacement therapy[3] and homology-directed repair (HDR) correction of disease-causing mutations. Overall, development of synthetic nanoparticles hold promise to improve the safety, efficacy, and utility of CRISPR/Cas gene editing.

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COMPLEMENT MODULATION OF NANOMEDICINE PERFORMANCE IN HEALTH AND DISEASE

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The complement system is an integral constituent of the innate immune system orchestrating various protective, regenerative and inflammatory responses [1]. Nanomedicines can potentially trigger complement activation and as such the role of complement system in nanomedicine integrity performance and safety is multifaceted [1]. Thus, complement plays important roles in regulation and synchronization of nanomedicine opsonisation and phagocyte clearance. Our recent studies have shown that C3 opsonisation is continuous and variable *in vivo* and demonstrated a vital role for non-specific blood proteins in complement activation [2,3]. Indeed, some of these proteins generate necessary antigenic epitopes for docking of a few natural antibodies on some, but not all nanoparticles, which subsequently serve as target for nascent C3b attack and formation of C3bBb-properdin convertases. Dynamics of non-specific blood protein binding might therefore explain interindividual disparities in complement activation kinetics and opsonisation efficacy.

Nanoparticles are widely tested in vaccination strategies both as an antigen depot systems and adjuvants. Part of nanoparticle adjuvanticity may be related to their complement activation properties, since upon C3 cleavage, liberated C3d (in fluid phase and surface bound) can induce B lymphocyte activation for maintenance of long-term B cell memory [1]. Indeed, B cell complement receptors (CD21 and CD35) cooperate with B-cell antigen receptor to efficiently recognize C3dg-tagged nanoparticles/antigens and signal augmentation is achieved through CD19 recruitment. Complement also plays a role in negative selection of B lymphocytes, but the role of complement in tolerance to self-antigens are apparently restricted to those self-antigens that are evolutionary conserved.

Uncontrolled complement activation is deleterious to the host, causing cellular injury and contributing to the development of disease. For instance, we have shown that intratumoural complement activation by nanomedicines can promote tumour growth [4]. This is partly due to C5a liberation resulting in influx of immunosuppressive Treg cells. C5a also facilitate neutrophil recruitment to tumour microenvironment, which further help with cancer progression. C5a also stimulates the production of functionally active tissue factor in peripheral blood neutrophils, which results in tumour growth and metastases. Among its other roles, C5a can inhibit the production of IL-12 in macrophages and induce macrophage polarization by activation of the nuclear factor-κB. C1q is also capable of inducing macrophage polarization and suppressing macrophage NLRP3 inflammasome activation. C1q polarized macrophages express elevated levels of programmed death-ligand 1 and -2 and promote Treg proliferation. In addition to these, the possible role of nanoparticle-mediated intracellular complement (complosome) activation is poorly explored, but this may modulate cell physiology and metabolism in tumour microenvironment (as well as in adjacent health tissues) and initiate resistance to immunotherapies. On the other hand, controlled nanomedicine-directed complement activation could be beneficial in pathological conditions that will benefit from influx of immunosuppressive cells.

There are reports claiming a causal role for complement anaphylatoxins in adverse reactions to nanomedicines [reviewed in 5]. These reports have correlated nanoparticle-mediated complement activation in human serum to cardiopulmonary responses in pigs, and suggested the utility of the porcine model in nanomedicine safety assessment. Contrary to these, compelling evidence indicate a direct role for some macrophage sub-populations (and other immune cells) in infusion-related reactions independent of complement activation [1,5,6]. Our recent results with isolated porcine pulmonary intravascular macrophages further excludes the role of complement activation in bioactive mediator release on nanomedicine challenge, and therefore questions the scientific validity of the porcine model (and other cloven-hoof animals) in nanomedicine safety assessment, which is irrelevant to human cases [1,5]. Accordingly, we call for development of more accurate biomarker screening and diagnostic imaging to predict infusion reactions to nanomedicines in humans.

Finally, considering multifaceted roles of the complement system in modulating nanomedicine performance, development of species- and pathway-specific complement and complement-receptor inhibitors could open up new avenues for assessing the role of complement and complosome in nanomedicine safety [7]. Nevertheless, through concerted "nanomedicine structure-complement function" mapping we have been able to engineer a panel of complement-safe nanomaterials and nanopharmaceuticals [1,8].

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DEVELOPMENT OF NANOPARTICLES FOR CLINICAL USE: IMPORTANCE OF DEGRADATION AND EXCRETION

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There are huge expectations for the use of nanoparticles (NPs) to deliver therapeutics and for imaging of different diseases, such as cancer. Carefully designed experiments, both *in vitro* and *in vivo*, are essential to fully explore this technology. Despite many promising NPs being made during recent years, the biological studies performed with such NPs very often do not have the quality needed to support the conclusions drawn [1,2,3]. More interdisciplinary collaboration to improve the quality of such studies is required.

With a long experience from pharmaceutical R&D, I will discuss improvements that should be made in biological studies with NPs. The design of animal studies, including which time points to take samples and which parameters to analyse, is critical when aiming at developing drugs for clinical use [1,3,4]. Biodistribution, metabolism and excretion studies are extremely important not only to generate such data (e.g. for an imaging agent), but also to evaluate safety and to predict whether it is likely that the NPs studied ever can receive market approval for clinical use [3]. It should be noted that even small variations in NP structure can result in differential stress responses and mode of cell death [5].

It is of utmost importance that NPs made of non-endogenous substances are degraded and excreted. The impact of having biodegradable versus non-degradable NPs on toxicity studies, cost of development and the risk/benefit analyses one can expect pharmaceutical companies to perform, will be discussed [(3)].

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THE QUEST FOR NANOTECHNOLOGY PLATFORMS TO TARGET THE CENTRAL NERVOUS SYSTEM

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Neurological disorders affect the structure and/or the function of the brain or the spinal cord, which together form the central nervous system (CNS). There are over 600 known neurological disorders and for many of them treatment options are extremely limited. The blood-brain barrier (BBB) and the blood-cerebrospinal fluid barrier (BCSFB) represent major hurdles for the effective and timeous delivery of therapeutic agents to the CNS. The BBB is a highly selective membrane barrier at the brain microvessel level that controls transport between the systemic blood circulation and the CNS. The relatively low permeability of the BBB results from tight junctions between capillary endothelial cells. The BCSFB is a barrier between the blood and the cerebrospinal fluid (CSF) found in choroid plexus, and in this case, the endothelium is more permeable due to the lack of junctions, and tight junctions between epithelial cells of the choroid plexus are those that regulate the permeability of molecules from the blood stream to the CSF. It has been estimated that more than 98% of small-molecule and ~100% of large-molecule neurotherapeutics cannot cross these barriers to reach concentrations within the therapeutic window in the CNS. Crossing the BBB and the BCSFB remains a major difficulty in the development of drugs for the treatment of CNS diseases. In this scenario, the design of novel strategies to increase drug bioavailability in the CNS is called for. In this presentation, I will introduce and discuss two new strategies investigated in my laboratory to actively target neurotherapeutics to the CNS: (i) the exploitation of the folic acid receptor pathway in the choroid plexus epithelium[1] and (ii) the surface-modification of nanoparticles with highly biostable shuttle peptides that target the transferrin receptor in the BBB.[2]

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SMALL SIZES FOR THE SMALL: NANOMEDICINE CHALLENGES AND OPPORTUNITIES IN PEDIATRIC CANCER

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Nanotechnology made sound contributions to the treatment of disease in general and cancer in particular due to the ability to target drug-loaded nanomaterials by the enhanced permeation and retention effect (passive targeting). Furthermore, the design of nanocarriers surface-modified with ligands recognized by receptors overexpressed in specific cell types (e.g., cancer cells) is an extensively investigated (though still unrealized in the clinical practice) strategy for active targeting and reduce off-target accumulation and toxicity; e.g., sugared nanocarriers improved the diagnosis and chemotherapy of solid tumors overexpressing glucose transporters (e.g., breast cancer). Conversely, the investigation of nanomedicines for the therapy of pediatric disease is very scarce due to a very fragmented pharmaceutical market, the limited availability of appropriate disease models and ethically questionable clinical trials[1,2]. For example, the research at the interface of pediatric cancer and nanomedicine usually employs adult cancer models that show extremely limited clinical relevance. Our research group investigates different polymeric nano-drug delivery systems for the treatment of infectious and neurodegenerative diseases and cancer in children using different administration approaches with special interest in self-assembly nanocarriers. In this presentation, I will initially present the challenges faced in pediatric nano-drug delivery and then, present the different strategies investigated in our laboratory to design novel nanobiomaterials with focus on the targeting of drugs to pediatric solid tumors.[3-5]

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ENGINEERING RESPONSIVE NANOPARTICLES AND DEVICES AGAINST BIOFILM INFECTIONS

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Biofilms are dense bacterial colonies that may adhere to the surfaces of medical devices and are major contributors to infections. These colonies are characterized by a self-produced matrix of extracellular polymeric substances (EPS). The extracellular matrix typically consists of polysaccharides, proteins, nucleic acids and lipids and has both structural and physiological functions. It is actually due to the resilience of the EPS that biofilm infections are

very difficult to be treated and, in fact, antibiotic resistance can be transferred readily in biofilms through quorum sensing.
In this talk, we will highlight the next generation of smart medical devices that fight and prevent biofilms and their related infections utilizing responsive nanomaterials. This is explored employing a nanomanufacturing process with proven scalability and reproducibility, flame aerosol technology, to assist rapid technology transfer to industry. We employ flame direct nanoparticle deposition on substrates and combine nanoparticle production and functional layer deposition in a single-step with close attention to product nanoparticle properties and assembly of devices. Specific focus lies on three distinct strategies against biofilms. Specifically, we will explore: (a) the potential of nanoparticle coatings on the surface of medical devices that exhibit inherent antimicrobial properties, (b) how local temperature increase caused by external stimuli (near-IR light and/or magnetic fields) on plasmonic photothermal or superparamagnetic hyperthermal nanoparticles affects biofilm formation, and (c) the potential of photocatalytic nanoparticles upon light activation and whether such coatings on medical devices exhibit the desired anti-biofilm activity.

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INFUSION REACTIONS AS CRITICAL SAFETY BARRIERS: MODELS, MECHANISMS, FUTURE DIRECTIONS

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Exposure of certain nanomedicines to blood can lead to hypersensitivity reactions, also known as infusion reactions (IRs). The mild-to-severe manifestations can escalate into life threatening anaphylactic shock in a small percentage of hypersensitive man (<0.1%), presenting a safety barrier to the use of many promising nanodrugs. Pigs provide a highly sensitive animal model for IRs that can also been used for preclinical safety assessment, although there are views challenging the human relevance of the model and its utility in preclinical safety evaluation of nanomedicines. The presentation will review the pros and cons of the pig and other animal models of IRs, summarizing the experimentally derived evidence supporting the human relevance and utility of the model for safety prediction. The mechanism of IRs both in pigs and man involves two or more “hits” on the immune system, one being complement (C) activation that stimulate a variety of anaphylatoxin receptor-containing cells, and another is C-independent direct stimulation of allergy-mediating immune cells that secrete vasoactive mediators, including pulmonary intravascular macrophages (PIM cells in the lung of pigs). Recent experiments showing T-cell independent (Type II) immunogenicity of PEGylated nanoparticles that entail accelerated blood clearance of nanoparticles with IR enhancement (1) highlight a possible future use of the model for immunogenicity-related IR assessment. Likewise, examples will be shown for the use of the model for the development of safe human infusion protocols (2). In sum, pigs provide a hazard identification model for nanodrug-induced IRs that also allows studies on reaction mechanisms and strategies for the prevention of severe IRs.

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DELIVERY OF NEW MODALITIES, RECENT TECHNOLOGY TRENDS AND SOLUTIONS

WOUTER TONNIS MÜLLERS

New modalities, beyond small molecules and large monoclonal antibodies, are attracting a lot if interest. These new modalities bring forward certain drug delivery challenges of which some can be solved with nanomedicine. New technology trends and solutions will be presented around the delivery of new modalities.

PLATFORMS FOR THE DESIGN OF ENGINEERED NANOSTRUCTURES FOR THERAPY AND MULTIMODAL IMAGING APPLICATIONS

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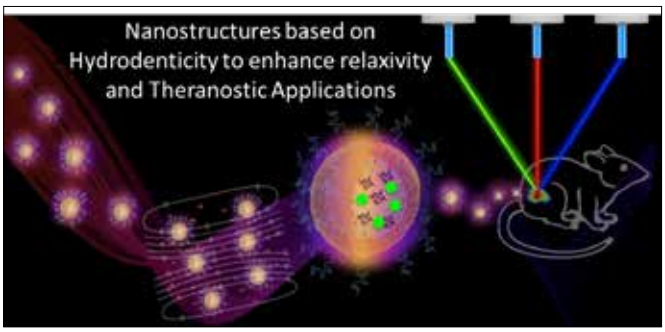

Recently, rational design of a new class of contrast agents (CAs), based on biopolymers (hydrogels) and natural materials, have received considerable attention in Magnetic Resonance Imaging (MRI) diagnostic field. Several strategies have been adopted to improve relaxivity without chemical modification of the commercial CAs, however, understanding the MRI enhancement mechanism

remains a challenge. Furthermore, the combination of more Imaging Modalities(Multimodal Imaging)[1, 2] and its integration with the therapy (Theranostics) [2] is acquiring more attention because it allows integration of the strengths of individual modalities while overcoming their limitations and provides more accuracy in the response. A multidisciplinary approach is used to highlight the basic principles ruling biopolymer-CA interactions in the perspective of their influence on the relaxometric properties of the CA. Changes in polymer conformation and thermodynamic interactions of CAs and polymers are investigated at the molecular level to better understand the involved phenomena[3]. Furthermore, different strategies based on microfluidic, high-pressure homogenization and calorimetry are presented enabling the production of different complex architectures[4-6] with multimodal imaging and theranostic properties. A significant highlight will be given to the effect of the hydration of the hydrogel structure on the relaxometric properties and its application to the nanomedicine field, called Hydrodenticity. A comparison *in vitro* and *in vivo* among different techniques and architectures is possible regarding impact of the interaction between the biopolymers and the tracers to design rationally further nanovectors with improved properties and able to overcome many biological barriers[6-11].

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REGULATORY CONSIDERATIONS FOR DRUG PRODUCTS CONTAINING NANOMATERIALS DURING THE PRODUCT LIFECYCLE

KATHERINE TYNER

In 2017, the United States Food and Drug Administration (FDA) published a draft guidance for industry entitled “Drug Products, Including Biological Products, that Contain Nanomaterials." This guidance has been developed to provide industry and other stakeholders with the Agency’s current thinking for the development of human drug products that contain nanomaterials. The Center for Drug Evaluation and Research (CDER) within FDA does not define or categorize drug products containing nanomaterials and follows the same regulatory processes as for drug products not containing nanomaterials. Adequate characterization of the nanomaterial, understanding of its intended use and application, and how it relates to the product quality, patient safety, and efficacy is considered by CDER to be a suitable framework for evaluating nanomaterials in pharmaceutical products. This presentation will discuss how the guidance applies from initial product development through generic development with an emphasis on identifying analytical techniques used to characterize and control the drug product. Current regulatory science on these complex drug products will also be discussed.

ADVANCING NANOMEDICINES INTO THE CLINIC

MARK B. VAN ELDIJK, Ardena (ChemConnection)

Ardena is at the forefront of the nanomedicine landscape. This fast-evolving field uses nanoscale or nanostructured materials to impart unique pharmacokinetic and therapeutic effects such as enhanced dissolution rate and oral bioavailability, targeted delivery, enhanced efficacy and reduced toxicity. In addition to evaluation of nanomedicines for treatment of diseases such as cancer and autoimmune disorders, nanoparticles are also explored for imaging purposes. The control of materials in the nanometer size range requires scientifically demanding chemistry, analysis and manufacturing techniques. Our nanomedicine expertise encompasses process and analytical development, GMP manufacturing, formulation and dossier development. We provide our expertise as a contract development and manufacturing organization to navigate nanomedicine products into the clinic.

LARGE SCALE GMP PRODUCTION OF LIPOSOMES

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Based on the number of products and ongoing clinical trials with liposomes, liposomes can be considered as the most successful drug-carrier system in nanotechnology so far[1]. The development of products like Doxil® and AmBisome®[2] addressed the hurdles, which had to overcome, making the reproducible large-scale cGMP production of these liposome products possible. Besides manufacturing factors like, for instance, efficient incorporation of the drug in the liposomes, control of the particle size and particle size distribution of the liposomes, handling of solvents during production, the large-scale availability of pharmaceutical grade phospholipids was certainly another unlocking key success factor enabling large-scale production of liposome products. For liposome products natural phospholipids, derived from soybean and egg yolk and synthetic phospholipids are being used. When required also cationic lipids may be applied. This seminar briefly reviews production steps and manufacturing methods to encapsulate hydrophilic or lipophilic compounds and addresses the selection of the (phospho)lipid components to enable large-scale cGMP production.

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POLYPEPTIDE-BASED CONJUGATES AS VERSATILE THERAPEUTICS

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Polypeptides are already playing a major role on a number of different relevant areas such as nanomedicine[1]. The physico-chemical parameters of a polypeptide-conjugate, and hence its biological

performance, are defined by an intricate interplay of multiple structural factors. This highlights the need for detailed structure-activity relationship studies to develop the hierarchical strategies of polypeptide conjugate design. However, structural complexity also represents a unique opportunity, since small changes at the structural level might endow nanomedicines with outstanding and unexpected biological performance[2].In our group, we have overcome the main classical limitations for the synthesis of defined polypeptides using precise controlled reactions followed by an adequate characterization yielding to well-defined polypeptidic architectures by NCA polymerization techniques[3]. In addition, post-polymerization techniques allow us the introduction of a variety of functionalities yielding a set of orthogonal reactive attachment sides[4]. Using these techniques and following a bottom-up strategy we have been able to obtain star-based polypeptide architectures with the capacity to self-assemble yielding supramolecular nanostructures with interesting properties[5]. This strategy together with an adequate polymer-drug linker design[6] enabled *in vitro* and *in vivo* evaluation, revealing a lack of toxicity, an enhanced *in vitro* cell internalization rate and significantly greater terminal and accumulation half-life *in vivo* together with a significant lymph node accumulation5. These results allow us to envisage these systems as promising nanocarriers for therapeutic or diagnostic applications, especially in anticancer treatments including lymph node metastasis and cancer immunotherapy. Proof of Concept for metastatic breast cancer6 and for immunotherapy design in melanoma will be also shown as well as the use of this self-assembled architectures in applications such as neurodegenerative disorders, spinal cord injury or acute kidney injury.

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THE MECHANOBIOLOGY OF LYMPH NODE TISSUES: IMPACT OF SWELLING ON IL-7 MEDIATED HOMEOSTASIS

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Lymph nodes are an integral part of the adaptive immune system. They are surrounded by a fibrous capsule and an inner medulla rich in dendritic, B- and T-cells, as well as macrophages which are all embedded in a reticular extracellular matrix network assembled by fibroblastic reticular cells (FRCs). While much has been learned about the physiology of lymph nodes regarding their key functions to filter the lymph and to identify and fight infections, many questions remain how the immune reaction involving so many different cell types is orchestrated and fine-tuned in response to acute inflammation to prevent overreactions or a failure to respond. Here we asked whether novel nanotechnologies allow us to learn more about the orchestrating role of extracellular matrix in naïve and swollen lymph nodes. With a newly developed nanoscale mechanosensory probe that differentially binds to relaxed versus stretched fibronectin fibers, we tested the impact of lymph node swelling on fibronectin fiber tension within the lymph node reticular networks. We found that fibronectin fibers are highly tensed in naïve and adjuvant swollen lymph nodes, but that fibronectin fiber tension is lost in virally infected lymph nodes [(1)]. Why is this finding significant? As fibronectin contains many binding sites for growth factors, cytokines and other ECM molecules, stretching of fibronectin fibers might expose some of those binding sites, while destroying others [(2)]. We also found recently using an *in vitro* stretch assay that Interleukin (IL)-7, which plays a central role in the adaptive immune system, binds more strongly to stretched than to relaxed or enzymatically cleaved fibronectin fibers [(3)]. Others found that IL-7 is more potent in the ECM-bound state compared to its soluble counterpart. Taken together, this suggests that Il-7 is less potent in virally infected, than in naïve and adjuvant swollen lymph nodes. Different to all other cytokines, IL-7 does not activate immune cells, but keeps immune cells under homeostasis, particularly B-cells and resting naïve and memory T cells, while upregulating their proliferation. Destruction of the tensed fibronectin fiber network by viral infections in lymph nodes is thus expected to release Il-7, which then stops hindering the immune cells to get activated. Discoveries how the mechanobiology of ECM and mechanical forces might impact the immune response are prone to open new therapeutic avenues.

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NANOMEDICINE TODAY – 'QUALITY BY DESIGN' OR 'TRIAL AND ERROR'?

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For many years, nanocarrier systems have been developed to deliver cancer therapeutics more efficiently to a specific site of action. With a rising number of drug products being approved, the quality and safety criteria are becoming a key concern of the regulatory authorities globally [1].
Drug release testing is well-accepted in the early selection of formulation prototypes and during quality control. For nanomedicines novel methods such as the dispersion releaser (DR) technology enable a precise measurement of this essential characteristic even in presence of serum proteins [2]. However, in the area of nanomedicines and when considering the limitations of existing clinical data, the correlation between the drug release rate and the *in vivo* response is difficult to establish.
In this ongoing investigation, the plasma concentration-time profiles of nanocarrier formulations were systematically analyzed and a physiologically-based biopharmaceutics model was created [3]. The *in silico* model comprises a compilation of the existing human pharmacokinetic data and calculates the *in vivo* release rate of the nanocarriers. It was applied to the drug formulations Foscan®, Foslip®, Doxil®, and Myocet®, resulting in a comprehensive understanding of their pharmacokinetic behavior.
For formulations of the photosensitizer temoporfin, Foslip® and Fospeg®, an *in vitro* drug release assay was developed using the DR technology. The release conditions were based on the expected physiological dissolution pressure and revealed a prolonged release of the drug from pegylated and non-pegylated liposomes. Additionally, the size and stability of the liposomes
were monitored in presence of rat plasma. A vesicle size of 97 ± 3 nm for Foslip® and 97 ± 1 nm for Fospeg® was observed over the whole time period without any significant changes in the size or the number of particles. By using this sophisticated combination of *in vitro* and *in silico* methods, an optimal representation of the pharmacokinetic profile was achieved (see Figure 1).

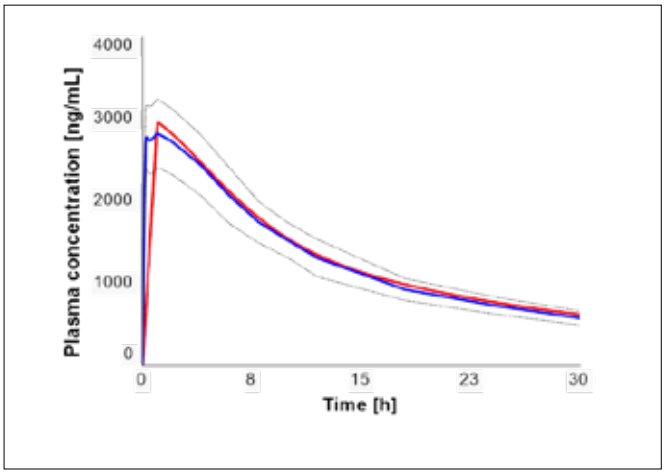

Figure 1: *Mean plasma concentration-time profile of the Foslip® (blue line) with upper and lower standard deviation profile (grey dotted lines) in comparison to the **in silico** simulation (red line).*

More recent efforts were focused on the evaluation of the formulation prototype NanoBB-1-Dox that has been evaluated in a phase I clinical trial [3]. The polymeric nanoparticle formulation was developed for the treatment of glioblastoma multiforme and was expected to deliver doxorubicin to the brain. To achieve this aim, a receptor-mediated transport of the nanoparticles was exploited [4]. However, the rapid release of the drug from the carrier reduces the fraction of the drug available for this passage across the blood-brain-barrier. And while the particles exhibit a high stability in presence of rat plasma (122.1±0.8 nm over 96 h), the *in silico* analysis reveals a race against time with regards to the drug release.
Learning from years of '*trial and error*', this verified *in silico* model brings a breath of fresh air to the nanocarrier delivery and enables the design of improved formulation prototypes optimized for clinical application in humans.

Keywords: doxorubicin, nanoparticles, *in vitro* release, pharmacokinetics.

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GUIDING MRNA FORMULATIONS FROM LABORATORY INTO CLINICAL TRIALS. LESSONS LEARNED FROM DEVELOPMENT AND OPTIMIZATION OF LIPOSOMAL FORMULATIONS.

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RNA-based therapeutics, which function by either silencing pathological genes through delivery of siRNA or expressing therapeutic proteins through the delivery of exogenous mRNA to cells, hold great potential for the treatment of various diseases. However, mRNA molecules are large, fragile and easily degrade. They do not readily cross plasma membranes to enter target cells and so a delivery solution is required.
Lipid nanoparticles (LNP) are the leading delivery systems for enabling the therapeutic potential of small interfering RNA (siRNA), mRNA for systemic applications or CRISPR. Lipid nanoparticles (LNPs), currently represent the most advanced platform for RNA delivery, which have now advanced into human clinical trials and their mRNA delivery safety profiles have been evaluated in human and non-human primates.
Lipid nanoparticle delivery platforms have been extensively investigated and optimized for the formulation of oligonucleotide drug products and provide a good basis for mRNA based systems. However, mRNA containing LNPs need to be treated differently than oligonucleotide containing LNPs, as particle structure has an impact with respect to stability upon processing conditions.
Data will be presented, which describe impact of process conditions on the generated particle size and homogeneity as well as encapsulation efficiency not just during the LNP formation process, but also during further downstream processing steps, like tangential flow filtration as well as sterile filtration process steps. A few examples of drug products and related processes will be shown, where special focus will be set on process parameters like pump characteristics, process temperature, solvents and their respective concentrations as well as shear forces in filtration processes.

DEVELOPING POLYMERIC NANOFORMULATION FOR NEURODEGENERATIVE DISORDERS VIA INTRANASAL DELIVERY

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Intranasal administration has appeared as an alternative non-invasive way to bypass the BBB and target drugs directly to the central nervous system [1]. Due to the large surface area and highly vascularised mucosa of nasal cavity, as well as the close vicinity between the brain and the nasal cavity, nose-to-brain drug delivery can be a revolutionary approach in targeting brain disorders [2].

Amyotrophic lateral sclerosis (ALS), is a fatal neurodegenerative disease that affects the motor neurons, leading to paralysis and death eventually. At present, edaravone (EDV) is one of the only two approved drugs for the treatment of ALS, acting as a potent free radical scavenger and antioxidant to slow the advance of ALS [3].

We have recently developed polymeric nanoparticles to intranasally deliver EDV. We hypothesised that using nanocarriers to formulate the drug specifically for nasal delivery can enhance the drug loading, alter the biodistribution, reduce the toxicity and thus improve the therapeutic index.

METHODS

EDV-loaded PEGylated PLGA nanoparticles (NP) or oil-cored nanocapsules (NC) were formulated using nanoprecipitation method [4]. NP-EDV and NC-EDV formulations were optimised and characterised in terms of their physiochemical properties, encapsulation efficiency, drug release profiles and shelf-life stability. Cytotoxicity and protective effects against oxidative stress were examined *in vitro* using BV-2 mouse microglial cells. Nanoformulations were labelled with a near infrared dye DiR to assess the organ biodistribution profile by *in vivo* and *ex vivo* optical imaging following intravenous or intranasal administrations. The amount of drug distributed to major organs including blood was quantified by liquid chromatography–mass spectrometry (LC-MS).

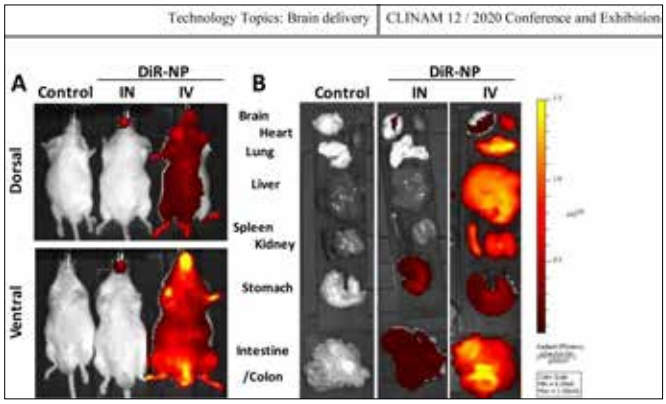
RESULTS

NP-EDV were measured smaller in size compared to oil-cored NC-EDV (70.2±2.2 nm vs 149.1±2.1 nm) and slightly less negatively charged (-12 vs -18 mV). Both of them exhibited comparable sustained release profiles that ~50% drug was released over 8 h in PBS or in the presence of 50% serum, analysed by HLPC. Great shelf-life stability was obtained up to 30 days. In vitro studies confirmed both NP-EDV and NC-EDV reduced the oxidative-stress-induced toxicity and the protection effect was more prominent in the case of NP-EDV. Optical imaging showed NP-EDV accumulated in most of the vital organs and tissues at 4 h post iv injection. In contrast, uptake limited to brain, stomach and intestine/colon were found in mice received NP-EDV at 4 h post intranasal injection (Fig. 1). Quantitative analysis by LC-MS confirmed the uptake of EDV in the brain via NP-mediated intranasal delivery.

CONCLUSION

EDV-loaded PLGA-PEG nanoparticles and oil-cored nanocapsules were formulated with uniform size, good stability and favourable release profiles. The resulting nano-formulations effectively protected microglial cells against oxidative stress. As demonstrated by *in vivo* optical imaging and quantitative LC-MS analysis, the brain uptake of EDV-loaded nanoparticles indicating its potential as a novel non-invasive nose-to-brain delivery system of EDV for the treatment of ALS.

Fig. 1: *In vivo* and *ex vivo* organ biodistribution of DiR-labelled NPs in mice after intravenous (iv) or intranasal (in) administration. (A) *Representative whole-body in vivo images obtained at 4 h post-injection.* **(B)** *Representative ex vivo images of excised organs at 4 h post-injection. Mice were iv or in injected with PBS, or DiR-NP-EDV. At 4 h post injection, whole body perfusion with 0.9 % saline was performed and major organs were harvested. All images were obtained by IVIS Lumina III (λ_{ex}: 740; λ_{em}: 790 nm).*



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KNOWLEDGE MANAGEMENT AND HEALTH DATA STANDARDIZATION EFFORTS AT FDA TO ENHANCE THE UTILITY AND VALUE OF REAL WORLD EVIDENCE FOR PUBLIC HEALTH

FRANK F WEICHOLD,

The current health care business model has brought ever-increasing care costs while population life expectancy is declining in the US over the past years. Harnessing the digital revolution by building a dynamic regulatory environment throughout the entire lifecycle of medical products, including pre and post-approval epidemiology and surveillance, offers the opportunity for better return on investments, particularly toward preventive care. Accurate modeling and simulation of individual and public health expectations and outcomes require high data quality and provenance, in addition to access, re-usability/inter-operability and security. Convergence of data collection standards and knowledge management in clinical research, clinical practice and standard of care are obligatory goals. Despite the mandatory validation of clinical lab tests, the concept of semantic interoperable and standardized laboratory test derived data is not sufficiently implemented yet. Currently, mobile health and telemedicine, all and any sensor or detector derived data sets are similarly hampered by the lack of source data traceability, once engulfed by the e-health record systems. This results in problems with source data validation in context of evidence generation, with data transformation errors and re-coding into different reporting systems, including LOINC (logical observation identifiers codes and names) in regulatory submissions. Progress in ongoing multi-agency/stakeholder efforts to improve the quality, interoperability, utility and portability of electronic laboratory data (i.e., *in vitro* diagnostic [IVD] data) through the harmonized implementation of semantic data interoperability standards

that have been appropriately qualified will be presented. The role of FDA in working with stakeholders in consortial settings, as well as advantages and opportunities for open consensus "learning" standards will be discussed.

CELLULAR FATE OF NANOPARTICLES DELIVERED TO THE MURINE LUNG: A NEW ROLE OF MACROPHAGES?

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Pulmonary drug delivery has been widely accepted as optimal route for treatment of lung diseases. The cellular fate and behavior of drug nanomedicine or nanoparticles (NM/NPs) in the lung is not only critical to drug efficacy, but also important for understanding of nanotoxicology. Resident alveolar macrophages are recognized as the first line of cellular host defense and are mainly responsible for clearing allergens, microorganisms, and foreign micro-sized particles via phagocytosis. Numerous studies have suggested that particle size plays a dominant role in controlling the cellular fate of NM/NPs in the lungs. While larger particles with size over 200 nm are readily phagocytized by alveolar macrophages, smaller particles (less than 200 nm) are able to escape initial phagocytosis[1]. Those findings are typically found by performing 2D stereological analysis in lung slices and bronchoalveolar lavage (BAL) cells using fluorescent or electron microscopy and by quantitative tissue radioanalysis. Recently we introduced cutting-edge imaging techniques such as phase-contrast x-ray imaging and tissue-cleared light sheet fluorescent microscopy (TC-LSFM) for *in vivo* visualization of delivery dynamics in real-time and 3D *ex vivo* single-cell resolution co-mapping of lung morphology and nanoparticle biodistribution in the intact murine lungs, respectively[2-3]. This indeed provided several previously unappreciated insights into the characteristics of pulmonary NP delivery and distribution, for example, the significant difference in NP 3D end-delivery distribution pattern for intratracheal instillation of the bulk liquid versus aerosol inhalation[2]. Moreover, leveraging TC-LSFM for evaluation of the temporal profile of NP (re-)distribution we unveiled new features of NP biokinetics such as redistribution of proximally deposited particles to the periphery of the lungs most likely due to macrophage uptake and subsequent migration into the more distal regions of the lung (Figure 1a and b). Abundant evidence on macrophage-mediated migration (a previously unknown feature) was further provided here by multi-parameter analysis of lungs from macrophage (GFP-) reporter mice using TC-LSFM, immunostained precision cut lung slices, as well as flow cytometry and microscopic examination of cells lavaged from the these lungs. Additionally, multicolor immunostained TC-LSFM imaging of intact lung has also been established in this study (Figure 1c, d, and e), opening new avenues for unraveling the interplay between NM/NPs and immune cells, vasculature system, nervous system, lymphatics *etc.,* in 3D with cellular resolution. The multi-technology approaches developed here are of utmost importance for understanding NM/NP therapeutic efficacy and fate or the toxicology profile *in vivo*.

Keywords: Pulmonary dynamic delivery, 3D lung imaging, Nanoparticle (re-)distribution, Macrophage-mediated migration, Targeted drug delivery.

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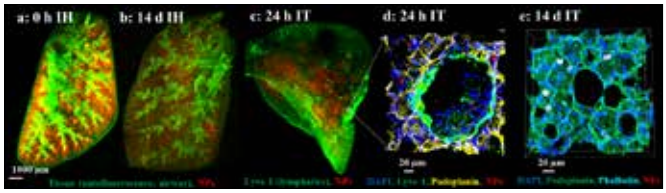
Figure 1: *3D mapping of nanoparticle redistribution pattern in left murine lung at 0 h and 14 days after ventilator-assisted aerosol inhalation (IH: a and b) or at 24 h after intubated instillation (IT: c) with tissue-cleared whole lobe immunostaining using LSFM. (d) and (e) show the NPs was confined in the macrophages sitting close to but not in the lung lymphatic endothelium in precision cut lung slice.*



PRODRUGS AND ASSOCIATED OPPORTUNITIES IN LOCALIZED DRUG SYNTHESIS

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Prodrugs are cunning chemical entities with masked therapeutic activity that can be revealed via (enzymatic) bioconversion for site specific activity. Over the past decade, we have explored enzyme-prodrug therapy using implantable biomaterials to achieve localized drug synthesis and do so using enzyme-containing surface coatings, hydrogel beads, and electrospun fibers – for diverse biomedical applications.

In our recent work, we realized that prodrug conversion can be most successfully performed using endogenous enzymes that are overexpressed or that become accessible in diseased tissues and specifically cancer. For this, we developed extended scaffold glucuronides that with enhanced delivery to the tumor volume. To identify the lead structure, we screened molecular, macromolecular, and supramolecular prodrugs.

In our most recent work, we turned our eyes to prodrugs that are associated with and are trafficked by mammalian cells. In one aspect, these cell-associated prodrugs function as a "kill switch" mechanism, to deactivate the engineered cell on demand. In another aspect, engineered cells work as cellular Trojan horses for delivery of prodrugs to the tumor volume.

In all of the above applications, key to our achievements was in the design of prodrugs that mask toxicity of the incorporated drug and release the toxin in a site-specific manner, when instructed by the enzymatic fingerprint of the biomaterial or that of the tumor tissue, or when triggered externally for an on-demand deactivation of cells.

IS PROTEIN CORONA FORMATION IN PLASMA AN INTRINSIC PROPERTY OF ALL NANOPARTICLES?

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The current understanding of nanoparticle-protein interactions indicates that the particle surface rapidly adsorbs proteins upon introduction into a living organism. The resulting induced formation of a protein corona dictates the fate of nanoparticles in mice and men. The present study focuses on core-crosslinked nanoparticles with long circulation times, differing in the hydrophilic polymer material namely poly(N-2-hydroxypropylmethacrylamide) (pHPMA), polysarcosine (pSar) and polyethylene glycol (PEG). To investigate protein corona formation, the nanoparticles were each incubated in human blood plasma and separated by asymmetrical flow field-flow fractionation (AF4). Notably, multiangle laser light scattering showed no detectable differences in particle size or polydispersity upon incubation and isolation from plasma. By SDS-PAGE in combination with the very sensitive silver staining, almost no further proteins could be detected. Label-free quantitative proteomics was additionally applied to analyze the composition of the traces of protein corona. For nanoparticles with pSar and PEG as the hydrophilic shell, no significant enrichment of proteins (compared to coeluting proteins) was observed, while the enrichment of some proteins was detected for pHPMA. These findings illustrate there are synthetic nanoparticles void of protein corona formation. This absence assures in the case of CriPec® docetaxel a truly beneficial pharmacokinetic profile in patients.

DESIGN OF CARRIERS FOR PROTEINS AND RNA MOLECULES

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Biological drugs, including proteins and RNA-based polynucleotides, are taking an increasing space in the industry pipelines. Despite their potency, the difficulties of these macromolecules for overcoming biological barriers and reach the intracellular targets have limited their full exploitation.
Fortunately, the continuously improved understanding of the biological barriers and the molecular biology associated to pathological conditions is paving the way for a more comprehensive and rational design of protein formulations based on the use of nanotechnology. Our laboratory, with a long-track experience in the formulation of macromolecules using polymer nanoparticles, has significantly contributed to this field. As an example, in the 90's we were the first to report that nanoparticles made of either PLA-PEG or chitosan were efficient vehicles for the transmucosal delivery of proteins, antigens and polynucleotides. The result of our subsequent efforts is an array of nanotechnologies, which make use of polymers and lipids and can be used to deliver biologicals across mucosal surfaces, and to facilitate their intracellular delivery following parenteral administration.
In my presentation, I will focus on the design of carriers for proteins and RNA molecules that could be used in different therapeutic areas: (i) nasal nanovaccines, taking HIV as an example, (iii) nose-to-brain delivery of RNA, (iv) delivery of mAb targeted to intracellular onco-proteins, as new oncological treatments.
Overall, our experience in this field has benefited from integrative approaches adopted by specifically designed consortia. Hopefully, the results of these cooperative efforts will help to accelerate the progress of a rational design of protein-based nanomedicines.
More information about these projects can be found at:
http://www.usc.es/grupos/mjalonsolab/

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Anticancer drug delivery: Ana Cadete, Ana Olivera and Dolores Torres from the USC, Spain and Gema Moreno and Angela Molina from the "Universidad Autónoma de Madrid", Spain.
Vaccine delivery: Jose Crecente, Tamara Gómez from the USC and Ma Luo and Francis Plummer from University of Manitoba, Canada.
Nose-to-Brain delivery: Eleni Samaridou from USC and Hannah Walgrave and Evgenia Salta from VIB, Belgium.
The research activity has been founded by the Horizon 2020 Program the European Comision (grant agreement # 646142 – NANOPILOT and grant agreement No. 721058- B-SMART), by MINECO- PCIN-2017-129/ AEI, under the frame of EuroNanoMed III, and by The National Institutes of Health (NIH) (Grant Number: R01AI111805),

CLINAM

European Foundation for Clinical Nanomedicine

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Basel, October 26–28

ABSTRACTS POSTERS

THE COMBINATION OF SICD47 AND DOXORUBICIN IN A LIPID NANOCARRIER CLEARS SOLID TUMOURS IN MOUSE MODEL VIA TUMOUR MACROPHAGE ACTIVATION

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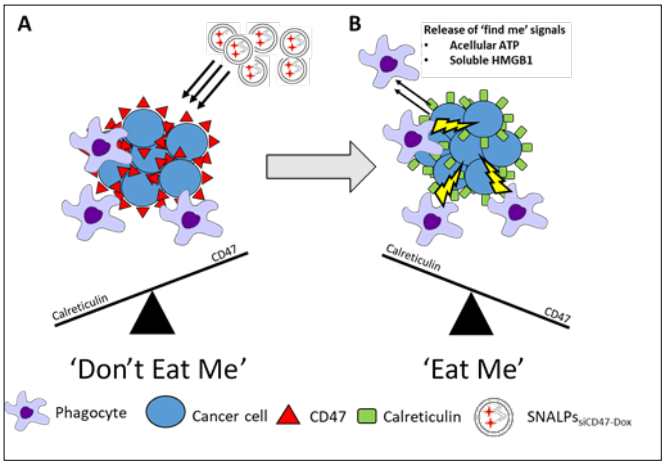
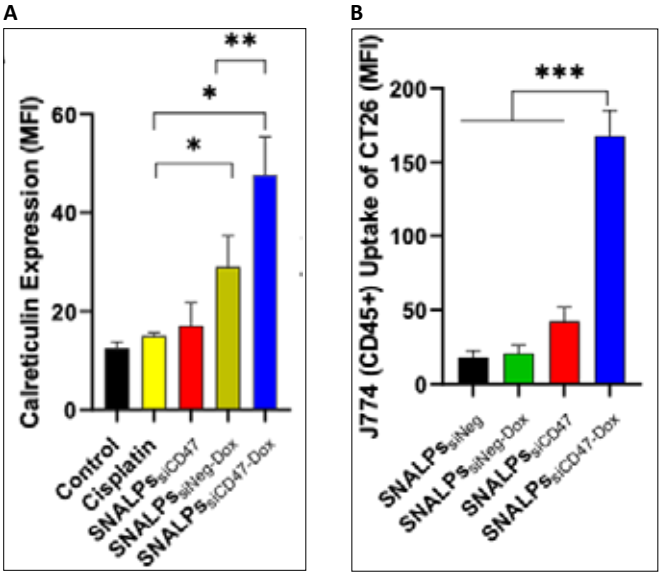
Introduction: Doxorubicin is a potent cytotoxic agent that can induce immunogenic cell death (ICD), an apoptotic phagocytic mechanism which directs the host immune system to engulf cancer cells [(1)]. Generally, ICD involves the overexpression of cellular surface calreticulin, the release of different DAMPs such as ATP, HSPs and HMBG1. Although ICD is very potent in stimulating the immune system to combat cancer cells, its effect is thought to be dampened by CD47 overexpression on cancer cells [(2)], as a strategy to evade the clearance by the host immune system. Therefore, blocking CD47 by using either monoclonal antibodies or siRNA could have a promising therapeutic outcome. Administration of anti CD47 monoclonal antibodies intravenously may result in rapid dilution and toxicity through off target effects [(3)]. In this study, we aimed to fabricate a lipid nanocarrier capable of encapsulating both doxorubicin and siCD47 as a combinatory immunotherapy strategy to eradicate colon cancer.

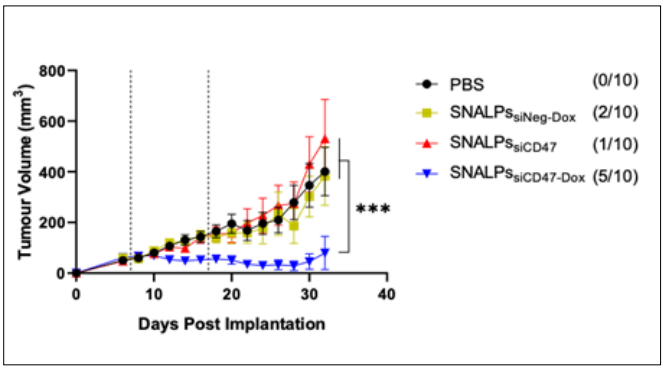


Proposed mechanism described in this study. *Many tumours express high levels of CD47 as a 'don't eat me' signal to avoid the recognition by host immune system (A). CD47 is counter balanced against calreticulin surface expression which acts as pro-phagocytic 'eat me' signal. The surface calreticulin is considered as a hallmark of immunological cell death (ICD) and is induced by doxorubicin. Therefore, the preparation of SNALPs for the co delivery of: siCD47, knocking down CD47, and Doxorubicin, to induce ICD, facilitated to tumour cells is hypothesised to have a synergistic effect through increased phagocytosis and subsequent immune activation (***B***).*

Methods: Different SNALPs with or without doxorubicin were fabricated by ethanol injection method and characterized for particle size, zeta potential and entrapment efficiency for both doxorubicin and siRNA. The cellular uptake of both siRNA and doxorubicin, as well as, the transfection efficiency were assessed in CT26 cells. The tumour accumulation and therapeutic efficacy of the combinatory therapy were tested in CT26 bearing mice and compared to monotherapy to prove the hypothesis.

Results: The optimized cationic serum stable SNALPs had a particle size 122.33±6.65 and entrapment efficiency > 65% for both siRNA and doxorubicin. SNALPs improved the cellular uptake of both doxorubicin and siRNA in dose and time dependent manner with augmented cytotoxicity against CT26 cells by 3.8 fold over the solution. The SNALPs were successfully able to knockdown CD47 by approximately 70% in 48 h. siCD47 and doxorubicin co-loaded SNALPs induced surface calreticulin expression and subsequently amended macrophage-mediated phagocytosis of cancer cells. The combination therapy showed a complete remission of tumour in 50% of the tested animals while the remaining animals showed significantly lower tumour Volume as compared to either monotherapy.





The co-delivery of doxorubicin and siCD47 into SNALPs induced surface calreticulin expression, macrophage phagocytosis and consequently the therapeutic outcome.
*The ability of both siCD47 and doxorubicin containing SNALPs to alter expression of calreticulin on CT26 cells was tested using flow cytometryTM. Data is expressed as mean fluorescence intensity and SD (n=3-6) (A). Statistical analysis was performed using a student's T test followed by Mann Whitney post-test * p<0.05 **p<0.005. CT26 cells were labelled with CellTraceTM before being incubated with different SNALPs for 48 h. The cells were collected and co cultured with J774 macrophage cells for 6 h. Cells were harvested and stained with anti-mouse CD45 monoclonal before being acquired on a FACs Calibur flow cytometer. Relative MFI of CD45+ J774 cells is shown in (B). Bars represent the average of mean fluorescence intensity and SD (n=3). One way ANOVA followed by Tukey's post-test ***p<0.001. In vivo assessment of SNALPs were conducted in CT26 tumour bearing BALB/c mice (n=10 per group). On day 7 and 17 (dashed lines) mice were i.v. injected with different tested treatments. Tumour size was monitored, and the numbers of mice clearing the tumour are recorded in parenthesis beside each group (C). Statistical analysis was performed using 2 way ANOVA followed by Tukey post-test, ***p<0.001, data points represents the mean and SEM.*

Conclusion: Combining siCD47 with a doxorubicin as an ICD inducing drug SNALPs results in potent synergy in anti-tumour therapy and potentially could resolve certain types of 'immunogenic' solid tumours.

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PH-SENSITIVE TAT-DECORATED PEGYLATED LIPOSOMAL SILYBIN: SYNTHESIS, IN VITRO AND IN VIVO ANTI-TUMOR EVALUATION

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The delivery systems significantly increased efficacy of chemotherapeutics through enhanced targeting and control on the drug release patterns however, biological barriers of tumor microenvironment greatly influence the spreading and penetration of nanomedicine within the tumor. PEGylation for example hinders efficient nanoparticle cell interaction. Thus, to achieve a balance between the longevity of nanocarriers in circulation and optimum cellular association, PEG-detachable systems could be designed to respond to various tumor specific stimuli. We used SLB, a polyphenolic flavonoid known for outstanding pharmacological activity which shows low bioavailability and intensive metabolism. Though SLB formulation developed with enhanced oral absorption, few have enabled parenteral application of this compound. We report herein the fabrication of a PEG-detachable silybin (SLB) liposome decorated with TAT-peptide. For this, Acyl hydrazide-activated PEG_{2000} was prepared and linked with ketone-derivatized DPPE via an acid labile hydrazone bond to form $mPEG_{2000}$-HZ-PE. TAT peptide was conjugated with a shorter PEG_{1000}-PE spacer and efficacy of coupling was monitored by TLC using silica plates and TAT-PEG_{1000}-PE was visualized with iodine vapor exposure (Fig 1).

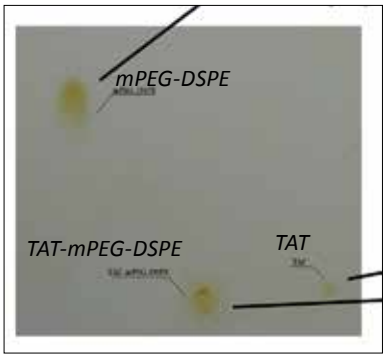

Fig 1.
Representative TLC patterns of different reactants and products. TLC was done using silica plates and mobile phase of chloroform: methanol: water (90:18: 2% v/v) and iodine vapor exposure.

TAT peptide conjugated with a shorter PEG_{1000}-PE spacer was post-inserted into PEGylated liposome (DPPC:SPC:Chol). The patent-based method was used to prepare SLB-SPC complex to be incorporated into liposomes with high loading efficiency. Briefly, SLB in an aprotic solvent, acetone, was mixed with SPC overnight under stirring, the mixture was concentrated in vacuum and diluted with n-hexane and the precipitated pale yellow complex was collected by filtration and dried under vacuum at 40 °C. SLB association within SLB-SPC lipid complex greatly enhanced its encapsulation efficiency in liposome bilayers which stably reached up to 50% (Table 1).

The presence of 10% FCS in release medium increased SLB release from pH-sensitive liposomes compared to non pH-sensitive counterpart. We observed that $mPEG_{2000}$-HZ-DPPE liposome significantly enhanced cell killing due to the PEG detachment over time in 4T1 cancer. The presence of TAT in the pH-sensitive formulation ($mPEG_{2000}$-HZ-DPPE/TAT-PEG_{1000}) significantly promoted cellular association due to TAT exposure in longer incubation time and greatly enhanced cytotoxicity (Table 1).

The pH sensitivity and shielding effect of long PEG chain on TAT peptide was investigated using DiI liposome and FACS analysis. Pretreatment to the lowered pH enhanced cellular association of pH-sensitive TAT-modified liposome due to the cleavage of hydrazone bond and TAT exposure.

Formulations	Diameter [a] (nm)	PDI [b]	Zeta potential (mV)	Encapsulation (%)	IC50 (μg/ml) 72 h
$mPEG_{2000}$-DSPE	78±4	0.23±0.01	-16.5	50.6	28.36 ± 7.9
$mPEG_{2000}$-DSPE/TAT-PEG_{1000}	79±4	0.23±0/01	-15.3	43.7	21.2 ±4.2
$mPEG_{2000}$-HZ-DPPE	123±3	0.20±0.02	-6.8	40.6	6.72 ± 3.9[b**]
$mPEG_{2000}$-HZ-DPPE/TAT-PEG_{1000}	124±5	0.14±0.01	-5.0	34.4	6.03 ± 3.6[c*]
Silybin in DMSO	-	-	-	-	2.69± 1.2

[a] p<0.05 when compared to $mPEG_{2000}$-DSPE

[b] p<0.01 when compared to $mPEG_{2000}$-DSPE

[c] p<0.05 when compared to $mPEG_{2000}$- DSPE/ TAT-PEG_{1000}

Table 1
Characteristics of different pH-sensitive and non-pH-sensitive silybin liposomes Each value represents Mean ± standard deviation (n = 3)

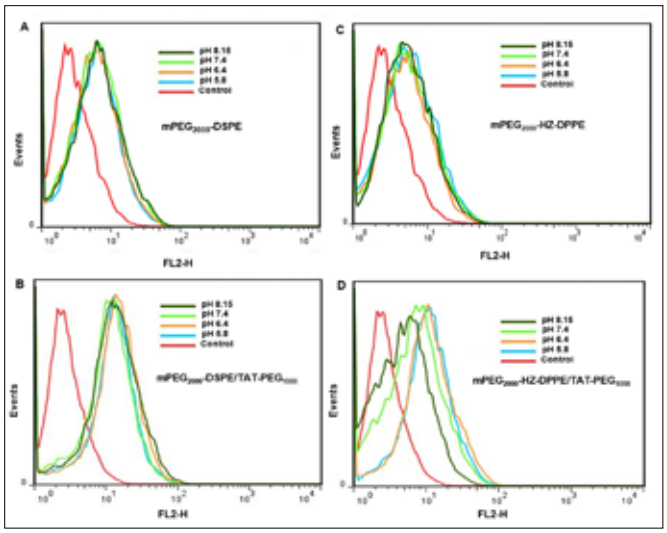

Fig 2
Flow cytometric analysis measuring pH-sensitivity of DiI labeled SLB entrapped liposomes in 4T1 cells at 37 °C. Liposomes were diluted 1:10 in PBS at pH values 5.8,6.4, 7.4 and 8.15, and samples were added into the cell culture wells, in triplicate. Results, expressed as Geometric mean fluorescence intensity (MFI), represented mean ± S.E.M. of three independent experiments.

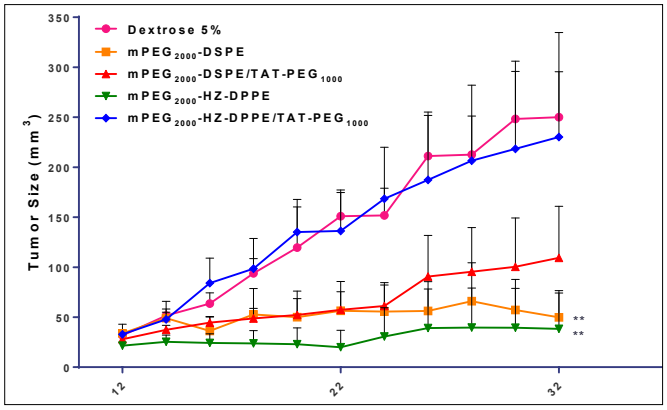

Figure 3
Inhibition of tumor growth in female BALB/c mice bearing 4T1 tumor upon injection with pH-sensitive SLB liposomes (10 mg/). Data are pre-

*sented as mean ± standard error mean of 5 mice /group. **, p<0.01, when compare to the Dextrose 5%. In vivo* results were promising with the pH-sensitive liposome which detach PEG moieties upon exposure to the acidic tumor microenvironment and by enhancing cellular uptake greatly retarded tumor growth and prolonged the survival of 4T1 tumor-bearing BALB/c mice. While TAT modification was effective *in vitro* in improving cellular uptake, it largely contributes to the fast removal of liposome from blood circulation and consequently diminished efficacy of formulation in animal study. This could be due to TAT exposure in the dynamic blood circulation and strong non-specific interaction of nanocarriers with RES before reaching the tumor tissues.

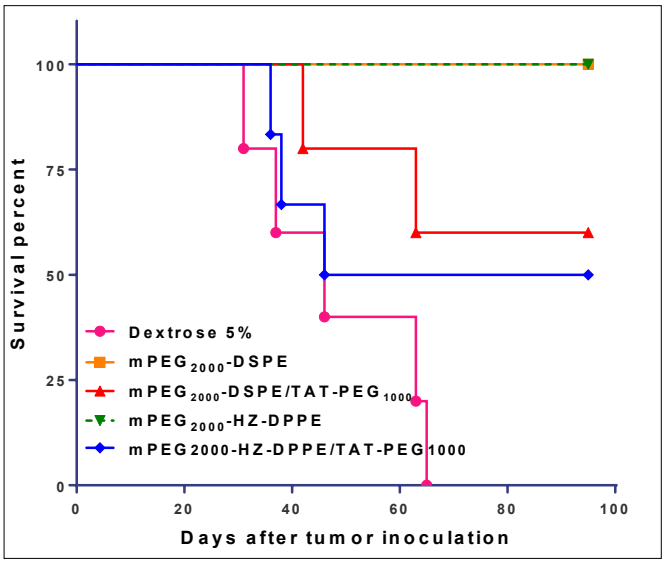

Figure 4
in vivo survival experiments in female BALB/c bearing 4T1 tumor in vivo.

Important conclusion can be drawn from results: formulations either devoid of TAT or those with hidden TAT in the circulation results in better tumor inhibition responses. This has been clearly proven in $mPEG_{2000}$-DSPE/TAT-PEG_{1000} treated animals in which TAT moieties are covered with a stable PEG chain, thus limiting TAT exposure in blood circulation. Improved tumor growth inhibition response was observed in this group of animals which was comparable to that of $mPEG_{2000}$-DSPE which lacks TAT moieties. Thus, despite promising *in vitro* results, the dynamic blood flow environment might result is PEG detachment in $mPEG_{2000}$-HZ-DPPE/TAT-PEG_{1000} formulation, exposing highly interactive TAT molecules that were supposed to be shielded by the PEG chains. Consequently, there might be a great chance of either opsonization of the nanocarriers by reticuloendothelial (RES) system or the non-specific strong binding of TAT surface moieties with cells of the non-tumor organs, resulting in reduced bioavailability in tumor area.

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PREPARATION OF MSC-DERIVED EXOSOMES TAGGED WITH APTAMER AGAINST MYLEIN AS A THERAPEUTIC FOR MULTIPLE SCLEROSIS

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Mesenchymal stem cell-derived exosome is a safe and effective delivery platform with a potential capacity to exert immunomodulation effect and peripheral tolerance toward auto-reactive cells via bearing regulatory and tolerogenic molecules. Inflammation and neurodegeneration are the clinical manifestation of multiple sclerosis (MS). In order to fight against MS, the efficient choices are the ones, which prevent inflammation and induce remyelination. In this regard, the previously reported LJM-3064 aptamer which showed considerable affinity toward myelin and demonstrated remyelination induction was employed as both targeting ligand and therapeutic agent. Thus, in the current study, the carboxylic acid-functionalized LJM-3064 aptamer was covalently conjugated to the amine groups on the exosome surface through EDC/NHS chemistry. The obtained results showed that the aptamer-exosome bioconjugate could promote the proliferation of oligodendroglia cell line (OLN93) *in vitro*. Moreover, *in vivo* administration of the prepared aptamer-exosome bioconjugate in female C57BL/6 mice as a prophylactic measure produced a robust suppression of inflammatory response as well as lowered demyelination lesion region in CNS, resulting in reduced *in vivo* severity of the disease. The prepared platform employing exosome-based nanomedicine as a novel approach for managing MS would hopefully pave the way to introduce a versatile approach toward an effective clinical reality.

Keywords: "Multiple sclerosis (MS)" "Mesenchymal stem cell" "Exosome" "Aptamer" "LJM-3064"

One of the well-known classes of secreted biological nanovesicles is exosomes, which are released by different cells. These vesicles play an important role in intercellular communication by delivering different molecules such as proteins, RNAs and micro RNAs [1]. Although synthetic drug delivery systems have been used for many years, exosomes as natural lipid-based carriers have potentially great innate properties for delivery of molecules either *in vitro* or *in vivo*. Their considerable advantages over other platforms are their low immunogenicity, their ability to be loaded with biological materials such as proteins, they are well-tolerated *in vivo* and possess high delivery efficiency [2-4]. Recent studies represent exosomes as an ideal drug delivery system with desirable properties [2]. In this regard, the capability of MSC-derived exosomes to decrease apoptosis and lymphocyte infiltration, to inhibit auto-reactive lymphocyte proliferation and to promote anti-inflammatory cytokines secretion, has been reported. Additionally, it has been demonstrated that they could potentially induce peripheral tolerance and immune responses modulation.Some studies indicated that MSC secretome could be a capable therapeutic agent for the inflammatory and degenerative diseases such as MS and rheumatoid arthritis [5]. In the current study, we isolated and implemented the exosomes-derived MSC as a potent carrier to deliver therapeutic LJM-3064 aptamer. Therefore, we evaluated the effects of MSC-derived exsosomes decorated with LJM-3064 aptamer on remeylination processes and immunomodulatory activity in myelin oligodendrocyte glycoprotein (MOG35-55)-induced mouse multiple sclerosis model (EAE).

MATERIALS AND METHOD

Conjugation of aptamer to exosomes

The 5' modified COOH LJM-3064 aptamer (40 nt) was used in order to conjugate the LJM-3064 aptamer to the surface of exosomes. Activation of aptamer carboxyl groups was conducted through EDC/NHS chemistry to covalently conjugate the aptamer to the

amine-containing molecules on the exosomes surface as follow: N-hydroxysuccinimide and 1-ethyl-3-(3-carbodiimide dimethyl-aminopropyl) were separately dissolved in 1 mL of PBS, and then both solutions were mixed. Four microliters of the aforementioned solution were added to 100 µL of 5' modified COOH LJM-3064 aptamer, and then added to PBS pH 7.4 and stirred for 4 h. In the next stage, exosome solution was added to the activated aptamer at 5% w/w ratio of aptamer/exosome and incubated for 24 h at room temperature while stirring. Finally, the reaction mixture was poured into the Amicon centrifugal ultra-filter and centrifuged at 9000 g for 10 min, to remove free aptamer from exosome-bound aptamers (Exo-APT).

Induction and evaluation of experimental autoimmune encephalomyelitis (EAE)

EAE induction was performed using Hooke Laboratories EAE induction kit according to the manufacturer's protocol. Briefly, the Female C57BL/6 mice were actively immunized by injection of an emulsion of MOG35-55 and complete Freund's adjuvant subcutaneously in each flank. Also, 250 ng pertussis toxin was injected intraperitoneally at the time of induction and 48 h later. All mice were daily weighed and monitored for clinical manifestations of EAE and their scores were recorded using a scale ranging from 0 to 7, in conformity with the following system: grade 0, no obvious clinical symptom; grade 1, partial loss of tail tonicity; grade 2, complete paralysis of tail; grade 3, abnormal gait with flaccid tail; grade 4, paralysis of hind legs; grade 5, hind limb palsy with partial immobility of hind body; grade 6, hind and forelimb paralysis; and grade 7, death or moribund. At the end of the study, each group was analysed for the onset day of disease, maximum mean clinical score (MMCS), and cumulative disease index, and the data were compared with those observed in the control group.

RESULTS AND DISCUSSION

Synthesis of Exo-APT bio-conjugate

To verify that the LJM-3064 aptamer was covalently conjugated to the surface of the exosomes, two experiments were conducted. First, the ATTO-labeled LJM-3064 aptamer was co-incubated with exosomes in the presence of EDC and NHS, and then the fluorescence intensity was analyzed by flow cytometry. The second experiment was agarose gel electrophoresis analysis which indicated successful aptamer conjugation on exosomes as the aptamer-conjugated exosomes did not show any mobility on agarose gel due to their ultra-high molecular weight (Figure 1B).

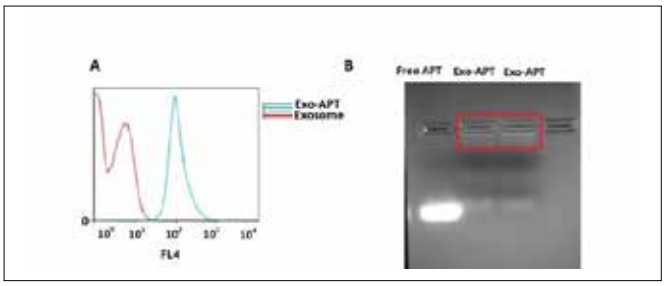

Figure 1. *Analysis of ATTO-labeled-aptamer-conjugated exosome (Exo-APT) by flow cytometry (A); Agarose gel 12% electrophoresis evaluation of aptamer-conjugated exosome (B).*

aAnalysis of clinical manifestation of EAE in both Exo and Exo-APT treated mice

In the current study, the effect of both Exo and Exo-APT on EAE model *in vivo* was evaluated via prophylactic and therapeutic approaches. As depicted in Figure 2, the first sign of clinical manifestation of EAE in control group was began with tail paresis on day 11.50±0.7, reaching a maximum mean clinical score (MMCS) of 5.5±0.28 on day 15 (Figure 5, A). In contrast, when mice received Exo as a prophylactic strategy, disease onset took place on day 12.50±0.7 and disease severity lessen on day 15 in comparison with control, with a mean clinical score (MCS) of 3.625 ±0.55 (Figure 2, A), while maximum mean clinical score (MMCS) reached 5.16±0.16 on day 22.

In this regard, in mice receiving Exo-APT with an equal dose of exosome as a prophylactic measure, disease onset started on 12.50±1.29 and disease severity was significantly reduced, with a mean clinical score (MCS) of 2.25 ±0.75 on day 15 in comparison with control (P=0.038). Non-significant differences were observed between all treatments groups except the Exo-APT-PR, which was significantly different from Exo-PR.

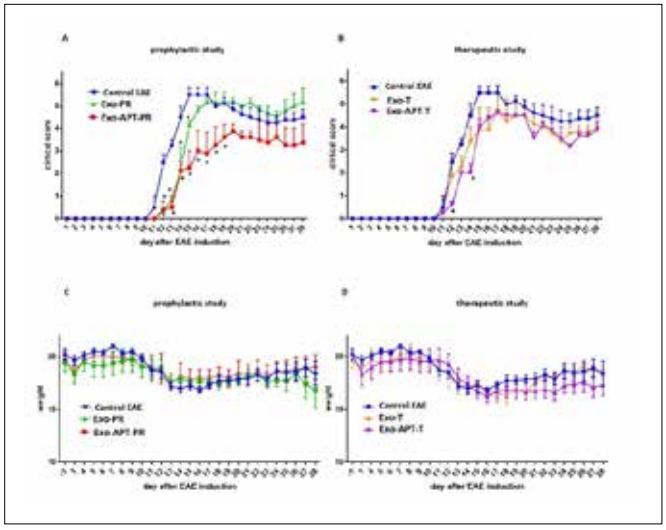

Figure 2. *EAE disease course in C57BL/6 mice after treatment with either Exo or Exo-APT. All mice were monitored for clinical manifestation of EAE, and the results were presented as the mean clinical scores (A, B); the mean body weight of treated mice during the experiment up to 28 days (C, D). Data were presented as mean ± SEM.*

It should be noted that the maximum mean clinical score (MMCS) of mice receiving prophylactic Exo-APT was 3.8±0.31 on day 20 showed a significant decrease compared to that of control group (P=0.028). (Fig. 2).

CONCLUSIONS

In the current study, MSC-derived exosomes exhibited immunomodulatory properties and when administrated as prophylactic treatment, reduced the MS clinical score. Additionally, our results demonstrated that surface functionalization of exosomes with LJM-3064 aptamer produced synergistic immunomodulatory properties and remyelination effect. In this regard, it was confirmed that prophylactic administration of Exo-APT significantly decreased the ameliorating disease severity by reducing Th1 response and increasing Treg population leading to the lowest inflammation and recruitment of inflammatory cells into the CNS. The obtained results were further supported by significant reduction in demyelination and pathological scores. The prepared bio-conjugated as a therapeutic and curative platform indicated neuroprotective properties as evidenced by increase in the number of total fibers and improvement in the thickness of the myelin sheaths (g-ratios).The importance of the work and the significance of the achievements should be highlighted in conclusion part. Avoid repeating the abstract as the conclusion.

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LIPOSOMAL PEPTIDE-BASED VACCINE COMBINED WITH LIPOSOMAL CELECOXIB FOR MELANOMA TREATMENT

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Introduction: Melanoma as one of the most immunogenic cancers, is considered as a good candidate to develop the immunotherapy treatment (1). In several studies, it has been shown that liposomal peptide-based vaccines can defeat cancer cells proliferation. To enhance the effectiveness of vaccination against cancer, we considered additional strategies. In this study, we evaluated the effect of combination of liposomal celecoxib with dendritic cells matured by melanoma antigen, gp-100 peptide for melanoma treatment. The therapeutic efficacy of this combination was evaluated in B16F10 bearing C57BL/6 mice. In this study, celecoxib as a cyclooxygenase-2 inhibitor would help to eliminate inhibitory mechanisms of tumor microenvironment and gp-100 peptide would enhance MHC I class presentation and cytotoxic T cell activation. Generally, we peruse three goals in this study: Determine the role of liposomal formulation as delivery system for drugs with low water solubility, understand the role of Dendritic cells in tumor growth, and evaluate the anti-tumor effects of cyclooxygenase-2 inhibitors

Methods: Liposomal formulations containing gp-100 peptide and celecoxib were prepared and characterized (2). C57BL/6 mice bearing B16F10 melanoma tumors were vaccinated with different formulations of gp-100 peptide in combination with administration of liposomal celecoxib. Immunological tests such as ELISpot assay, flow cytometry and cytotoxicity assay were performed on splenocyte suspensions, and the remaining mice were evaluated for tumor growth and survival analysis. The results were analyzed using GraphPad Prism software.

Results: In this study, therapeutic combination of liposomal celecoxib and gp-100 demonstrated an effective immune response and tumor regression in C57BL/6 mice bearing B16F10 melanoma (figure 1). The combinational therapy of dendritic cells + liposomal gp-100peptide + liposomal celecoxib leads to a significant amount of IFN-γ secretion and increased number tumor infiltrated lymphocytes (TILs) and cytotoxic activity.

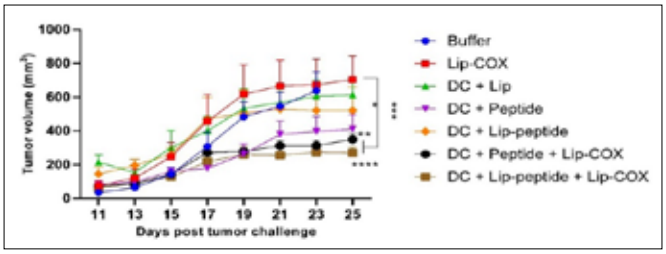

Figure1: *In vivo therapeutic efficacy of different liposomal formulations and tumor volume (mm3) of each mouse in each treatment group were evaluated and compared with Buffer groups. The values are means of tumor size ± SEM.*

Conclusion: Taken together, our data supports the rational development of combination of liposomal gp-100 matured dendritic cells and liposomal celecoxib to overcome the immunosuppressive tumor microenvironment, improve immunologic response and enhanced therapeutic outcomes in melanoma model. All in all, this combination could be employed as a promising vaccine to generate potent CTL anti-tumor immune responses that could be beneficial to treatment of melanoma.

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CIBER-BBN: A SUCCESS STORY OF COLLABORATION BETWEEN DIFFERENT RESEARCH INSTITUTIONS IN THE NANOMEDICINE FIELD IN SPAIN

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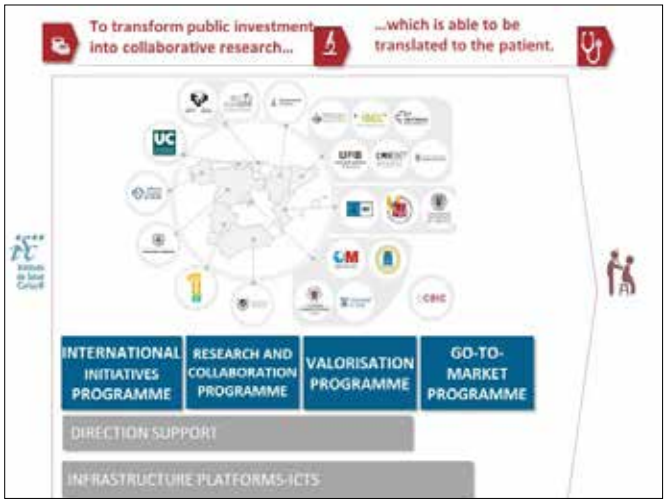

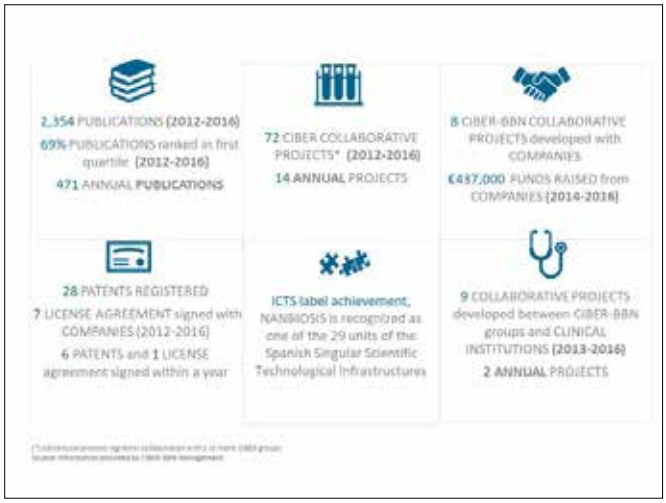

Figure 1. *(1) CIBER-BBN's programmes map; (2) Main scientific and technological CIBER-BBN results (2012-2016).*

Translation to the clinical practice of novel nanomedicines, medical devices and other nanotechnologies and tools for biomedicine is a complex process that requires specific knowledge and experience. Over the last decade, nanomedicine has seen its deployment as a fully-fledged sector within an organised and growing community, supported by national and European policies for funding research and translation. As a result, there exists currently a mature and excellent research community working at cutting-edge technologies. However, translation into clinical applications is still slow.

Within the Biomedical Research Network (CIBER-BBN), forty-six groups of internationally recognized scientific and technological high level collaborate in projects with the purpose of conducting both translational research and industrial transference. CIBER-BBN counts with its own scientific programme that is divided into three different areas: i) Bioengineering, ii) Biomaterials and iii) Nanomedicine. Focused on these three research fields, early stage development projects of new biomedical tools are designed, taking into

account from the very beginning new and advanced technologies for manufacturing processes and advanced characterization and regulation aspects to guarantee the success and viability of the developed technologies.

The new Master Plan 2018-2021 has defined five strategic activities: a) International Projects and Initiatives, b) Research and Collaboration, c) Valorisation, d) Industrial Transference and e) Go-to-market. The objective is to guide excellent basic and applied research through the innovation process to develop new nanomedicine products/applications/tools. Moreover, the close collaboration with experts on Nanosafety, regulatory issues, investors and consultancy experts help us in the preparation of business plans for the creation of new spin-offs and start-up companies.

Examples of some of our initiatives are the calls raised within CIBER-BBN, launched in January 2018: a) the Intramural collaborations 2018-2021 call and ii) the Early Stage projects call. As a result, 112 collaborative projects, with direct funding for the groups, and 28 early stage projects, in which young researchers lead their own projects, are today ongoing. Results from previous calls (from 2012 to2016) have shown a very fruitful collaborative network. The fostering of the translation of early research of excellence in Nanomedicine is always the fixed and common objective of CIBER-BBN and its groups.

P62/SQSTM1 MEDIATED AUTOPHAGY MODULATION INDUCED DNA DAMAGE IN A549 CELLS ON EXPOSURE OF GRAPHENE OXIDE-CHLOROQUINE NANOCONJUGATE

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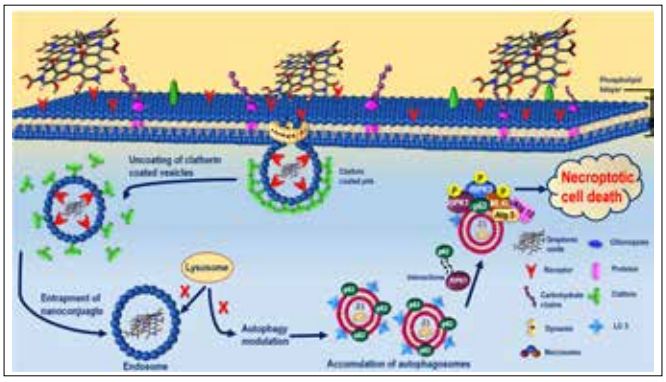

Cancer being the second leading cause of mortality & morbidity and has been accounted for 9.6 million deaths worldwide in the year 2018. According to recent GLOBOCAN 2018 report by International Agency for Research on Cancer (IARC), lung cancer is the leading cause of cancer associated deaths and causing 1.8 million deaths (18.4 % of the total) in 2018 worldwide. Conventional cancer therapy encompasses one or more modalities such as surgery, radiotherapy, hormone therapy and chemotherapy specifically for every cancer type. Despite of the research advances, unfavorable pharmacokinetics, acquired drug resistance, alterations in molecular targets, activation of prosurvival cell signaling cascades and ineffective induction of cell death limits the effectiveness of chemotherapy. Specifically, multidrug resistance and poor target recognition demands intense and collaborative efforts among clinician's, nanotechnologist, chemist and bio-engineers.

Recently, nanotechnology – based chemotherapeutics represent a new era of "cancer nanomedicine" providing enhanced molecular targeting and specificity, favorable pharmacokinetics and reduced side effects. Among various carbon based nanomaterials, graphene oxide (GO) owing to their extra ordinary and versatile surface properties have shown excellent potential in several preclinical studies. Recent reports on the biological properties of GO like autophagy modulation, specific molecular target recognition, enhanced specificity and better drug sensitization provides new paradigms to rest it's efficacy in cancer nanomedicine.

Cancer cells may exploit autophagy in response to cellular stress and/or increased metabolic demands for their rapid cell proliferation. On the other hand, inhibition of autophagy by nanomaterials or in combination with pharmacological inhibitors (like chloroquine, wortmannin, thapsigargin, matrine, Azithromycin and Verteporfin etc) could be an important strategy to greatly enhance the efficacy of conventional therapy platforms in multidrug resistant tumor. On the other hand, various factors like DNA repair activation mechanism by either autophagy or other complex cell signaling cascades like PARP-1 activation selectively helps cancer cells to maintain resistant to conventional chemotherapeutics drug. Hence, we envision the designing of Graphene oxide – Chloroquine nanoconjugate to specifically target DNA repair activation mechanism in A549 lung cancer cells through p62/SQSTM1 mediated autophagy modulation.

In the present study, we have conjugated Graphene oxide (GO) known to alter the autophagy responses and Chloroquine (Chl) an autophagy inhibitor and studied the genotoxicity and cell signaling pathways for cell death in human lung cancer A549 and normal lung BEAS-2B cell lines. Chloroquine (Chl) is a FDA approved drug for malaria and has also shown anticancer potential. Structural, functional and optical properties of GO, Chl and GO-Chl have been investigated using Raman, FTIR and UV- Vis spectroscopy respectively. The morphological aspect of GO and GO-Chl was analyzed using TEM, FESEM. Further, AFM measurements revealed the wrinkled morphology and formation few layer and monolayers (0.92–1.17 nm) of GO nanosheets. MTT assay exhibits significant cell death in A549 lung cancer cells on exposure with GO-Chl and negligible toxicity of BEAS-2B cell lines. DCFDA assay reveals that GO-Chl exposure enhance generation of ROS. Flow cytometry based Propidium Iodide (PI) assay reveals the plasma membrane disruption leading to alteration in the cell cycle and is attributed to ROS generation. Further, flow cytometry based Annexin V/PI assay for cell cycle analysis indicate towards the halts of the cell cycle at G1 phase and possible alterations in the DNA damage response due to exposure of GO-Chl nanoconjugate. The DNA damage in A549 cells was accessed using Comet assay. The result reveals a significant increase in Olive tail moment with increasing concentration of GO-Chl. Further, the autophagy response in A549 cells due to GO-Chl treatment is investigated through fluorescence microscopic analysis (MDC staining and GFP-LC3 plasmid), TEM observations and immunoblot analysis. Enhanced level of LC-3 I/II and Atg-5 markers signifies the autophagosomes formation and elevated expression of p62/SQSTM1 indicates the inhibition of autophagy at later stage. Further, the enhanced level of p62/SQSTM1 are expected to inhibit the DNA repair mechanism. The modulation of autophagy machinery with DNA damage pathways may provide a therapeutic window for next generation nanomedicine and GO-Chl nanoconjugate could act as potential therapeutic agent.

Keywords: Graphene oxide, Chloroquine, Autophagy, Necroptosis and DNA damage.

SCALABILITY AND REPRODUCIBILITY OF IRON OXIDE NANOPARTICLES (IONP'S) REVISITED AND UPGRADED FOR USE IN NANOMEDICINE

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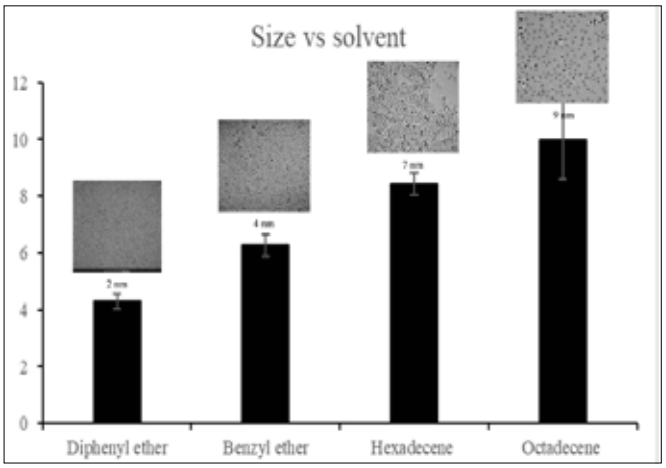

Figure 1 *The graph represents changes observed in size of nanoparticles with change of solvent*

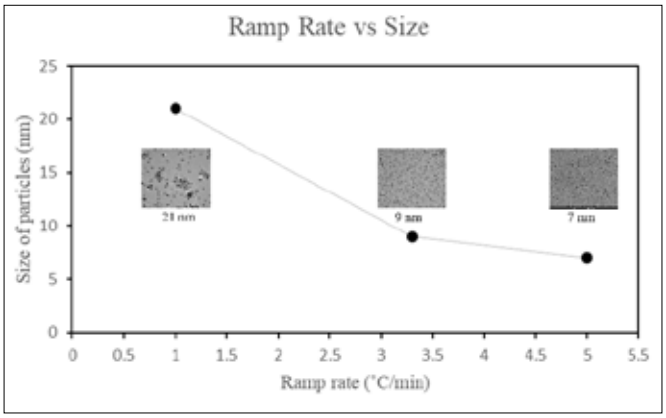

Figure 2 *The graph depicts changes observed upon changing the ramp rate while keeping remaining parameters constant*

Over the last decade, nanomedicine has shown exceedingly promising results which, if translated to be used as common clinical practices, hold the capability of revolutionizing the healthcare industry in the future. This successful translation from bench to bedside requires, in the first place, a very efficient synthesis process. There are numerous reports on nanoparticles syntheses, but almost none of them talks about the scalability or reproducibility, making these two factors the main roadblocks in bringing nanomedicine to the patient. In this poster, we discuss how existing protocols fare in terms of reproducibility as well as scalability, for one of the most used nanoparticles in the industry - iron oxide nanoparticles, before presenting our own method, which yields ultra-small nanoparticles with high reproducibility as well as scalability. Thermal decomposition method was chosen as mode of synthesis because it is the most commonly used for biomedical applications due to its several advantages, which include narrow distribution of size, good crystallinity as well as saturation magnetization. For the purpose of our study, we tried some major precursors, such as iron oleate and iron acetylacetonate because the reaction tends to proceed differently depending on the nature of the precursor[1]. We also used different solvents (like hexadecene, octadecene, benzyl ether, diphenyl ether) and ramp rates since the size and shape of the nanoparticles has been shown to be dependent on them as well[2,3]. Finally, the magnetic relaxivity of particles will be discussed since the particles we synthesized are to be used as contrast agent in magnetic resonance imaging (MRI). Our results exposed a few loopholes in different articles, which will be discussed further in the poster presentation. The characterization techniques used include x-ray diffraction (XRD), transmission electron microscopy (TEM), dynamic light scattering (DLS), magnetic relaxivity at 1.5T, etc.

Keywords: nanomedicine, contrast agents, MRI, nanoparticles

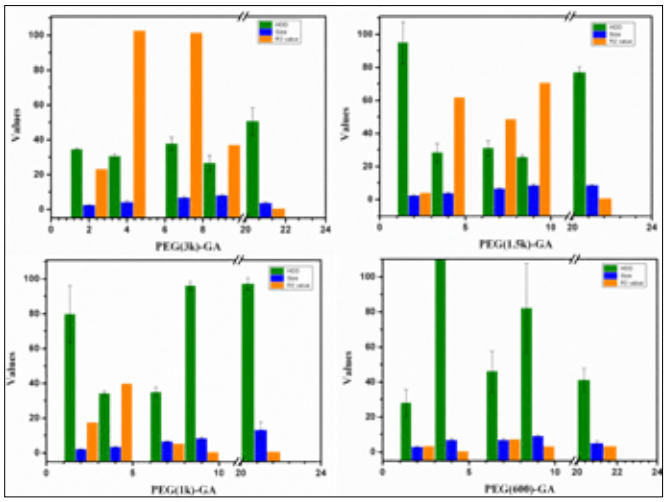

Figure 3 *The graphs show the relaxivity values of different sized nanoparticles (2,4,7,9 and 21) with PEG(x)-gallic acid ligand (x=3k, 1.5k, 1k, 600) at 1.5T*

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CO-DELIVERY OF CAMPTOTHECIN AND SURVIVIN SHRNA VIA TARGETED ROD-SHAPED MESOPOROUS SILICA NANOPARTICLE FOR SYNERGISTIC CANCER THERAPY

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INTRODUCTION

Colorectal cancer (CRC) is the world's third most prevalent malignancies with continuously increased incidence and mortality [1, 2]. The present research project is focused on investigation of the capability of AS1411 targeted rod mesoporous silica nanoparticles as a drug delivery systems and evaluate the synergistic effect the codelivery of camptothecin and Survivin shRNA-expressing plasmid to down-regulate survivin gene expression in nucleolin positive C26 cell line. We also assessed the therapeutic efficiency of the targeted platform *in vitro* and *in vivo*.

METHODS

Targeted and non-targeted pegylated mesoporous silica nanorods (PEG-MSNRs) were prepared according to the previous report with some modification [3] and employed to load camptothecin (CPT) and survivin shRNA expressing plasmid to form nanoplex. The formulation were evaluated invitro in terms of structural characterization, drug loading, controlled release efficiency, cytotoxicity, gene transfection and apoptosis. Moreover *in vivo* tumor growth, bio

distribution and survival in C26 tumor bearing mice were extensively tested.

RESULTS

Electron microscope images revealed that MSNRs were well prepared in rod shapes with an average length of 100 nm. PEG-MSNRs showed high loading capacity for CPT and iSur-pDNA due to their porous structures and net positive charge.
The results manifested that the developed Apt-PEG MSNRs@CPT/Sur targeted efficiently to nucleolin positive C26 cell lines and shows higher cytotoxicity than nontargeted PEG-MSNRs@CPT/Sur form.

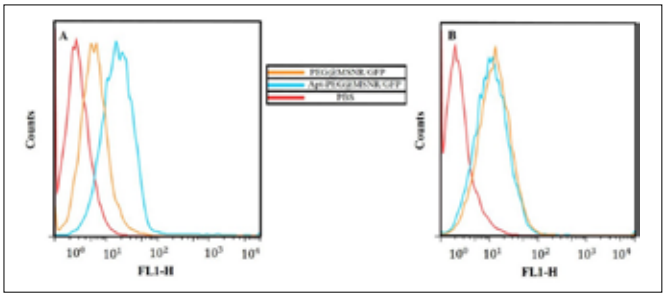

Figure 1: *Fluorescent intensity histogram to demonstrate cellular uptake of targeted and non-targeted formulation in A) C26 and B) CHO cell lines.*

Further investigations indicated that Apt-PEG MSNRs@CPT/Sur show higher apoptosis rate in C26 compared to PEG MSNRs@CPT/Sur, PEG MSNRs@CPT and free CPT.

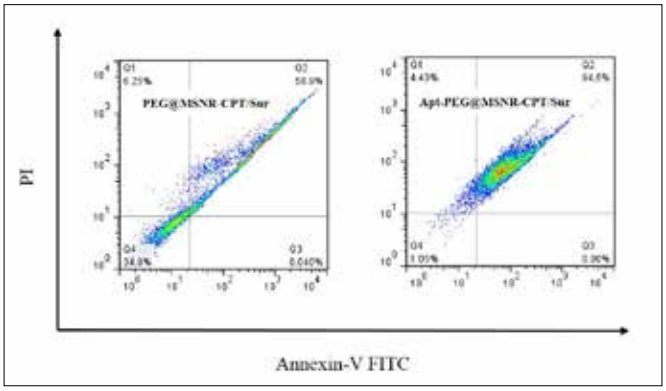

Figure 2: *Apoptosis effects of Apt-PEG MSNRs@CPT/Sur compare to PEG MSNRs@CPT/Sur on C26 cells lines.*

Conclusion: Obtained results demonstrated that the prepared nanohybrid has unique properties which might be a promising carrier for codelivery of chemotherapeutic drugs and gene drugs for synergistic cancer therapy.

Keywords*: mesoporous silica nanorods, Camptothecin, Survivin, co-delivery*

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THROMBOXAN-RELATED TRANSIENT HYPERTENSION IN RATS CAUSED BY AN AMPHOTERICIN-B-CONTAINING LIPOSOME

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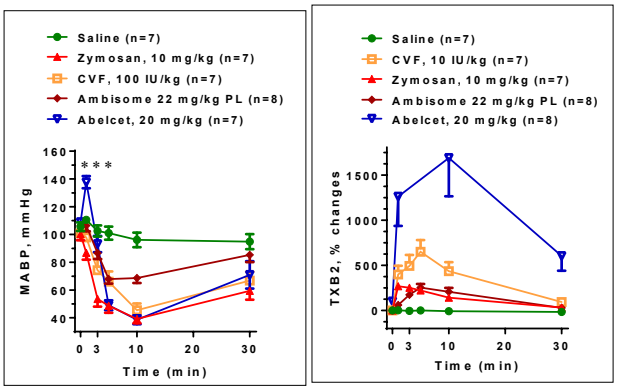
Background: Pharmacotherapy with nanomedicines can cause infusion reactions, which are acute, IgE-independent, considerably varying hypersensitivity reactions (HSRs). Complement (C) activation is one explanation for the HSRs, termed C activation-related pseudoallergy (CARPA). Recent studies raised the possibility of C-independent mechanisms, referred to as C-independent pseudoallergy (CIPA). The current study was designed to evaluate the side effects caused by two liposomal amphotericin B formulations with substantially different physicochemical characteristics (AmBisome and Abelcet) in anesthetized rats. AmBisome consists of small unilamellar liposomes, while Abelcet forms large multimicron ribbon-like lipid complexes.

Methods: Male Wistar rats (Toxicoop Ltd., Budapest, Hungary) weighing 270–350 g were anesthetized with pentobarbital (60 mg/kg i.p., Sigma Aldrich, Budapest, Hungary). Mean arterial blood pressure (MABP) was measured in the left carotid artery using a BPR-02 pressure transducer and an HG-01D amplifier (Experimetria Ltd., Budapest, Hungary). The data were collected using a PowerLab data acquisition system (ADInstruments Ltd., Oxford, United Kingdom) and were recorded on a desktop computer using LabChart data analysis software (ADInstruments Ltd., Oxford, United Kingdom). Blood (0.5 mL) was collected from the carotid artery prior to treatments (time 0) and at 1, 3, 5, 10, and 30 min after treatments (Ethical Approval: PEI-001/3948-6/2014).
Saline, AmBisome (22 mg/kg PL, Gilead Sciences Int., Ltd., Cambridge, England), cobra venom factor (10 U/kg, Quidel Co., San Diego, CA, USA), Abelcet (20 mg/kg, Sigma-Tau Pharmaceuticals, Inc. Gaithersburg, USA) and zymosan (10 mg/kg, Sigma Hungary Ltd., Budapest, Hungary) were injected over 1 min into the left femoral vein. Total plasma C3 concentration was measured using a MicroVue Pan-Specific C3 Reagent kit (Quidel, San Diego, CA, USA). Thromboxane B2 (TXB2) was measured by ELISA (Cayman Chemical, Ann Arbor, MI, USA). Blood cell counts were measured using an Abacus Vet5 hematology analyzer (Diatron MI Co., Budapest, Hungary).
All data presented are means ± SEM. Statistical analysis was performed using two-way ANOVA for repeated measurements followed by Dunnett's multiple comparisons test (GraphPad Prism v6; GraphPad Software, La Jolla, CA, USA).

Results: Abelcet was the only liposomal preparation that caused an initial increase in MABP, all other liposomes and also the C activators induced hypotension only. This unusual response was paralleled by an extreme increase in plasma TXB2 concentration, which considerably exceeded the TXB2 responses to other treatments. Thrombocytopenia was also the greatest after Abelcet administration. Abelcet also caused a 5-fold increase in plasma C3a concentration.

Conclusions: The two liposomal amphotericin B formulations caused contrary initial blood pressure changes in anesthetized rats, since Abelcet increased but AmBisome decreased blood pressure. The initial increase in blood pressure after Abelcet administration

seems to be related to the extreme increase in plasma TXB2 concentrations.



BIOMIMETIC MICROPILLARY-ON-A-CHIP TO MODULATE ENDOTHELIAL PERMEABILITY AGAINST NANODELIVERY SYSTEMS

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Microfluidic technologies provide the possibility to overcome the limitations of traditional 2D assays used in cell biology. By combining 3D cell cultures with flow systems that mimic the physiologically relevant conditions and functions of organs and tissues, microfluidic models have gained attention during last decade [1]. These devices referred to as lab-on-a-chip, present several advantages, such as reduction of samples' volume, reduction of costs, and fine control of the cell microenvironment [2]. In the field of biomedical applications, microfluidic systems are appered in drug testing, drug screening, and disease modeling platforms.

On the other sire looking at the advances in the nanotechnology area, a plethora of nanoparticles (NPs) have been fabricated, varying in size, shape, surface chemistry and stiffness. In order to facilitate the rapid screening of NPs in terms of transport and efficacy for multiple clinical applications, microfluidic systems offer an efficient and cost-effective opportunity [3]. Most NPs for clinical use are intravenously administered in the blood to reach the diseased tissue, whereby the first barrier to reach the target tissue is posing the blood vessel endothelium. Hence, the construction of biomimetic capillaries *in vitro* will provide an effective way to understand the vascular journey of NPs in the microcirculation.

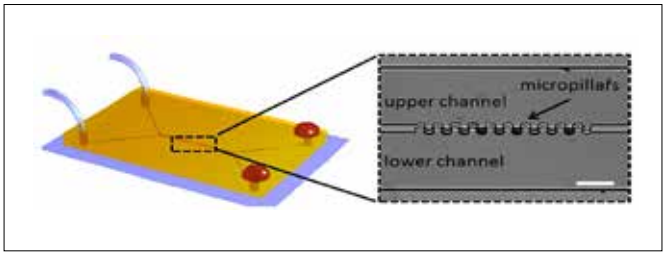

Figure 1. *Microfluidic chip with permeable walls.*

So far, blood vessel-on-a-cahip have been applied to study NPs margination, effect of vessel geometry, shear stress, vessel permeability and NPs translocation across the endothelium [4]. Although the integrity of the vascular barrier is often compromised within a tumor, drug delivery systems have to escape the vascular flow and cross the endothelial cell barrier. The barrier could be modulated by inducing the shrinkage of the endothelial cells with hyperosmotic solution, like mannitol, [5] or by selective activation of Adenosine Receptor A2A, via agonists like Lexiscan [6]. Here, a double-channel microfluidic system with a lower channel and an upper channel connected in the middle part by an array of micropillars that act as a membrane. The upper channel mimicks the vascular compartment, while the lower channel serves as the extravascular compartment, represented by a complex extracellular matrix (ECM) [7]. ECM components can influence cell polarity, metabolism, fate, and migration [a]. For this reason, it is important to design an ECM that mimics the *in vivo* features based on the biological components of the system. In this work, the ECM matrix, loaded in the lower channel, is composed by a mixture of collagen type I and Matrigel. The upper channel is seeded with Human Umbilical Vein Endothelial Cells (HUVECs) in order to build up a more biological relevant vessel-on-a-chip. Confocal fluorescence microscopic analysis, together with Scanning Electron Microscopy (SEM), revealed a continuous monolayer of endothelial cells adherent to the inner surface of the upper channel with continuous VE-Cadherin-containing junctions.

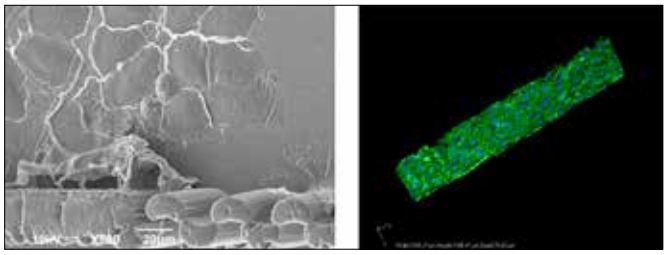

Figure 2. *Characterization via SEM and Confocal microscopy of the endothelium-lined microvessel on-chip.*

The micropillary-on-a-chip allows us to characterize, under dynamic conditions, the paracellular permeability of the vascular walls in real-time using a fluorescence microscope. 250 kDa Dextran (Dex) and 200 nm spherical beads have been injected in the vascular channel at a flow rate of 0.1 µL/min and thier diffusion was followed for a maximum of 10 minutes. In order to open the vascular walls and enhance the tissue accumulation of the two probes, endothelial cells were pretreated up to 30 minutes, with Mannitol 1 M or Lexiscan 1µM. After treatment, at specific time points, the paracellular permeability for Dex and beads was evaluated, as previously reported. As expected, the hypermeabilization effect related to the hyperosmotic solution of Mannitol is higher with respect to the opening induced by Lexiscan and it is also time-dependent. Conversely, the adenosine receptor agonist presents a hyperpermeabilization effect that peaked around 15 minutes of treatment and is stable up to 30 minutes.

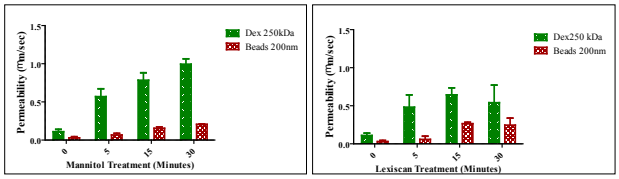

Figure 3. *Permeability modulation of the vasculature. On the left the hyperpermeabilization with 1M of Mannitol up to 30 minutes. On the right, the hyperpermeabilization reported with 1µM of Lexiscan at the same time points.*

Finally, a 3D-micro vascular model integrated with a complex ECM protein-based hydrogel was developed. This provides a physiological microenvironment for endothelial cells. Endothelial permeability was followed in real-time via fluorescence microscopy. Modulation of endothelial permeability was achieved via osmotic or receptor-mediated opening. These data demonstrate the ability to predict the behaviors of the vasculature under different stimuli and to investigate the capacity of NPs to permeate the endothelial walls and accumulate in the extravascular space.

Acknowledgments: This project was partially supported by the European Research Council, under the European Union's Seventh Framework Program (FP7/2007-2013)/ERC grant agreement no. 616695, by the Italian Association for Cancer Research (AIRC) under the individual investigator grant no. 17664, by the European Union's Horizon 2020 research and innovation program under the

Marie Skłodowska-Curie grant agreement No 754490, by the British Heart Foundation (BHF) grant RE/13/5/30177; the *Engineering and Physical Sciences Research Council* (*EPSRC*) grant EP/L014165/1 and the European Commission Marie Skłodowska-Curie Individual Fellowships 661369. The authors acknowledge the precious support provided by the Nikon Center; the Electron Microscopy and Nanofabrication facilities at the Italian Institute of Technology.

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FINE TUNING CORE CROSS-LINKED POLYMERIC MICELLES – IMPAIRING NANOPARTICLE FORMATION AND DRUG-CONJUGATION

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Polymeric micelles are conceived to improve bioavailability and pharmacokinetics and to reduce off-target toxicity of hydrophobic drugs (e.g. taxanes). Once assembled, micelles remain dynamic structures readily affected by drug loading and biological environment calling for additional stabilization. To achieve a more specific accumulation in diseased tissue, long-term circulation and triggered drug-release are key features for second-generation nanomedicines.[1]

This study introduces polypept(o)ides for facile and robust preparation of drug-conjugated core cross-linked polymeric micelles. Polypept(o)ides represent a novel class of copolymers that combine the shielding properties of polysarcosine, a PEG surrogate with identical solution properties but improved immunological profile,[2] with the diverse functionality of polypeptides.[3] For the synthesis of core cross-linked polymeric micelles, block copolymers of polysarcosine-block-poly(S-ethylsulfonyl-L-homocysteine) and polysarcosine-block-poly(S-ethylsulfonyl-(D/)L-cysteine) have been developed. In both cases, the S-ethylsulfonyl-protecting group allows for chemoselective disulfide bond formation upon micelle preparation by self-assembly.[4] In combination with hydrazide-modified cross-linkers, the process of nanoparticle synthesis and purification can thus be decoupled from drug-conjugation. As a result, pH- and redox-responsive paclitaxel-containing core cross-linked polymeric micelles have been prepared by this strategy (Dh 30-40 nm). While poly(S-ethylsulfonyl-L-homocysteine) encodes for an α-helical superstructure, poly(S-ethylsulfonyl-L-cysteine) tends to form antiparallel β-sheets. As reported previously, this feature can be exploited for secondary structure-directed self-assembly giving access to various morphologies.[5] At the same time, the superstructure-formation impacts the drug conjugation yield. Moreover, cross-linker architecture affects the circulation behaviour, as investigated using the zebrafish embryo model.[6]

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SIZE RATHER THAN TARGETING LIGAND AND THE INFLAMMATORY MEDIATOR USED, DOMINATE NANOPARTICLE TRANSLOCATION AND BINDING ACROSS THE DYSFUNCTIONAL ENDOTHELIUM

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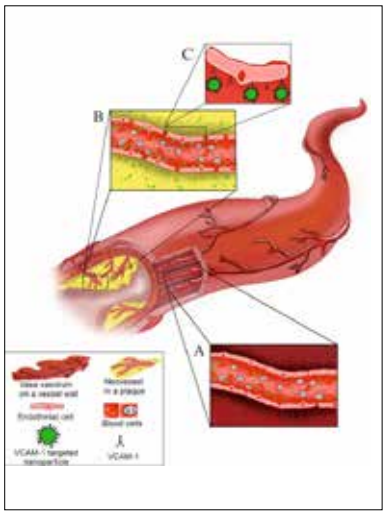


Fig.1. *(A) Structure of a vasa vasorum on a vessel wall. (B) Structure of a neovessel, which is sprout from vasa vasorum to a plaque. Nanoparticles can translocate from these neovessels to a plaque environment due to the leaky and compromised structure of these neovessels. (C) Upregulation of VCAM-1 receptors on endothelial cells of the neovessels leads to the binding of VCAM-1 targeted nanoparticles to the cells.*

Atherosclerosis is characterized by endothelial dysfunction, which is the most important pathological factor in this disease. Enhanced

permeability of dysfunctional endothelial layers on the main lumen as well as leakiness of the intraplaque neovessels can be considered as a promising strategy for passive targeting (*i.e*. non-specific targeting) of nanoparticles to atherosclerotic lesions. Moreover, dysfunctional endothelium and enhanced inflammatory responses in atherosclerosis provide numerous opportunities for active targeting (*i.e.* site-specific targeting) of nanoparticles. Among the most frequently-used targeting strategies, adhesion molecules such as vascular cell adhesion molecule 1 (VCAM-1), which shows upregulation and overexpression on dysfunctional endothelial cells and intraplaque neovessels, is a commonly utilized targeting approach (Figure 1). Understanding nanoparticle biophysicochemcial interactions with dysfunctional endothelial cells is important in the design of optimal nanoparticles that can traverse this barrier to atherosclerotic plaques. On the other hand, design of optimal nanoparticles, which show successful targetings and higher therapeutic effects *in vitro*, is a fundamental step toward successful *in vivo* tests. Since, *in vitro* screening models are the initial step and the most promising predictor for not only animal studies but also subsequent clinical trials involving patients. In this work, we have investigated the effects of a range of inflammatory mediators relevant to heart disease in order to assess their effects on endothelial cell tight-junction disruption and used this as a model to screen differently sized fluorescent polystyrene nanoparticles targeted to the VCAM-1 receptor using three known VCAM-1 binding peptides. It was the goal of this study to assess the most optimal nanoparticle properties for 1) VCAM-1 binding and 2) translocation across the inflammed and leaky endothelium. For this purpose, a confluent monolayer of HUVEC (Human emberical vein endothelial cells) was cultured on a Transwell® filter inserts (polyethylene terephthalate membrane,1 µm pore size) to mimic a healthy endothelial layer of a vessel wall. Subsequently, the endothelial layers were activated and stimulated by atherosclerotic inflammatory mediators to mimic a dysfunctional endothelium. For this purpose, three different inflammatory mediators (*i.e.* tumor necrotic factor alpha (TNF-α), interleukin 1 beta (IL1-β), and thrombin) were used based on our preliminary studies. Tight junction formation and tight junction disruption, which are respectively the indicator of healthy endothelial barrier and leaky endothelial layer, were visualized by VE-Cadherin immunostaining (Fig. 2.A). Furthermore, immunostaining of VCAM-1 was carried out to compare the expression of this cell adhesion molecule on the healthy and dysfunctional endothelial layers (Fig.2.B). As can be seen in Fig.2, the junction disruption and VCAM-1 expression for stimulated endothelium (dysfunctional endothelium) are higher than the healthy endothelium. Among the stimulated cells, TNF-α-stimulated cells and thrombin-stimulated cells show the highest and lowest effect, respectively.

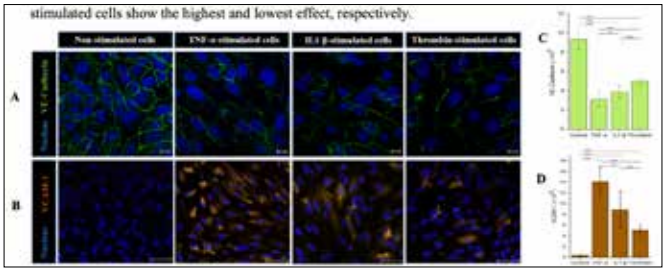
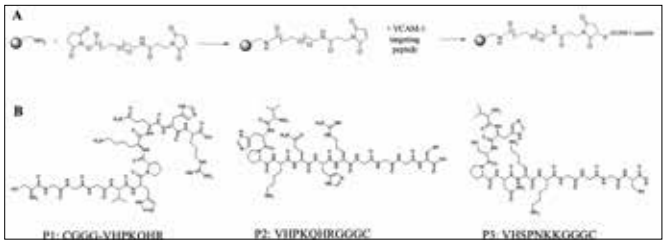


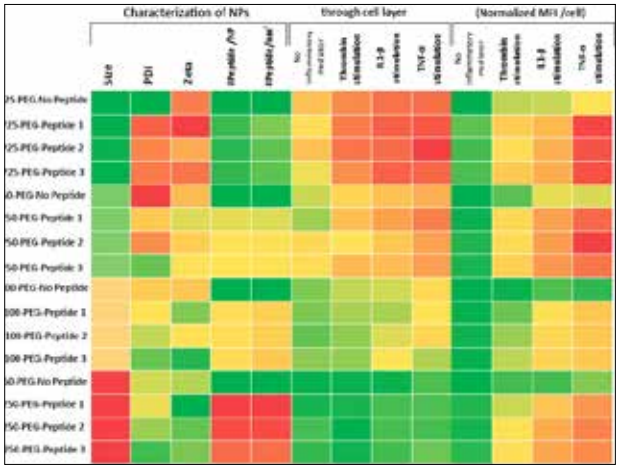
Fig.2. *(A) VE-Cadherin staining (scale bar 10 µm), and (B) VCAM-1 staining (scale bar 50 µm) of HUVEC cells before and after incubation with different inflammatory mediators. Quantification of (C) VE-Cadherin and (D) VCAM-1 signals.*

At the next step, a library of NH2-functionalized and Rhodamine-coupled nanoparticles with different sizes of 25, 50, 100, and 250 nm (abbreviated as NP25, NP50, NP100, and NP250) were pegylated as can be seen in Fig.3.A. Then, the pegylated nanoparticles were coupled with three different VCAM-1 targeting peptides (Fig.3.B).

Fig.3. *(A) Development of VCAM-1 targeted nanoparticles, (B) Chemical structure of VCAM-1 targeting peptides used in this study.*



After the development of the nanoparticle library, the size, polydispersity index (PDI), and zeta potential of the nanoparticles were measured using dynamic light scattering (DLS) technique. Furthermore, the number of peptides per nanoparticle ($\#_{peptide}/NP$) and surface density of peptides ($\#_{peptide}/nm^2$) were calculated based on the difference of the absorbance at 205 nm between peptide-coupled nanoparticles and bare nanoparticles. To investigate the transendothelial permeability of the nanoparticles through the healthy and dysfunctional endothelium, nanoparticles were added to the apical compartment of Transwell inserts. After 24 h incubation, the concentration of the nanoparticles in the basal compartment was measured based on the rhodamine fluorescence intensity of the translocated nanoparticles. In order to study the binding of the nanoparticles to healthy and dysfunctional endothelium, the excess nanoparticles were washed after 24 h incubation of the nanoparticles with endothelial layers. The cells were fixed, the nucleus was stained by Hoechst, confocal microscopy was performed, and the mean fluorescent intensity of rhodamine (MFI) and the number of nucleus for each sample were measured. As shown in Fig.4, the smaller nanoparticles show higher permeability through the dysfunctional endothelium. The percentages of translocated NP25 through the endothelial layers stimulated by different inflammatory mediators, are in the range of 3.6 to 5.3%. However, this range drops to 1.6 to 3.8%, 0.9 to 1.8%, and 0.2 to 0.7% for NP50, NP100, and NP250 respectively, highlighting the fact that smaller nanoparticles are better candidates for passive targeting of nanoparticles toward atherosclerotic lesions. Moreover, NP25 shows remarkably elevated binding to dysfunctional endothelium by active targeting of VCAM-1, compared to other NPs. The binding of VCAM-1 targeted NP25 to TNF-α-stimulated cells, IL1-β-stimulated cells, and thrombin-stimulated cells are respectively 18, 8, and 5 times higher than the binding of bare nanoparticles to un-stimulated cells. The result of these experiments, which indicates that the size of nanoparticles plays a crucial role in not only passive targeting but also active targeting of the nanoparticles, sheds more light on the design of more effective nanoparticles for atherosclerotic nanomedicine.



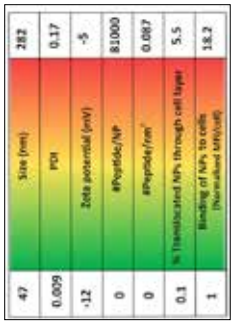


Fig.4. *(A) Heat map shows characterization of the VCAM-1 targeted nanoparticles (size, PDI, zeta potential, number of peptides per nanoparticle, and surface density of the peptides), percentage of the translocated nanoparticles through the healthy and dysfunctional endothelium, and binding of the nanoparticles to the healthy and dysfunctional endothelium. (B) Heat map parameters.*

CYCLOSPORIN A LOADED LIPID NANOPARTICLES FOR THE INTRAVENOUS TREATMENT OF RETINOPATHY OF PREMATURITY

MARILENA BOHLEY

Retinopathy of prematurity (ROP) is among the leading causes of childhood blindness in both the developing and developed world with increasing incidence due to an increased survival of infants born at very early gestational ages [1]. Visual impairment and blindness are caused by massive damages of the retina due to enormous delocalization of blood vessels in the posterior eye [2]. Mechanistically, the vascular endothelial growth factor (VEGF) was identified as a key regulator for blood vessel proliferation and hyperpermeability [3]. Currently, the standard therapeutic treatment consists of either peripheral retinal ablation with laser or invasive intravitreal VEGF inhibitor injections [4]. With the generalized VEGF knockdown seeming to be beneficial in contrast to the destructive, complication causing, and less efficient laser treatment [5]. However, the profound and durable suppression of VEGF successfully inhibits the pathologic angiogenesis, VEGF is of utmost importance for the physiologic developmental angiogenesis and vital for the overall ocular homeostasis and integrity since VEGF biologically affects numerous cell types in the retina such as Müller cells, astrocytes, photoreceptors, and retinal pigment epithelial cells [6,7]. Consequently, we reasoned that a systemic cell-specific, VEGF modulating nanotherapy could be a major accomplishment in the treatment of retinopathy of prematurity.Here, we use nanoparticles that can be administered intravenously and allow for efficient drug delivery to the retina by specific accumulation in endothelial cells and retinal pigment epithelial (RPE) cells as a systemic cell specific drug delivery system. For intracellular anti-VEGF therapy, the nanoparticles were loaded with the well-known and widely used immunosuppressant Cyclosporin A (CsA). Cyclosporin A is not only able to modulate immune responses, but interferes at multiple sites with the VEGF signaling pathway and additionally possesses anti-inflammatory activity, thereby being the perfect drug candidate for the treatment of retinopathy of prematurity [8,9]. Here, we investigate whether CsA loaded nanoparticles are able to efficiently inhibit retinal neovascularization by modulating the VEGF levels using the mouse model of retinopathy of prematurity (Fig. 1).

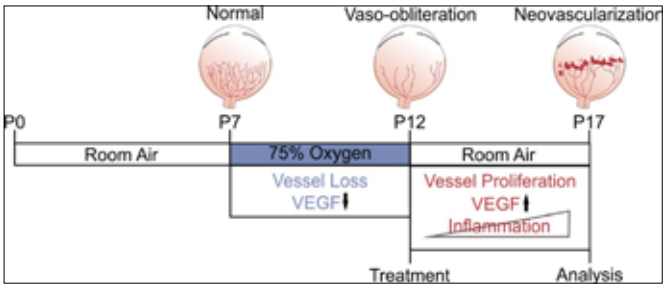


Fig. 1. *Cartoon depicting the pathogenesis and intervention regime in the mouse model of retinopathy of prematurity.*

In mice with retinopathy of prematurity the CsA loaded nanoparticles showed tremendous effects on the suppression of neovascularization, efficiently reducing the extent of vessel proliferation down to level comparable with healthy mice (Fig. 2).

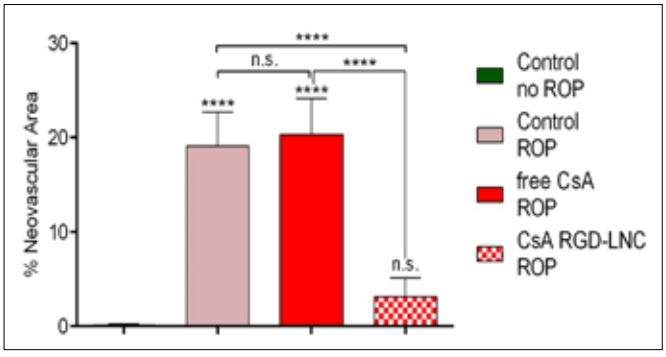


Fig. 2. *Quantification of the relative neovascular area in retinae at P17 after the treatment with free CsA or CsA loaded RGD-LNC at P12.*

As the free CsA had no effect on the neovascularization, we hypothesized that this must be due to the poor bioavailability of the drug in the posterior eye. To verify our hypothesis, the drug content in the posterior eye after the intravenous application of either free CsA or CsA loaded particles was investigated (Fig. 3).

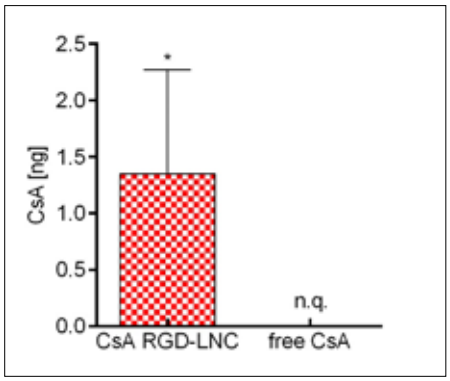


Fig. 3. *UHPLC-MS measurements of the drug content in the posterior eye at P17 after the intravenous application of either free CsA or CsA loaded RGD-modified nanoparticles at P12 of mice with ROP.*

The analysis revealed that detectable amounts of drug are only present in the case of drug loaded nanoparticles, indicating that the nanoparticle delivery system is mandatory to achieve sufficient levels of drug in the area of pathogenesis.

Here, we propose a single dose of intravenously injected CsA loaded nanoparticles as a novel treatment regimen for retinopathy of prematurity. In addition to that, the therapeutic concept of cell specific nanoparticles in combination with an anti-angiogenic, anti-inflammatory drug could be a potent treatment option for other neovascular diseases like proliferative diabetic retinopathy and wet age-related macular degeneration.

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ANTIBODY CONJUGATION STRATEGIES TOWARDS AN AIMED CARGO DELIVERY AND IMPROVEMENT OF NANOVACCINES

MAXIMILIAN BRÜCKNER, RICHARD DA COSTA MARQUES (SHARED POSTER)

Antibody functionalized nanoengineered drug delivery systems have the ability to overcome current challenges for immunotherapy due to their high specificity towards the targeted body region. Furthermore, a profound understanding of the interaction of nanocarriers with the targeted cells and the subsequent cellular trafficking is crucial for a successful treatment and benefit from a specific targeting strategy. However, no antibody-bound nanocarrier has been clinically approved to date. This may be a result of the conjugation strategy that influences the spatial orientation of the attached antibody on the nanocarriers´ surface. Nevertheless, various conjugation strategies for a sufficient attachment of an antibody onto a nanocarrier have been proposed. Therefore, the choice of an appropriate bioconjugation chemistry determines the success of an antibody functionalized nanocarrier system. Here, the surface amine groups of cross-linked starch iron oxide nanocarriers were conjugated with two different bi-functional cross-linkers via NHS chemistry and compared with each other. Each cross-linker contains a special functional group for the attachment of modified CD11c monoclonal antibodies. On one side, maleimide carrying linkers were conjugated to Traut's thiolated antibodies via thiol-maleimide chemistry and on the other side linkers containing DBCO were attached to azide-modified antibodies via copper-free click chemistry. The binding affinity of the antibody nanocarrier conjugates towards a murine dendritic cell line (DC2.4) was analyzed by flow cytometry. Here, different antibody amounts on the nanocarrier could induce a dendritic cell uptake for both conjugation strategies. However, blocking experiments further highlighted the importance of the orientation of the antibody on to the nanocarriers´ surface. Though, maleimide synthesized conjugates presented their antibodies randomly on the surface, while the antibodies attached via copper-free click chemistry are oriented. To evaluate the *in vivo* properties of the antibody modified nanocarriers, targeting experiments with mouse plasma were performed and it was proven that the biomolecular corona does not diminish the targeting efficiency. Lastly, early investigations on intracellular trafficking were conducted to examine a directed approach to deliver the cargo to specific and desired compartments.

SHUTTLE PEPTIDE MODIFICATION IMPROVES THE ACCUMULATION OF AMPHIPHILIC POLYMERIC NANOPARTICLES IN THE BRAIN OF MICE

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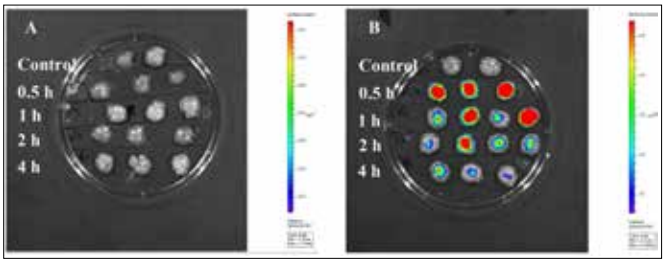

Figure 1. *Biodistribution of (A) pristine and (B) peptide-conjugated particles as captured in the brains of ICR male mice by IVIS after 0.5, 1, 2 and 4 h after intravenous injection.*

The treatment of diseases of the central nervous system (CNS) is a highly challenging owing to the presence of the blood-brain barrier (BBB), a primary physiological barrier, which precludes the crossing of a broad spectrum of drugs into it. In this scenario, the design of novel strategies to increase drug bioavailability in the CNS is called for. Aiming to pave the way to a more efficient delivery of drugs into the CNS, a full protease-resistant peptide coined shuttle peptide that targets the transferrin receptor (TfR) highly expressed in the brain blood vessels has been designed and its ability to cross the BBB demonstrated [(1)].

Polymeric micelles (PMs) are nanostructures formed by the self-assembly of polymeric amphiphiles and that display hydrophilic and hydrophobic domains. PMs have been extensively investigated to encapsulate and target poorly water-soluble drugs. In this work, we initially modified the surface of multimicellar nanoparticles (size of 200 nm) produced by the hydrophobization of a chitosan (CS) backbone with poly(methyl methacrylate) (PMMA) and poly(acrylic acid) (PAAc) [(2,3)] with this shuttle peptide. After a comprehensive characterization of the nanoparticles, we assessed their cell compatibility and permeability in BBB endothelium monolayers *in vitro*. Results showed that the modification increases the apparent permeability of the nanoparticles by 4-fold. Finally, biodistribution studies upon intravenous injection to ICR male mice confirmed the ability of this peptide to increase the CNS bioavailability of the nanoparticles (**Figure 1**).

Acknowledgements. This work was funded by the Ministry of Health of Israel and the 7th Framework Programme-European Commission (project EuroNanoMed-II Cure2DIPG).

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DIRECT ASSESSMENT OF NANOPARTICLE HYDROPHOBICITY

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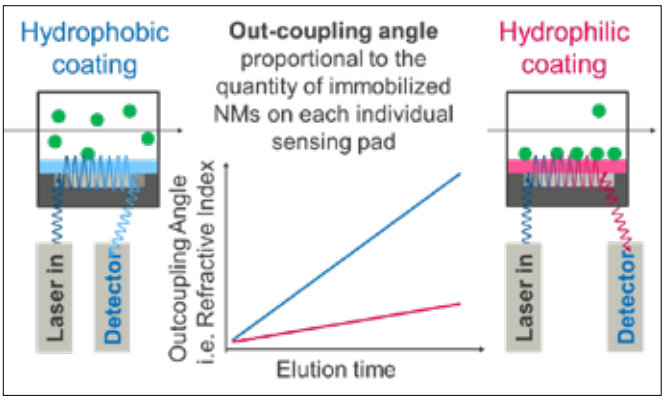

Figure 1*: Schematic representation of the Waveguide Interrogated Optical System detection system for assessing particle hydrophobicity as a function of their affinity to defined surfaces.*

With the objective of improving efficacy, physicochemical properties and pharmacokinetic profiles of pharmaceutical substances, nanodrugs are being extensively investigated. Therapeutic compound uptake, biodistribution, and assimilation have always been major challenges for pharmaceutical companies. For nanodrugs, these parameters are strongly impacted by the surface properties of the nanoparticles such as surface charge and hydrophobicity. Currently, no technique enables straightforward assessment of the hydrophobicity of nanomaterials and nanodrugs, thus increasing costs of optimization for therapeutics. In the frame of the European ACEnano project, CSEM is developing innovative characterization techniques to evaluate the hydrophobicity of nanoparticles. Here we present the assessment of hydrophobicity of polystyrene based nanoparticles with various surface functionalizations. We could successfully show that with a new surface-based method, hydrophobicity can be characterized and compared, which in turn can be used to develop a qualitative comparison chart for nanoparticles.

PHYSICAL CHARACTERIZATION OF LIPOSOMAL DRUG FORMULATIONS USING MULTI-DETECTOR ASYMMETRICAL-FLOW FIELD FLOW FRACTIONATION

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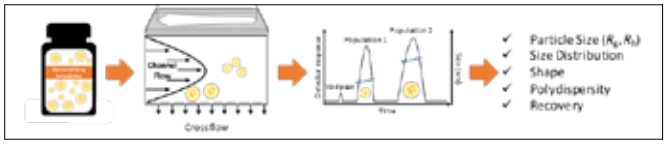
Liposomal formulations for the treatment of cancer and other diseases are the most common form of nanotechnology enabled pharmaceuticals (NEPs) submitted for market approval and in clinical application today. The accurate characterization of their physical-chemical properties is a key requirement; in particular, size, size distribution, shape, and physical-chemical stability are key among properties that regulators identify as critical quality attributes. Here we develop and validate an optimized method, based on multi-detector asymmetrical-flow field flow fractionation (MD-AF4) to accurately and reproducibly separate liposomal drug formulations into their component populations and to characterize their associated size and size distribution, whether monomodal or polymodal in nature. In addition, the results show that the method is suitable to measure liposomes in the presence of serum proteins and can yield information on the shape and physical stability of the structures. The optimized MD-AF4 based method has been validated across different instrument platforms, three laboratories, and multiple drug formulations following a comprehensive analysis of factors that influence the fractionation process and subsequent physical characterization. Interlaboratory reproducibility and intra-laboratory precision were evaluated, identifying sources of bias and establishing criteria for the acceptance of results. This method meets a documented high priority need in regulatory science as applied to NEPs such as Doxil and can be adapted to the measurement of other NEP forms (such as lipid nanoparticle therapeutics) with some modifications. Overall, this method will help speed up development of NEPS, and facilitate their regulatory review, ultimately leading to faster translation of innovative concepts from the bench to the clinic. Additionally, the approach used in this work (based on international collaboration between leading non-regulatory institutions) can be replicated to address other identified gaps in the analytical characterization of various classes of NEPs. Finally, a plan exists to pursue more extended interlaboratory validation studies to advance this method to a consensus international standard.



Keywords
Field Flow Fractionation, Liposome, Complex Drug, Particle Size, Physical-Chemical Characterization, Method Validation, Standardization, Regulatory

POINT OF CARE TEST DEVICE FOR THE MULTIPLEXED DETECTION OF CHRONIC OBSTRUCTIVE PULMONARY DISEASE (COPD) BIOMARKERS IN SPUTUM

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Chronic obstructive pulmonary disease (COPD) is the world third leading cause of death and its burden is steadily increasing. COPD is characterised by an accelerated decline of lung function and by repeated acute exacerbations (AECOPD) that present with excessive sputum secretion and often microbial or viral infections. In order to prescribe an appropriate treatment, sputum is collected to detect the presence of infection. Routine bacteriology analysis, however, usually requires days, delaying dangerously the therapeutic decision.
Inflammation is a key underlying mechanism of AECOPD [1] triggered by bacterial and viral respiratory infections. Persisting airway

inflammation entails the secretion by the human immune system of pro-inflammatory cytokines like interleukin-8 (IL-8) and tumour necrosis factor-α (TNF-α), as well as specific enzymes such as myeloperoxidase (MPO) [2,3] . Study of these inflammation biomarkers led to recognise AECOPD and discriminate acute exacerbations due to bacteria from those due to viral agents and non-infectious causes [4]. Recently, technically demanding diagnostic approaches for early lung cancer detection focused on DNA, proteins, and microRNA biomarkers in sputum [5]. This work reports on the development of a compact point-of-care test device that allows for the simultaneous detection of IL-8, TNF- α and MPO in sputum that would help in the early diagnosis of COPD. An initial step for effective sample preconcentration making use of tailor-made magnetic nanoparticles loaded with antibodies to the three different biomarkers is also implemented. The device, although comprises state-of-the-art technological approaches, as it is based on a reusable electrochemical transducer array and a disposable paper microfluidic component, is quite unique in the combination of both components to carry out the amperometric detection of the biomarkers in the pre-concentrated samples in less than 10 min. The electrochemical transducer array comprises five gold two-electrode electrochemical cells fabricated on silicon substrates by microelectronic technologies whereas the paper microfluidic component relies on a wax printing technique to define five microfluidic channels that can be easily aligned onto the electrochemical transducer array. A scheme of both components and a picture of the actual device is shown in **Figure 1**.

The device performance relies on a sandwich immunoassay format for each biomarker that includes an enzyme-labelled antibody conjugate for carrying out the amperometric detection of the corresponding immunoassays. These are carried out on to the magnetic nanoparticles, which flow along the fluidic channels and are captured by small magnets placed close to the electrochemical cells. The use of an enzyme label, together with an adequate substrate produces an electroactive product that could be detected on the electrochemical cells by amperometry. Working in this way, the three biomarkers could be detected and preliminary analytical studies produced calibration curves for the three biomarkers, and example being shown in Figure 2. As a conclusion, a proof-of-concept of the POCT architecture has been developed and tested that demonstrated the potential for the simultaneous detection of three biomarkers in sputum related to COPD diseases. It is envisaged that such analytical device could be a valuable asset in the rapid detection and monitoring of COPD and related exacerbations, as well as other high-incidence diseases for which specific biomarkers had been identified.

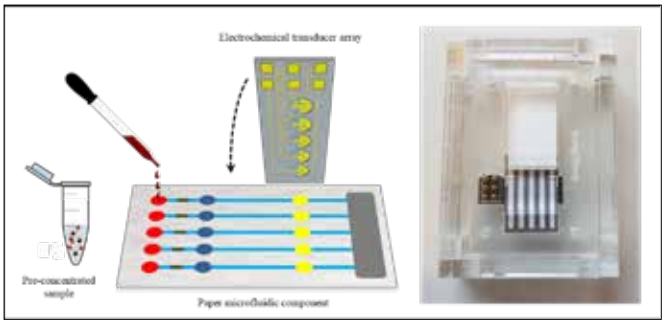

Figure 1. *Left: Schematic representation of the electrochemical transducer array and the paper component and how they are arranged in the device. Right: Picture of the actual device.*

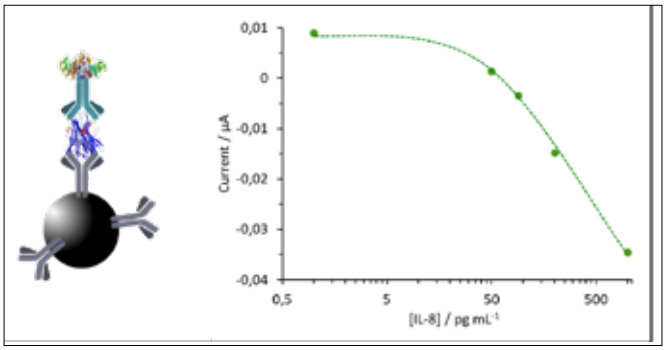

Figure 2. *Left: Schematic representation of the immunoassay formats implemented for the detection of the three biomarkers and (right) calibration curve of IL-8 using the POCT device.*

Acknowledgements

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PERSONALIZED NANOMEDICINE DELIVERY: THE IMPACT OF HEPARIN ON THE CELLULAR UPTAKE

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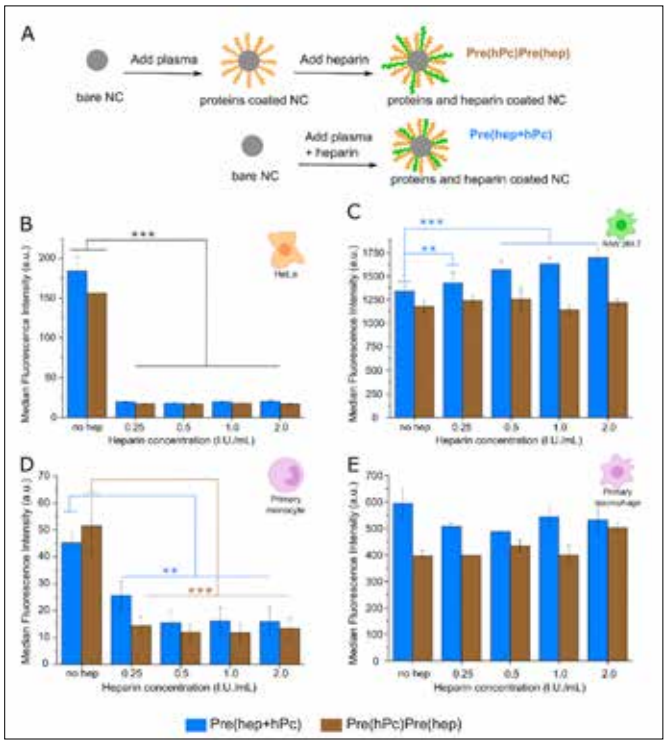

Figure 1. *Preparation and cellular uptake of pre-coated nanocarriers (NC) with plasma and heparin. A) B) The pre-coating procedure is detailed for the two conditions investigated. B-E) The cells were incubated for 2 hours with the pre-coated NCs in serum-free media. The*

*extent of the cellular uptake was quantified by flow cytometry. The error bars represent the standard deviation of a biological triplicate. p-values <0.001 were identified with ***, while p-values <0.01 were identified with ** as calculated with the ANOVA one-way model.*

Nanomedicines are an attractive option for the treatment of cancer and other diseases. This is due to the lower systemic toxicity achieved, compared to conventional drugs, and to the possibility for targeted delivery. Yet, the interaction of these nanomedicines with their environment is not fully characterized. Here, we investigated how heparin could influence the cellular uptake of nanocarriers. Heparin was chosen due to its extensive use as an anticoagulant in the clinic.
Initially, we pre-coated nanocarriers with plasma and heparin in different conditions to assess the effect the timing of the introduction of heparin in the system would have on the cellular uptake of the nanocarriers [1]. We observed that the concomitant presence of heparin and plasma during the protein corona formation affected significantly the cellular uptake of nanocarriers in RAW 264.7 macrophages, HeLa cells, and primary monocytes as shown in Figure 1. Then, we moved on to a clinically relevant system, and incubated the cells with a solution containing liposomes, serum, and heparin. We observed that the heparin concentration modulates strongly the cellular uptake extent of positively charged liposomes in cancer cells and blood phagocytes.
From this studies, we concluded that heparin is responsible for the change in cellular uptake extent observed in our experiments. Thus, we recommend to carefully plan the timing of the injection of heparin and to choose wisely the nanomedicine administered to heparinized patients.

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MODIFIED GOLD NANOPARTICLES AS GENE EDITING ADJUVANTS IN CANCER CELLS

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The p53 tumor suppressor gene is mutated in more than 50% of human cancers, while the remaining 50% of tumors exhibit alterations in pathways regulating p53 functions [1]. Some TP53 mutations encode for mutant p53 (mp53) proteins, referred as “gain of function” (GOF), which acquire many oncogenic properties that sustain cancer progression [1]. Hence, novel approaches aimed to inhibit the expression of mp53 proteins could represent a valid therapeutic approach for patients bearing mp53 associated tumors. The recently developed CRISPR/Cas-based strategies have meant a breakthrough in the field of gene editing, since they allow a much more efficient manipulation of DNA sequences as compared to previous editing tools. The CRISPR/Cas system consists of a Cas endonuclease in complex with a guide RNA, which is complementary to a target DNA. Following guide recognition, the endonuclease generates a break on the DNA that can be repaired through two pathways: non-homologous end-joining and homology-directed repair, less efficient but required for precise editing [2]. Although the correction of disease-causing mutations through CRISPR-mediated gene editing has great therapeutic potential, the safe and efficient delivery of the molecules involved in this edition remains a major challenge [3]. In this regard, gold nanoparticles (AuNPs) have been largely exploited in biomedicine as drug delivery systems, because of their low toxicity, biocompatibility, and stability [4]. This was due because the surface of AuNPs can be modified with a variety of bioactive molecules, such as chemotherapy drugs, nucleic acids, and proteins.

In our study, we developed a therapeutic system based on CRISPR-Cas9 technologies, which can reduce the level of mp53 proteins and generate unspecific insertions and deletions (indels) in the TP53 gene leading to a decrease in cell viability and apoptosis in cancer cells (**Figure 1**). To further increase the efficacy of the system, we improved the overall editing process by inhibiting critical genes involved in DNA repair (e.g Kun70, Kun80, XCCR4) using therapeutic nucleic acids as antisense or gapmers. Notably, preliminary results showed that this approach was able to reduce the expression of mp53 protein, suggesting a higher therapeutic potential of our CRISPR system when combined with the interference of DNA repair-related genes.

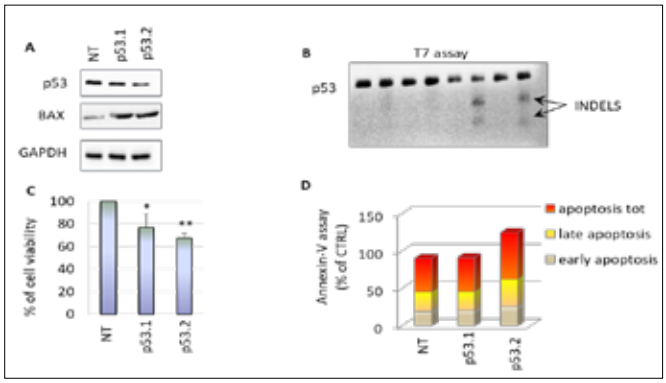

Figure 1. *A) Inhibition of p53 protein by Cas9. B) Indels generation C) Cell viability studies D) Annexin-V assay of PANC-1 cells after the treatment.*

Concomitantly, we prepared AuNPs with Turkevich’s method [5] and modified them with tailored PEI-based molecules developed in our group. Such structures containing stimulus-sensitive linkers can interact with negatively charged nucleic acids, ease their translocation into the cells, promote the endosomal escape and carry the nucleic acids in the cytoplasm. To further improve the stability of the nanostructures and their circulation time *in vivo*, polyethylene glycol (PEG) also has been added in selected batches (**Figure 2**).

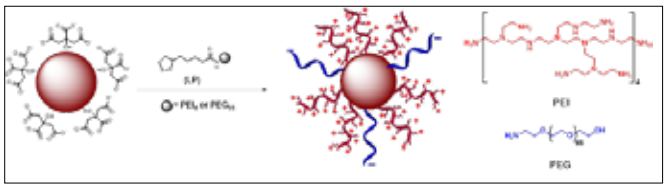

Figure 2. *Synthesis of AuNPs (generation 1) with Turkevich’s method*

Such AuNPs have been evaluated for their efficiency in nucleic acid binding performing gel retardation assay for fluorescence oligonucleotides (e.g. 6-FAM) (**Figure 3**). Later on, we observed that these nanostructures can delivery nucleic acids targeting autophagy genes in cancer cells. Thus, the nanostructures developed here will serve as a multifunctional platform to deliver therapeutic nucleic acids (e.g. plasmids, gapmers, siRNAs) in cancer cells to improve gene editing of key oncogenes involved in cancers (e.g. TP53) providing more effective and selective therapies against mp53-associated cancers.

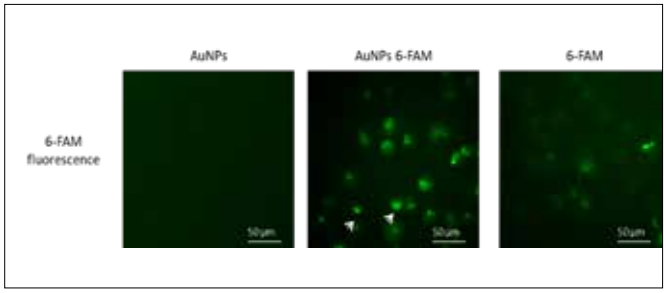

Figure 3. *Fluorescent microscopy images of Panc-1 treated with AuNPs functionalized with 6-FAM*

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ANTIGEN-CONJUGATED CHITOSAN NANOCAPSULES FOR MUCOSAL VACCINE DELIVERY AGAINST PNEUMOCOCCAL INFECTIONS

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INTRODUCTION

Streptococcus pneumoniae is an opportunistic pathogen that colonizes human upper respiratory tract and causes pneumonia, meningitis and septicemia. This pathogen presents cell membrane proteins such as PsaA (pneumococcal surface adhesin A) involved in adhesion and nasopharyngeal colonization processes [1]. The ubiquitous presence of PsaA in all pneumococcal serotypes and effectiveness as immunogen has made it an attractive choice as an antigen candidate.

Chitosan-based nanosystems are interesting technologies for antigen delivery, enhancing the immunogenicity of nasally administered vaccines [2]. The objective of this work has been the synthesis and characterization of chitosan nanocapsules chemically conjugated with the PsaA antigen, as well as the study of their biocompatibility and interaction with different primary cultures of human immune cells.

EXPERIMENTAL

The protein mPsaA was expressed in *E. coli* BL21 (DE3) strain. Chitosan-maleimide and PsaA-S-acetylthioacetate (SATA) coupling was performed according to Prasanna et.al. [1] and the resulting conjugate was characterized by ^{1}H-NMR and circular dichroism. Nanocapsules were prepared by solvent displacement and characterized using a Nano/Zeta sizer ZEN and field emission-SEM regarding their size, surface characteristics and morphology. Association efficiency of PsaA was measured by the microBCA assay. Colloidal stability was studied by NTA in simulated nasal fluid in the presence/absence of mucin, using freshly prepared and freeze-dried formulations.

Human monocyte-derived dendritic cells were generated from buffy coats using GM-CSF and IL4. Their morphology, viability and their activation/ maturation were evaluated (7-AAD, MTS, FACS, confocal microscopy) after their incubation with blank and antigen-conjugated chitosan nanocapsules. Uptake and allogenic response was measured by FACS using fluorescent nanocapsules and by the quantification of CD4+/CD25+ and CD8+/CD28+ signals in dendritic cells and T lymphocytes, respectively.

RESULTS AND DISCUSSION

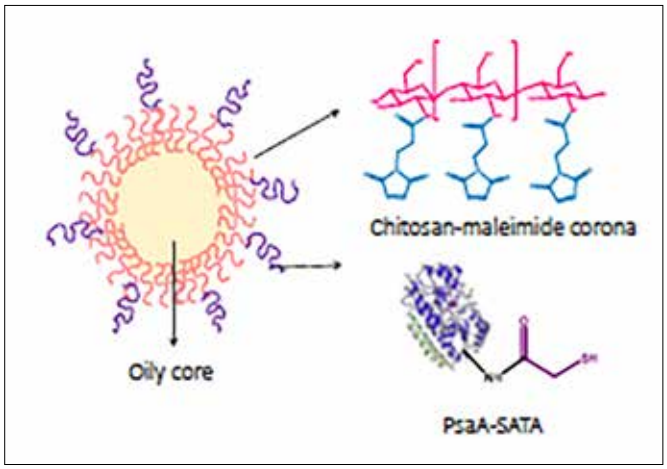

Fig. 1 *Schematic representation of the chemically conjugated PsaA-chitosan nanocapsules*

^{1}H-NMR and circular dichroism analysis confirmed the efficient conjugation between chitosan-maleimide and PsaA-SATA. Nanocapsules prepared with/without antigen-conjugated chitosan presented a homogeneous distribution of spherical shaped particles, within the size range of 250-300 nm and had positive surface charge. Association rates were three times higher compared to nanocapsules encapsulating PsaA without polymer-protein conjugation.

The nanocapsules were stable in simulated nasal fluid in the presence/absence of mucin and in cell culture media (37ºC, 24 h). They could be freeze-dried and reconstituted without altering their original physico-chemical properties. Antigen-conjugated nanocapsules showed low toxicity, together with efficient recognition and internalization by human monocyte-derived dendritic cells and macrophages. The nanocapsules also showed the capacity to activate allogenic responses in T lymphocytes through the specific maturation of dendritic cells, resulting in CD4 (CD4+/CD25+, 25% activation) and CD8 T lymphocytes (CD8+/CD28+, 20% activation) compared to immature DC.

CONCLUSIONS

The thiol-maleimide conjugation between the polymer and the antigen produced nanocapsules with high stability and greatly improved association efficiency, enabling surface presentation of PsaA for efficient immune cell recognition and processing. Complementary studies on cytokine secretion profiles and the evaluation of antigen-specific responses are currently underway to further evaluate the potential of these nanocapsules as vaccine delivery systems.

ACKNOWLEDGEMENT

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MICELLAR-BASED, MULTIFUNCTIONAL POLY-CARBONATES AS VERSATILE (IMMUNO-) DRUG CARRIERS

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Classical small molecular therapeutics can provoke immense side effects and systemic responses apart from their site-of-action. Especially for novel (cancer) immunotherapeutics or advanced antibiotics, there is an urgent need for smart drug delivery systems to overcome these issues. Advanced biodegradable polymer carriers may efficiently deliver highly active drugs to their target site and omit off-target effects.

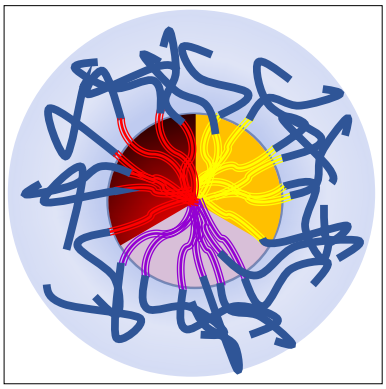

Figure 1 *Synthesis and application of core-shell nanoparticles based on biodegradable polycarbonates. Multiple polymerization techniques as well as different functional monomers are applied to obtain tailor-made carrier systems for the delivery of various therapeutic molecules.*

For that purpose, polycarbonates are introduced as novel attractive, biodegradable materials for advanced drug delivery. Selective chemical modification of these polymers is a necessity to obtain tailor-made, multifunctional carriers with optimized properties for individual therapeutics. Therefore, functionalizable polymers were synthesized and fabricated into nanogels with covalently attached drugs/dyes for immunostimulation. Covalent incorporation of positive charges supports the delivery of therapeutic oligonucleotides. Amphiphilic polycarbonate block copolymers with benzyl side chains were employed for π-π-stacking promoted encapsulation of novel aromatic antibiotics.
Latest attempts towards responsive degradation features upon internal or external stimuli are currently ongoing.

SPHERICAL NANOPARTICLES FOR THE DELIVERY OF METHOTREXATE TO ATHEROSCLEROTIC PLAQUES

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Atherosclerosis is a chronic inflammatory disease affecting the blood vessel walls. Its pathogenesis is based on the transformation of macrophages into foam cells, following the uptake of oxidized low density lipoproteins (oxLDL). Recent discoveries found that methotrexate (MTX), a folate antagonist originally developed for the treatment of malignancies, also possesses anti-inflammatory activity and can modulate cholesterol transport in macrophages. However, the use of low-dose free methotrexate did not reduce cardiovascular events in the recent CIRT trial [1]. Low solubility in physiological solution, short half-life and its toxicity limit the use of free MTX. In this work, a new MTX nanocarriers is presented to improve MTX based therapies by ameliorating its bioavailability and reducing its toxicity. To efficiently load MTX into nanocarriers a lipid-based prodrug has been realized by liking MTX to 1,2-distearoyl-sn-glycero-3-phosphoethanolamine. This lipid-MTX was then used as a constituent of spherical nanoparticles: Liposomes (LIP) and Spherical Polymeric Nanoparticles (SPNs) [2]. LIP were prepared by thin layer evaporation (TLE) while SPNs were synthetized using a sonication-emulsion technique. Nanoparticles present comparable features. For the lipidic nanoparticles, the size is 174±2 nm (PdI: 0.15 ± 0.0007), and Zeta Pot is equal to -48 ± 0.02 mV; for the polymeric nanocarrier, the size is 208±2 nm (PdI: 0.15 ± 0.02), and Zeta Pot is equal to 45.8 ± 0.02 mV. MTX encapsulation efficiency (EE%) was found to be 70±5% for Lip-MTX and 1.5±0.2% for SPNs-MTX (**Figure 1**).

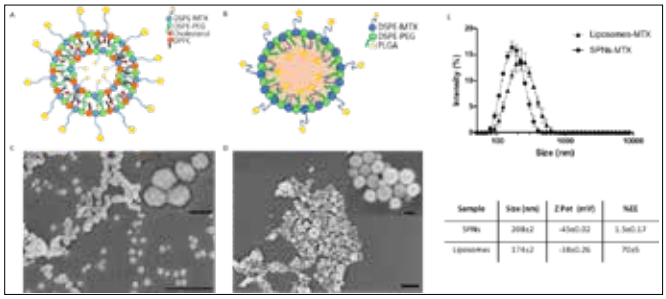


Figure 1. *Schematic representation of Liposomes(A) and SPNs(B). SEM images of Liposomes (C) and SPNs(D), scale bar 500 nm, scale bar magnification 100 nm.*

Foam cells from rat bone marrow derived monocytes (BMDM), upon treatment with oxLDL were used as a model to test the two formulations. TEM and confocal imaging were used first to analyze oxLDL accumulation into lysosomes. In order to visualize oxLDL uptake this molecule was conjugated to DIL (**Figure 2**).

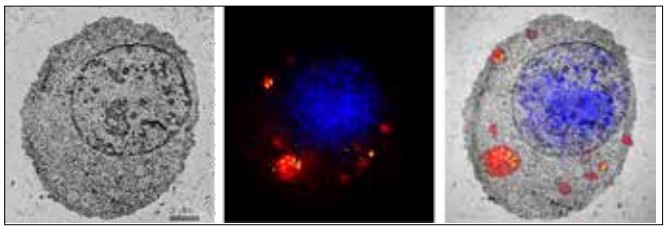
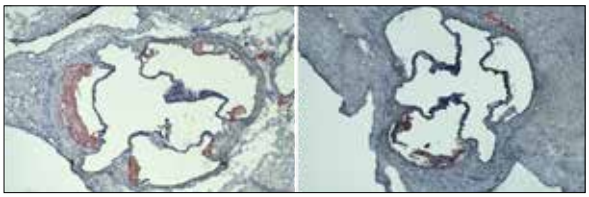


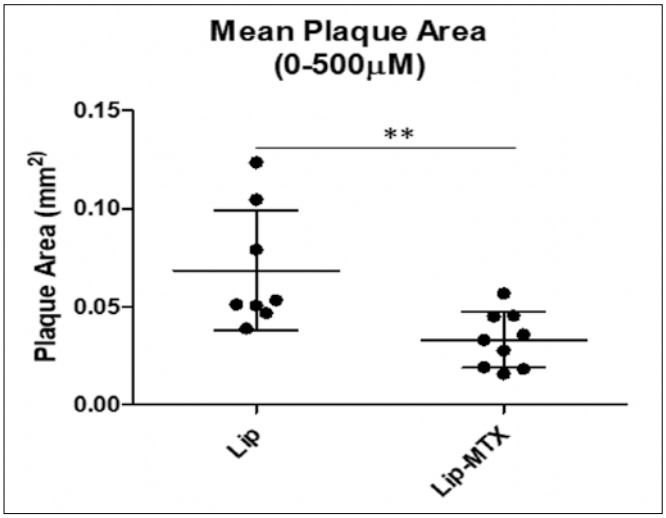
Figure 2. *Characterization of Foam cell: overlap between TEM images and confocal images.*

The reversion of foam cells to macrophages was investigated upon treatment with Lip-MTX, SPNs-MTX and free MTX, as a control. Imaging analysis revealed a reduced amount of oxLDL into foam cells in the samples treated with Lip-MTX and SPNs-MTX. Both Lip-MTX and SPNs-MTX were able to induce a decrease in cholesterol amount after 24 hours. The efficacy of the treatment was also proved by gene expression analysis. RT-PCR showed down-regulation of CD36 and SRA-1 genes (foam cell markers) [3] and up-regulation of the reverse cholesterol transporter (ABCA1) [4] in foam cells treated with the nanoformulations. Lip-MTX and SPNs-MTX reduce also inflammatory gene expression of the pro-inflammatory IL-6, IL-1β and TNFα. Cytotoxicity tests of Lip-MTX and SPNs-MTX were performed on BMDM, cells showed a good tolerance to the treatment at the used doses. Lip-MTX were used *in vivo* in murine experimental atherosclerosis. ApoE-/- mice, fed with high-fat diet for 28 days were treated for 4 weeks (once every three days) and

plaque burden measured. Results show that this treatment reduces the plaque area supporting the concept that a systemic delivery of MTX particles may constitute an effective strategy to inhibit early atherogenesis (Figure 3).

Figure 3. *Treatment with nanoparticles: (A) empty liposomes (Lip) and (B) liposomes-MTX (lip-MTX). Representative photomicrographs of oil red O (ORO)-stained aortic sinuses (bar=500 µm). Graph showing the effect of Lip and Lip-MTX treatment on mean lesion area (bar=500 µm).*





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DUAL FUNCTIONAL THERANOSTIC NANOPARTICLES FOR RHABDOMYOSARCOMA GENE THERAPIES AND REAL-TIME EVALUATION OF THERAPEUTIC EFFECTS

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Rhabdomyosarcoma (RMS) is the most common soft tissue sarcoma and is extremely aggressive and highly malignant. The majority of cases occur in children, and most commonly appear in the arms or legs. Currently RMS is treated with aggressive chemotherapy, as well as surgery and/or radiation in many cases. The aggressive treatments often cause long term after effects, and can even cause permanent life-altering disabilities. Alternative treatment modalities are urgently needed.

The aim of this work is to build a dual-functional theranostic nanoparticle with a pH-activated-release therapeutic core. siRNA will be delivered into tumour cells and the therapeutic effects monitored through non-invasive NIR signals. The synthesized dual-functional nanosystem will target the release of the siRNA and suppress the growth of the cancer cells, and the NIR signal should decrease as the tumour shrinks. This project will set up the foundation of an efficient dual functional nanosystem which could be clinically applied in the future.

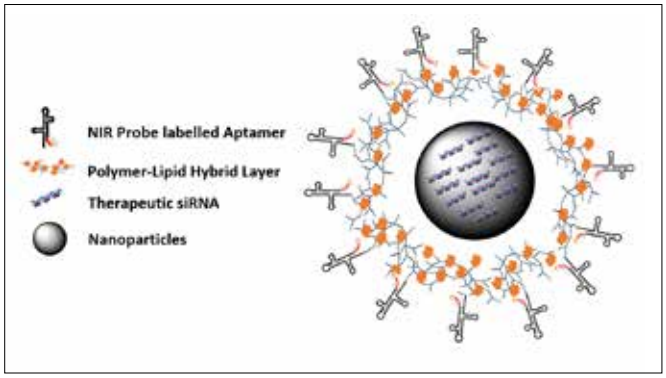

Figure 1. *Schematic diagram of the designed nanosystem*

Two types of nanoparticles based on silica and gold have been synthesized as the core of the system. The surface of both types of nanoparticles was functionalized with thiol bonds in order to carry, and permit targeted release of siRNA. The synthesized nanoparticles were characterized with various different techniques. Raman fingerprints of —SH at 2580 cm^{-1} was observed for validation of surface thiol bonds.

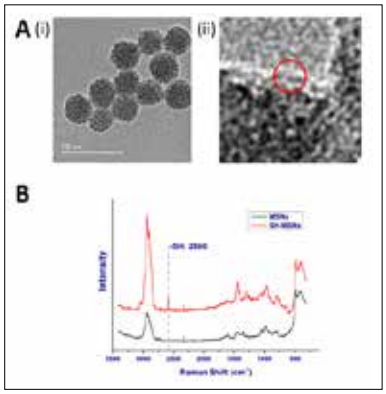

Figure 2. *Surface Characterization of Synthesized SH-MSNPs*
A: TEM images of (i) synthesized 50 nm SH-MSNPs, (ii) Enlarged surface thiol bond layer of SH-MSNPs. B: Thiol bond was detected using Raman Spectroscopy. A unique 2580 cm-1 peak was shown in SH-MSNPs, but not in MSNPs.

Aptamers were chosen as the targeting ligand for our nanosystem. Compared to antibodies, aptamers are much smaller, with higher affinity and specificity, and lower immunogenicity. Furthermore, it is easier and cheaper to manufacture aptamers. The binding tar-

get for aptamer generation was Neural EGF-Like 1 (NELL1). This was chosen based on previous microarray and microscopy results which suggested that it was overexpressed on the surface of target rhabdomyosarcoma cells. Oligos have been isolated through the lab designed assay, and the binding affinity has been tested using Surface Plasmon Resonance (SPR).

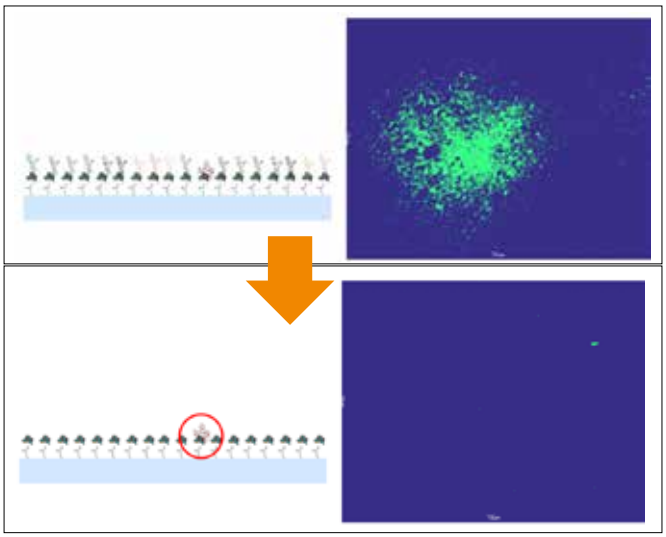

Figure 3. *Aptamer selection based on conjugated FAM fluorescence Representative fluorescence showing the FAM labelled-oligo library incubated coverslip under microscope after (A): no washing, (B): After 5th round of washing. The decrease of the fluorescent signal represented the oligos with lower binding affinities to NELL1 being removed from the surface during the washing process.*

The presentation will highlight the mechanism of our innovative nanosystem with some experimental results, which will show the innovation of the targeted release, real-time visualization of therapeutic effect, and accelerate the clinical use of nanoparticles for gene therapies.

PROGRAMMED SELF-ASSEMBLY OF PEPTIDE BASED INHIBITOR TOWARD HYPOXIC CANCER THERAPY

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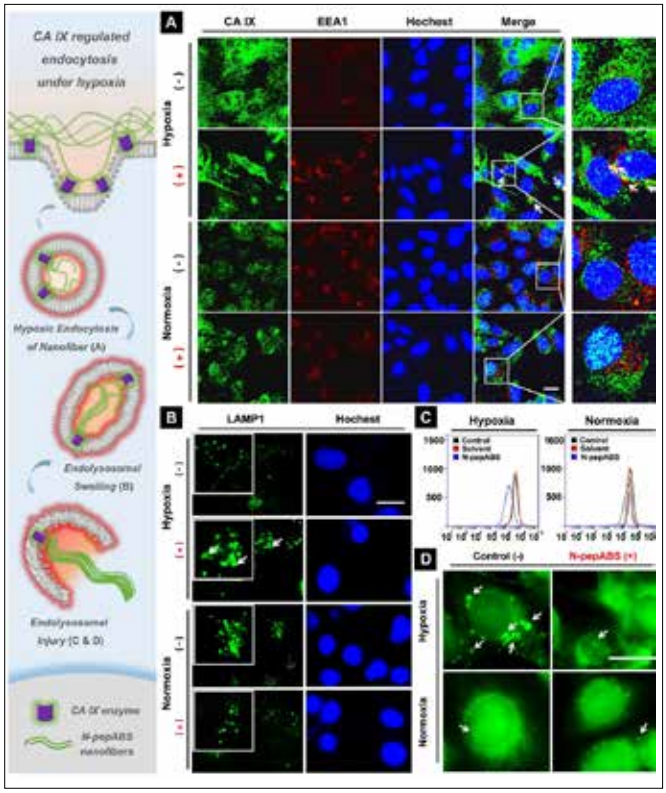

Fig. 1. *Nanofibers promote CA IX-regulated endocytosis under hypoxia. (A) CA IX regulated endocytosis of Nap-phe-phe-lys-CA inhibitor nanofibers has appeared under hypoxia after 24 h treatment of 500 μMNap-phe-phe-lys-CA inhibitor (+) or medium control (-), with scale bar represented as 20 μm; We further observe subsequent (B) endolysosomal swelling and (C, D) intracellular acid vesicles injuries after 48 h treatment of 500 μMNap-phe-phe-lys-CA inhibitor under hypoxia, with scale bar represented as 20 μm; Then blockage of protective autophagy has been detected after 48 h treatment of 500 μMNap-phe-phe-lys-CA inhibitor*

Approachability to oxygen is critical for most of organism, and instability of oxygen will affect the cell function. In limitation of oxygen the cells alter their pathway to low oxygen consumption. Hypoxia is a feature of many type of solid cancers that generating intratumoral oxygen gradients and change the cell metabolism, therefore cause tumor therapy resistance.

CA IX enzyme that overexpressed on the surface of several kinds of hypoxic tumor cells, is one of the hypoxia marker which shows limited expression in normal tissue and has associated with cancer progression, metastasis, and impaired therapeutic response.Small molecules of CA inhibitor modified with short peptide successfully achieve CA IX targeted self-assembly that localize CA inhibitors on hypoxic cancer cell surface and enhance their inhibition efficacy and selectivity.Taking advantage of extracellular active site of CAIX, this enzyme was successfully inhibited by short peptide self-assemble conjugated to traditional small molecule inhibitor of CAIX. Due to strong ligand-receptor binding after targeting CAIX, pericellular self-assembling of short peptide will enhance the CAIX inhibition

Besides, CA IX-related endocytosis also promotes selective intracellular uptakes of these nanofibers under hypoxia, by which nanofiber structure upgrades with decreasing pH values. This subsequently cause intracellular acid vesicles 28 damage and protective autophagy blockage

Moreover, *in vivo* applications exhibit considerable efficacies of anti-metastasis, and anti-angiogenesis in breast tumor, which has accomplished remarkable enhancements of antitumor efficacies for Doxorubicin administration. Based on our achievements this system can efficiently target hypoxia cancer cell and works as a promised CAIX inhibitor.

Keywords:hypoxia; self-assembly; CAIX; peptide; nanofiber, cancer cell,CAIX inhibitor

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COMPARATIVE IN VIVO TRACKING OF EXTRACELLULAR VESICLES FROM NUCLEAR, FLUORESCENCE AND BIOLUMINESCENCE APPROACHES

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The development of extracellular vesicles (EVs) for therapeutic applications requires an in-depth understanding of their *in vivo* biodistribution and pharmacokinetic profile. This work presents a comprehensive comparison of nuclear, fluorescent, and luminescent imaging technologies to identify the ideal *in vivo* EV tracking approach.

METHODS

EVs were purified from Expi293F cells conditioned medium (CM) by differential centrifugation followed by iodixanol density gradient separation and characterised by NTA, TEM and western blots. **Expi293F-derived EVs were either engineered Expi293F** to express mCherry or NanoLuc (Nluc) reporter proteins; or labelled with Indium[11] (as [In^{111}]DTPA) or Xenolight DiR post-EV isolation. CT26 tumour-bearing BALB/c mice were administered a single intravenous dose of 1x 10^{11} EVs followed by imaging at 1h, 4h and 24h using SPEC/CT and IVIS systems. Tissue distribution and blood circulation profile of EVs were analysed up to 24h after injection.

RESULTS

Whole body live imaging of Nluc-EVs in mice yielded limited signal to enable accurate visualization. DiR-EVs yielded better detection sensitivity than Nluc-EVs, but [In^{111}]DTPA-EVs gave the best sensitivity and resolution. Whole body imaging of mCherry-EVs yielded too much nonspecific background auto-fluorescence which resulted in indifferent images from control mice. Ex vivo organ quantification of [In^{111}]DTPA- and DiR-labelled Expi293F-derived EVs showed highest accumulation in the liver and spleen, followed by kidneys or lungs at 24h post-dosing. Tumour accumulation was only observed with [In^{111}]DTPA-EVs, gradually increasing up to ~2% ID at 24 h. Interestingly, Nluc-EVs showed highest accumulation in the lungs, followed by spleen, although the EVs were of similar sizes and not aggregated. Blood circulation profile was able to be obtained from [In^{111}]DTPA- and Nluc-EVs, showing rapid clearance of EVs from circulation with less than 10% ID detected in blood 15 after administration. Excretion profile was only obtainable from [In^{111}]DTPA-EVs, which showed 1-1.5% ID being cleared in the urine and faeces respectively.

CONCLUSION

Nuclear imaging offers the highest sensitivity and resolution for *in vivo* EV tracking both in terms of whole body live imaging and ex vivo tissue quantification. This however requires specialised set up and equipment for the radiolabelling and imaging work. Optical methods are more accessible but have a more limited tissue penetration and sensitivity, which can be optimised with right selection of the dye. This study also warrants the possibility of major alteration in EV biodistribution following exogenous expression of reporter proteins, which should be validated prior to undertaking *in vivo* tracking studies.

FIGURES

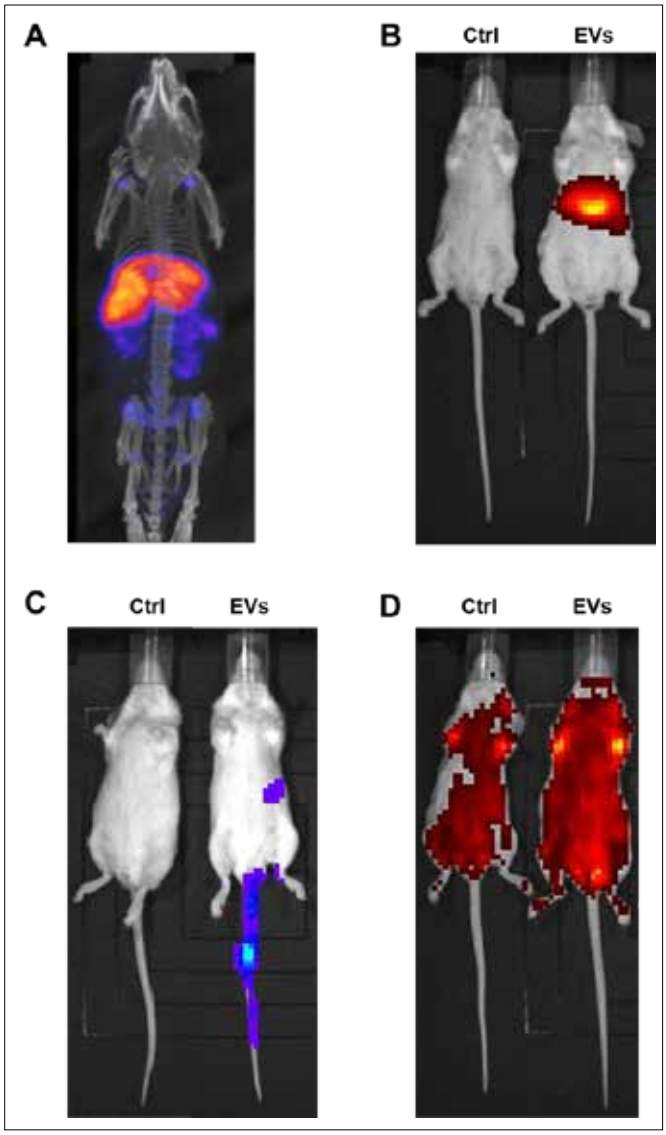

Fig. 1 Whole body live imaging of Expi293F-derived EVs in CT26 tumour-bearing Balb/c mice. *Animals were injected intravenously with 1x10^{11} of either* **(A)** *[^{111}In]DTPA-EVs,* **(B)** *DiR-EVs, (C) Nluc-EVs and (D) mCherry-EVs and imaged after 24 h post-injection.*

SYNTHESIS AND REPRODUCIBILITY OF FUNCTIONALIZED HYDROXYLETHYL STARCH (HES) AND PROTEIN NANOCAPSULES BY INVERSE MINIEMULSION AND THEIR BIOLOGICAL PROPERTIES

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Immunotherapy has been established as a successful method for cancer treatment and the combination with nanotechnology is anticipated to be the next step towards personalized modern medicine.[1, 2] The human immune system consist of many different cell types whose regulation is extraordinarily complex. Using the cytokine IL-2, T-cells can specifically be addressed and their growth and

differentiation can be stimulated.[3,4] Since regulatory T-cells (Tregs) are involved in tumor-associated tolerance processes and Treg function is partly controlled by STAT3-mediated mechanisms, the controlled delivery of encapsulated STAT3-inhibitors in nanocarriers could represent one example of the intervention into a tumor-associated tolerance mechanism (Figure 1).[5]

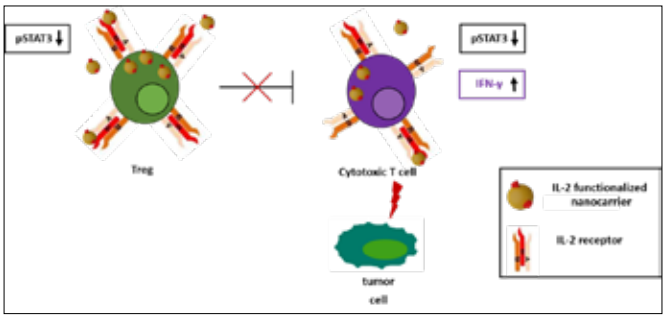

Figure 1: *IL-2 functionalized nanocarriers could inhibit Treg cells and lead to tumor elimination.*

Still one of the biggest challenges in nanomedicine is the synthesis and surface modification of defined nanocarriers in a reproducible manner. Depending on the nanocarrier material, the composition of the surface in respect to charge, polarity, adsorbed impurities and stoichiometry of end groups can be difficult to determine and are never identical from batch to batch.[6]
Herein a nanocarrier-system consisting of HES (hydroxylethyl starch) nanocapsules, which is able to address T cells, as a result of an extensive surface modification, is presented and the complexity of the accurate synthesis is exemplified. The nanocapsules are prepared in an inverse miniemulsion process and are further functionalized by introduction of dibenzocyclooctyne (DBCO) groups for copper-free click chemistry. By azidation of the cytokine interleukin-2 (IL-2) and subsequent 1,3-dipolar cycloaddition with the beforehand functionalized HES nanocapsules, tunable amounts of IL-2 can be attached to the nanocapsules (Figure 2).

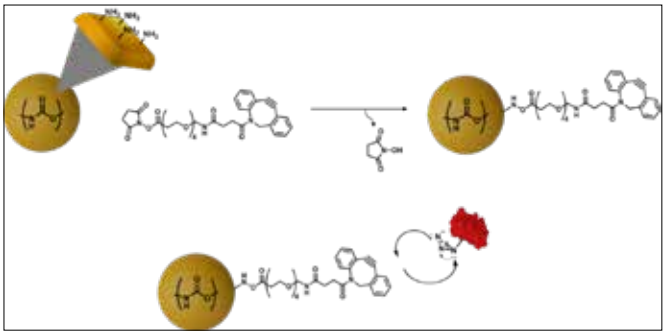

Figure 2: *Introduction of DBCO onto the surface of HES nanocapsules and the subsequent 1,3-dipolar cycloaddition of the nanocapsules with azidated IL-2.*

Cell proliferation experiments showed adjustable proliferation of T cell-like CTLL-2 cells dependent on the offered IL-2 concentration on the nanocapsule's surface upon binding of the nanocapsules onto the IL-2 receptor of the cells. The findings demonstrate that by reducing the amount of IL-2 on the nanocapsule surface, a dose-dependency is achieved, which is important for a precise addressability of specific subpopulations of T-cells.
Surface modification is a common method to endow a nanocarrier with a biological function, but sometimes the material itself can enable the nanocarriers to provoke a specific reaction inside cells. Proteins can be utilized as nanocapsules since they show excellent degradability and biocompatibility as well as their native function can be preserved during nanocapsule formation. For example Paßlick *et al.* presented that nanocapsules consisting of ovalbumin, a protein derived from chicken egg white, can react as an adjuvant boosting the immune system.[7]
Moreover, the type of crosslinker might play an important role in terms of cell and protein interaction. We show that protein nanocapsules with narrow size distributions and low protein adsorption upon contact with blood serum can be synthesized by inverse miniemulsion and interfacial crosslinking of the protein using triazolinediones (TADs) as powerful dienophiles and enophiles, which smoothly perform electrophilic aromatic substitutions and Diels-Alder reactions under ambient conditions.[8] The physicochemical and biological properties of TAD-crosslinked protein nanocapsules were compared with conventional diisocyanate crosslinked nanocapsules. The results showed that the type of crosslinker reaction controls the cell uptake of protein nanocapsules towards cancer cells and the interaction with blood proteins since the crosslinkers react with different functional groups within the proteins leading to different surfaces of the protein nanocarriers (Figure 3).

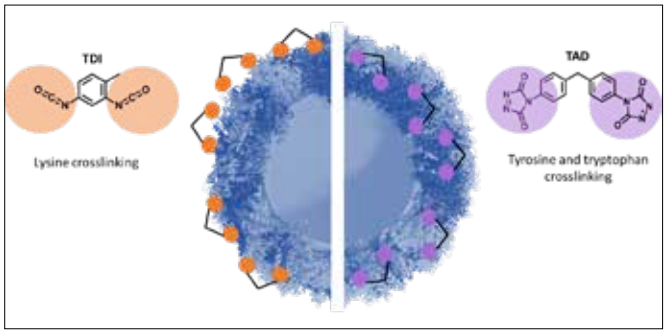

Figure 3*: TDI and TAD crosslinking during protein nanocapsule formation lead to different protein structures of the nanocapsules.*

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LIPOSOME SURFACE FUNCTIONALIZATION WITH SITE-SPECIFIC MODIFIED ANTIBODIES VIA BIO-ORTHOGONAL CLICK CHEMISTRY

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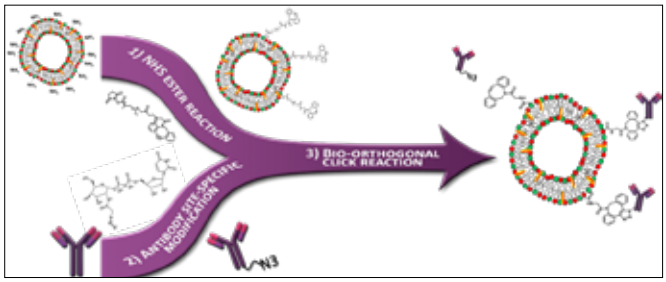

Scheme 1: *Schematic step-by-step illustration of liposomes' surface*

functionalization with DBCO groups via NHS-ester reaction and subsequent site-selective antibody attachment by click-chemistry.

The modification of liposomal surfaces, especially coupling with targeting ligands, is interesting for a mass of applications and a variety of chemistries (e.g. maleimide–thiol coupling) bring this into reality. In the conventional approaches, the surface functionalization mostly takes place before the amphiphilic molecules (block-copolymers, lipid etc.) complete the self-assembly, by introducing specific functional groups directly into the amphiliphilic molecules. However, there are mainly three challenges still existing: 1) the introduced functional groups may react with loaded cargo; 2) natural carriers like extracellular vesicles should be functionalized making chemical pre-assembly modification impossible; 3) the attached targeting antibodies are not specifically conjugation-site controlled, which will dramatically decrease the antibody targeting efficiency. Here, we would like to present the site-specific coupling of antibodies to the surface of amino group-terminated liposomes *via* bio-orthogonal copper-free click chemistry after liposome self-assembly. [1] The present primary amino groups were functionalized with a linker carrying a strained alkyne group for a bio-orthogonal strain-promoted alkyne–azide cycloaddition (SPAAC) reaction. Compared to common strategies for antibody modification, site-specifically functionalized antibodies with azide moieties along the Fc region to avoid interference with the antigen binding sites are prepared. The liposome surface functionalization reaction was optimized by precisely analyzing the number of available functional groups (both amine and alkyne), which often represents a challenge for self-assembled systems. Furthermore, by applying the strain-promoted azido-alkyne cycloaddition (SPAAC) the use of Cu(I) as a catalyst is avoided - an important advantage considering the known deleterious effects associated with copper in cell and protein studies.

Keywords: self-assembly, surface functionalization, click chemistry, antibody specific modification

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RAMAN SPECTROSCOPY, A SENSITIVE METHOD FOR BONE QUALITY EVALUATION. ALTERNATIVE TO HISTOLOGY

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Resume: Our days it has been involved a large number of surgical techniques involving the implantation of various types of bone graft and /or bone substitutes in order to achieve periodontal regeneration. Despite positive observations in animal models and successful outcomes reported for many of the available regenerative techniques and materials in patients, including histologic evidence, robust information on the degree to which reported clinical improvements reflect **true periodontal regeneration remains just limited**. Bone quality is a matter of mineral content (mature / immature bone ratio) and of structure as well. Regarding bone quality before and after healing period (sinus lift bone augmentation), investigation was performed by RAMAN technique. There were evaluated following peaks, before and after healing period **(Fig. 1)**:

- 430 – 450 cm^{-1} (v_2, PO_4^{3-});
- 955 – 960 cm^{-1} (HPO_4^{2-}, immature bone);
- 960 – 965 cm^{-1} (mineral bone, mature bone);
- 1023 cm^{-1} ($P_2O_7^{4-}$; PPi, inorganic pyrophosphate)

The normalized peak intensity values, are related to the compounds concentration. Octacalcium phosphate (**OCP**, $Ca_8(HPO_4)_2(PO4)_4{\cdot}5H_2O$,) is considered very important because it is regarded as an *in vivo* precursor of HA. [3] Trying to find traces of transformation of OCP to HA, the presence of HA nano rods and plate-like HA particles can be utilized as signs of bone augmentation process and a good quality future bone.

The goal of our future study is that a correlation must be established between RAMAN spectra and bone main organic / inorganic fractions value in order to obtain a one-step complete investigation. Method easily can be adapted for *"in vivo"* bone quality evaluation, being much less invasive method then the well-known.

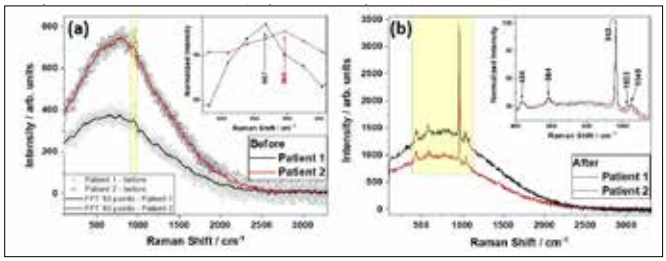

Fig.1. *Raman spectra for bone samples (a) before treatment, (b) after treatment. (E. Gatin et al, Raman spectroscopy: Application in Periodontal and Oral Regenerative Surgery for Bone Evaluation IRBM 40 (5), 2019)*

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DESIGN AND DEVELOPMENT OF INORGANIC NANOPARTICLES FOR RADIO-ENHANCEMENT THERAPY

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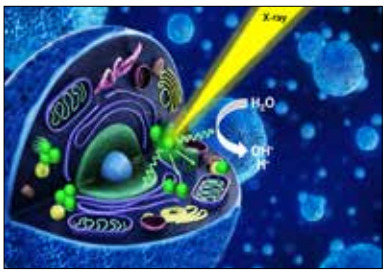

Figure 1: *Schematic illustration of the principle of Nanoparticle-enhanced Radiotherapy*

Nanoparticle-based radio-enhancement has the prospect to improve cancer cell eradication by amplifying the damage caused by (X-ray) irradiation through ejection of secondary particles (electrons) leading to further oxidative stress (Figure 1). Gold nanoparticles are a natural choice because of their high atomic number and biological compatibility and are therefore most researched nanoparticles. However, due to the heterogeneous results and the lack in mechanistic understanding, these particle systems have not yet entered clinics. Most recently, nanoparticles based on more exotic hafnium dioxide have shown promising results in first clinical studies. There is increasing evidence that radio-enhancement efficacy does not solely dependent on high atomic numbers but involves a complex cascade of secondary reactions.
Here, we will present a comprehensive nanoparticle-based radio-enhancement study including a selection of inorganic oxide nanoparticles (TiO_2, ZrO_2, HfO_2) with comparable morphologies, sizes and surface chemistries. We compare the conventional wet chemistry synthesis technique to the liquid flame spray pyrolysis which allows effective scale up to meet clinical and industrial demands. We will report on uptake, subcellular distribution and radio-enhancement effects in radio resistant tumor cell lines using clinically relevant exposure settings. We also show how the nanoparticles can be tailored to incorporate multimodal imaging possibilities for a theranostic approach[1]. Our comprehensive analysis will pave the way for a more rationalized nanoparticle design and development for nanoparticle-based radio-enhancement studies.

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POLY(PROPYLENEIMINE) DENDRIMERS AS CARRIERS OF ANTICANCER ADENOSINE NUCLEOTIDES

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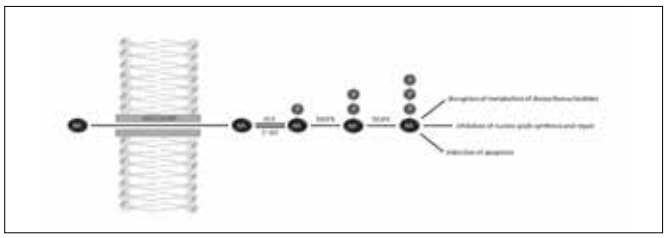
Nucleoside analogues (NAs) have been in clinical use for over 50 years. Developed as antiviral agents, they were among the first therapeutics introduced for the medical treatment of cancer. For haematological malignancies, adenosine and cytidine analogues are most commonly used. These compounds exhibit a great variety of cytotoxic activities, including disruption of the metabolism of natural nucleotides, inhibition of replication and repair processes of DNA and induction of apoptosis.All nucleoside analogues share common intracellular transport and metabolic pathways that include facilitated transfer through the cell membrane and activation by intracellular kinases. As hydrophilic molecules, the drugs cannot penetrate the cell membrane by passive diffusion, and require the presence of specialized nucleoside transporters enabling cell entry. Inside the cell, NAs are progressively phosphorylated to active triphosphate forms, which is essential for their cytotoxic action (Figure 1). Such complex metabolism causes the exposure of these drugs to a wide range of potential resistance mechanisms. These include reduced expression of nucleoside transporters, which leads to inefficient uptake and accumulation of therapeutics inside the cancer cells, and decreased activity of intracellular kinases, resulting in inadequate conversion of nucleosides to their active forms.

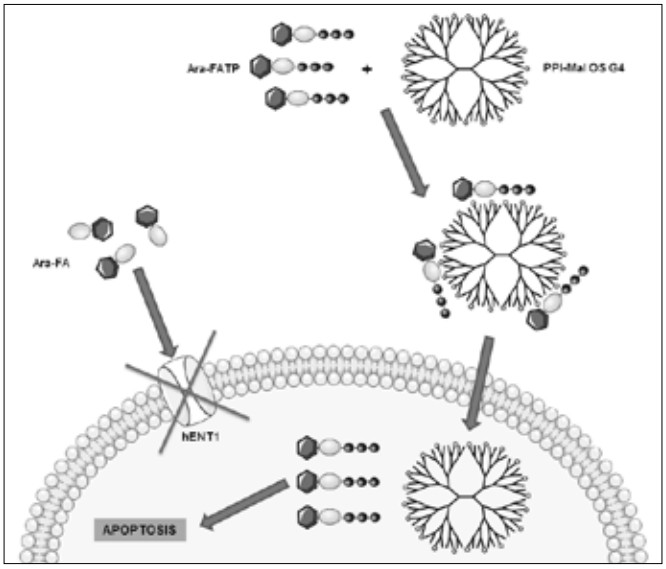
Fig. 1: *Metabolism and mechanisms of action of nucleoside analogues. NA: nucleoside analogue; P: phosphate group; hENT/hCNT: human equilibrative/concentrative nucleoside transporter; dCK: deoxycytidine kinase; NMPK: monophosphate kinase; NDPK: diphosphate kinase; 5'-NT: 5'-nucleotidase (source:* [1]*).*



For the circumvention of these obstacles and the enhancement of efficacy of anticancer chemotherapy based on adenosine analogues, the direct intracellular delivery of active metabolites by nanocarrier systems has been proposed. Numerous studies confirmed that highly-branched dendrimers of well-defined structure are superior to other nanoparticles with regard to drug delivery. Dendrimers provide high loading capacity, as well as improved solubility and biodistribution of the drugs. Their nanometric size and globular shape favour cell entry and prolong blood circulation time. While cationic dendrimers form stable non-covalent complexes with negatively-charged compounds, their inherent toxicity hampers their direct clinical application. Thus, surface covalent modifications, including glycosylation, have been introduced in order to reduce the positive charge of the dendrimers. Since adenosine analogues are most often used as antileukemics, the use of glycodendrimers is especially justified: leukemic cells usually overexpress surface lectin receptors with high affinity for carbohydrate ligands, enabling receptor-mediated endocytosis and decreasing the detrimental side effects on healthy tissues.The present work aimed at characterisation of complexes of adenosine nucleotides and poly(propyleneimine) dendrimers of the 4th generation (PPI G4) (unmodified or partially surface-modified with sugar moieties for improved biocompatibility), and the assessment of their applicability in anticancer therapy.
In the first stage of research, isothermal titration calorimetry and zeta potential titration have been applied for the determination of stoichiometry and thermodynamic parameters of interactions between PPI dendrimers and adenosine-5'-triphosphate (ATP) as a model adenosine nucleotide. It has been shown that PPI dendrimers possess the ability to efficiently interact with nucleoside

triphosphates and to form stable complexes via electrostatic interactions between the ionized phosphate and amino groups on the nucleotide and the dendrimer, respectively. The complexation process is spontaneous, enthalpy-driven and depends on buffer composition and pH. The determined optimal nucleotide:dendrimer ratios have been used in subsequent studies [2].
Further experiments in *in vitro*-cultured leukemic cell lines (CCRF, THP-1, U937) using complexes of maltose-modified PPI dendrimer (PPI-Mal OS G4) and fludarabine triphosphate (Ara-FATP) have shown that Ara-FATP has limited cytotoxicity towards investigated cells compared to the nucleoside form (Ara-FA), but complexation with the glycodendrimer significantly increases its activity. Moreover, it has been shown that the transport via hENT1 transporter is a limiting step for the toxicity of Ara-FA, while complexation with PPI-Mal OS G4 allows Ara-FATP to kill cells even in the presence of hENT1 inhibitor (Figure 2). Thus, the use of glycodendrimers for drug delivery may allow to circumvent the cellular resistance associated with decreased transporter activity. Finally, it has been demonstrated that complex formation does not change the specific intracellular action of Ara-FATP, preserving its ability to inhibit nucleic acid synthesis and induce apoptosis via the intrinsic pathway [3].

Fig. 2: *Intracellular transport of fludarabine in the form of nucleoside (Ara-FA) and triphosphate (Ara-FATP) in a complex with the PPI-Mal OS G4 dendrimer in the presence of the hENT1 inhibitor (source: [3], modified).*



Since these experiments do not provide a direct proof of the hENT1-independent intracellular transport of the nucleotide by the dendrimer, in the next stage of research a novel synthesis technique for radioactively-labelled fludarabine triphosphate has been devised, allowing to demonstrate the ability of nucleotide-glycodendrimer complex to deliver the drug into the cells without the involvement of membrane nucleoside transporters [4].
Considering these promising results, in the further course of studies, clofarabine (CAFdA), an adenosine analogue drug of the second generation, has been applied to verify the hypothesis that PPI dendrimers may serve as universal carriers for therapeutic nucleotides. Using surface plasmon resonance and molecular modelling to elucidate the properties of drug-dendrimer complexes, it has been shown that clofarabine triphosphate (CAFdATP) exhibits significantly different molecular interactions with PPI dendrimers in comparison to Ara-FATP, leading to different therapeutic outcome of the complex (decreased rather than increased cytotoxicity). It has been hypothesized that stronger interactions of CAFdATP with PPI dendrimers and its ability to mask surface positive charge of these macromolecules may decrease the capacity of the complexes to cross cell membrane and release the drug [4].
In the final stage of studies, zeta potential measurements, ultrafiltration, and asymmetrical flow field-flow fractionation have been applied for the determination of surface electrostatic potential and stability of nucleotide-dendrimer formulations. Indeed, CAFdATP has shown a significantly higher tendency to mask positive surface charge of PPI dendrimers than Ara-FATP, and to form stronger complexes regardless of environmental conditions. These results support the hypothesis of potentially reduced cellular uptake and intracellular release of CAFdATP from the complex, which may decrease the delivery potential of PPI macromolecules in case of this drug [5].
In conclusion, the performed studies indicate the potential of the application of PPI dendrimers as carriers for adenosine nucleotide drugs in order to enhance their intracellular concentration, leading to improved therapeutic outcome. However, it is crucial to note that the stability and properties of nucleotide-dendrimer complexes strongly depend on the environmental conditions and the chemical structure of the drug, which should be taken into account during the design of both *in vitro* and *in vivo* studies.

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SPECIFIC SILENCING OF MICROGLIA TLR4 AND CD11B IN RAT BRAIN BY SIRNA-DELIVERING NANOPARTICLES

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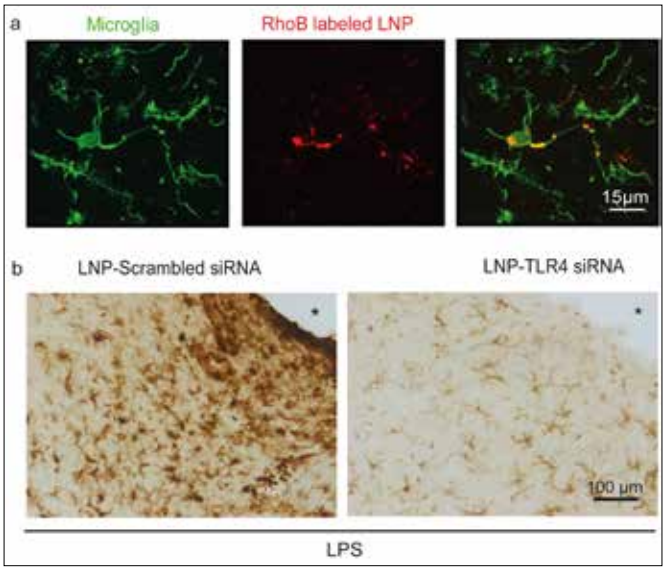
Microglia are the brain innate immune cells that are responsible for maintaining brain homeostasis by surveying the environment, sensing invading pathogens and phagocytizing dead neurons and cellular debris, thus eliciting an innate immune response. Current technologies, like the Cre-loxp recombination system, only allow the knockdown of microglial genes in the entire brain, which limits the study of microglia function in specific brain regions. For specific manipulation of microglial gene in targeted brain regions, we introduced a novel method, using a lipid and polymer hybridized nano-carrier (LNP) as a tool, to specifically and locally silence microglia genes. To validate this gene silencing system, we used cationic ε-polylysine co-polymer nanoparticles and absorbed Toll-like receptor 4 (TLR4) siRNA or CD11b siRNA on their surface (NP-siRNA), and then coated the NP-siRNA with a lipid bilayer (LNP-siRNA). Physical characterization showed a uniform and stable preparation of the LNP-siRNA with a diameter around 100 nm. In the BV2 microglia cells, the silencing efficiency of the LNP-TLR4 siRNA at a concentration of 100 nM reached 81% at the protein level after

24h incubation, as evaluated by western blotting and immunocytochemical staining. We then tested the inflammatory response of LNP-TLR4 siRNA-treated BV2 cells after stimulation with lipopolysaccharide (LPS). We found that cytokine (TNFα, IL-6 and IL-1β) mRNA levels in LNP-TLR4 siRNA-treated BV2 cells were much lower than in the control group after stimulation with LPS. To further evaluate the silencing efficiency and functional relevance, we injected LNP-TLR4 siRNA (20 h, 100 nM, using LNP-scrambled siRNA as control) into the rat mediobasal hypothalamus and analyzed the microglial immune response following an intravenous injection with LPS. We observed a significantly reduced activation, as reflected by the total number of microglia (analyzed by ionized calcium binding adaptor molecule 1 immunoreactivity (iba1-ir)), in the LNP-TLR4 siRNA group as compared to the LNP-scrambled siRNA control group. Our results suggest that the LNP-siRNA approach is a promising new technique for spatially and temporally manipulating microglia activity in the brain.

a) Specific uptake of LNP (RhoB labeled, red) by microglia (iba1-ir, green) following intracranial injection for 4 hours.
b) Less activated microglia (iba1-ir) in the LNP-TLR4 siRNA group in response to LPS comparing to the LNP-scrambled siRNA control group.



AUTOMATED REVIEW OF THE SCIENTIFIC LITERATURE ON NANOMEDICINES

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Regulatory decision making suffers from the limited availability of the information on the safety and quality of innovative health products such as nanomedicines. On the other hand, thousands of publications addressing nanotechnology-enabled medical applications are released every year. A systematic review of the scientific literature could provide the lacking data, supporting the release of regulatory guidance on data requirements and decreasing the uncertainty for product developers. However, monitoring of the scientific literature, which is constantly increasing, and processing of high amount of data requires the use of automatic tools that can speed up the process and reduce a bias.
Different automatic tools exist already for certain steps of a systematic review procedure such as search refining, de-duplication or abstract screening, but no tools are available for the most time-consuming tasks which are evaluation of article quality and information extraction. In order to identify specific toxicity effects induced by nanotechnology-enabled products we used a battery of available automatic tools for initial steps of the systematic review process and we developed a new tool for the automatisation of the information extraction for both, quality assessment and result exploration (Figure 1).
The segmentation of the article into sections, based on the metadata of the article's PDF and Natural Language Processing approaches, allowed further application of text mining techniques ensuring the relevance and accuracy of the extracted information. A set of criteria related to nanomaterial characterisation was applied in order to score and rank the articles. Furthermore, based on the developed ontology, the most reported toxicity effects were extracted and mapped against different types of nanomaterials (Figure 2). Such knowledge is of critical importance for the development and regulatory assessment of nanotechnology-enabled products.
Importantly, this exploratory work has confirmed the feasibility of a systematic review of the literature using a battery of automatic tools, and as such can be used in future for multiple purposes.

Figure 1: *Schematic representation of the main steps of the systematic review performed using automatic tools.*

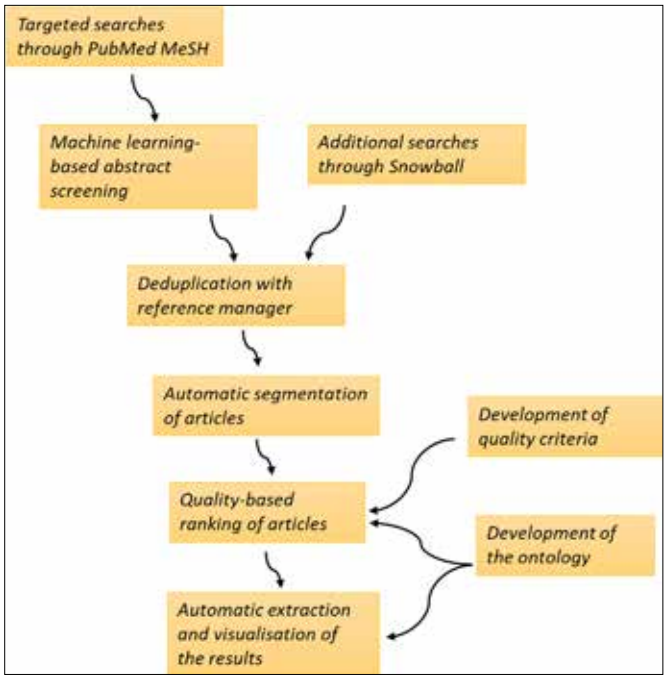
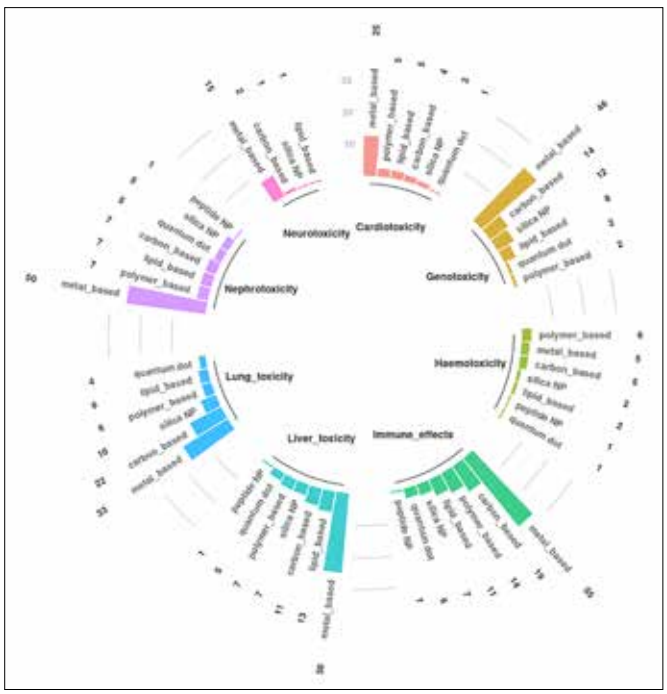


Figure 2: *Most reported toxicity effects induced by different types of nanomaterials.*



ACKNOWLEDGMENT

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PH-RESPONSIVE NANOGELS DERIVED FROM SQUARIC ESTER AMIDE-BASED PRECURSOR POLYMERS FOR DRUG DELIVERY APPLICATIONS

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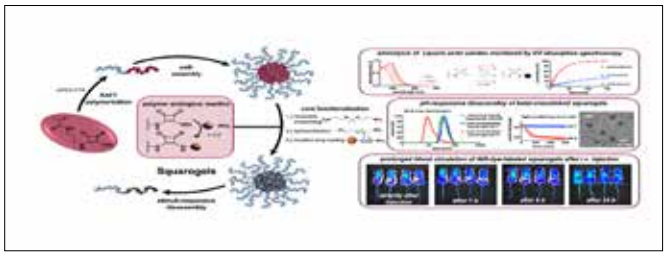
Spontaneous self-assembly of amphiphilic block copolymers is a straightforward method of accessing well-defined supramolecular topographies in the nanometer regime. However, unless stabilized by additional physicochemical crosslinks, such polymeric architectures remain in a dynamic equilibrium with their single unimer chains and become responsive towards disintegration upon dilution below their critical micelle concentration. For applications in complex systems, for instance as "nanomedicines" for *in vivo* applications, drug loaded polymeric micelles need to withstand further competitive interactions with e.g. amphiphilic compartments of the blood system. Therefore, controlled stabilization of nanosized self-assemblies is a key requirement to avoid rapid disassembly and premature drug release under such conditions.[1]
To address such requirements, we have been developing reactive amphiphilic polymers forming precursor polymeric micelles that can further be derivatized and stabilized inside their cores and, thus, transformed into hydrophilic nanogels.[2]
To promote robust and selective nanogel fabrication also under aqueous conditions, we introduce (meth)acrylate/(meth)acrylamide-based monomers with pendant squaric ester monoamide groups. Such groups are known to enable conjugation to primary amines under aqueous conditions but are also resistant to radical polymerization conditions.[3] We therefore polymerized squaric ester monoamide based methacrylates via reversible addition fragmentation transfer (RAFT) polymerization and self-assembled the resulting block copolymers into precursor micelles (Fig.1). Their amine-reactive cores could sequentially be derivatized by primary amines into polymeric squaric bisamides monitored by UV-Vis spectroscopy. Thereby, conjugation with hydrophilic bisamines as well as water-soluble primary amines contributed to core-crosslinking and core hydrophilization, respectively, affording so-called squarogels.
Degradation and drug release kinetics of the squarogels have been fine-tuned by the use of pH-degradable ketal-crosslinkers as well as pH-responsive maleic anhydride-based linkers for covalent drug conjugation. In vivo, this installed pH-responsibility will contribute on the one hand to disassembly of the squarogels into soluble unimers and on the other hand to the effective release of the unconjugated drug under endosomal pH conditions. Interestingly, the resulting squarogels generally showed high blood stability as well as long circulation properties in the blood stream after i.v. administration. Therefore, squarogels can be considered as versatile polymer-based nanoarchitecture platform for controlled and effective polymeric drug delivery applications.

Fig.1: *Squaric ester monoamide derived methacrylates can be block copolymerized into self-assembling amphiphilic reactive precursor block copolymers that are subsequently fabricated into pH-degradable squarogels with prolonged blood circulation and drug delivery properties.*



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TEMOZOLOMIDE ACID LOADED NANO-PARTICLES: A PROTEIN-BASED PLATFORM FOR GLIOMA THERAPY

DINA HELAL[1,2], Nadia Rouatbi[2], Julie Wang[2], Shunping Han[2], Mona M.A. Abdel-Mottaleb[1], Amany O. Kamel[1], Gehanne A.S. Awad[1], Ahmed-Shawky Geneidy[1], Khuloud T. Al-Jamal[2] *
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Brain cancer is one of the most leading causes of death in the world. The brain–blood barrier (BBB) represents the main challenge for chemotherapy. The metabolically active tumours are hungry for nutrients thus albumin intake into tumour tissues is greatly increased for use as a source of amino acids and energy for cancer cells. Albumin-binding proteins, such as SPARC (secreted protein acidic and rich in cysteine) and glycoprotein 60 are known pathways for albumin uptake by tumours. Temozolomide (TMZ) is the first line drug for treatment of glioma. It however suffers from rapid degradation leading to inability to deliver an effective dose to tumours besides the non-selective uptake and systemic toxicity. TMZA is the carboxylic acid derivative of TMZ and is one of the active forms of TMZ. TMZA has improved physical stability compared to the prodrug TMZ. We hypothesise that Human Serum Albumin (HSA) nanoparticles encapsulating TMZA (TMZA-HSA-NPs) will offer improved drug stability and targeted delivery leading to sufficient concentrations reaching glioma, enhanced efficacy and reduced side effects.

Methods: TMZA loaded HSA-NPs were prepared by desolvation method as shown in Figure 1A. A 3^2 factorial design was adopted to study the effect of formulation process parameters (drug amount and sodium cholate concentration) on responses namely the particle size (PS) and drug loading (DL%). Formula showing the highest drug loading was then tested for cytotoxicity on BL6 (brain cancer stem cell line) and GL261 cells (glioblastoma cell line) using MTT assay. HSA NPs were labelled by Alexa 488 NHS to investigate cellular uptake of the NPs in both cell lines.

Results: TMZA was successfully loaded in HSA-NPs. Increasing TMZA amount led to an increase in DL% and a particle size re-

duction (Figure 1B). The highest DL% achieved was ~5% with PS ~112nm, confirmed by TEM (Figure 1C). The prepared NPs showed comparable toxicity to free TMZA for both cell lines (Figure 1D), suggesting that formulation process did not compromise drug chemical stability. HSA labelled NPs showed preferentially high internalization by BL6 and GL261 cells (Figure 1E).

Conclusions: HSA NPs loaded with TMZA of suitable DL% and PS characteristics were prepared. Functional TMZA was delivered to cell *in vitro* and showed pronounced cell killing. Fluorescently labelled NPs could internalize in glioblastoma and brain cancer stem cell lines. These results demonstrate that TMZA-HSA-NPs offer an attractive nanocarrier that warrant additional *in vivo* testing in intra-cranial glioblastoma mouse model.

Acknowledgements: Dina Helal is a Newton Mosharafa Fellow.

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(2) Chen Fang, Kui Wang, Zachary R. Stephen, Qingxin Mu,Forrest M. Kievit, Daniel T. Chiu, Oliver W. Press and Miqin Zhang Temozolomide Nanoparticles for Targeted Glioblastoma Therapy. ACS Appl Mater Interfaces. 2015, 7(12), 6674–6682

Learning objectives: Optimize the particle size and TMZA loading in HSA NPs Study the cytotoxicity and cellular uptake of TMZA-HSA-NPs Evaluation of the cellular uptake of HSA-NPs by different types of brain cancer cells

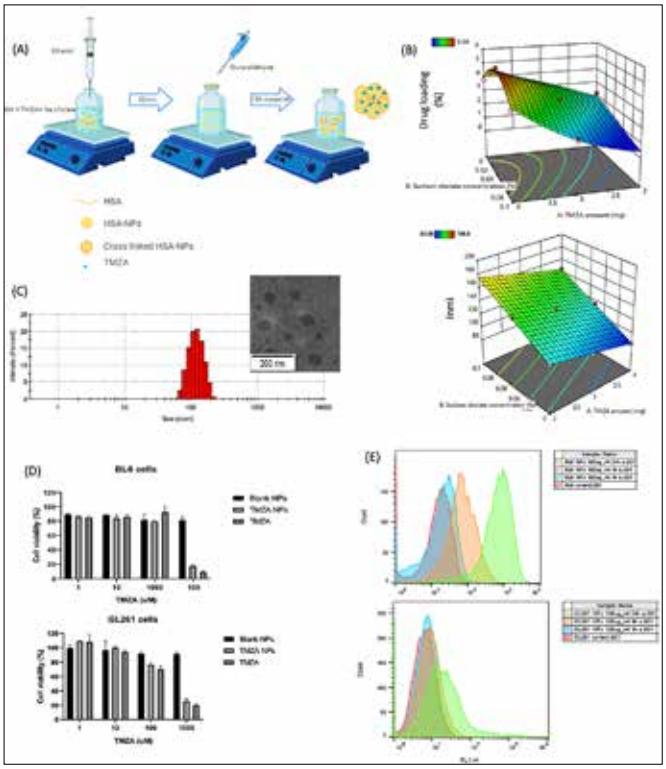

Figure 1. *(A) Preparation of HSA NPs by desolvation method. (B) DOE of TMZA-HSA-NPs showing the effect of TMZA amount and Na cholate concentration on DL% and particle size. (C) Representative image of the size distribution by DLS and TEM.(D) Cytotoxicity of Blank NPS, TMZA-NPs and free TMZA by MTT assay. (E) Cellular uptake of HSA-NPs by BL6 andGL261 cells.*

Presenter Biography: Dina Helal is a Newton Mosharafa Fellow and a joint PhD student at the Institute of Pharmaceutical Science at KCL (Al-Jamal's lab) and Ain Shams University, Egypt. Her project focusses on designing albumin and polymeric nanoparticles for brain delivery.

IMPACT OF LINEAR-POLYETHYLENEMINE LENGTH IN STRUCTURE-FUNCTION CORRELATIONS OF RNA/POLYPLEXES

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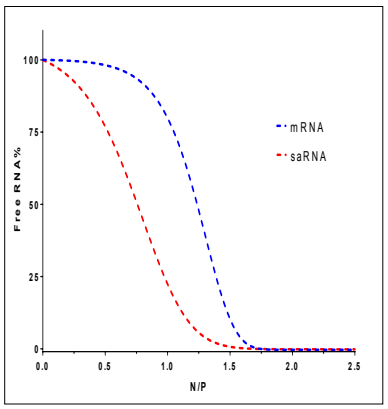
Messenger RNA (mRNA) technology is promising to revolutionize therapeutic approaches in a variety of fields, including cancer immunotherapy, protein substitution and infectious disease vaccination[1]. Besides the canonical un-modified mRNA (uRNA), other engineered mRNAs such as self-amplifying RNA (saRNA) and chemically modified mRNA (modRNA) have demonstrated to be promising candidates, because they lead to much higher expression of the protein of interest than identical doses of unmodifiedmRNA[2,3].
Regardless of the mRNA platform, therapeutic applications of the RNAs require tailored delivery vehicles to protect it from degradation, control uptake mechanism and foster specific spatio-temporal release of functional cargo. This demands structural and functional understanding of the delivery vehicles at different levels, including the biological performance of such. Among the different types of non-viral vehicles, polyplexes based on linear polyethylenimine (PEI), are very promising candidates. Linear PEI poses a high chain flexibility and density of positive charges, which is responsible for both the outstanding complexation of nucleic acid and intrinsic endosmolytic activity. The latter plays a fundamental role in the specific spatio-temporal release of the cargo of such vehicles. The impact of molecular weight of PEI(s) has been previously described[4]. However, most of these studies are based on PEI(s), which were chemically not comparable due to the synthetic route and de-acetylation degree. Moreover, most of the PEI(s) in these studies are obtained from the ring-opening polymerization of aziridine, which leads to an intrinsic high polydispersity of the PEI(s) species[5,6]. Here, the impact of linear PEI chain length in the structure-function coherencies within mRNA/PEI polyplexes, was systematically investigated. Linear PEIs were obtained from full de-acetylation of poly-2-ethyl-oxazoline(s) of different size. This offers a significantly improved control of the polymer species over the classical aziridine synthesis and enables final polymers species with very discrete dispersity (D<1,2).

METHODS

PEI-Polyplexes were assembled from either mRNA, saRNA or modRNA. Characterization of the delivery vehicles included RNA content, size, binding profile and further structural morphologies provided from Small-Angle X-Ray Scattering (SAXS) and circular dichroism (CD) measurements. Biological activity was evaluated both *in vitro* and *in vivo* using luciferase encoding RNAs.

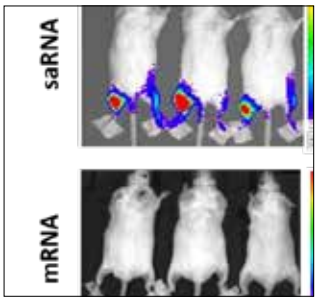


uRNA and saRNA binding stoichiometry with PEI formulation was investigated. *Binding profile of 500r.u linear-PEI to uRNA and saRNA showed differences. Further, saRNA required a lower N/P ratio to be completely bound compared to the shorter and less structurally complex uRNA.*

RESULTS

Structural and conformational changes of formation of PLX products from the different types of RNA were tracked. Aspects of the formulation protocols, such as the charge ratio during particle formation, were correlated with structure changes of the particles and folding of uRNA, modRNA and saRNA, as a function of the linear

PEI(s) size. Significant differences in the overall biological performance of these particles were found. Interestingly, while *in vitro* delivered PLXes containing saRNA, uRNA or modRNA-polyplexes exhibited comparable biological activity, *in vivo* delivery of saRNA-PLX resulted in significant higher transfection activity than in the case of uRNA-PLX or modRNA-PLX.



Intramuscular application of luciferase coding-uRNA or saRNA PEI-Polyplexes. *One single injection of 1µg RNA in Mus Musculus, i.m., N/P12, 500r.u linear-PEI. While saRNA-PLXs could achieve functional expression, no signal could be observed in the uRNA-PLX.*

Our results show that cationic driven complexation vehicles possess a high complexity regarding structure-function correlations. These findings underline that a detailed understanding of nanoparticle structure is essential for control of the cellular and cytosolic processes. Based on such insights, development of improved, tailored formulations for specific therapeutic purposes may be enabled. Further investigation will be necessary to obtain a full understanding of the *in vivo – in vitro* correlation inside such systems.

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BIOORTHOGONAL AND MULTICOMPONENT ENCAPSULATION INTO PROTEIN NANOCAPSULES VIA METAL-FREE AZIDE-ALKYNE CLICK CHEMISTRY

NATKRITTA HÜPPE

Immunotherapy targets therapeutic vaccination by either activating or suppressing immune responses. Therapeutic vaccination against tumor diseases remains challenging in cancer treatment. One reason is insufficient stimulation of dendritic cells by the vaccine which is crucial for a successful vaccination effect.[1, 2] Studies reported the phenomenon of superadditive stimulation where the use of different PRR ligands in combination trigger diverse signaling adaptor molecules and enhance their stimulatory capacity.[3, 4] While common formulations cannot sufficiently trigger T cell responses, nanocarriers have the advantage to protect the payload from the outer environment and can be designed to deliver to the targeted site and release their cargo with a certain stimuli.[5, 6] Loading the antigen and adjuvants into one nanocarrier enables an all-in-one delivery with high local concentration of all components and therefore enhancement of DC stimulation. The challenge for nanomedicine is here an encapsulation of several cargoes with high efficiency and without effecting the cargoes activity. Used adjuvants offer different activation properties to achieve a superadditive DC-stimulation, but they also have different chemical characteristics, which complicate a multicomponent encapsulation into one nanocarrier (Fig.1).[7-9]

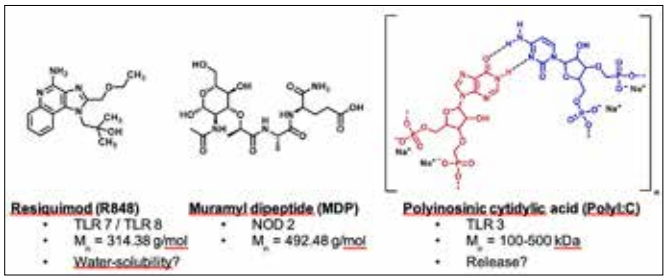


Figure 1. *Chemical and biological characteristics of adjuvants, resiquimod, muramyl dipeptide and polyinosinic cytidylic acid.*

The inverse miniemulsion approach is used for the encapsulation of the adjuvants into nanocapsules. The capsule shell is formed through an interfacial crosslinking at the water droplet interface. The cargoes are dissolved in the aqueous dispersed phase and are therefore encapsulated by the shell formation.[10] Synthetic polymers as shell material often lack biocompatibility and degradability wihout producing toxic side products.[6] Proteins offer both, compatibility and degradability, and are ideal as nanocarrier material.[11] Azide-alkyne click chemistry enables chemoselective cross-linking. When using an activated ester (i.e. hexanediol dipropiolate), cross-linking of the carrier material proceeds metal-free and bioorthogonal, optimal for encapsulation of sensitive cargoes without changing their activity (Fig.2).[12]

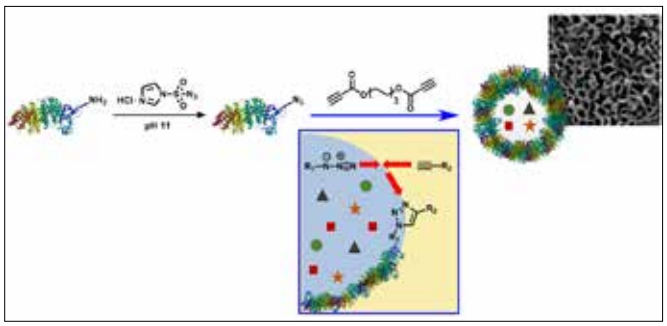
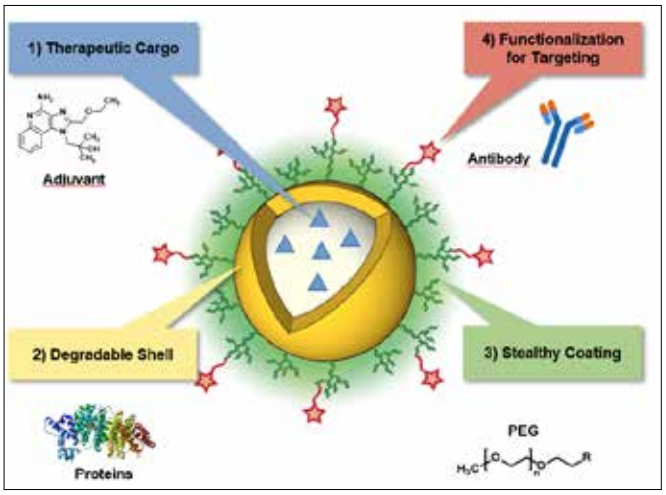


Figure 2. *Formation of protein nanocapsules and multicomponent encapsulation of water-soluble drugs via interfacial azide–alkyne click reaction in inverse miniemulsion.*

Encapsulation of multiple adjuvants into protein nanocapsules is achieved with high loading capacity. The developed nanovaccine triggers strong superadditive stimulatory potential on dendritic cell stimulation. This nanocarrier approach offers encapsulation of various water-soluble cargo and the use of any bio-based shell material such as tumor-related antigens. Furthermore, the capsule surface can be modified with stealth and targeting moieties to incease blood circulation time and dendritic cell targeting. Combined with their low biocompatibility and cytotoxicity, the introduced protein-nanocapsule *via* azide-alkyne click chemistry provide a promising platform for designing tailored nanovaccines for tumor treatment (Fig.3).

Figure 3. *Schematic Representation of protein nanocapsules for targeted drug delivery.*



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DEVELOPMENT OF NANOPARTICLE -BASED MRNA CARRIER SYSTEMS FOR TRANSIENT IMMUNOMODULATION OF IMMUNE CELLS.

ISABELL KEIL

Nanoparticle (NP) -based carrier systems are a promising tool for non-viral gene transfer. Especially the targeted delivery of mRNA as a therapeutic in cancer immunotherapy is interesting, as it allows the transient expression of factors for modulation of immune cells. For mRNA to become therapeutically active, several parameters need to be met: low immunogenicity of RNA, high stability, high targeting and translation efficiency. Several modifications of synthetically produced mRNA led to a significant decrease in immunogenicity, improvement of intracellular stability and translation. However, pharmacologically optimized NP-based mRNA carriers and ligands need to be validated, to ensure targeted and stable mRNA delivery.

The aim of this study is the identification of NP-based mRNA carriers for targeted delivery into leukocyte subpopulations, such as monocytes, T-, B- and NK cells in the human or murine system.

Therefore, we investigated potent NPs as carriers and different ligands for targeted delivery.

NP-based mRNA carriers were evaluated based on in-house produced cationic charged polymer- and lipid-based structures, known as polyplexes (PLXs) and lipid nanoparticles (LNPs). Antibodies and derivates are identified as potent ligands for particle functionalization. Functionalized NPs can be used to deliver mRNA into distinct immune cell populations and to facilitate their transient immunomodulation. For conjugation of ligands to NP surface, we work with electrostatic interactions or copper-free click reactions. Physicochemical compositions and functional properties of NPs are characterized, in order to optimize an efficient mRNA delivery *in vitro* and *in vivo*.

Focusing on target and unspecific transfection efficiency, dose-dependency and viability of transfected cells, we established a transfection assay in human peripheral blood mononuclear cells (PBMCs) and murine splenocytes. We successfully detected specific transfection of functionalized NPs, by investigating the expression of several reporter-mRNAs via flow cytometry. To more closely mimic the human situation, we adapted the established transfection assay of human PBMCs using whole blood. With this, we were able to counteract coagulation of whole blood and to get further insights into the impact of natural blood components on NP characteristics and their subsequent efficiency. In addition, we are using fluorochrome-labeled mRNA as a tool for the validation of new ligands and to study cell binding efficiency of (non-) functionalized NPs.

As we already identified potent ligands for human and murine targets, selection of a lead candidate for *in vivo* studies will be the next step.

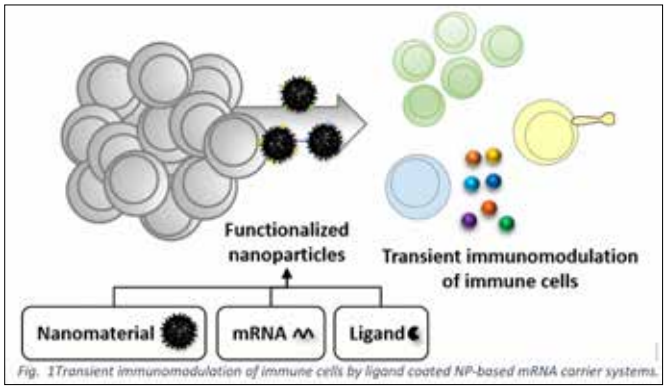

Fig. 1Transient immunomodulation of immune cells by ligand coated NP-based mRNA carrier systems.

MICELLAR-BASED, MULTIFUNCTIONAL POLYCARBONATES AS VERSATILE (IMMUNO-)DRUG CARRIERS

PATRIC KOMFORTH,[1] Christian Czysch,[1] Alexander Fuchs,[1] Thi Thuy Duong Dinh,[1] Bernhard Biersack,[2] Jonathan Schupp,[3] Matthias Bros,[3] Stefan Naumann[4] and Lutz Nuhn[1]
Max Planck Institute for Polymer Research,[1] 55128 Mainz, Germany, in collaboration with Department of Dermatology, University Medical Center Mainz,[3] Institute of Polymer Chemistry, University of Stuttgart[4] and Department for Organic Chemistry, University of Bayreuth.[2]

Classical small molecular therapeutics can provoke immense side effects and systemic responses apart from their site-of-action. Especially for novel (cancer) immunotherapeutics or advanced antibiotics, there is an urgent need for smart drug delivery systems to overcome these issues. Advanced biodegradable polymer carriers may efficiently deliver highly active drugs to their target site and omit off-target effects.

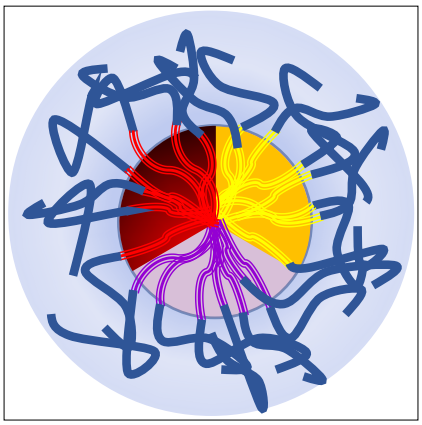

Figure 1 *Synthesis and application of core-shell nanoparticles based on biodegradable polycarbonates. Multiple polymerization techniques as well as different functional monomers are applied to obtain tailor-made carrier systems for the delivery of various therapeutic molecules.*

For that purpose, polycarbonates are introduced as novel attractive, biodegradable materials for advanced drug delivery. Selective chemical modification of these polymers is a necessity to obtain tailor-made, multifunctional carriers with optimized properties for individual therapeutics. Therefore, functionalizable polymers were synthesized and fabricated into nanogels with covalently attached drugs/dyes for immunostimulation. Covalent incorporation of positive charges supports the delivery of therapeutic oligonucleotides. Amphiphilic polycarbonate block copolymers with benzyl side chains were employed for π-π-stacking promoted encapsulation of novel aromatic antibiotics.
Latest attempts towards responsive degradation features upon internal or external stimuli are currently ongoing.

AUTOMATED NERVE FIBRES IDENTIFICATION AND MORPHOMETRY ANALYSIS WITH NEURAL NETWORK BASED TOOL IN MATLAB

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Analyses of nerve histology are core assays in basic and applied research and even in clinical setting. Detailed report on nerve morphology may unbiasedly indicate the current state of a peripheral nerve. Manual method requires trained technician and is a time-consuming procedure. Available plugins to well known image processors are limited in use and data outcomes. Thus, the aim of the study was to create a. tool for for fast and repeatable analysis of a nerve section image. As a results we get very high precision of analysis in shorter time.
Keywords. Axon evaluation, Neural network

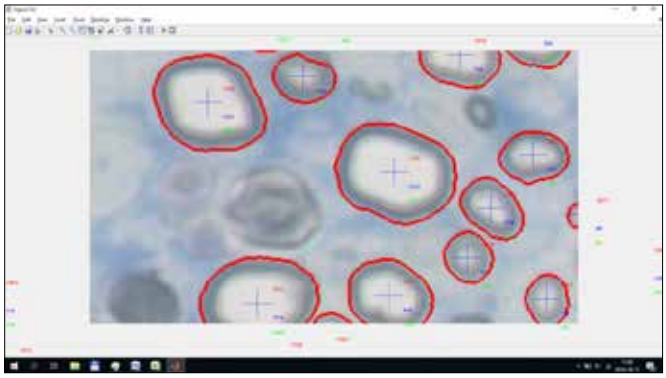

Figure 1. *Image shows result of marking axons and myelin sheaths Results*

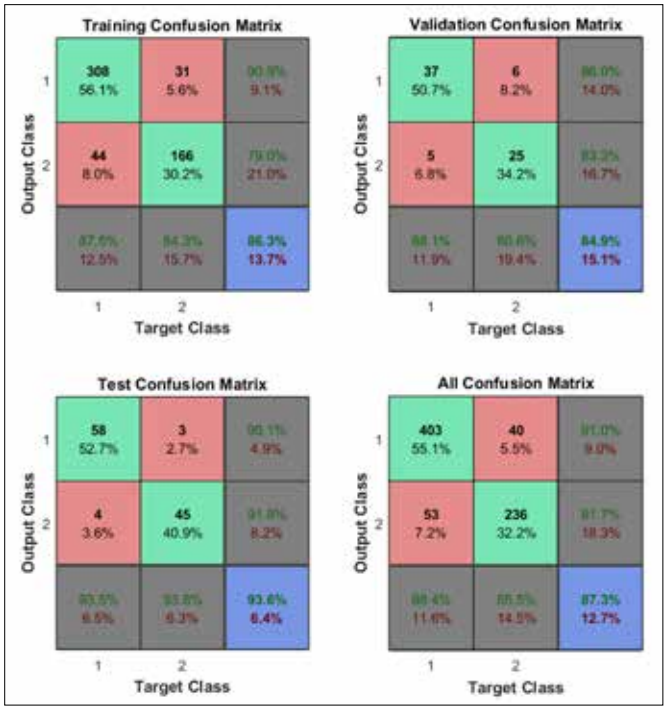

Figure 2. *Confusion matrixes from applied Neural Net Pattern Recognition tool*

Introduction

Preclinical research of neuroregeneration requires assessment of nerve fibers histology. The most reliable results contain histomorphometry parameters of axons and myelin sheaths. Manual methods are prone to human error and are time consuming. Computer-based techniques may improve the above and deliver the accuracy comparable with a experienced researcher. The study aimed to prepare a neural-network MATLAB script, with a novel approach to contrast-based method used for whole nerve section analysis.
Materials and methods
Semithin sections of a rat sciatic nerve stained with toluidine blue were a input data. They were scanned at 40x magnifications. Firstly, the analysis includes grey-scale conversion and contrast improving modifications e.g. CLAHE. Unlike other described methods, which

analyze objects after binarization, we worked with the grey-scale image. Objects found with regionprops function were then filtered. Our protocol offers manual verification and removal of false-positive objects. We also applied Neural Net Pattern Recognition from Matlab for filtering results. Then, a rectangle enclosing each border of an object was defined. From the center to the edge of the acquired area line segment was lead and histogram on this length was set. The border of each axon was determined in the point of highest contrast between neighbour pixels. The borders of myelin sheaths were defined likewise. This operation was repeated 360 times, around the analyzed nerve cell (radial histogram scanning and thresholding). The method resembles manual analysis since local contrast is used to mark the axon.
Finally, the main morphometric parameters may be determined (radius, G ration, thickness, area etc.). Results were compared with manual measurements of 28 random nerve images, estimated by 3 researchers in ImageJ. Comparison to manual data was shown as ratios: script: manual.
After optimization of brightness and contrast parameters, axon area overlay at level 1,010 and 1,009 of the myelin sheaths was achieved. Paired T-test showed no significant differences between these two methods (p-value = 0,321). Use of Neural Net enables to reach 93,6% of accuracy of axons recognition versus 3 researchers. A run time of the script is about 12 times shorter than the manual method

Discussion

Other similar methods consist of binarization, which doesn't allow to define borders of the nerves precisely. The script presented shows a new approach to this case. It is based on reconstructing the way of evaluation done by the scientist. The presented script shows a new approach to this case. Our idea is based on reconstructing the way of evaluation done by the scientist Owing to this innovative idea, the higher level of accuracy of computerized evaluation is possible. The most depending factor is optimalising the differentiation between correctly and uncorrectly marked borders. Further researches focus on increasing the efficiency of this protocol

Conclusion

The presented script performs an accurate analysis of nerve sections on a grey-scaled image. It overcomes bias of a binarization. The method decreases time of analysis and remains repeatable.

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CHEMOTHERAPEUTIC DRUG SELECTIVE NANOPARTICLES AND THEIR APPLICATION IN A POINT-OF-CARE THERAPEUTIC DRUG MONITORING DEVICE

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The decision-making in chemotherapy nowadays depends on standard methods that are liquid chromatography followed by mass spectroscopy (LC-MS/MS)[1] or capillary chromatography; both are labour- and cost-intensive and can be performed only in dedicated hospitals and laboratories. This lead to a minimal therapeutic drug monitoring in patients and hence that 30-60% of drugs are administered without clinical benefits.
We developed prototypes for point-of-care devices to quantify doxorubicin and SN-38 basing on highly sensitive fluorescence read-out of differences in emission of parental drug and its metabolite[3]. For paclitaxel we developed highly selective nanoparticles which were incorporated in a microfluidic lab-on-a chip device (optofluidics based)[2] for read-out
The proposed therapeutic drug monitoring should reduce adverse effects by too high drug dose, detect under-treatment as the administered drug dose does not reach the minimum effective concentration the therapeutic outcome and reduced health care costs.

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IN VITRO TESTING OF LOCALIZED TREATMENT OF PRIMARY LIVER CANCER USING NANOPARTICLES

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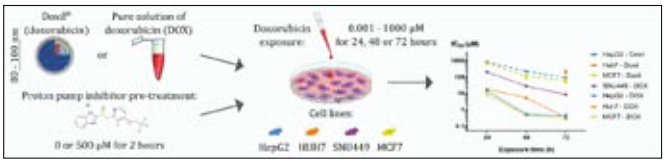
Figure 1. *A graphical abstract showing the main work flow of this study as well as the measured IC_{50} values of the different cell lines.*
Introduction:



Primary liver cancer was the sixth most commonly diagnosed cancer and the fourth leading cause of cancer death worldwide in 2018. Hepatocellular carcinoma (HCC) is the most common type of primary liver cancer worldwide, with about 75-85% of all primary liver cancers being HCC. [1]

Today there exists multiple both localregional and systemic treatment strategies for HCC. Among them, transarterial chemoembolization (TACE) has been the standard care in intermediate-stage HCC. This locoregional treatment method consists of locally infusing a drug delivery system with a chemotherapeutics through the hepatic artery that feeds the tumor. One of the most commonly used chemotherapeutic agents of this locoregional treatment of HCC is doxorubicin (DOX). [2].

Novel pharmaceutical formulations for parenteral administration of DOX has been developed with the objectives to reduce common and serious side effects, such as cardiotoxicity, as well as increase the overall anti-tumor effect. The first such nanoformulation was approved in 1995, Doxil®, which is based on a liposome technology covered with a layer of polyethylene glycol coating. [3].

As DOX is a weak base it can easily be trapped in the acidic conditions found in late stage endosomes and lysosomes in the cell. To counter this, some studies have found that by co-administering proton pump inhibitors such as lansoprazole they are able to increase the pH of these acidic environments, thereby limiting the amount of DOX sequestered. In a previous study by Tannock et al., it was shown that by exposing the cells to a two-hour pretreatment of lansoprazole, the dose needed to inhibit half of the cells (IC_{50}) was drastically lowered. [4]

Human cancer-derived cell lines are the most widely used *in vitro* models to investigate the effect of drugs on cancer mechanistically and to compare the *in vitro* potency between treatments. Here, a panel of different cancer cell lines, representative of the natural heterogeneity observed in primary tumors, were used.[5] The three HCC cell lines selected in this study were HepG2, Huh7 and SNU449, as well as the breast cancer cell line MCF7.

In this study, the primary objective was to investigate the difference in anti-tumor effect to DOX exposed as a solution or a liposome based nanoformulation (Doxil) of three commonly used liver cancer cell lines as well as in a breast cancer cell line. Secondly, the anti-tumor effect exposure of DOX as a solution or nanoformulation (Doxil) when pretreated with lansoprazole was examined. Thirdly, the total intracellular concentration of DOX was determined and correlated to the anti-tumor effect.

Methods and materials:

Three HCC cell lines, HepG2, Huh7 and SNU449, were selected based on published data concerning IC_{50} values of the most commonly used HCC and hepatoma cell lines. The cell lines were selected based on sensitivity to DOX, with HepG2 and Huh7 being more sensitive, while SNU449 is described as the most drug-resistant commercially available cell line which is not genetically engineered to be chemo-resistant. [6]

A breast cancer cell line, MCF7, was also included in the study to reflect the current usage of Doxil, which is indicated for treatment of breast cancer in Europe and Canada.

The cells were treated with monotherapies of DOX solution or Doxil either with or without a pretreatment of lansoprazole. DOX stock solution was serially diluted to concentrations in the range of 0.001–1000 µM in cell media, while Doxil was diluted to concentrations of 0.1 to 1000 µM. Plates were incubated in an incubator for the desired exposure time (24, 48 or 72 hours). Cell viability was monitored via a resazurin reduction assay.

To control for toxicity of DMSO, the solvent used for the DOX and lansoprazole stock solutions, a separate control study was performed on all cell lines. The cells were exposed to cell media serially diluted with DMSO to concentrations in the range of 0.01-10 % (v/v) DMSO.

To determine the intracellular concentration of DOX after treatment, the cells were treated with their calculated IC_{50}-values, based on the pooled results of the cell viability assays.

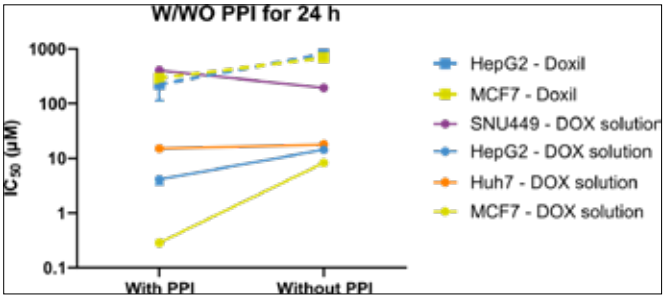
Results and conclusions:

Cell viability and IC_{50} values vary greatly depending on the cell lines, with especially SNU449 having a high resistance to both formulations, as expected from literature. The calculated IC_{50} values can be seen in the figure 1 above.

The impact of pretreatment with lansoprazole varies depending of the cell line as well as use of either Dox or Doxil, as can be seen in figure 2. It seems that the use of lansoprazole pretreatment lowers the IC_{50}-values of most cells treated with Doxil, with a few exceptions. Over all, it shows a statistically significant decrease in IC_{50} values in around half of all experiments.

This study is currently still ongoing and a complete set of results will be published in the spring of 2020.

Figure 2. *The difference in calculated IC_{50} values with and without lansoprazole pretreatment when treated with DOX or Doxil for 24 hours.*



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A NOVEL BIONANOTECHNOLOGY PLATFORM FOR THE DEVELOPMENT OF BEST-IN-CLASS ENZYME THERAPEUTICS.

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Over the past 30 years, 30 therapeutic enzymes have been approved by EMA and FDA (1/4 of all approved biologics). Therapeutic enzymes are mostly used for the treatment of both rare genetic disorders and cancers, through enzyme replacement therapies and tumor metabolism targeting, respectively. However, existing therapeutic enzymes exhibit efficacy issues (lack of systemic stability,

low residence time) and toxicity issues (up to 80% anti-drug reactions, immunogenicity).
Perseo pharma has developed a therapeutic enzymes platform based on the immobilization and the shielding of enzymes on silica nanoparticles (the enzzen® technology). This technology exploits the self-assembly of silane building blocks at the surface of enzymes to grow an organosilica layer, of controlled thickness, that fully protects the enzyme (Figure 1). There is no release of enzymes, the nanomedicine is designed to keep its integrity throughout its time in the body.

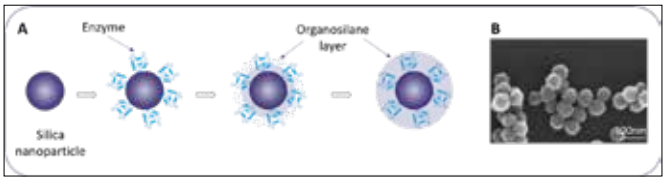

Figure 1: ***A****. Schematic representation of PER001 manufacturing steps (Patent WO2015/014888).* ***B****. SEM capture of nanomedicine.*

Perseo Pharma initiated a non-clinical proof of concept with a first compound, PER001, corresponding to the native form of an approved enzyme to which the enzzen® technology has been applied. In this presentation we report preclinical results for this nanomedicine. We show that the enzzen® technology provides the enzyme with key features including a long-lasting stability of the biocatalytic activity, and a protection from antibody recognition as well as from external stresses. In addition, we report an unperturbed enzyme activity in biological fluids (plasma and whole blood). Results of biocompatibility studies show that increased concentrations of PER001 exert non-cytotoxic effects on both HepG2 and LLC-PK1 cell lines. Moreover, no hemolysis is detected in the therapeutic range of concentrations, making them suitable candidates for various biological applications.
Altogether, these results validate the enzzen® plateform for the further development of enzyme therapeutics by Perseo pharma.

ASSESSING INTER-INDIVIDUAL ACTIVATION OF THE COMPLEMENT SYSTEM TO INCREASE THE SAFETY OF LIPOSOMAL FORMULATIONS

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Figure 1 *Application of an in vitro assay to assess the complement activation throughout the drug development process*

The development and standardisation of predictive toxicological tests assessing the immunotoxicity of smart nanomaterials is in particular challenging due to inter- and intra-species variations of the immune system.
It is acknowledged that different types of nanomedicines have the potential to induce the complement activation related pseudo-allergy (CARPA) syndrome[1], regulators from all over the world including the European Medicine Agency (EMA), the American FDA as well as the Japanese MWHL recommend the assessment of CARPA in preclinical toxicity testing strategies[2]. However, the existing safety evaluation guidelines such as the ICH S8[3] have not yet included standardised methods allowing to assess CARPA and/or the underlying mechanisms such as complement (C) system activation[4].
Therefore, the development of a predictive, quick and easy to use immunoassay is relevant throughout the whole development pipeline of the formulation[5] (figure 1).
This research activity aims to evaluate systematically the impact of inter-individual variations of the complement system for the standardisation of toxicological tests (figure 2). We have selected the iC3b fragment as a key biomarker of the complement activation and assessed its quantity in an enzyme immunoassay (EIA) after treatment with various nanomaterials. In particular, essential assay parameters such as the selection of negative and positive controls, the concentration ranges as well as incubation times with the test materials were critically analysed. After treatment with different substances and materials (e.g. cobra venom, Cremophor-EL, branched polyethyleimine, liposomes and doxorubicin hydrochloride), the iC3b levels of pooled serum were compared to the serum from individual donors.

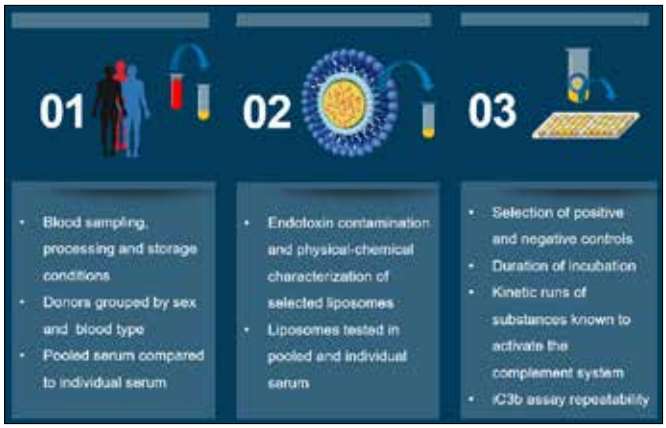

Figure 2 *Flow chart – testing activation of the C-system*

Based on the results presented in figure 3, the variability of complement activation found in serum from different donors show that commercially available pooled serum may represent only partially the potential of a liposomal formulation to activate the complement system.

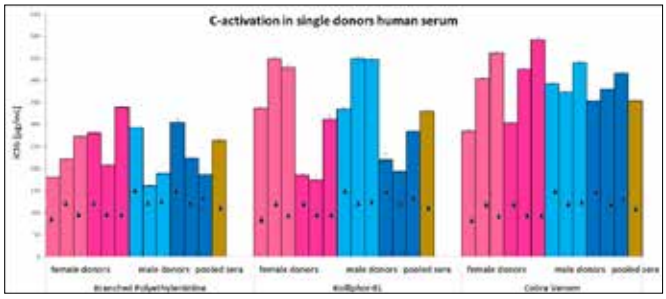

Figure 3 *Positive controls (branched polyethylenimine, kolliphor-EL and cobra venom) comparison in single donors human serum (pink - female donors; blu - male donors) and commercially available pooled serum (yellow). Histograms represent the mean (N=3) of iC3b complement fragment production in selected biological matrices. Data are shown as means of individual donors ± St. Dev. The black triangles are representing the iC3b levels of the negative controls.*

The results show that an assay based on pooled serum can be either over- or under predictive when it comes to an individual risk assessment. An under predictive result can have serious consequences on a small group of patients who can have severe or fatal reactions after the treatment with smart nanomaterials.
For this reason, it would be advisable to monitor individual immune responses through an easy to use medical device. Such devices can be based on microfluidic systems that only use low blood volume

and short experimental times to evaluate the immunogenicity of smart nanomaterials.

Acknowledgments: We gratefully acknowledge the financial support of the JRC's Exploratory Research program. We would also like to thank the staff of the JRC Medical service for their technical support and the blood donors.

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IMPROVED INTESTINAL RESIDENCE TIME OF NANOSYSTEMS USING NOVEL CHITOSAN BIOSCAFFOLDS

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Introduction: The aim of this work has been to develop an innovative drug delivery platform based on tailored nanoemulsions doped into biodegradable scaffold. Such system has been designed to improve local intestinal delivery of nanomedicines after oral administration through the release of the selected drug-loaded nanoemulsions (NE) from the mucoadhesive chitosan (CH) matrix (Figure 1).

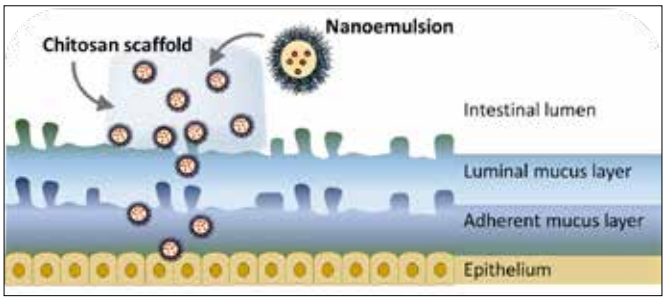

Figure 1: *Representation of the nanoemulsion-doped into chitosan scaffold and its interaction with intestinal mucosae.*

Methods: Nanoemulsion (NE) was obtained by emulsion phase inversion (EPI) technique and optimized by a mixture design. The crystallinity and fluidity of the NE shell were investigated using differential scanning calorimetry (DSC) and X-ray diffraction both in colloidal suspension and in dried state following freeze-drying and spray-drying [1]. The chitosan (CH) scaffold doped with NE was obtained using the freeze-casting technique [2,3] and a structural analysis using scanning electron microscopy (SEM) and optical images was performed. To evaluate the behavior of the release rate of NE associated to scaffold *in vitro* release studies were carried out in the biorelevant intestinal fluid (FaSSIF-V2) [4]. Cytotoxicity of NE was assessed on HCT-116 colon cancer cells by means of (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) and Lactate Dehydrogenase (LDH) assays following 24h of incubation with different NE concentrations. Finally, fluorescent-labeled NE and CH-NE were administered as physical mixture and as re-hydrated scaffold by oral gavage to healthy mice to evaluate their intestinal residence time.

Results: NE were optimized and loaded with the model drug tacrolimus by an experimental design. NE converted into dried powders using both spray-drying and freeze-drying techniques, recovered their initial properties following reconstitution in water. Structural analysis carried out suggested that in its dry state the NE shell developed into crystalline, while it became amorphous when in suspension [1]. CH scaffolds loaded with NE (CH-NE) were successfully obtained (Figure 2-A) and using microscopy techniques (SEM and optical microscope) we were able to characterize the cell-walls structure of the system. The presence of NE improved the mechanical strength of the scaffold thanks to the interactions occurring between particles and polymer chains. The NE release rate *in vitro* was highly dependent on the scaffold structure and composition: a sustained NE release was achieved inFaSSIF-V2 at high CH scaffold concentrations (28% of NE release in 2h, reaching 50% after 8h and then no further release was observed until 72h). Cytotoxic studies in HCT-116 cell line showed a time- and concentration-dependent inhibition of cell viability upon incubation with blank nanosystems. Following 24h of incubation NE displayed cytotoxicity at the higher tested concentration (156 µg/mL). *In vivo* fluorescent imaging study following oral gavage of NE and CH-NE shown an increased retention in the colonic area of CH-NE (up to 6h) in comparison to the NE which was rapidly cleared from the intestine (Figure 2 B and C).

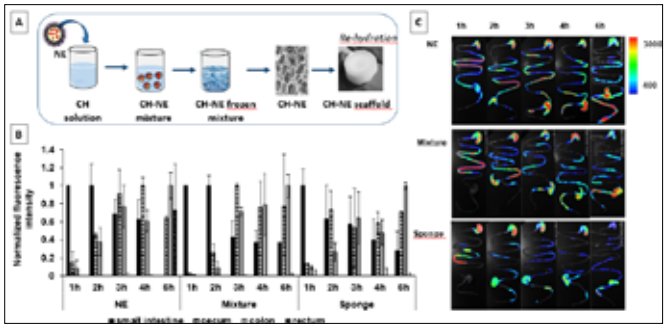

Figure 2: *A) Formulation of nanoemulsion-loaded scaffold. B) Semi-quantitative fluorescence analysis of small intestine, cecum, colon and rectum following oral administration of different systems. C) Ex vivo fluorescent imaging of GI tract of mice following oral gavage of nanoemulsion (NE), NE-loaded chitosan mixture (Mixture), NE-loaded chitosan sponge (Sponge) in at time 1h, 2h, 3h, 4h and 6h.*

Conclusions: The results here presented demonstrated the potential of the developed nanocomposite scaffold as a versatile tool for sustained intestinal drug delivery. We believe that the features set of CH scaffold and NE makes this system uniquely attractive for the development of advanced intestinal dosage form for prolonged intestinal drug delivery.

Acknowledgement: *This research has received funding from ANR, HyDNano project (ANR-18-CE18-0025-01).*

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ROBOTICS IN MAGNETIC DRUG TARGETING

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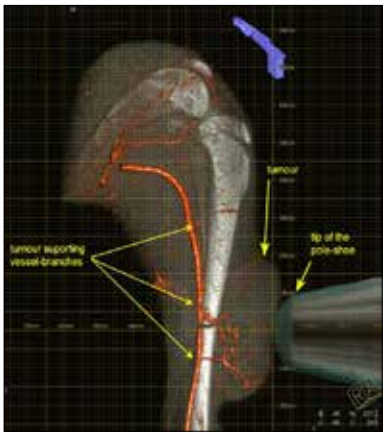

Fig. 1: *Graphical superimposition of an angiography of the left, tumor-bearing hind leg of a rabbit with the tip of the electromagnet during treatment. Result in this case: the tumor receded completely in the lower part, but continued to grow in the upper part.*

Magnetic drug targeting promises the possibility of treating diseases such as cancer and arteriosclerosis more effectively and with fewer side effects [1]. In various studies it has even been shown *in vivo* in comparison to normal systemic drug administration that superparamagnetic iron oxide nanoparticles (SPIONs) can be used to magnetically enrich the nanoparticles, but also the drugs in the diseased areas [2, 3]. However, the ongoing trials have also shown that in individual cases it was not possible to clarify exactly why the accumulation worked very well in one treatment and why it did not work in the other treatment [3]. There is, however, a very high probability that the magnet was not positioned correctly (Fig.1) or that more than one position would have been needed for a proper enrichment. To find this out, standardized models and technical systems are needed to position the magnet reproducibly and exactly in relation to the diseased area. However, in the same way, these technical systems later are needed for the treatment of patients. Therefore, we here propose the use of a high precision robotic system for this very important challenge.

PERSPECTIVE AND PREVIOUS WORK

Robotics intelligent algorithms and artificial intelligence penetrate more and more areas of human life. In medicine, this offers great opportunities to develop completely new therapeutic concepts. In the context of Magnetic Drug Targeting we propose combining different medical devices to act hand in hand to make this new and promising therapeutic approach even more effective and precise in order to accelerate clinical implementation.

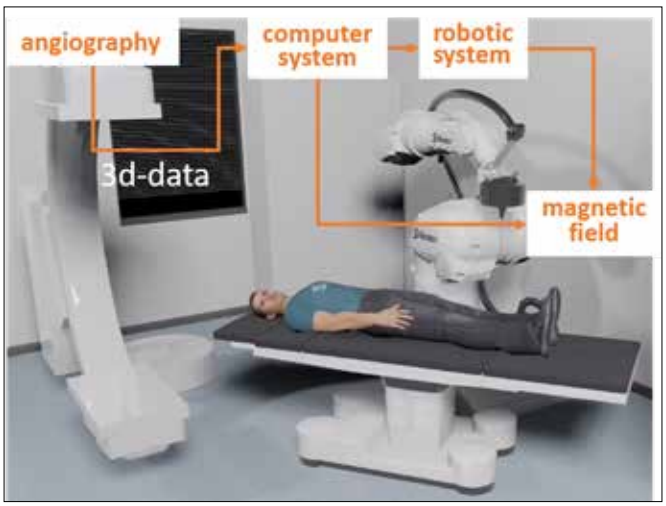

Fig. 2: *Perspecitve of the intended data flow for an optimal positioning of a magnetic field to the diseases tissue of a patient.*

Our vision is a three-step procedure. First, we want to acquire three-dimensional data of the tumor and its supporting vasculature or an arteriosclerotic plaque. In a second step, this data is transferred to a computer, which is trained to recognize the relevant supporting vessels and is able to calculate the position or positions of the magnetic field for an optimal accumulation and distribution of the nanoparticles and the drug in the diseased area. In a third step, the computer systems transfers the relevant positions to a robotic system, which maneuvers the magnet and by that, the magnetic field to these 3d-coordinates (Fig. 2).
To date, we were able to equip an animal operation theatre with an angiographic system (Artis Zee Floor®, Siemens Healtheniers, Forchheim, Germany.), which is currently routinely used for acquiring 3d-data of tumor bearing rabbits or rabbits with arteriosclerotic plaques. Additionally, we were able to install a high precision industrial robot (TX200, Stäubli Bayreuth GmbH, Bayreuth, Germany), which is equipped by the help of Toolcraft GmbH, Georgensgmünd, Germany, with a highly potent electromagnet.
Next steps will be the implementation of a adjustable positioning routine for the robot and the development of corresponding 3D-flow-models for conducting standardized and reproducible accumulation experiments. After that, we are going to begin to implement the 3D-space-data of the models, acquired by the angiographic system into the positioning routine and finally the system is going to be tested and verified in animal trials.

CONCLUSIONS

Magnetic Drug Targeting could be a very effective treatment for several diseases. Nevertheless, up to date a precise and reproducible positioning of the magnetic field is not possible. This proves that one important factor for a successful translation of MDT to the clinics cannot be achieved without a corresponding medical device development. Therefore, we did install an angiographic system capable of acquiring 3d-data of e.g. tumors or arteriosclerotic plaques and a high precision robotic system, which is equipped with a strong electron magnet.
However, before such a system can be used in a clinical setting a multitude of challenges have to be solved. Among these are the development of suitable algorithms for transferring the 3d-data of the diseased tissue to the magnetic field positioning robot. But also safety issues have to be solved, so that it can be insured, that such a potent system does not accidentally injure medical personal or the patient.

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COUPLIN G RATIOMETRIC LUMINESCENCE THERMOMETRY WITH HYPERTHERMIA FOR LOCAL INTRACELLULAR HEATING AS AN ADVANCED CANCER HYPERTHERMIA THERAPY

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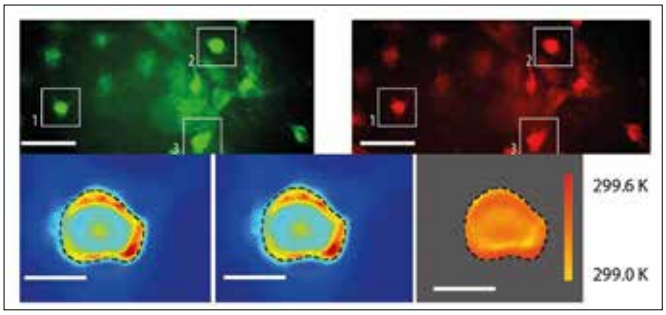
Nowadays, nanotechnology attracts a great deal of attention for a wide range of use as biomedical applications. For this purpose, different nanosystems have been designed for imaging, therapy or for the combination of both, as theragnostic agents. Among all of these, nanoparticles forlocalized thermal heating, involving magnetic, plasmonic or phonon-induced heating, appear as promising and powerful noninvasive techniques for biomedical applications. Such heating systems can be used as stimuli for controlled drug released, remote control of single-cell functions, or hyperthermia therapy of cancer, among others. However, the efficiency of such local heating systems requires to be able to finely measure local temperature at the direct proximity of nanoheaters (NHs). A solution consists to use nanothermometers, i.e thermometers able to sense temperature at such low scale as nanoparticles.Among all of them, luminescent lanthanide-based thermometers their versatility; they present narrow emissions, high quantum yield, and possibility to tune the emission from UV to IR.

In a previous study, we already shown that lanthanide complexes coupled with NHs are able to sense temperature in dead cells the system in the context of the European project Horizon 2020 HYPTEMPCELL. For this, we intent to optimize both the heating ability of the NHs and the emission/stability of the luminescent lanthanide-based nanothermometers used. Exposing the nanosytem to an AC magnetic field, we are able to sense the local heating at the periphery of the NHs, with a faster response in comparison with the temperature given by the thermocouple inserted in the medium. The next step will be to realize the same inside living cells, and to determine if local intracellular heating is able to provoke cells' apoptosis.



Up: Imaging of Tb3+ (left, in green) and Eu3+ (right, in red) emissions from cell-internalized multicore beads. Down: images of OK cells treated with the nanoparticles.

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SOMETIMES LESS IS MORE: ANTIBODY AMOUNT DETERMINES THE BIODISTRIBUTION AND DENDRITIC CELL UPTAKE OF PEPTOBRUSH NANOPARTICLES IN VIVO

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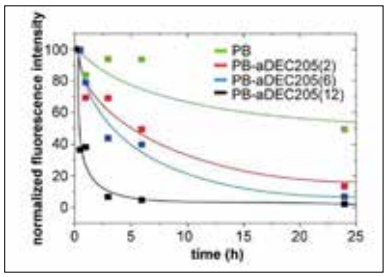

Figure 1. *The extent of liver accumulation of systemically applied PB-antibody conjugates correlates with their antibody density: CW800-labeled PB formulations were systemically injected into mice. Blood was retrieved at the time points indicated, and contents of PB formulations were quantified by measurement of fluorescence intensities.*

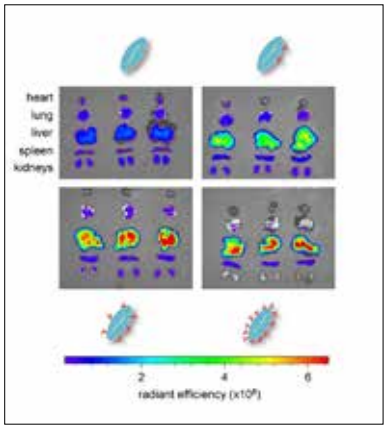

Figure 2. *The extent of liver accumulation of systemically applied PB-antibody conjugates correlates with their antibody density: fluorescence imaging of organs retrieved from mice treated with the various PB formulations 24 hpi.*

Despite considerable progress in the design of multi-functionalized nanoparticles (NP) to deliver drugs in a cell type-focused manner, their systemic application still often results in unwanted liver accumulation. The exact mechanisms contributing to this general observation have not been fully elucidated yet. Here we asked for the role of cell targeting antibody density per NP as a determinant of NP liver accumulation. We used sarcosine-based peptobrushes (PB) [1] which in an unconjugated form remain in the circulation for a long time (>24h) due to low unspecific cell binding. PB were labeled with a near infrared dye, and were conjugated with average numbers (2, 6 and 12) of antibodies specific for the dendritic cell (DC) selective surface receptor DEC205 [2]. We assessed the time-dependent biodistribution of PB-antibody conjugates by *in vivo* imaging and flow cytometry (Fig.1). We observed that PB-antibody conjugates were trapped in the liver [3]. The extent of liver accumulation of PB-antibody conjugates correlated with the number of attached antibodies (Fig. 2). In addition, liver endothelial cells are mostly responsible for retaining aDEC205 coated PB, via their Fc receptors [4] . Accordingly, PB-antibody conjugates with an average of only two antibodies per NP showed lowest liver entrapment, and engaged DC in spleen and lymph nodes at highest

extent. Altogether, our study underlines that liver endothelial cells play a yet scarcely acknowledged role in liver entrapment of antibody-coated NP, and that low antibody numbers on synthetic NP are both necessary to minimize liver accumulation and sufficient for specific cell targeting in other organs *in vivo*.

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MULTIDETECTOR FIELD-FLOW FRACTIONATION TECHNIQUES FOR THE CHARACTERIZATION OF LIPOSOMAL DRUG FORMULATIONS

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Field-Flow Fractionation (FFF) belongs to the flow-based separation techniques, where separation of dissolved, suspended and dispersed sample constituents in the size range of approx. 1 nm to 20 µm is achieved within a thin, ribbon-like channel without stationary phase [1].

In FFF, separation of different sample constituents is induced by a force field that is applied perpendicular to the channel flow, which transports the sample toward the channel outlet and further to the respective detectors. Depending on the applied force field, FFF can be divided into different subtechniques. In Asymmetrical Flow FFF (AF4), a second flow, called cross flow, enables separation according to hydrodynamic size. By superimposing the cross flow field with an electrical field, Electrical Asymmetrical Flow FFF (EAF4) additionally enables the separation according to charge thus gaining access to electrophoretic mobility and Zeta potential of the sample. In Centrifugal FFF (CF3), a centrifugal force field induces separation according to mass respectively density of the sample. Due to its wide applicability and gentle separation conditions, FFF and its subtechniques have become a powerful characterization tool for nano-sized samples particularly in the field of bio- and nanomedicine [2,3] including the characterization of liposomes [4,5].

Two FFF application examples for the characterization of liposomes are presented here. In the first study, CF3 coupled with UV-detection and offline microscopy was used to quantify the amount of non-encapsulated drug in a liposomal drug formulation by separating the free drug from the filled liposomes (Figure 1).

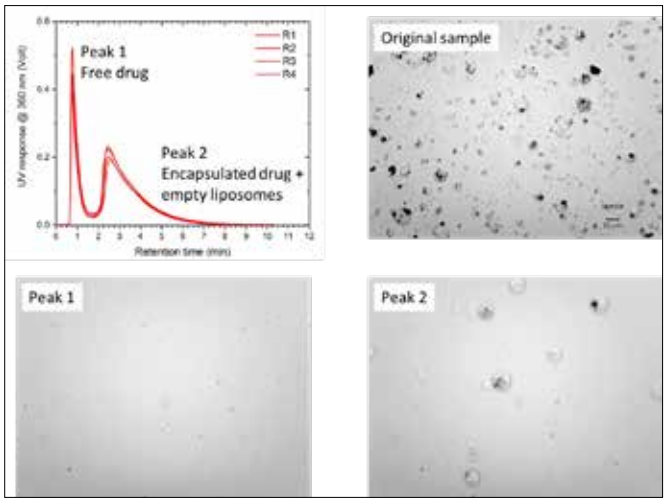

Figure 1: *Top left: CF3-UV fractogram of the liposomal drug product indicating separation of free and encapsulated drug; top right: optical micrograph of the original sample; bottom left: optical micrograph of a CF3 fraction collected from peak 1; bottom right: optical micrograph of a CF3 fraction collected from peak 2.*

In the second study, EAF4 coupled with UV-, Multi Angle Light Scattering (MALS) and Dynamic Light Scattering (DLS) detection was used to characterize a commercial liposomal drug formulation (Liposomal Doxorubicin HCl) in 10 mM phosphate buffer, pH 6.9. This approach enabled access to the size distribution both from MALS (Radius of gyration Rg = 15-43 nm) and DLS (Radius of hydration Rh = 28-33 nm) as well as the zeta potential (-24 mV) of the sample under physiological conditions (Figure 2).

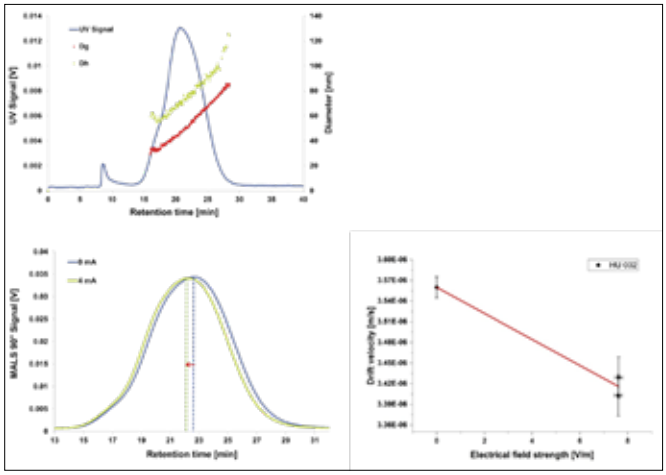

Figure 2: *Top: EAF4-UV-MALS-DLS fractogram of the Liposomal Doxorubicin HCl sample (Rg: red trace, Rh: green trace; bottom left: Observed retention time shifts in presence (green) and absence (blue) of an electrical field; bottom right: Diagram of total drift velocity vs. applied electrical field in order to determine electrophoretic mobility and Zeta potential (Smoluchowski approximation) of t he sample.*

Both studies clearly highlight the capabilities of field-flow fractionation techniques for the comprehensive physico-chemical characterization of liposomes as they give valuable insights into crucial properties such as drug loading efficiency, size distribution and surface zeta potential. This not only facilitates a better understanding of the behavior of such promising drug delivery systems especially under physiological conditions, but furthermore allows a more rational synthesis approach thus enabling tailor-made liposomal formulations for particular bio- and nanomedical applications.

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Prof. Y. Barenholz, Head of Membrane and Liposome Research Lab at Hebrew University Hadassah Medical School in Israel, is gratefully acknowledged for providing the Liposomal Doxorubicin HCl sample.

CONTACTPOINTNANO.CH: A STRATEGIC INITIATIVE TO TRANSFER THE NANOSAFETY KNOWLEDGE TO SWISS SMES

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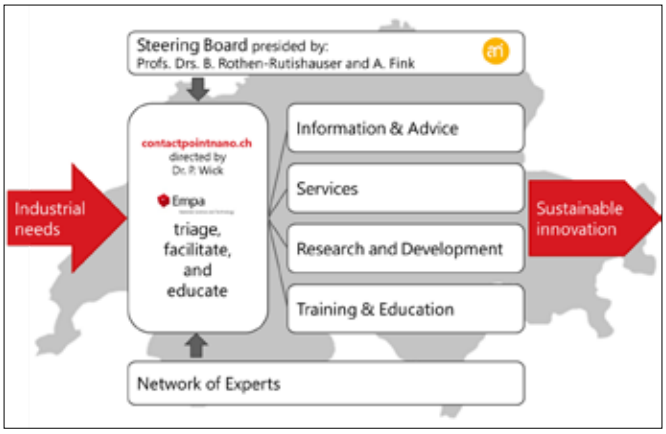

Figure 1. *Organization of the contactpointnano.ch*

As an independent national contact point, contactpointnano.ch pools and structures the scientific and regulatory expertise available in Switzerland on the safe handling of synthetic nanomaterials – from production and application to disposal. Its objective is to efficiently convey tailored high-quality information to companies (established companies, SMEs and start-ups), thus accelerating the transfer from invention to innovation and allowing Swiss companies to remain competitive in an international environment. contactpointnano.ch is based on a broad network of proven experts and ensures a qualified and independent transfer of knowledge. It anticipates topics for regular workshops on current matters, organizes information events and facilitates the exchange of experience and know-how in the area of nano-innovation, nano-safety, and the evolution of Swiss and international regulatory framework.
conatctpointnano.ch provides tailored contacts to experts who: i) can convey information on nanomaterials, their safe handling as well as relevant regulatory provisions, ii) offer expert opinions and procure access to services, testing and analyses, iii) are active in the field of research and development. At contactpointnano.ch companies will find competent partners who will assist them directly or point them to available knowledge resources and agencies.
Further details can be found on: www.contactpointnano.ch.
You can also direct your query to: contactpointnano@empa.ch.

CORRELATION OF INFUSION SPEED AND OCCURRENCE OF COMPLEMENT ACTIVATION RELATED HYPERSENSITIVITY REACTIONS DURING ABELCET INFUSION IN RATS

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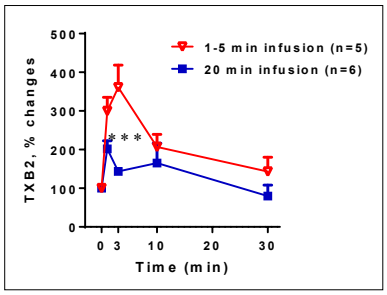
Introduction
Nanomedicine-induced hypersensitivity reactions (HSRs) can manifest as serious adverse events during clinical use, limiting widespread application of the drug. A common, empirical approach to reduce the phenomenon is to slow down the infusion speed, and, hence, extend the duration of drug administration [(3)]. However, it is not known how the reduction of drug exposure to blood influences the complement (C) activation-related and C-independent pathways of pseudoallergy (CARPA and CIPA). The goal of the current study was to assess the relative impacts of infusion speed reductions on parameters of CARPA and CIPA in rats, using Abelcet, a commercially available amphotericin B-containing lipid complex, as a model of reactogenic lipid based nanomedicines.

Materials and methods
Male Wistar rats weighing 380-450 g were anesthetized with pentobarbital (60 mg/kg i.p.). The right carotid artery and the left jugular vein were cannulated to measure the mean arterial blood pressure (MABP) and for drug administration. MABP and heart rate (HR) were continuously registered (PowerLab and LabChart, ADInstruments, Budapest, Hungary). All rats were treated with Abelcet at 10 mg/kg i.v. infused over either 1 or 5 min, or over 20, referred to as rapid and slow infusion rates, respectively. The pooling of 1 and 5 min values under the "rapid infusion" group was ethically justified on the basis of lack of significant differences between the corresponding 1 and 5 min values of all measured endpoints. Blood samples were drawn before and at 1, 3 10 and 30 mind after the end of infusions.

Results
Rapid or slow infusions of Abelcet led to sudden rises of plasma TXB2 concentrations already at 1 min post-injection and reached peak at 3 min, which peak was significantly higher in the rapid infusion group than in that in the slow infusion group (**Fig. 1A**). The Abelcet-induced thrombocytopenia was also significantly more expressed after rapid infusion compared to that in the slow infusion group at 1 and 3 min (**Fig. 1B**). On the other hand, white blood cell count was similarly decreased in both groups at 1 and 3 min, but compensatory leukocytosis at 30 min developed only in the rapid infusion group, not after slow infusion (**Fig. 1C**).



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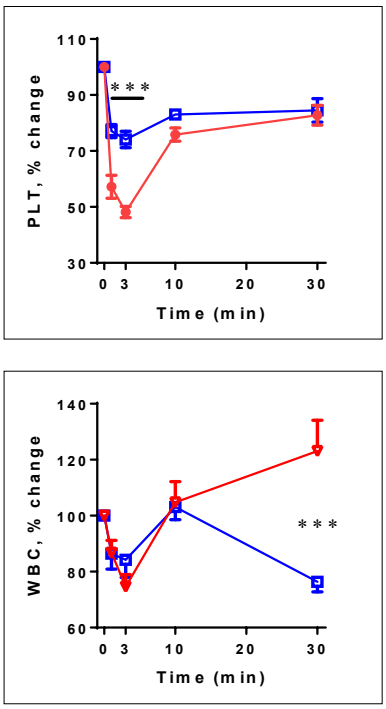

Figure 1*: Effects of Abelcet (10 mg/kg i.v.) on* **A)** *plasma thromboxane B_2 (***TXB2***) concentration* **B)** *blood platelet (***PLT***) and* **C)** *white blood cell (***WBC***) counts in rats after 1 or 5 min infusions and after 20 min infusion. The line in the figure B shows the duration, when PLT count in the two groups are statistically significantly different. Two-way ANOVA and Sidac post hoc test. *** = p<0.001.*

Abelcet (10 mg/kg, i.v.) caused a large increase in plasma C3a concentration in both groups. However, while peak plasma C3a concentration was elevated 5-fold in the 1-5-min infusion group it changed only 2.3-fold in the 20-min infusion group (**Fig. 2A**). Abelcet caused a transient hypotension at both infusion rates. However, the peak effect was larger and the duration of hypotension was longer in the 1-5-min infusion than in the 20-min infusion group (**Fig. 2B**). As a result, MABP was significantly different in the two groups from 16 to 24 min. Abelcet also caused a small tachycardia but is was similar in the two groups (not shown).

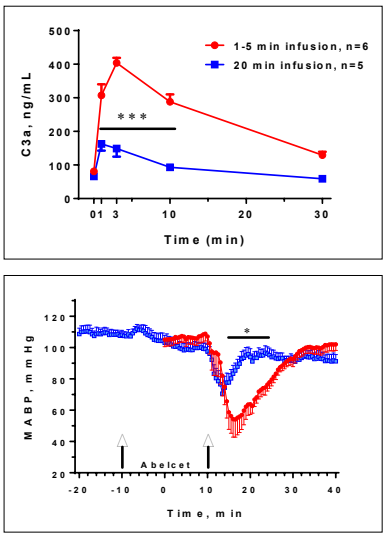

Figure 2. *Effects of Abelcet (10 mg/kg i.v.) on* **A)** *on the plasma C3a concentration and* **B)** *the mean arterial blood pressure in rats (n=5-6). Abelcet was administered 1 or 5 min prior to the second arrow (1 or 5 min infusion), or between the two arrows (20 min infusion). The line in the figures show the duration, when the parameters in the two groups are statistically significantly different. Two-way ANOVA and Sidac post hoc test. *, *** = p<0.05, 0.001.*

Discussion and conclusions

We have shown previously that the amphotericin B-containing liposomal drug, AmBisome, caused CARPA in rats, characterized by hypotension, a rise of plasma thromboxane B2 (TXB2) concentration, a stable metabolite of TXA2, C activation and blood count changes [(1)]. The current study confirmed and extended the previous findings as Abelcet, another amphotericin B-containing lipid based nanomedicine caused similar effects in anesthetized rats. In keeping with the low sensitivity of rats to nanoparticle-induced hemodynamic changes, the reactogenic dose of Abelcet was about 10-fold higher than the reactogenic dose of similar nanomedicine in pigs, which mimics the sensitivity of hypersensitive humans to these drugs [(1)].

The current results clearly show that the rate of Abelcet infusion has a significant impact on the symptoms of HSRs. These observations have a clear translational relevance, since they suggest that it is possible to use the rat model to establish whether decreasing the infusion rate of a test drug or agent would lower the risk of HSRs, and if yes, it enables to develop safe infusion protocols just as it has been done in pigs [(2)].

The current findings on the rise of C3a in parallel with all other physiological changes provide evidence for the involvement of complement activation in the HSR observed. Furthermore, the finding that C3a production was infusion-speed dependent just as the other measured endpoints suggests that that the primary mechanism of HSR was CARPA, rather than CIPA. Nevertheless, the different pattern of WBC changes following rapid and slow infusion cannot be explained with C3a production and points to a more complex mechanism that involves C-independent mechanism, i.e. CIPA.

In conclusion, large bolus doses of Abelcet, an amphotericin B-containing liposome, caused typical symptoms of HSRs in rats, whose mechanism is consistent with the "double hit" explanation of these reactions, one hit being C-mediated and the other independent of C activation. The model can serve to explore the effects of infusion speed on these reactions.

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IN VIVO ASSESSMENT OF THE METAL-ORGANIC FRAMEWORK FORMULATION SIL@NANOMIL-89 IN A MONOCROTALINE MODEL OF PULMONARY ARTERIAL HYPERTENSION.

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Introduction: Nanomedicine is an attractive, promising and fast growing field that uses nanoparticles to serve as drug carriers for targeted drug delivery. We are working on the idea that targeted drug delivery could be therapeutically useful in the treatment of pulmonary arterial hypertension (PAH) and have focused on the highly porous, imageable iron-based metal-organic framework (MOF); nanoMIL-89 as a platform. PAH is an aggressive and incurable disease with high morbidity/mortality rates. Currently available PAH drugs work by either promoting vasodilation or inhibiting vasoconstriction however, they are limited by the systemic side-effects[1]. We have prepared nanoMIL-89 and found it to be relatively non-toxic when tested in cells *in vitro* and in control rats *in vivo*[2]. We have gone on to charge nanoMIL-89 with the PAH drug sildenafil to produce sil@nanoMIL-89. We found sil@nanoMIL-89 to be relatively non-toxic to cells, to release sildenafil in aqueous buffer and to mimic the vasodilator effects of sildenafil on vessels *in vitro*[3]. Here we report a pilot study where we have assessed the effects of nanoMIL-89 and sil@nanoMIL-89 in a rat monocrotaline model of PAH *in vivo*.

Methods: Rats were treated with monocrotaline for one week. At Week 2 rats were given either nanoMIL-89, which served as a vehicle, or sil@nanoMIL-89 (50mg/kg) intravenously twice a week for two weeks after which plasma was collected and organs harvested. Levels of nanoMIL-89 and sil@nanoMIL-89 were estimated by iron concentration in organ homogenates, right heart hypertrophy (an indirect measure of PAH) was calculated using the '*right ventricle/ left ventricle +septum ratio*' and endothelin-1 (a marker and mediator of PAH) and sildenafil levels were measured using ELISA.

Results: Plasma levels of sildenafil were detectable up to 4 days after injection of sil@nanoMIL-89. In MOF treated rats iron levels were highest in spleen, liver and lung, with stronger effects seen with sil@nanoMIL-89 than nanoMIL-89; which could be due to enhanced uptake due to the vasodilation. Endothelin-1 was reduced in animals treated with sil@nanoMIL-89 although right heart hypertrophy was not different between the groups (Figure 1).

Conclusion: Sil@nanoMIL-89, the first imageable metal-PAH nanodrug passively accumulates in the lung, releases sildenafil and reduces endothelin-1 levels *in vivo*. Whilst these results are promising, they are at the pilot stage and higher powered studies are required to assess the efficacy of Sil@nanoMIL-89 in this and other models of PAH.

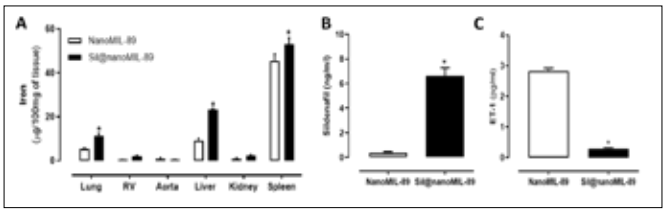

*Figure 1: Measurement of iron (i.e. nanoMIL-89 or sil@nanoMIL-89) deposition and accumulation in tissues and organs (A), Sildenafil levels from the plasma (B) and plasma levels of endothelin-1 (C) of the two groups after two weeks of treatment with either nanoMIL-89 or sil@nanoMIL-89. Statistical significance for n=3 animals from one experiment was determined by (A) two-way ANOVA followed by Bonferroni post-test and (B and C) t test followed by Mann-Whitney test. Statistical significance was assumed where *p<0.05.*

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COMPOSITE SCAFFOLD MADE OF HYDROXYAPATITE COATED PLA NANOFIBERS CONTAINING BMP-2 LOADED LIPOSOMES TO IMPROVE BONE REGENERATION

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Introduction

Current clinical treatment of critical bone defects is associated with considerable limitations and complications. The goal of the present study is to develop a simple biomimetic scaffold platform to provide a 3D microenvironment with controlled release property of an osteogenic peptide to enhance osteogenesis of mesenchymal stem cells (MSCs) and improve the healing process of the defected tissue. To this aim, we fabricated electrospun scaffolds conjugated to nanoliposomes encapsulating BMP-2 peptide. After physicochemical characterization of the designed nanoliposomal formulation and the scaffold, we evaluated osteogenic differentiation of the MSCs *in vitro* and *in vivo*.

Methods

Various nanoliposomal formulations were prepared by film rehydration and membrane extrusion method. Surface charge and zeta potential of the nanoparticles were measured using dynamic light scattering. Then, release pattern of each formulation was evaluated and liposomes were covalently conjugated to the scaffold. Afterwards, surface morphology, water contact angle and mechanical properties of the designed platform was analyzed. Finally, adipose-derived MSCs were seeded onto the scaffold and cytocompatibility and differentiation properties of the platform was evaluated measuring the DNA content, gene expression, alkaline phasphatase activity and calcium content. Thereafter, the performance of the designed scaffold platform was investigated in subcutaneous rat model.

Results

The selected nanoliposomal formulation was negatively charged and 129 nm in size. Its encapsulation efficiency was 30%. Besides, the selected formulation could release up to 80% of the encapsulated cargo in a sustained manner for 18 days (figure 1).

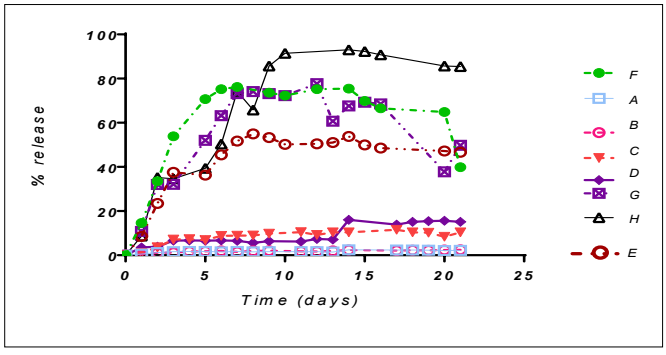

Figure 1. *Release profiles of different BMP-2 encapsulated liposomal formulations*

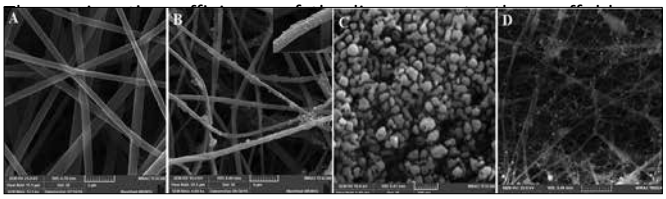

Figure 2. *Fig1. SEM image of (A) electrospun nanofibers, (B) nano-HA-coated nanofibers, (C) HA nanoparticles and (D) SEM image of liposome-loaded scaffolds (liposcaffolds).*

The *in vitro* results demonstrated that the designed platform was cytocompatible and significantly could enhance differentiation of MSCs to osteoprogenitor cells. The ectopic bone formation model also showed that the primary ossification centers were increased significantly compared to the control (Figure 3).

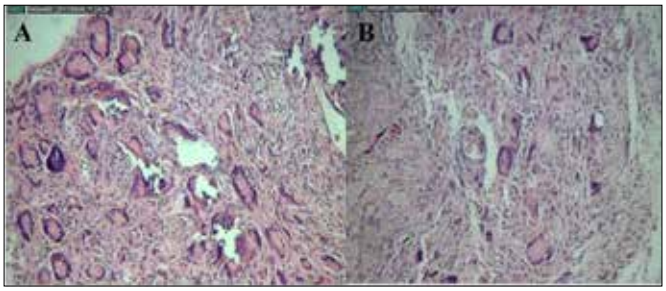

Figure 3. *H&E staining of subcutaneously implanted scaffolds. (A) Liposcaffolds-HA (B) scaffold-HA-peptide.*

Conclusion

The sustained release pattern of the peptide from the scaffold and the appropriate physicochemical properties of the platform led to significant induction of the osteogenesis demonstrating the potential application of our fabricated scaffold in promoting bone regeneration.

Keywords: 'Nanoliposome', 'Bone tissue engineering', 'MSC', 'BMP-2 peptide'

NORMALIZATION OF TUMOR MICRO ENVIRONMENT WITH DEXAMETHASONE INCREASES CISPLATIN-LOADED NANO-CARRIER DELIVERY AND EFFICACY IN METASTATIC BREAST CANCER

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ABSTRACT

Nanocarriers (NCs) accumulate in tumors through the enhanced permeability and retention (EPR) effect [1]. As NCs and other nano-sized therapies are in clinical practice, translatable strategies that increase the magnitude of their accumulation and penetration while reducing heterogeneity of microdistribution may improve treatment outcomes. One such strategy to increase and conform NC penetration and accumulation involves normalizing the non-cancerous components of the tumor, which collectively are known as the tumor microenvironment (TME) [2]. Without normalization, tumor vessel function is compromised because the TME promotes vessel leakiness and compression [2].

Dexamethasone is a glucocorticoid steroid with anti-inflammatory properties used to treat many diseases, including cancer, in which it helps manage various side effects of chemo-, radio-, and immunotherapies. Here, we investigated the tumor microenvironment -normalizing effects of dexamethasone in metastatic murine breast cancer (BC). We found that low dose dexamethasone normalizes vessels and the extracellular matrix, thereby reducing interstitial fluid pressure, tissue stiffness, and solid stress. Also, dexamethasone increases the tumor accumulation and efficacy of ~30 nm polymeric micelles containing cisplatin (CDDP/m) against murine models of primary BC and spontaneous BC lung metastasis, which also feature a TME with abnormal mechanical properties. These results suggest that pretreatment with dexamethasone before NC administration could increase efficacy against primary tumors and metastases.

METHODS

Orthotopic models for murine mammary tumors were generated by implantation of $5x10^4$ 4T1 or 4T1-luc and $1x10^7$ MDA-MB-231 mouse mammary cancer cells in 40 µl of serum-free medium into the third mammary fat pad of 6-week old BALB/c or BALB/c nu/nu female mice, respectively. Treatment was initiated when tumors reached ~90mm^3. Dexamethasone at 3mg/kg was administered daily from days 0 through 8. CDDP/m at 1mg/kg was administered by retro-orbital injection during sedation with isoflurane on days 2, 5, and 8. The tumors were measured every 1-2 days using calipers by an investigator blind of the treatment groups.

RESULTS

Dexamethasone normalizes tumor vessels dose-dependently

Vascular endothelial growth factor (VEGF) is the major driver of the pathophysiology of tumor vessels and blocking it promotes vascular normalization [3]. Dexamethasone reduces VEGF expression in murine models of brain cancer [4], so we hypothesized it would do so in BC. We treated immunocompetent BALB/c mice bearing orthotopic 4T1 BC daily for 4 days with 3 or 30 mg/kg dexamethasone. Dexamethasone reduced the levels of VEGF as assessed by immunofluorescence staining of tumor sections (Figure 1A,B). We next tested the effect of dexamethasone on vessel structure by using histology. Dexamethasone at 30 mg/kg reduced the microvessel density (Figure 1C,D), whereas dexamethasone at 3 mg/kg avoided pruning yet increased vessel maturity, as indicated by the association of NG2+ pericytes with CD31+ endothelial cells (Figure 1E,F).

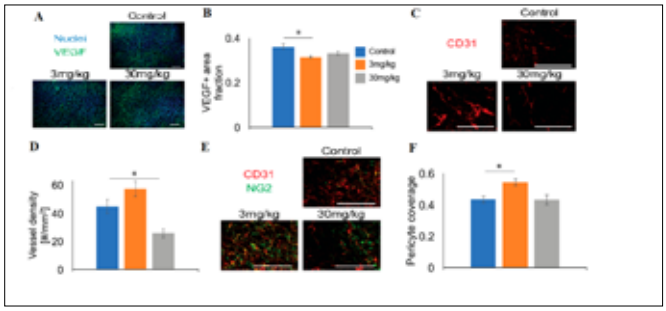

Figure 1. *Dexamethasone induces vascular normalization. (A) Representative images of VEGF (green) with nuclear counterstain (blue) immunofluorescence. (B) Quantification of area fraction positive for VEGF immunofluorescence staining. (C) Representative images of CD31 (red) immunofluorescence marking tumor vessels. (D) Immunohistological quantification of the tumor vessel density, as assessed by the number of vessels normalized to the image area. (E) Representative images of CD31 (red) and NG2 (green) immunofluorescence marking endothelial cells and pericytes, respectively. Yellow areas indicate colocalization of both cell types. (F) Quantification of the pericyte coverage of microvessels, as assessed by the fraction of CD31-positive staining area that is also positive for NG2.*

DEXAMETHASONE NORMALIZES THE ECM AND MECHANICAL TME.

We then tested whether dexamethasone normalizes ECM. We focused on collagen I and hyaluronan because they have been identified as matrix components that contribute to solid stress and vessel compression. With histological staining we found that dexamethasone reduced levels of hyaluronan (Figure 2A,B) and not collagen I (Figure 2A,C) in the tumors. Based on these results, we tested whether the mechanical TME was normalized. We found that 30

mg/kg dexamethasone reduced tissue stiffness, as measured by the elastic modulus, whereas 3 mg/kg only produced a trend (Figure 2D). On the other hand, by using the tumor-opening assay, which indicates the amount of residual solid stress held within the tumor tissue by the amount it opens after cutting, we confirmed that both doses reduced solid stress (Figure 2E). A larger opening is associated with more solid stress. Furthermore, we found that dexamethasone reduces interstitial fluid pressure in 4T1 tumors (Figure 2F). Thus, the results from the histology, and mechanical experiments suggest that dexamethasone normalizes the ECM in tumors by reducing hyaluronan levels.

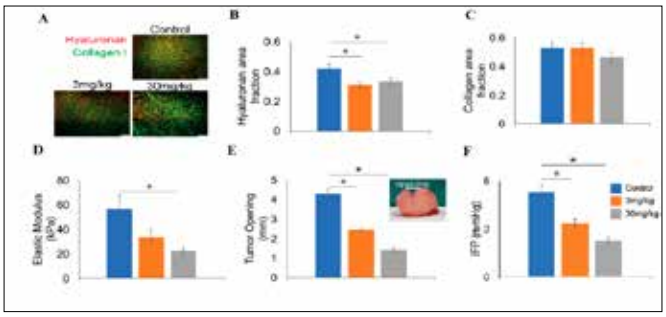

Figure 2. *Dexamethasone induces ECM normalization. (A) Representative images of hyaluronan (red) and collagen I (green) immunofluorescence. Yellow areas indicate colocalization of both ECM components. (B) Quantification of area fraction positive for hyaluronan immunofluorescence. (C) Quantification of area fraction positive for collagen I immunofluorescence. (D) Tumor tissue elastic modulus, which is a measure of stiffness. (E) Solid stress levels, which was assessed by length of the tumor opening after cutting the tissue. (F) Interstitial Fluid Pressure levels.*

DEXAMETHASONE INCREASES EFFICACY OF NCS IN METASTATIC BC

After confirming that dexamethasone normalizes vascular structure, ECM, and vessel function, we next hypothesized that dexamethasone would affect the rate NCs transport across tumor vessels and penetrate toward cancer cells. Thus, to test our hypothesis, we used CDDP/m, which are ~30 nm. Firstly, we tested the therapies in a primary tumor growth delay study against orthotopic 4T1-luc tumors, with an end point of days until 1000 mm^3 tumor volume. We found that CDDP/m (1 mg/kg) monotherapy increased the number of days for the tumors to reach 1000 mm^3 compared to control (Figure 3A). Combining dexamethasone (3mg/kg) with CDDP/m (1 mg/kg) significantly increased this time period compared to that with CDDP/m monotherapy (Figure 3A). Thus, dexamethasone enhances the efficacy of CDDP/m against 4T1 primary tumors.

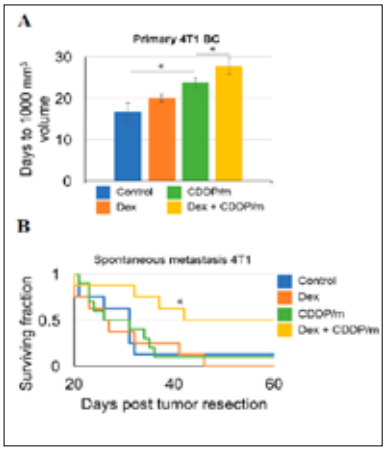

Figure 3. *Dexamethasone increases the efficacy of 30 nm CDDP/m in 4T1 tumors. (A) Quantification of tumor growth rate. Graph of the number of days between treatment initiation and 1000 mm3 tumor volume. When tumors reached ~90 mm3, they were size- and time-matched into control, dexamethasone monotherapy, CDDP/m monotherapy, and dexamethasone and CDDP/m combination. (B) Animal survival in mice with spontaneous metastases, which developed after surgical primary tumor resection at 300 mm3. Mice were size- and time-matched into control, dexamethasone monotherapy CDDP/m monotherapy, and dexamethasone and CDDP/m combination.*

DEXAMETHASONE INCREASES EFFICACY OF NCS IN PULMONARY BC METASTASES

Next, we investigated the effects of the combination of dexamethasone and CDDP/m on lung metastases. To mimic the clinical treatment protocol of metastatic disease and produce spontaneous metastases, we surgically removed 4T1 primary BC tumors when they reached ~300 mm3. Then, after waiting 2 days post-surgery for the mice to rest and metastases to develop further, we administered two cycles of CDDP/m with daily dexamethasone. In these mice, we found that only the combination of dexamethasone (3mg/kg) and CDDP/m (1mg/kg) provided a survival advantage (Figure 3B). Thus, dexamethasone increases the efficacy of CDDP/m against BC pulmonary metastasis.

DISCUSSION

Our results demonstrate the value of TME normalization in improving the efficacy of NCs. By identifying dexamethasone as a therapy that normalizes both vessels and the ECM, we confirm that the combination of normalization strategies is effective in potentiating the efficacy of cytotoxic NCs in models of metastatic BC.

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PLATELET DEPLETION OF HUMAN WHOLE BLOOD REVEALS THAT PLATELETS POTENTIATE THE RELEASE OF IL-8 IN A THROMBIN-DEPENDENT MANNER

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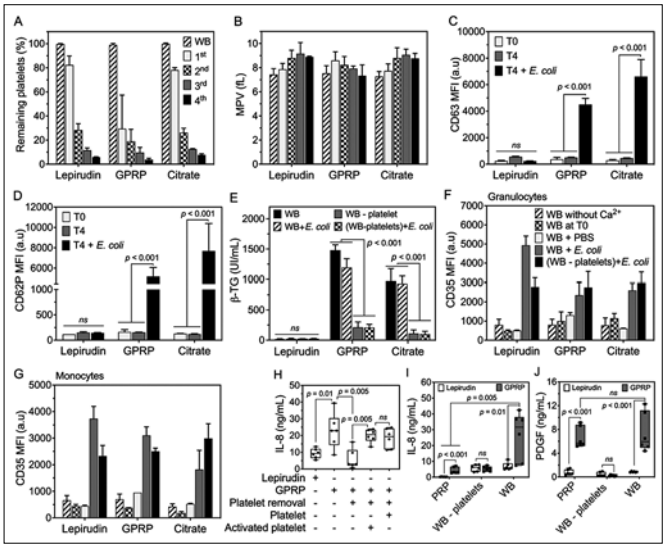
Background. The release of inflammatory cytokines including the chemokine IL-8 is a cornerstone of the cellular inflammatory host response to various structures, including nanoparticles used for drug delivery. Recently, we found that thrombin modulated the plasma-release of inflammatory cytokines in response to bacterial incubation in whole blood anticoagulated with the fibrin polymerization inhibiting peptide GPRP. Whether thrombin directly activated leukocytes or mediated activation via thrombin-dependent activation of platelets remains unresolved. The specific thrombin inhibitor lepirudin enabled us to study the effect of thrombin as compared with the GPRP in this complex human whole blood model.

Objectives. We aimed to investigate the contribution by platelets in the release of IL-8 in a human whole blood environment under optimal anticoagulation approaches for this particular purpose.
Methods. Human whole blood was anticoagulated by either the fibrin-inhibitory peptide, GPRP, or the thrombin-inhibitor, lepirudin, in combination with 0.7% (w/v) citrate. A third anticoagulant used was full strength 3.2% citrate as comparison. Platelets were separated by repeated centrifugations from the whole blood into platelet-rich plasma, which simultaneously depleted the whole blood fraction from platelets and plasma. The activation of platelets and

leukocytes were characterized during the platelet-depletion process. Cellular activation and cytokine response were compared between whole blood, platelet-depleted blood, and platelet-rich plasma upon bacterial incubation with E. coli for 15 minutes for CD activation markers (through flow cytometry) and ELISA measures of b-thromboglobulin or four hours at 37 °C for cytokine response (through multiplex).

Results. Platelets were not activated during the separation process from whole blood but responded to E. coli-incubation after re-calcification with 6.25 mM Ca2+. Similarly, granulocytes and monocytes remained functionally intact during the separation process. Plasma levels of a number of cytokines, including IP-10, IL-1b, IL-6, IL-1Ra, MIP-1a, MIP-1b, and IL-8 were significantly reduced in platelet-depleted blood as compared to whole blood, but recovered after the reconstitution of platelets or when incubated with the supernatant of activated platelets. The leukocyte fraction and platelets were found to each separately contribute to IL-8-elevation at around 5 ng/mL; however, if combined, the release of IL-8 increased to 30 ng/mL. This process was dependent on thrombin since the levels of IL-8 remained at 5 ng/mL in whole blood if thrombin was blocked.

Conclusions. We conclude that: (i) platelets can be depleted from whole blood by combining the GPRP-anticoagulant with citrate, and (ii) the selective release of IL-8 into plasma was mediated via thrombin-dependent activation of platelets which stimulates the synthesis and release of IL-8 from the leukocyte-fraction.



Overview of the study. (A) Platelet counts and (B) mean of platelet volumes (MPV) during the platelet depleting process. Abbreviation in A and B: whole blood (WB), first (1st), second (2nd), third (3rd), and forth (4th) centrifugation. Platelet activation was measured by the expression levels of CD63 (C), CD62P (D) on platelet surface using flow cytometry, and by (E) soluble platelet activation marker, beta-thromboglobulin (b-TG), using ELISA. Abbreviation in C and D: the expression level of the markers immediately after collection (T0), after four hours (T4), and after four hours, then incubated with E. coli (T4 + E. coli) after recalcification. Abbreviation in E: whole blood (WB), whole blood incubated with E. coli (WB + E. coli), platelet-depleted blood (WB – platelet), and platelet-depleted blood incubated with E. coli (WB-platelets + E. coli). Activation of granulocytes (F) and monocytes (G) was evaluated by the expression level of surface marker, CD35, during the platelet-depleted process using flow cytometry. Abbreviation in F and G: whole blood after collection without adding Ca2+ (WB without Ca2+), whole blood when Ca2+ added (WB T0), whole blood with Ca2+ added and incubated with PBS (WB + PBS), whole blood with Ca2+ added and incubated with E. coli (WB + E. coli), platelet-depleted blood with Ca2+ added and incubated with E. coli (WB – platelets + E. coli). In figures A-G, bars are separated by anticoagulant lepirudin and GPRP combined with 0.7% citrate and citrate alone (3.2%) and are without calcium unless indicated that Ca2+ has been resupplied. Plasma source of (H-I) IL-8 and (J) PDGF release upon E. coli-incubation. Abbreviations: platelet-rich plasma (PRP), whole blood (WB), platelet-depleted blood (WB – platelets).

NANOPARTICLE-EVALUATION IN HUMAN WHOLE BLOOD MODELS EX VIVO: THE OVER-LOOKED EFFECTS OF ANTICOAGULANTS

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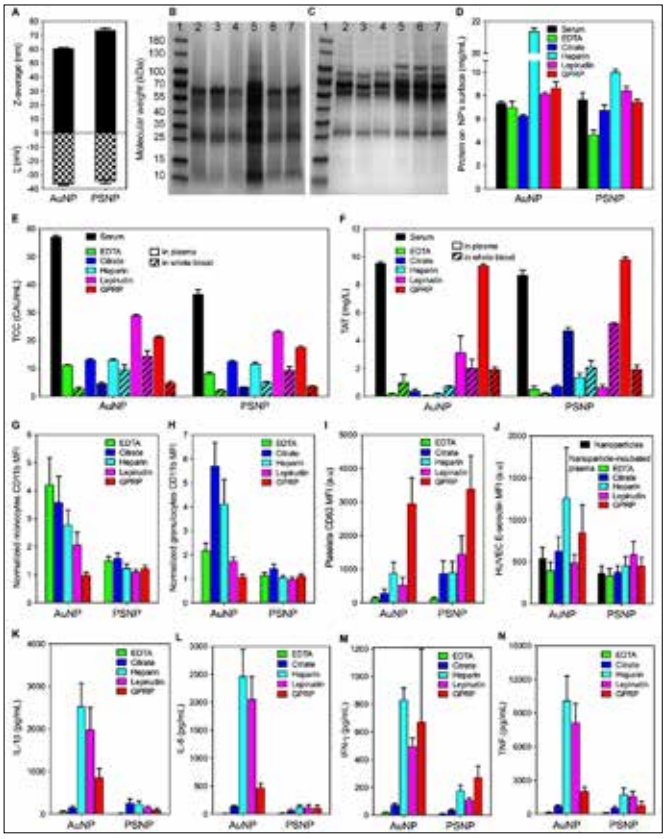
Background: In contact with protein-rich media, nanoparticles acquire a double layer of proteins known as the protein corona, which defines their biological identity rather than its designed synthetic identity. It is well-known that the composition of the protein corona is influenced by physicochemical properties of nanoparticles. While nanoparticle properties have been extensively researched, the property of the protein corona is poorly examined.
Objectives: Herein, we aimed to investigate how various anticoagulants influences the composition of the protein corona, which may affect the subsequent inflammatory and thrombotic response. The anticoagulants investigated included the calcium chelators EDTA and citrate, the multifunctional coagulation inhibitor heparin, the highly specific thrombin inhibitor lepirudin, and the fibrin polymerization blocking peptide GPRP. The hypothesis was that differences in anticoagulant mechanism may lead to the difference in protein composition of the corona.
Methods: Plasma was prepared from human whole blood anticoagulated with either: EDTA (10 mM), citrate (3.2%), heparin (10 units/mL), lepirudin (50 µg/mL), or GPRP (8 mg/mL). Gold nanoparticles (AuNP) and polystyrene nanoparticles (PSNP) at 50 nm were incubated with these plasmas or human serum with a nanoparticle's surface area density in the mixture of 100 cm^2/mL. After incubation, the protein composition of the corona was profiled, and the activation of both the complement and the coagulation system was evaluated. In addition, the effects of these nanoparticles on immune cells in *ex vivo* human whole blood anticoagulated with the different anticoagulants were characterized. Finally, plasma cytokines induced by nanoparticle incubation in whole blood was profiled, and their effects on endothelial activation were measured by flow cytometry.
Results: Protein composition of the corona was different between the anticoagulants. Proteins from heparin-anticoagulated plasma bound to the surface of nanoparticles at the highest amount, as characterized by both gel electrophoresis and BCA (bicinchoninic acid) protein assay. The two nanoparticles activated the complement and coagulation systems differently in both plasma and whole blood, with the lowest activation level observed with EDTA and citrate. AuNP activated granulocytes and monocytes in an anticoagulant-dependent manner, and stronger than PSNP in all cases. Similarly, cytokine releases induced by AuNP's incubation were at a higher level than PSNP's incubation, and dependent on the anticoagulants used. In contrast, while still in the anticoagulant-dependent manner, the two nanoparticles activated platelets at similar levels. Incubation with plasma collected from nanoparticle-incubated whole blood induced activation of human umbilical vein endothelial cells stronger than direct incubation of the cells with nanoparticles.
Conclusions: Our data illustrate the impact anticoagulants have on nanoparticle-protein corona properties. It highlights the importance to choose the proper anticoagulant in biocompatible tests of materials, and that it is crucial to understand the effect a particular anticoagulant has on the biological system intended to study.
Overview of the study. (A) Hydrodynamic diameter (z-average) and zeta potential (ζ) of gold nanoparticles (AuNP) and polystyrene nanoparticles (PSNP). Gel electrophoresis images of proteins

abound to the surface of AuNP (B) and PSNP (C). Abbreviation: protein ladder (lane 1), serum (2), EDTA plasma (3), citrate plasma (4), heparin plasma (5), lepirudin plasma (6), and GPRP plasma (7). (D) Proteins bound onto the surface of nanoparticles as quantified by microBCA protein assay. (E) Terminal complement complex (TCC), a marker of complement activation, induced by the incubation of nanoparticles with either plasma or whole blood anticoagulated with different anticoagulants, as indicated. (F) Thrombin-antithrombin (TAT), a marker of coagulation activation, induced by the incubation of nanoparticles with either plasma or whole blood anticoagulated with different indicated anticoagulants. The activation of monocytes (G), granulocytes (H), and platelets (I) was quantified by detecting activation markers (as indicated on the respective y-axis) by flow cytometry after incubation of nanoparticles in whole blood anticoagulated with indicated anticoagulants for 15 minutes. (J) Plasma collected after a four hours-incubation of nanoparticles with whole blood was used to incubate with endothelial cell (HUVEC) and HUVEC-activation was quantified by flow cytometry after a three hours-incubation. Of 27 cytokines profiled, IL-1β (K), IL-6 (L), IFN-γ (M), and TNF (N) are represented at varied levels after incubation of nanoparticles with whole blood anticoagulated with indicated anticoagulants for four hours.



IN VIVO ANALYSIS OF HYALURONIC ACID-BASED NANOPOLYMERSOMES FOR BREAST CANCER TREATMENT

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In the current research, the fabrication of hyaluronic acid-based polymersomes for targeted delivery of doxorubicin is presented. Thus, doxorubicin was encapsulated in aqueous compartment of hyaluronan-polycaprolactone (HA-PCL) polymersomes using nanoprecipitation method. In our previous study, the therapeutic index of the prepared formulation was studied in metastatic breast cancer model *in vitro*. In this study, we reported the *in vivo* analysis of the hyaluronic acid-based polymersomes in breast cancer treatment. The size of obtained polymersomes was 146±9.6 nm with encapsulation efficiency and loading content %55 and %3.6, respectively. The obtained results demonstrated that the HA-PCL polymersomes release of DOX in a sustained manner. Much better features of the fabricated formulation in terms of *in vivo* antitumor efficacy and wider tumour tissue necrosis and bio-distribution in comparison with PEG-PCL-DOX nanoparticles suggested that HA-PCL-DOX can potentially reduce off-target effects due to its targeting capability.

Keywords: Nanopolymersome, Doxorubicin, Hyaluronic acid, Breast cancer, Polycaprolactone

Introduction: Breast cancer is one of the most commonly diagnosed cancers and the second cause of cancer related death among women globally [1]. In the current study was performed to develop novel hyaluronic acid-poly caprolactone (HA-PCL) nanopolymersomes. Doxorubicin was encapsulated in the aqueous compartment of nanopolymersomes *via* nanoprecipitation procedure for preparation of the sustained release of DOX for cancer therapy. Afterwards, cytotoxicity effect and cellular uptake were evaluated in MCF-7 and 4T1 cell lines. Moreover, the therapeutic index and biodistribution of DOX-loaded HA-PCL polymersomes were evaluated *in vivo* on 4T1 metastatic mouse breast cancer model.

Materials and Method: Hyaluronic acid solution (Mw= 5000 g/mol) was added to a round-bottom flask, then 1,2-ethylenediamine was drop-wisely added to the solution of HA. Then, the mixture was reacted for 4 hours. Subsequently, sodium cyanoborohydride was added to the mixture in an ice-bath and continuously stirring of the final reaction mixture was allowed to carry out at ambient temperature for 18 h [2]. Next day, the obtained solution was evaporated by rotary evaporator to eliminate excess diamine and water. Thereafter, the crude product was dialyzed against PBS buffer at 4 °C for 48 hours. Afterwards, the purified polymer was freeze dried in order to prepare amine-functionalized HA.

The aminated hyaluronic acid was dissolved in dimethylformamide (DMF) and then N, N-diisopropylethylamine was added at 100 °C and stirred for 24 h. The final product was dialyzed against distilled water for two days for removing unreacted HA, and then lyophilized by freeze-drying for 24 h and kept at −20 °C for further use. The structure of final product was verified using ^{1}HNMR spectroscopy. The existence of amide linkages in HA-PCL was featured implementing Fourier transform infrared (FT-IR) spectrophotometer. Blank HA-PCL NPs and doxorubicin-loaded HA-PCL polymersomes were prepared using the nanoprecipitation method. HA-PCL copolymer was dissolved in dimethylsulfoxide, subjected to sonication for 30 minutes in 60 ºC, while doxorubicin was dissolved in deionized water, separately. The organic phase was added dropwise to the hydrophilic phase under probe-sonication. Then, the solution was allowed to stir for 2 h at 60 ºC and dialyzed against 2 L deionized water for 6 h for elimination of solvent and free doxorubicin. The final formulation was freeze-dried. The calculation of doxorubicin content of polymersomes was performed using the provided standard curve of DOX by UV spectrophotometer at 480 nm.

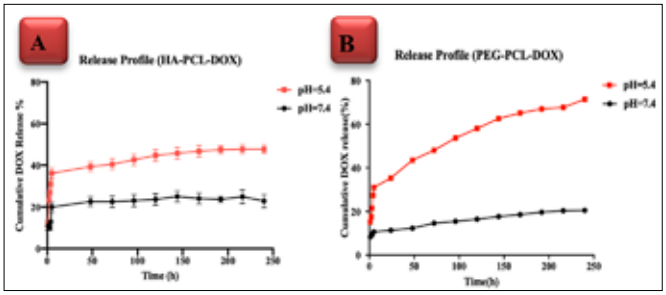
The *in vitro* release patterns of anticancer agent (DOX) from HA-PCL-DOX and PEG-PCL-DOX nanoparticles in either citrate buffer (pH=5.5) or phosphate-buffered saline (PBS, pH=7.4) were examined.

In vivo therapeutic efficacy was done in accordance and prior approval of Institutional Animal Care and Use Committee of the School of Pharmacy, Mashhad University of Medical Sciences. For the *in vivo* evaluation, four to six-week old female BALB/c mice were purchased from Pasteur Institute in Iran (Tehran, Iran) and maintained under conventional conditions in the laboratory animal care facility. Then, we established mouse tumor model by local subcutaneous injection of 4T1-tumor cells (4×10^{5} cells/ in 80 µL PBS solution) into the right flank of each mouse. After 8 days of injection, mice were distributed into four groups (n=5), control, free DOX, PEG-PCL-DOX and HA-PCL-DOX, and they received 100 µL of either free DOX, HA-PCL-DOX or PEG-PCL-DOX (DOX equivalent: 5 mg/kg) *via* a single tail intravenous administration. For negative control group, the injection of NaCl solution (0.9% w/v) was performed. The calculation of tumor volume for each mouse was made after measurement of following parameters: largest diameter, smallest diameter, as well as the depth of the tumor. After that, we entered

these parameters into a × b × w/2 equation where “a” and “b” are largest and smallest diameters of tumor, respectively, and “w” is its depth. Moreover, the toxicity assessment of free DOX, HA-PCL-DOX, and PEG-PCL-DOX NPs was performed by measurement of the body weight and survival rates. Animals were euthanized 30 days after the intravenous administration of free DOX, HA-PCL-DOX NPs and PEG-PCL-DOX NPs (n=5, 5 mg/kg DOX equivalent). After euthanasia of all mice, the kidneys, lungs, spleen, liver, heart, and tumor were collected, following three times washing with PBS and fixed in a 10% formalin solution. Tissues that have been formalin-fixed and paraffin-embedded were sectioned (5 µm diameter) and staining procedure with hematoxylin and eosin (H&E) was used. The images were prepared at 10× and 40× magnification.

Results and Discussion: Doxorubicin encapsulated nanopolymersomes based on HA-PCL copolymer (HA-PCL-DOX), was prepared through nanoprecipitation technique. Based on our results, DOX was encapsulated in HA-PCL polymersomes with encapsulation efficiency (EE) and loading content (LC) of %55 and %3.6, respectively. DOX encapsulation in PEG-PCL copolymer was performed implementing double emulsion method with EE and LC of %26 and 1.3%, respectively. Size distribution of polymersomes was determined to be 146.2±10.0 nm (polydispersity index =0.12, zeta potential=-42.1 ± 0.3) for HA-PCL-DOX and 106±21 nm (polydispersity index=0.2, zeta potential=-9.2±0.4) for PEG-PCL-DOX. The diameters of either HA-PCL-DOX or PEG-PCL-DOX are less than 200 nm, considering that particles smaller than 200 nm are capable of being accumulated at the tumor site via EPR effect thereby reducing the systemic adverse effects and improving the pharmacokinetics of entrapped drugs. Furthermore, high negative value of zeta potential is crucial for nanoparticle stability. Owing to the presence of hyaluronic acid on the HA-PCL nanopolymersome surfaces, the resultant HA-PCL-DOX NPs has a high negative charge.

Figure 1. *Release profile of HA-PCL-DOX (A) and PEG-PCL-DOX (B) in PBS, pH 7.4 and citrate buffer, pH 5.5. (n = 3, error bars represent standard error of the mean).*



The potential of DOX-loaded HA-PCL nanoparticles to improve growth suppression of 4T1 tumor was assessed in ectopic model of 4T1 tumor-bearing BALB/c mice after single-dose intravenous administration of either HA-PCL-DOX, PEG-PCL-DOX or free DOX (DOX equivalent concentration: 5 mg/kg). Obtained results demonstrated a significant inhibition of tumor growth after administration of either HA-PCL-DOX or PEG-PCL-DOX when compared with free DOX and control group which was likely due to EPR. Moreover, the significant tumor inhibition effect of HA-PCL-DOX in comparison with PEG-PCL-DOX was attributed to targeting capability of HA-PC-DOX to CD44 receptor of 4T1 tumor cells which increased the accumulation of the formulation within the tumor (Fig. 2A).

As a clinical sign of toxicity, the body weight of mice was monitored in all groups. According to the results, mice injected with either free DOX or PEG-PCL-DOX were demonstrated a body weight loss 3 days post-administration in comparison with mice in HA-PCL-DOX and control groups which further confirmed the systemic toxicity of free DOX and PEG-PCL-DOX. The significant inhibition of tumor growth in HA-PCL-DOX-treated mice eventuated in statistically longer median survival time when compared with either PEG-PCL-DOX, free DOX or saline treated groups. The survival percentage of 4T1 tumor-bearing mice injected by either PEG-PCL-DOX, HA-PCL-DOX or free DOX with administration of a 5 mg/kg dose during a 35-day period after administration were presented in Figure 2B. In this regard, only one mouse remains alive with either 5 mg/kg free DOX or saline administration after 26 and 25 days, respectively. Three out of five mice receiving PEG-PCL-DOX stayed alive up to day 35. However, all mice in the HA-PCL-DOX- treated group remained alive until day 35. The shorter time of survival for the free DOX-treated group when compared with the group injected by HA-PCL-DOX (statistically significant) could be ascribed to severe toxicity of free DOX. On the other hand, our observations did not exhibit any significant difference between median survival times in mice injected by either free DOX or saline.

Figure 2. *(A) Tumor growth (B) Survival patterns of ectopic model of 4T1 tumor in mice receiving intravenous injection of either HA-PCL-DOX, PEG-PCL-DOX or free DOX (equivalent DOX concentration: 5 mg/kg, n=5);*

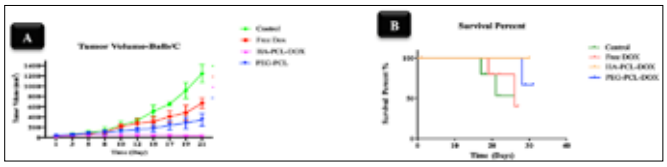
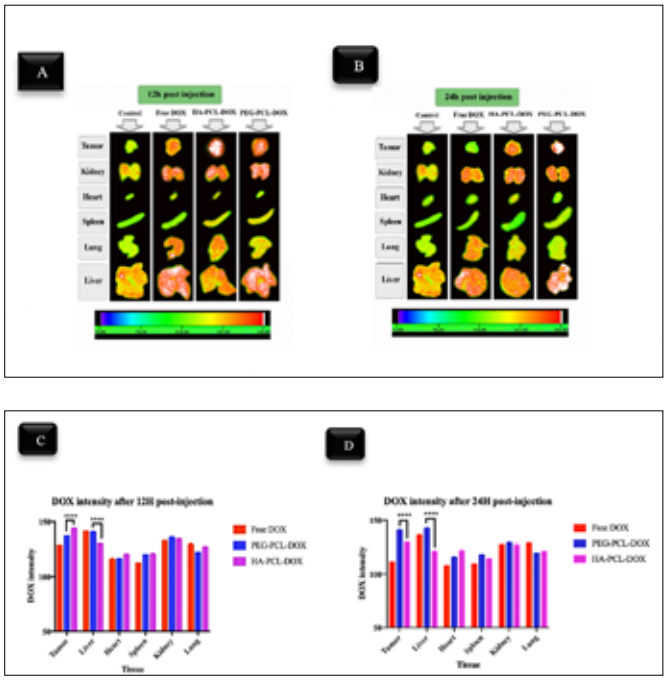


Figure 3. *Ex vivo fluorescence imaging of mice organs as well as tumor tissue. 12 h post-injection (A) and 24 h post-injection (B) of free DOX, HA-PCL-DOX and PEG-PCL-DOX. The intensity of DOX in mice organs and tumor tissue 12 h post-injection (C) and 24 h post-injection (D) of free DOX, HA-PCL-DOX and PEG-PCL-DOX.*



Conclusions: Herein, we developed a hyaluronan-PCL polymersomal delivery system for the targeted DOX delivery to CD44 positive murine 4T1 and human MCF-7 mammary cancer cell lines. The synthesized formulation could carry the drug with high encapsulation efficacy to the target site. It was accumulated in cancer cells at higher levels than in normal cells compared to free DOX and PEG-PCL-DOX formulation. Moreover, *in vivo* evaluations demonstrated that the HA-PCL-DOX had significant higher therapeutic index in comparison with PEG-PCL-DOX while illustrating the same safety profile. Thus, the developed hyaluronan-PCL polymersomes with targeting ability could be considered as an ideal candidate for DOX delivery in cancer treatment.

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TARGETED MMP-2 RESPONSIVE SN38-LOADED POLYMERSOMES FOR COLORECTAL CANCER THERAPY

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In the current study, polylactide (PLA) was conjugated to polyethylene glycol (PEG) *via* a small peptide sequence, PVGLIG which is a substrate for the tumor-associated enzyme matrix metalloproteinase-2 enzyme. The chimeric triblock polymer of PEG-b-PVGLIG-PLA was used to form nanoscale self-assembled polymersomes. Subsequently, the anticancer drug, SN38 was loaded into polymersomes through single emulsion method and the prepared polymersomes were characterized by DLS, AFM and SEM. The hydrophobic SN38 was loaded with 70 encapsulation efficiency and 5% loading content providing monodispersed spherical nanoparticles with 172 nm dimension. The prepared polymeromes provided sustained release of SN38 under physiological condition but augmented release following exposure to MMP-2 enzyme. Afterwards, AS1411 aptamer was conjugated to the surface of the polymersomes in order to provide guided drug delivery. *In vitro* cytotoxicity against C26 cells (+nucleolin) demonstrated significantly higher toxicity for the targeted formulation in comparison with the non-targeted one. *In vivo* study employing C26 tumor model in mice showed higher therapeutic index of MMP-2 responsive polymersomal formulation in comparison with the non-responsive one. It could be concluded that the prepared polymersomes bearing cleavable peptide sequence between their blocks and targeting ligand on their surface, provide interesting features as intelligent drug delivery system against cancer.

Keywords: Polymersome; SN38; MMP2; Enzyme responsive; AS1411

Introduction

The incidence of colon cancers has been steadily increased up to 1.8 million cases in 2018 [1]. Poor therapeutic index along with undesirable side effects after administration is the main drawback of the chemotherapy treatment approach [2]. SN38 which is more potent than its prodrug irrinotecan, is a topoisomerase 1 inhibitor [3]. Clinical application of SN-38 hindered by impediments such as low water solubility and poor stability due to the conversion of its active form to an inactive form at physiological pH [4]. Stimuli-responsive nanomaterials are one the interesting platform for providing intelligent drug delivery system. Carriers based on stimuli responsive materials initiate payload release upon exposure to relative stimuli comprising enzyme, temperature, salt, heat, pH, and electrical fields. Owning to the crucial role of various enzymes in cell regulation process and their overexpression in particular cancer tissue, therefore enzyme-responsive systems as a versatile generation of the carrier were proposed. Matrix metalloproteinases (MMPs) are zinc-containing endopeptidase and calcium-dependent proteolytic enzymes responsible for digest numerous constituents of the extracellular matrix [5]. In the current study, we conjugated polyethylene glycol (PEG) to polylactide (PLA) *via* the MMP2-cleavable peptide sequence to form intelligent MMP2-responsive self-assembled polymersomes for delivery of SN38. Then, we covalently attached a DNA aptamer AS1411 to the surface of SN38-encapsulated MMP-2 responsive polymersomes. Afterwards, the physicochemical aspects of the fabricated nanoplatforms and their therapeutic index was evaluated *in vitro* and *in vivo*.

Materials and Method

Synthesis of PEG-peptide-PLA triblock copolymer

PLA was dissolved in dichloromethane and EDC, NHS was added to the solution. The reaction mixture was stirred overnight at room temperature while protecting from light. Then the synthesized NHS-PLA was precipitated using cold diethyl ether and washed three timed with diethyl ether: methanol. The final activated PLA was vacuum dried and kept at -20˚C until further use. At the next stage, the activated NHS-PLA was conjugated to the N-terminal of peptide in the COOH-PEG-peptide conjugate. In this regard, NHS-PLA was dissolved chloroform and then PEG-peptide conjugate was added. The reaction mixture was stirred overnight at room temperature. The synthesized PEG-peptide-PLA copolymer was precipitated using cold diethyl ether and washed three timed with diethyl ether: methanol.

In vivo study

The antitumor efficacy of SN38 polymersomes was investigated in Female BALB/c mice. Briefly, BALB/c mice were subcutaneously inoculated with C26 cells. When the mean tumor size reached 15 ±6 mm3, mice were randomly divided into five groups (5 mice in each group) and received 0.2 ml of either normal saline 0.9% as negative control, irinotecan, SN38-pep-NPs, Apt-SN38-pep-NPs and SN38-NPs (5 mg/kg equivalent SN38) via tail veins injection. Mice weight and tumor sizes were monitored up to 30 days' post-injection.

Results and Discussion

Synthesis of PEG-Peptide-PLA

PEG-Peptide conjugate was attached to the synthesized COOH-PLA through amide bond formation implementing EDC/NHS chemistry. The prepared PEG-Peptide-PLA triblock was purified *via* precipitation method by diethyl-ether and methanol mixture. ^{1}HNMR spectrum of the triblock was illustrated in Figure. All resonances for PEG (3.6 ppm), PLA (1.5 and 5.1 ppm) and peptide (2.5 and 2.1 ppm) could be assigned confirming the synthesis of triblock copolymer. Using the integration of the relative molecular weights and peaks, the conjugation efficiency of PEG-Peptide to PLA-COOH was estimated to be approximately 88%.

In vitro drug release

The release pattern of SN38 from SN38-NPs in the presence or absence of MMP-2 enzyme showed the identical profile thus verifying the non-responsiveness of this platform. Similarly, the release pattern of SN38 from SN38-pep-NPs demonstrated 7-folds higher drug release in the presence of MMP-2 enzyme confirming the MMP-2 responsiveness of the developed platform (**Figure 1**). It should be noted that both systems in the absence of MMP-2 enzyme showed the sustained controlled release of encapsulated SN38 since lower than 10% of cargo released up to 240 h. The sustained-release property of the system is a result of the polymersome stability and rigid bilayer. On the other hand, the vesicles showed significant degradation and cargo release in the presence of MMP-2 enzyme. In this regard, the cancer associated overexpressed enzyme MMP-2 has been previously reported to cleave the aforementioned peptide substrate within a various nanostructures assembled from diblock copolymers, when the substrate was exposed on the particle surface [12].

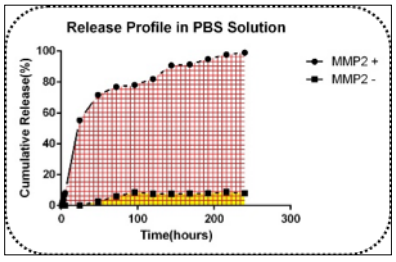

Figure 1. *Release study of the PEG-pep-PLA in the presence and absence of enzyme.*

In vivo study

In the next stage, the efficiency and intelligency of the SN38-pep-NPs in compassion with Apt-SN38-pep-NPs was evaluated on C26 subcutaneous tumor model in BALB/c mice. After tumor induction, the capability of the prepared SN38-NPs, SN38-pep-NPs, Apt-SN38-pep-NPs and irinotecan formulations were assessed by dividing mice into five groups. In this study, the following regimen was performed: two doses of intrave-

nous administration of irinotecan, SN38-NPs, SN38-pep-NPs, Apt-SN38-pep-NPs and saline with 3-day time interval. Tumor growth rate in terms of mean tumor size is illustrated in **Figure 2.**

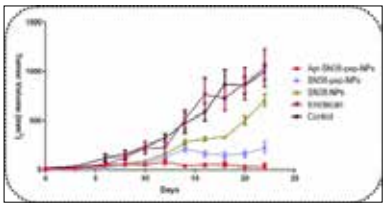

Figure 2. *Tumor weight of mice receiving different formulations.*

The obtained results demonstrated that a two doses of intravenous administration of either Apt-SN38- pep-NPs or SN38-pep-NPs was significantly more effective in tumor growth regression in comparison with SN38-NPs and this is likely due to responsive and intelligent behavior of Apt-SN38-pep-NPs or SN38-pep-NPs at tumor site in the presence of MMP-2 enzyme. Moreover, the highly improved tumor-inhibition efficacy of Apt-SN38-pep-NPs compared with SN38-pep-NPs could be ascribed to the Apt-SN38-pep-NPs binding to nucleolin on the C26 cells, thus probably postponing clearance of targeted formulation from tumor site.

The toxicity of each treatment was further assessed by analyzing its effects on body weight loss (BWL). The body weight-change curve in **Figure 3** shows that weight loss was less than 5% for mice treated with SN38-pep-NPs. BWL occurred in the SN38-pep-NPs treated group might have been due to the slight toxicity of the formulation dosage.

Late BWL was observed in the irinotecan-treated group 15 days' post-administration without recovery during 30 days because of irinotecan liver processing and higher toxicity of its active metabolite when circulating throughout the body. However Apt-SN38-pep-NPs treated mice compared to SN38-pep-NPs treated mice did not show any BWL during experiment which may be ascribed to its modified biodistribution and higher pharmacokinetic efficiency.

Conclusion

The present study, demonstrates the *in vitro* and *in vivo* efficacy of AS1411 aptamer-conjugated, SN38-loaded PEG-pep-PLA nanopolymersomes as nucleolin-targeted and MMP-2 responsive platform against C26 cells as models of colorectal cancer. In this study, the targeted, SN38-loaded PEG-pep-PLA nanopolymersomes demonstrated favourable efficacy against C26-tumor growth, based on the implementing of model C26 cells *in vitro* and *in vivo*. Similar strategies might be implemented to develop targeted intelligent system that increase the overall therapeutic index of encapsulated drug.

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MULTIFUNCTIONAL TRIBLOCK-COPOLYMERS AS POTENTIAL TRANSFECTION AGENTS

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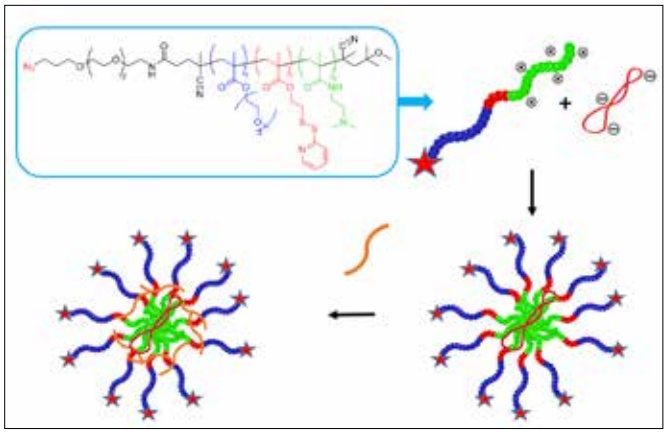
Over the past decades the controlled manipulation of the genome and proteome has established itself as very promising approach for many therapeutic applications. Nevertheless, effective *in vivo* transfection of target cells is a difficult task due to many physiological barriers and the imminent degradation of unprotected DNA and RNA in the blood stream. Using polymers as protecting vessels is a promising way for a safe transport and delivery of genetic information to the target cells. For this purpose, polymer based transfection agents have to work as functional, stimulus responsive system to be most effective combining shielding of the payload during transport, stability of the polymer / DNA or RNA complex (polyplex) with the possibility to release the active component after cellular uptake. This makes triblock copolymers attractive, which combine blocks for shielding and DNA complexation with an additional reactive block, which allows reversible stabilization and destabilization. Such triblock copolymers have been synthesized previously by Heller et al1 based on polypept(o)ides and showed to be effective as transfection agents.

RESULTS AND DISCUSSION

Based on these considerations we synthesized triblock-copolymers by RAFT polymerization, in which each block is designed to take a different task. Using an azide bearing chain transfer agent (CTA), the polymer can be modified in a post polymerization reaction by Alkyne-Azide Click reactions. Concerning the monomers, triethylenglycole metharcrylate (MEO3MA), pyridyldisulfideethyl methacrylate (PDSM) and pentafluorophenole methacrylate (PFPMA) were used. MEO3MA, due to the PEG-like structure should shield the polyplex from enzymatic degradation and mediate water solubility. PDSM can be reversibly crosslinked. These crosslinks can be cleaved reductively, hence stabilizing the polyplex while transporting the payload and promoting its release in the reductive milieu of the endosome.

Primary amines can be used in a selective amidation reaction at the PFPMA side chain. Therefore the third block can be modified with small molecule components and / or asymmetric diamines like N,N-dimethyl-ethylene diamine (DMEDA). The tertiary amine group of DMEDA is partially protonated under physiological conditions, therefore the third block can interact with DNA or RNA and form complexes of certain stability.



The RAFT polymerization of the new methacrylate based triblock-copolymer could be successfully established to synthesize tailor-made copolymers with narrow PDI. Thereby parameters like overall molecular mass, the ratio of hydrophilic, reactive and hydrophobic block and the individual number of repetition units could be varied freely. In addition to that, the activity of the reactive disulfide units has been proved in the final block copolymer. The formation of polyplexes with plasmide DNA (pDNA) has been investigated

with agarose gel electrophoresis at different N/P ratios to investigate on the polyplex stabilities. The formation of nano sized polyplexes with narrow distribution of the hydrodynamic radii has been checked by dynamic light scattering. Furthermore, different cross-linkers were used to further stabilize the polymer / DNA conjugate and the synthesized polyplex could be modified by fast and efficient DBCO-Alkyne-Azide Click reaction. Work on such polyplexes to transfect dendritic cells is in progress.

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P(DHPMA) BASED POLYMERSOMES FOR NANOMEDICAL APPLICATION

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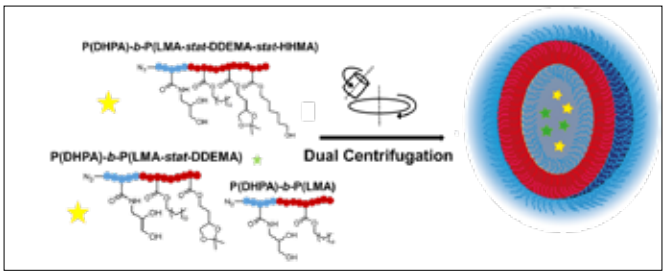
Over the past decades Polymersomes were investigated regarding their potential in nanomedical applications.[1] Polymersomes are made from amphiphilic blockcopolymers which undergo self assembly and form vesicular nanoparticles.[2] The amphiphilic blockcopolymers form a bilayer structure with an aqueous core similar to biological liposomes. Thus Polymersomes with a hydrophilic core and a hydrophobic membrane have a great potential for the encapsulation of various cargo.

RESULTS AND DISCUSSION

In order to create Polymersomes, amphiphilic diblock-copolymers were synthesized by RAFT blockcopolymerization using an azide bearing chain transfer agent (CTA). This offers the possibility for modifying the surface of the formed Polymersomes by Alkyne-Azide Click reactions. Following this approach Dyes or targeting moieties could be introduced on the surface of the nanoparticles. For the Synthesis of the Diblockpolymers the monomers, pentafluorophenole methacrylate (PFPMA), lauryl methacrylate (LMA), 2-(2,2-dimethyl-1,3-dioxolane-4-yl)ethyl methacrylate (DDEMA) and hydroxyhexyl methacrylat (HHMA) were used.

The hydrophilic component DHPA, due to the HPMA-like structure should act as a shielding part reducing interactions with biological enviroment.3 P(LMA) acts as the hydrophobic part, forming the bilayer membrane. In between the hydrophobic block two more components P(DDEMA) and P(HHMA) were integrated statistically, enhancing the pH sensitivity of the Polymersomes and their release behaviour .[4,5]



The RAFT polymerization of the diblockcopolymer with statistical components could be successfully established. The formation of the Polymersomes was prepared by asymmetric dual centrifugation. The purification of the nanoparticles was performed via HPLC in water.

The surface modification of the Polymersomes was performed with DBCO functionalized Dyes or Biotinconjugates using Alkyne-Azide Click reaction. Hydrophilic Dyes and the more hydrophobic compound Doxorubicine were successfully encapsulated into the Polymersomes. First *in vivo* experiments regarding the biodistribution of fluorescent-labelled Polymeresomes were conducted.

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OXIDATIVE TOXICITY IN DIABETES MELLITUS: ROLE OF NANOPARTICLES AND FUTURE THERAPEUTIC STRATEGIES

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Diabetes mellitus is one of the most common chronic medical conditions in the world. Increasing evidence suggests that chronic hyperglycemia can cause excessive production of free radicals, particularly reactive oxygen species (ROS). Free radicals play important roles in tissue damage in diabetes. The relationship between exposure to nanoparticles (NPs) diabetes has been reported in many previous studies. Evaluation of the potential benefits and toxic effects of NPs on diabetic disorders is of importance. This review highlights studies on the relationship between NPs and oxidative stress (OS) as well as the possible mechanisms in diabetic animal models and humans.

Keywords: Nanoparticles; Diabetes mellitus; Oxidative stress

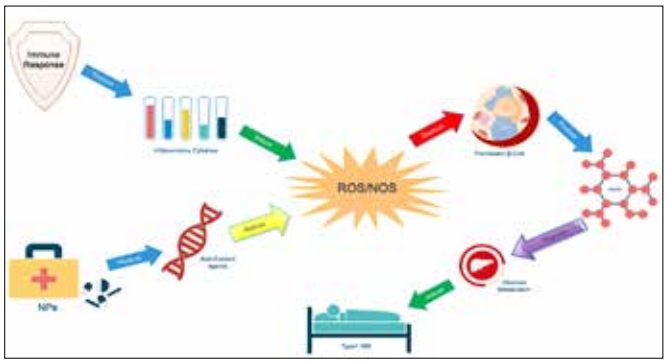

Figure1. *Schematic representation of nanoparticles as a vehicle for therapeutics and their innate antioxidant properties for the effective treatment of type 1 Diabetes mellitus (DM).*

ANTI-CANCER ACTIVITY OF A PH- AND THERMO-RESPONSIVE SILICA NANOPARTICLES ENCAPSULATED DOXORUBICIN (DOX) AND AVOCADO (PERSEA AMERICANA) SEED EXTRACT (PAX) ON 2D AND 3D HUMAN LIVER TUMOUR MODELS

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Avocado (*Persea aAmericana* Mill.) seeds are by-products in food processing and manufacturing. Recently, the pharmacological effects including anti-oxidant and cytotoxic activities of triterpenoids and polyphenols, plant secondary metabolites, were reported (1-2). Here, we aimed to develop a novel dual-therapy platform constructed by mesoporous silica-based nanoparticles encapsulated DOX and PAX (DP-MSNs) for liver cancer treatment. A significant anti-cancer activity from avocado seed extract on human liver cancer cells (HepG2 cells; $p<0.001$, n=3) was observed, *in vitro*, using both 2D and 3D human liver tumour models. Avocado seeds were, first, extracted by a maceration method with water and 50% ethanol. Next, the extract compositions were screened and quantified by chromatographic and spectrophotometric techniques, respectively.Anti-cancer activities of both extracts were measured using 2D-culture and 3D-liver spheroid model constructed from HepG2 cells and its drug-resistant counterpart. The 50% inhibition concentrations (IC_{50}) were determined on both non- and drug-resistant HepG2 models using viability assay and % reduction of tumour size. The anti-cancer mechanism of the extract was also elucidated. All physicochemical properties of DP-MSNs including particle size, surface charge density, % drug encapsulation, % drug release and their anti-cancer activity were observed and quantified.
Avocado seeds were extracted by the decoction method using water and maceration method using 50% ethanol solution with % yield at 8.82 and 11.12, respectively. Polyphenols, the major secondary metabolites, were detected and quantified. Avocado extracts exhibited specific thin layer chromatographic (TLC) fingerprint with the presences of phenolic compounds. The total phenolic of aqueous (A-PAX) and ethanoic extracts (E-PAX) were 11.81 and 13.38 g% gallic acid equivalent (g% GAE), respectively. Both A-PAX and E-PAX exhibited anti-cancer activity against HepG2 cells in dose-dependence manner with IC50 at 136.08 and 75.68 µg/mL, respectively. To enhance the efficacy and the stability of the E-PAX, MSNs were formulated with the addition of poly (N-isopropylacrylamide-co-acrylic acid (3-4). A dual thermo- and pH-responsive polymer (5-6), was used to encapsulate DOX and E-PAX in order to control anti-cancer drugs release at the target site. Mesoporous silica-based nanoparticles (E-PAX-MSNs and DP-MSNs) presented drug loading capacities of E-PAX and DP at 16.00% and 31.27%, respectively. The particles diameter of non-encapsulated MSNs, E-PAX-MSNs and DP-MSNs were 71.65 ± 0.87, 131.20 ± 1.96 and 184.50 ± 1.60 nm, respectively. Anti-cancer activity of DOX, E-PAX-MSNs and DP-MSNs against both 2D-culture and 3D-spheroid model of HepG2 cells were compared. Results indicated that the accumulated nanoparticles within the 2D-culture and 3D-spheroid model (non-resistant and drug-resistant cells) could release DOX and PAX via an acidic pH and NIR laser activation, leading to a significant cytotoxicity as shown in Figure 1.
Finally, the dual therapy platform (DP-MSNs) exhibited significant higher anti-cancer activity than that of the single therapy platform (DOX and E-PAX-MSNs) by initiating the size reduction of 3D-liver spheroids in dose-dependent manner. This novel dual therapy platform might be a promising platform for effective liver cancer treatment.

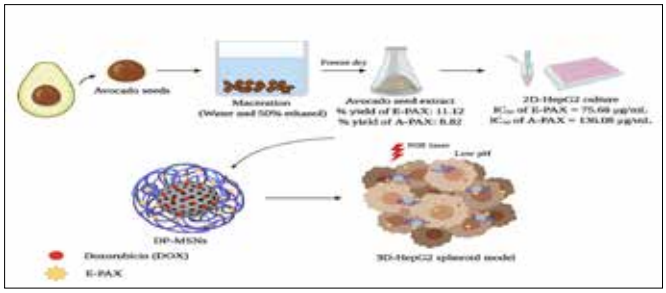

Figure 1 *Diagram summarized process of avocado seed extraction, anti-cancer activity of the extracts and the drug release mechanism of DP-MSNs in 3D-HepG2 spheroid model.*

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TARGETED DELIVERY OF ELESCLOMOL USING METAL ORGANIC FRAMEWORK EQUIPPED WITH EPCAM APTAMER AND FOLIC ACID FOR COLORECTAL CANCER THERAPY

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Cancer is the uncontrolled growth of cells in the body and is considered as one of the major causes of death globally. There are several cytotoxic chemotherapeutic agents used to treat cancer including elesclomol, methotrexate, 5-fluorouracil, cisplatin, tamoxifen, doxorubicin and others. Although billions of dollars have been spent on cancer research to develop these chemotherapies, it still remains a major illness for mankind partly due to the shortcomings of these therapies. These shortcomings include low targeting specificity, severe side effects (due to high doses) and poor pharmacokinetics. To avoid these drawbacks, anti-cancer drug delivery systems have been developed recently using nanocarriers including liposomes, micelles, polyelectrolyte capsules and others. One of the recent class of nanoparticles investigated for chemotherapeutic use are metal organic frameworks (MOFs) which are hybrid polymers that consist of metal ions or clusters and organic ligands. MOFs are used in many applications including gas/vapor separation, gas storage, catalysis, luminescent materials, and biomedical imaging. These structures have additional features that promote their use as drug carriers in the biomedical field. First, they are nontoxic, biodegradable and have the ability to carry high loadings of the anti-neoplastic agent due to their porous nature. Also,

they have well-defined crystalline structures that can be characterized by different analytical techniques and their sizes are suitable to control their *in vivo* drug release. In this study, we developed EpCAM-targeted PEGylated MOFs for controlled release of elesclomol. Afterward, the efficacy of the prepared system was evaluated *in vitro* and *in vivo*

Methods

Elesclomol has been synthesized using the primary substance of s-(thiobenzoyl) thioglycolic acid [(1),] in two steps. Condensation of this acid using methyl hydrazine in basic condition has slowly led to produce the intermediate material of N-methyl benzo thio hydrazide [(2)]. Hydrazide has then reacted with malonyl chloride [(3)] to achieve elesclomol [(4)]. During the reaction of metal salts with Elesclomol at equal concentrations, transition metal complexes of 5a, 5b, and 5c have been developed. Elesclomol and its metal complexes 5a, 5b and 5c have been completely characterized via FT-IR, NMR and HRMS. X-ray analysis has been done for 5c (Figure 1).

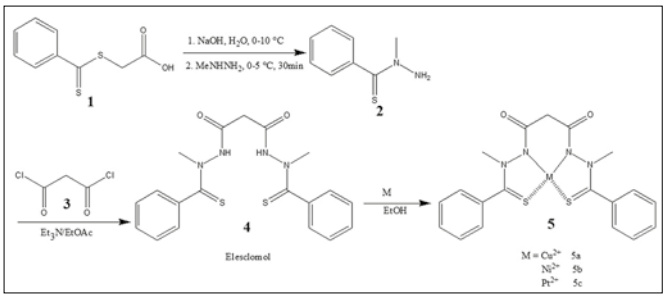

Figure 1. *Scheme of elesclomol synthesis*

After the synthesis of MOF-Drug-PEG-EpCAM Apt (Figure 2), the composition and pore structures were thoroughly studied with various spectroscopic and microscopic methods such as FT-IR, TGA, SEM-EDS and BET techniques.

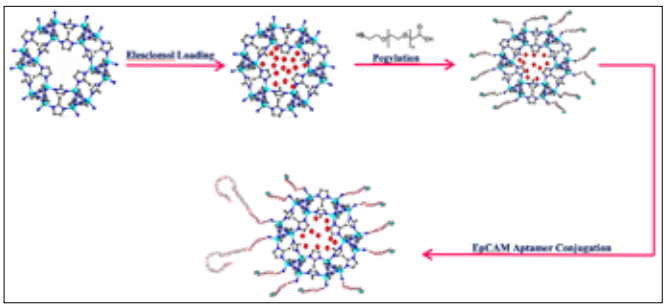

Figure 2. *Schematic of synthesis the drug delivery system*

Results

After characterization of the synthesized compound, the potential efficiency of antitumor activity of elesclomol loaded on MOF nanoparticles (ZIF-90) decorated by bifunctional PEG and EpCAM Aptamer was investigated using some bioassays consist in:

1. Drug loading & *In vitro* drug release measurements
2. *In vitro* cytotoxicity experiments
   2.1 MTT assay
   2.2 Cellular uptake by flow cytometry
3. Investigation of cellular binding of aptamer
4. Cellular uptake by fluorescence microscopy and
   1. *In vivo* CT scan imaging
   2. *In vivo* optical imaging

Keywords: Elesclomol; Metal-Organic Frameworks (MOFs); MTT; MRI.

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SITE-SPECIFIC NANOBODY CONJUGATION FOR TARGETED DRUG DELIVERY TO PROTUMORAL TUMOR-ASSOCIATED MACROPHAGES

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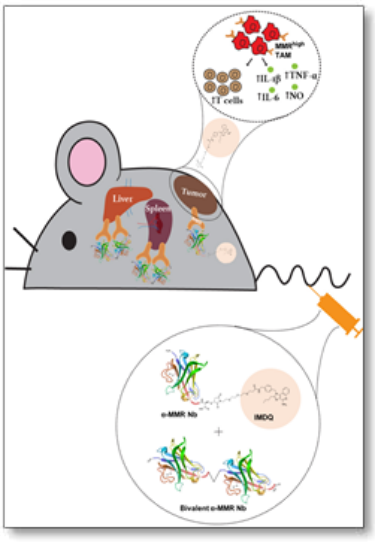

Figure 1*: Re-polarization of immunosuppressive TAMs using site-specifically modified anti-MMR/CD206 nanobodies.*

Nanobodies are one of the smallest available single chain antigen binding fragments derived from camelid heavy chain-only antibodies. With a molecular weight of about 15 kDA they are 10 times smaller than conventional antibodies. They can be produced recombinantly and genetically engineered to provide chemical functionalities for site-specific protein modification. In this study, nanobodies were used to target the macrophage mannose receptor (MMR, CD206) overexpressed on tumor-associated macrophages (TAMs). Those type of immune cells govern chronic cancer-associated inflammations and establish immunosuppressive tumor micromilieus. Strategies to re-polarize TAMs and trigger an antitumoral activity can be followed by using the targeting potential of anti-MMR/CD206 specific nanobodies engineered with a C-terminal cysteine. They can be site-specifically modified via maleimide chemistry under reducing conditions without interfering with their internal disulfides. Thus, one single fluorescent dye can be coupled to the nanobody, for instance, to monitor the recruitment of TAMs into immunosuppressive cancers. Additionally, immune modulating small molecules can be ligated to the nanobodies to stimulate the immune system of the tumor microenvironment after systemic injection. Alternatively, nanobodies can further be attached to the surface of nanogels loaded with multiple immune modulating molecules in order to trigger TAM repolarization after peritumoral injection. In summary, we believe that our nanobody approach may pave the road for targeted modulation of pro-tumoral TAMs during cancer immunotherapy.

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FLUORESCENCE CORRELATION SPECTROSCOPY STUDIES OF NANOCARRIER-BASED DRUG DELIVERY SYSTEMS

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Using nanoparticle-based carriers is an extremely promising way for the administration of therapeutic agents, such as drug molecules, proteins and polynucleotides. The nanocarriers can be used to increase the solubility of hydrophobic compounds, to protect their cargo from the environment, and if properly functionalized, to deliver it only to specific target cells and tissues. However, in order to get full advantage of this approach one needs a careful characterization of the nanocarrier systems at all stages of the drug delivery process: from their formation, to the possible interactions with e.g. plasma proteins in the blood stream all the way to the drug release in the cytoplasm of the target cells. In this regard, fluorescence correlation spectroscopy (FCS) offers a powerful and universal tool to measure the hydrodynamic radius, local concentration and fluorescence brightness of the studied fluorescent species thus allowing quantification of encapsulation efficiency, aggregation behavior, and kinetics of cargo release. In this contribution, we will present recent results on monitoring biootrhogonal reaction between antibody and a nanoparticle in blood plasma, quantifying a nanocarrier loading efficiency and drug release, as well as characterization of nanocarriers in full blood.

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CANNABINOID-CONTAINING POLYMERS AS NOVEL NANO DELIVERY PLATFORMS

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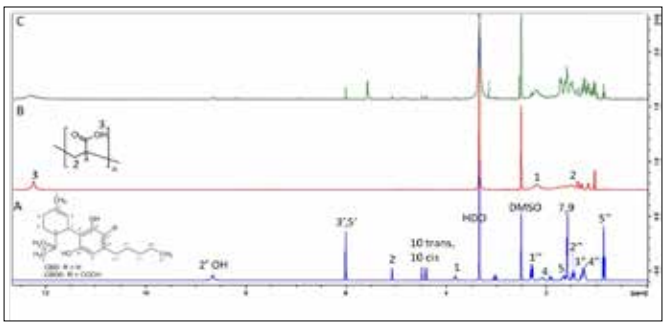

Figure 1. *^{1}H-NMR spectra of CBD (A), PAA(B) and the synthesized PAA-CBD conjugate (C). As shown in C, the PAA-CBD conjugate presents the peaks of both CBD and PAA. ^{1}H-NMR spectra were recorded on a 600.55 MHz spectrometer in deuterated DMSO.*

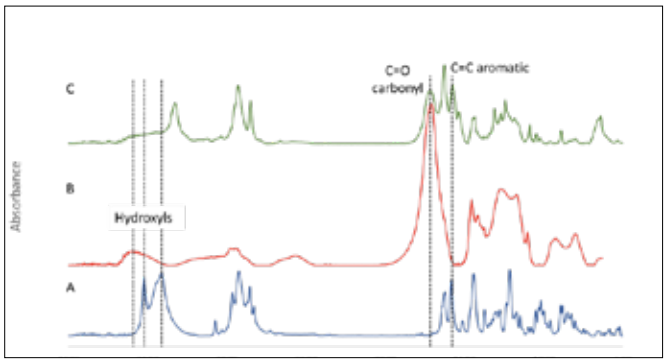

Figure 2. *FTIR spectra of CBD, PAA and PAA-CBD conjugation (A-C respectively) using KBr reveals that the conjugation was successful. FTIR spectra were recorded in an Equinox 55 spectrometer 1000 and 4000 cm^{-1} with 32 scans and a signal resolution of 4 cm^{-1}.*

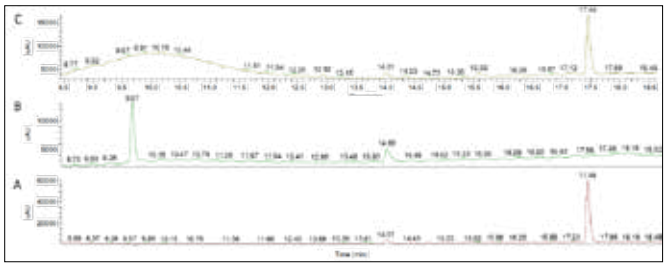

Figure 3. *HPLC-UV chromatograms of CBD (A) PAA (B), and the synthesized PAA-CBD conjugate (C). The CBD and the PAA exhibit sharp peaks at retention times 17.45, 9.67 min respectively in contrast to the conjugate peak that obtain broadening because the extent in the mass ranges.*

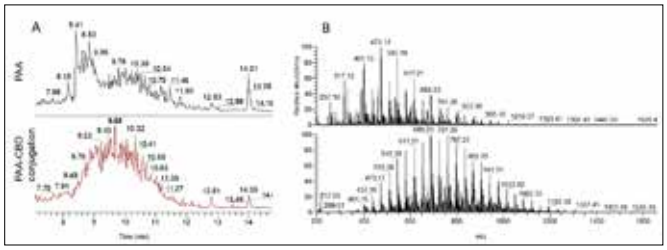

Figure 4. *LC-MS (A) negative ion mode chromatogram and (B) mass spectra (full mass range of ms 200.00-3000.00 of retention times 8-12 min) of PAA (top) and the synthesized PAA-CBD conjugate (bottom). The confirmation of the conjugation can be seen in different chromatogram shape of peaks between 8-11.8 minutes (A) and in mass shift in B, m/z of 257.10-803.30 for PAA and m/z of 401.15-1193.38 for conjugated polymer.*

Several approaches have been utilized for the delivery of hydrophobic drugs. Using micelles or polymeric nanoparticles (NPs) as drug carrier, is a very appealing approach. Nevertheless, their drawbacks include poor chemical stability and disintegration in systemic circulation, thereby limiting their clinical use[1,2]. Another approach is polymer-drug conjugation, where a hydrophobic drug is attached to a high molecular weight water-soluble backbone such as poly(ethylene glycol) (PEG). This approach often suffers from low drug loading[2].

Increasing evidence suggest on the therapeutic potential of *Cannabis* to treat a variety of diseases. Recently, considerable attention has focused on cannabidiol (CBD), a major phytocannabinoid in specific *Cannabis* chemovars. CBD is a non-psychotropic constituent of the plant that exhibits anticonvulsant, anti-inflammatory, and antioxidant properties. However its hydrophobic nature results in a poor bioavailability and absorption into the blood stream, hurdles in the CBD treatments[3-5]. In this research we propose a system that combines the advantages of drug encapsulation into polymeric nanoparticles and drug-polymer conjugation. We developed a delivery system where the hydrophobic CBD molecules are labily attached to a hydrophilic core. We further hypothesized that

by encapsulation of CBD into the formed conjugated particles we could achieve double release of CBD, both from the nanoparticles themselves and from the encapsulation.
Here we report on the first part of this research of design, including synthesis and characterization of the CBD-polymer conjugates, and the formation of NPs and their characterization. CBD was conjugated to the hydrophilic multifunctional polymeric backbone of poly(acrylic acid) (PAA) via a Steglich esterification reaction, resulting in amphiphilic conjugates. CBD, PAA and the synthesized CBD-PAA conjugates were analyzed by proton-nuclear magnetic resonance (^{1}H-NMR), Fourier transform-infrared spectroscopy (FTIR), ultra-high performance liquid chromatography UV (HPLC-UV) and liquid chromatography mass spectrometry (LC-MS) (Figures 1-4, respectively).
The NPs were produced by the nanoprecipitation and solvent evaporation method from ethanol solution in water. The size of the NPs was characterized by dynamic light scattering (DLS) and nanoparticle tracking analysis (NTA) at 25°C and 37°C. The average size that was measured was ~ 400 nm (DLS) and 118 nm (NTA). The NPS stability over time and at different temperatures was measured using DLS.
We concluded that PAA-CBD polymer conjugates were successfully synthesized and that the formed NPs in suspensions remained stable for more than five days. Moreover, we found that it is possible to prepare the NPs, to dry the suspension through freezdrying and to regenerate the NPs with simple addition of water even after two months. That make these NPs very appealing both in pharmacological and shelf-life aspects. The next steps of this research will be: measuring and calculating the amount of conjugated CBD per polymer; Investigating the stability and release properties of the CBD-based conjugates; Measuring the released CBD profile; Assessing the released CBD chemical stability and biological activity.

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STIMULATION OF IMMUNE CELLS WITH ADJUVANT-LOADED NANOPARTICLES IN THE CONTEXT OF MELANOMA THERAPY

JENNY SCHUNKE

In the context of medical applications, nanoparticles (NPs) are developed as versatile delivery systems for the treatment of tumors and infectious diseases. With their adjustable properties, nanoparticles enable protected drug transport and targeting of cells and tissues. Here, we have introduced a novel protein-based nanocapsule (NC) for effective, simultaneous delivery of antigen and adjuvant combinations to dendritic cells (DCs). The targeting of these professional antigen-presenting immune cells with NCs is of particular interest, since it allows guiding the immune response in a desired direction.
First, we were able to show that the combination of the TLR7/8 ligand resiquimod (R848) and the NOD2 ligand muramyl dipeptide (MDP) exerted superadditive stimulatory activity. Both adjuvants together, formulated in dextran-based NPs, triggered stronger DC stimulation than the combination of their soluble equivalents at equimolar concentrations. Furthermore, we encapsulated both adjuvants into protein-based NCs consisting of the model antigen ovalbumin (OVA), which allowed the combined delivery of antigen and adjuvants. These NCs showed a strong immunostimulatory potential towards DCs, which was analyzed by the expression of costimulatory surface markers, the secretion of pro-inflammatory cytokines, and the ability of accordingly treated DCs to mediate antigen-specific T cell responses.
In addition to high biocompatibility, our NCs are also characterized by good modifiability, which is of importance for planned *in vivo* approaches. By using this NC formulation as a therapeutic vaccine in the B16/OVA melanoma model we are going to investigate to which extent a DC-mediated anti-tumor immune response is triggered, and to evaluate the suitability of this vaccination platform in an OVA-independent B16/F10 tumor model.

SELF-ASSEMBLED NANOPARTICLES FOR STIMULATING ADAPTIVE AND INNATE IMMUNITY

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Introduction: Immunotherapy has gained increasing importance in clinical management of difficult-to-treat diseases including cancer and infections. Especially for cancer treatment, recent advances in immunotherapy using adoptively transferred T cells and monoclonal antibodies targeting the programmed death/ligand 1 (PD-1/PD-L1) axis have achieved ground-breaking clinical outcomes, including complete cure and long-term disease-free survival of late-stage cancer patients. Unfortunately, the remarkable therapeutic benefit of PD-1/L1 antibodies are only achieved in a fraction of patients. Hence, a huge unmet clinical need remains to render immunotherapy more efficiently in a broad population of patients. Drug interventions that stimulate adaptive and native immunity have been shown to potentiate cancer immunity. To address adaptive immunity, immunogenic cell death (ICD) has shown increasing potential. By ICD effect, dying cancer cells release antigens and danger-associated molecular patterns, which improve antigen presentation and cytotoxic T cell generation.[1] The immunomodulatory treatment turns “cold tumors” with low immunogenicity and poor immune infiltration into “hot tumors”, in which immunotherapy (e.g., checkpoint blockade therapy) exhibits the most optimal effectiveness.[2] The ICD effect of small molecule chemotherapeutics (anthracyclines, cyclophosphamide, and oxaliplatin) is often compromised due to their very low tumor disposition, which can be improved by nanomedicine-based tumor targeting.[1] The immunomodulation effect of nanomedicines can be further improved by pharmacological microenvironment modulators increasing tumor penetration of the nano-drugs, as well as infiltrating immune cells and immunotherapeutics such as checkpoint blockade antibod-

ies. In this study, self-assembled polymeric micelles stabilized by π-π stacking with enhanced loading capacities for multiple drugs and tumor targeting efficacy[3] are engineered with varying size to achieve optimal tumor penetration, which deliver anthracycline ICD inducers and pharmacological modulators to prime tumors for potentiating checkpoint immunotherapy. Moreover, imidazoquinoline-based toll-like receptor (TLR) 7/8 agonists have been shown to be potent innate immunity stimulants. However, they have severe side effects after injection due to leakage in the body.

Methods: The polymeric micelles are prepared from amphiphilic polymers synthesized by free radical or reversible addition-fragmentation chain transfer polymerization with different molecular weight via the nano-precipitation method. Various hydrophobic ICD-inducers and pharmacological tumor penetration enhancers are loaded in the micelles. The ICD induction and cytotoxicity are studied *in vitro*, and optimized formulations are injected in 4T1 tumor-bearing syngeneic mice to assess the immunomodulation effects and therapeutic efficacy in combination with anti-PD1 checkpoint blockade antibodies. Imidazoquinoline containing self-assembled nanoparticles were tested in a transgenic IFNβ+/Δβ-luc mouse model for its activation effect on innate immunity.

Results: Polymers with different molecular weights are synthesized, which form micelles with varying sizes between 30 and 100 nm. Hydrophobic compounds, (ICD-inducing) chemotherapeutics and pharmacological modulators, are efficiently loaded (loading capacity >20 wt%) in the micelles due to π–π stacking interactions. The PM induce significant ICD *in vitro* as characterized by calreticulin translocation and show high targeting efficiency *in vivo*. The micellar formulations induced complete tumor regression. In an immunocompetent mouse model of triple negative breast cancer (4T1), treatment with the nanomedicines induced enhanced infiltration of cytotoxic T cells in tumors. In addition, the imidazoquinoline containing self-assembled nanoparticles showed durable immunoactivation effect.

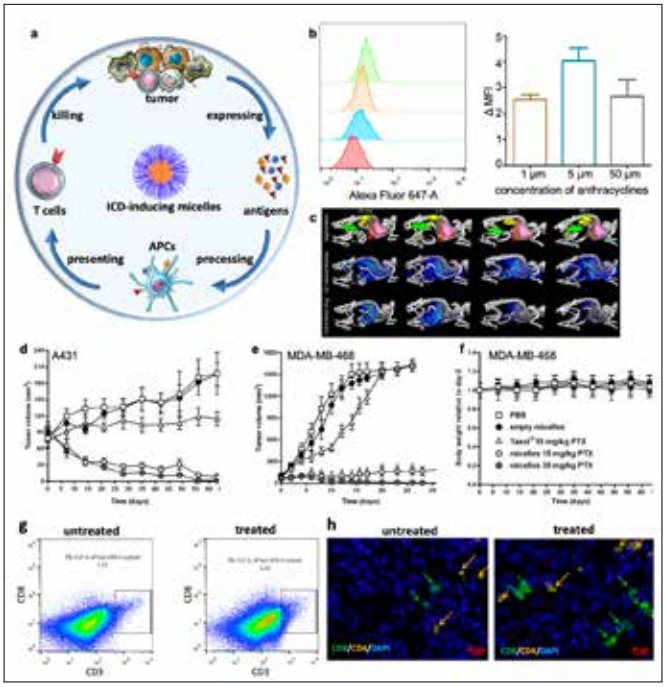

Figure 1: Self-assembled nanoparticles for stimulating adaptive and innate immunity. *Schematic illustration of the immune cycle which can be potentiated by ICD-inducing polymeric micelles (a). The ICD-inducing polymeric micelles induced significant calreticulin translocation of 4T1 cells in vitro assessed by flow cytometry (b) and efficiently targeted tumors as demonstrated by multimodal imaging (c). Paclitaxel-loaded polymeric micelles induced complete regression of both A431 (d) and MDA-MB-468 (e) with substantially prolonged survival (f). (g) Flow cytometry analysis of $CD^{3+}CD^{8+}$ cells in 4T1 tumor tissues after treatment, which was confirmed by fluorescence microscope (h).*

Conclusions: Self-assembled nanoparticles have been designed to stimulate adaptive and innate immunity, by inducing potent ICD and durable activation of the TLR7/8 pathway. These self-assembled nanoparticles that prime the tumor microenvironment are potential candidates in the setting of combination immunotherapy.

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A HIGH-LOADED LIPOSOME FORMULATION OF LAPATINIB DESIGNED BY EXPERIMENTAL DESIGN STRATEGY AND IT'S IN VITRO CHARACTERIZATION

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Breast cancer is a widespread cancer in women. As far as HER-2 amplification in these patients can lead to a more aggressive disease, targeting HER-2 can improve outcomes dramatically.

Lapatinib (Tykerb®) is a dual tyrosine kinase inhibitor, which was approved by USA food and drug administration in 2007 for the treatment of HER-2 positive metastatic breast cancer in patients having prior chemotherapy. It was also approved in combination with letrozol in 2010 for patients with hormone receptor and HER-2 positive breast cancer. The drug is poorly soluble in water (7 µg/ml), therefore absorption and bioavailability of oral dose is low and variable. On the other hand, lapatinib highly binds to plasma albumin (>99.9%) which can restrict its bioavailability. According to these features, for having a proper therapeutic response, patients should administer high daily doses of drug, which can cause serious side effects and limit its clinical use(1).

Liposomes are lipid-bilayer vesicles that can be used to carry different types of molecules. Biocompatibility, biodegradability, stability and non-immunogenicity are features of liposomes, which make them a good choice for encapsulating different compounds. Using liposomes as drug delivery systems can help improve the therapeutic effect and lower the side effects of the encapsulated drug(2).

Experimental design strategy is a good way to limit the number of experiments meanwhile, achieving the best outcomes by carrying out only the necessary experiments. Effect of various factors including phospholipids, cholesterol, pH, drug to lipid ratio, etc. on liposome size, zeta potential, poly dispersity index (PDI) and drug loading capacity can be investigated using this approach.

In the current study, we designed an experiment in order to have a liposome formulation containing lapatinib with suitable characteristics.

MATERIALS AND METHODS
EXPERIMENT DESIGN

A statistical experimental design based on D-optimal was carried out to investigate the effect of variables on liposome's characteristics. Tested variables were drug to lipid ratio (D/L), cholesterol content and final pH of the formulation. Total molar ratio was 50 mMolar for all formulations. Z-average, PDI and encapsulation efficacy percentage were evaluated as responses and data was analyzed using Design Expert software (version 10.0.4). According to the results, the formulation with optimal characteristics was picked and further procedures were done.

LIPOSOME PREPARATION

Briefly, phospholipids, cholesterol and LP (a chloroform-methanol stock solution) were mixed at the molar ratios presented in the Table1. The organic solvent was evaporated and then the formed film layer was hydrated with buffer solutions. Liposomal formulations were hydrated in a 65°C water bath in order to set the temperature over phospholipids Tm. Liposomes were then sonicated for 1 hour in a 65˚C bath sonicator. In order to exclude the un-entrapped drug the formulation was first slightly centrifuged for 2 minutes at 1000 g and then the supernatant was filtered through a 0.45 filter.

CHARACTERIZATION OF LIPOSOMES

Liposomes particle size and poly dispersity index (PDI) were determined by dynamic light scattering (DLS) using a particle size analyzer (Malvern Instruments, UK). The zeta potential of liposomes was determined after a 20-fold dilution in NaCl 10mM.
The concentration of the entrapped drug in liposomes was determined using a validated UV-Spectroscopic method.Furthermore, morphological characteristics of liposomes were determined by transmission electron microscopy (TEM). In order to assess the release profile of liposomes, 2 ml of the formulation was placed in a dialysis bag and dialyzed against PBS buffer (pH 7.4) plus 30% FBS at 37°C. Samples were collected at different time points and the amount of drug was measured by spectroscopy. Stability of the formulation was evaluated by assessing size distribution and loading efficacy at time points of 0,1,3 and 6 months at 4˚C (Table 2).

CELL CYTOTOXICITY ASSAY

Cytotoxicity of the formulation was assessed on TUBO cell line, which is a HER-2 positive breast cancer cell line. Then the optical density (OD) of samples was read at 550 nm using a Multiskan plus plate reader. The IC50 of formulation and free drug was calculated using PRISM software (Table 3).

RESULTS
LIPOSOMES PREPARATION AND CHARACTERIZATION

In current study, one formulation was selected out of 20 other tests, which were run according to the experiment design. A formulation consisting of HSPC/DPPG/mPEG2000-DSPE/cholesterol/LPT (molar ratio of 55/5/5/40) was prepared with total concentration of 50 mM. Particle size, PDI, Z-average and EE% are shown in Table1. TEM image of liposomes is shown as Figure 1, the size of the spherical shaped liposomes was about 100 nm and they were relatively uniform, which was consistent with data in Table 1.

Table 1 - *Characterization of Liposomes*

Particle Size				PDI	Zeta Potential (mV)	Drug Amount (µg/ml)	Encapsulation Efficiency Percentage
Z Average (nm)	Intensity (nm)	Volume (nm)	Number (nm)				
123.4	142.3	105.4	64.45	0.229	-12.2	818.2	93.8%

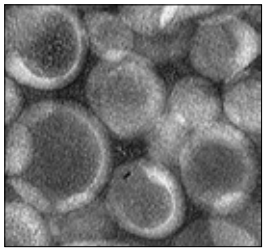

Figure 1- *Transmission electron microscopy (TEM) images of negatively stained liposomes containing lapatinib*

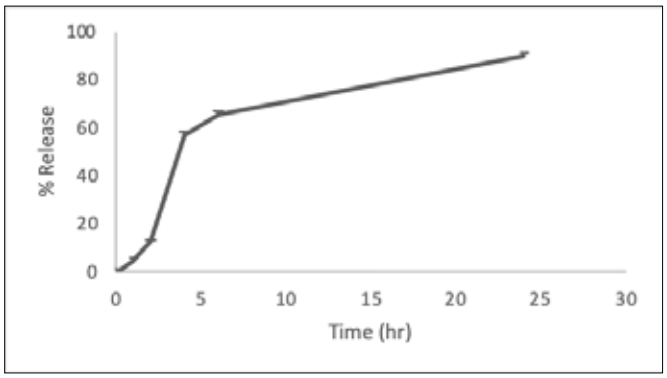

Figure 2- *Lapatinib release profile from liposome formulation at 37°C in PBS + 30% FBS medium*

Table 2- *Stability study of liposome formulation at 4°C*

Time intervals (months)	Z Average (nm) ± SD	PDI ± SD	% Encapsulation Efficiency ± SD
0	123.4 ± 1.48	0.229 ± 0.01	93.8 % ± 0.56
1	121.3 ± 1.89	0.212 ± 0.01	93.2 % ± 1.21
3	123.8 ± 1.3	0.207 ± 0.01	92.8 % ± 0.94
6	125.2 ± 0.6	0.234 ± 0.02	90.4 % ± 1.54

Cell Cytotoxicity Assay
IC50 of empty liposomes, free drug and liposomes containing LPT are shown in table 2.

Table 3 - *Cell Cytotoxicity Assay*

	Empty liposomes	Free LPT	Liposomes containing LPT
IC50	564 µM	19.27 nM	120.4 nM

CONCLUSION

In this study, a liposome formulation which had the best characteristics was picked out of 20 other formulations. Z-average, PDI and zeta potential values were satisfactory and were confirmed by TEM microscopy. The IC50 of liposomes was higher than the free drug, but they were capable of inhibiting cell growth successfully. It would be notable that this project was part of a bigger project; two other formulations are being characterized and their *in vitro* plus *in vivo* effects are being investigated. Complementary results will be published as soon as possible.

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MITOCHONDRIAL PHOTO FIELD-EFFECT TRANSISTOR: QUANTUM TUNNELING & PHOTO SENSITIVITY – MODEL EXPLAINING THE BRAIN DEATH DOGMA CHALLENGE

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Low level light therapy (LLLT) uses moderate light intensities (comparable to that of the sun prior to its attenuation by the atmosphere) delivered by red to near infrared (R-NIR) lasers or light emitting diodes (LED) operating in the 1 - 4 J/cm^2 dose window (Figure 1A) found to be effective both *in vitro* and *in vivo*. LLLT has an enormous clinical potential, alone and complemented with drugs and nanoparticles. LLLT started with the treatment of non-healing diabetic ulcers and continued with the treatment of dementia, stroke, traumatic brain injury, painful peripheral neuropathy, retinal disorders, depression, oral mucositis in cancer patients, burns,

inflammatory processes, infertility and cosmetic surgery (Figure 1B). LLLT is the tool of choice for oxidatively stressed cells, for instance, in an infected wound or the ischemic penumbra in a stroke, safeguarding accelerated cell proliferation and survival, respectively. Besides this list of exemplary clinical applications there is a growing number of exciting results achieved *in vitro* and in animal studies with a potential for translation in clinical trials. LLLT has an enormous potential in cell-based therapies where cell survival is critical [1]. In addition, LLLT has a substantial impact on the cosmetic use of LEDs. In October 2008 we published an article reporting for the first time on the use of an LED-based device in skin rejuvenation. The device used 670 nm LEDs and was initially designed to accelerate wound healing. The work initiated an unprecedented trend in the development and commercialization of LED-based devices for skin rejuvenation.

BRIDGING BETWEEN LLLT AND NANOMEDICINE

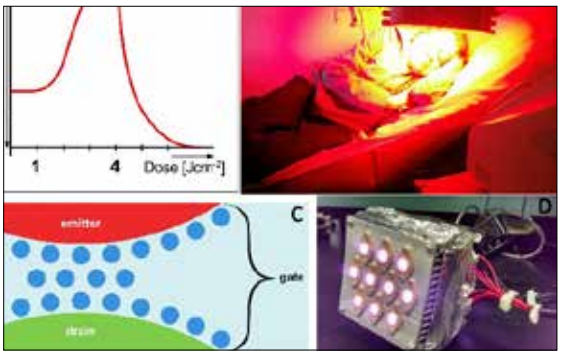
LLLT, a branch of laser medicine, shows excellent reproducibility both in the lab and clinical practice. The results could be explained by postulating the absorption of R-NIR photons by cytochrome c oxidase (COX) in mitochondria [2]. As documented [3] this theory is rather ill-defined and therefore it no longer can be counted as part of the theoretical framework proper. Inspired by laboratory experiments based nanotechnology we presented a new scheme to explain the interaction of R-NIR photons with cells [4]. The scheme involves the interaction of R-NIR photons with nanoscopic water layers (NWL) comprising 2-3 monolayers of H_2O and is relevant in nanomedicine. For instance, a 1 min exposure of cancer cells to non-invasive pulses of 670 nm laser light forced the cells to uptake anti-cancer drugs [5]. The carrier medium is the fraction of the cytosol which persists as bound water within the cell, i.e., NWL bound to membranes, organelles and macromolecules, predominantly hydrophilic surfaces in the intracellular space, which experience an instant volume expansion in response to the light pulse [6]. When the light is off, the cytosol consolidates and drugs or nanoparticles are sucked into the cell. As discovered later, the volume expansion is accompanied by a viscosity reduction; it is observed exclusively on hydrophilic surfaces [7], where NWL viscosities present values comparable to those of molasses. Interestingly, the mitochondrial nanoturbine (ATP synthase) rotates in such an environment with 9000 rpm.

Mitochondria run two vital functions: They supply energy in the form of adenosine triphosphate (ATP) and respond to oxidative stress by producing reactive oxygen species (ROS). While low levels of ROS are beneficial to the cell, high levels can drive the cells into apoptosis. Important in this context is the chemical constitution of ROS – in general oxygen with a negative charge. Extended bombardment with ROS accentuates the polarity of hydrophilic surfaces; they become more hydrophilic and the viscosity of NWL increases accordingly, in particular under confinement. This explains the effectivity of LLLT in oxidatively stressed systems. The light is instrumental in balancing the increased viscosity within and around the mitochondrial rotary motor. ATP upregulation under oxidative stress in response to LLLT is a well-documented phenomenon. The rotary motor is the first intrinsic target of the R-NIR photons.

MITOCHONDRIAL PHOTO FIELD-EFFECT TRANSISTOR

Translation of the effects of R-NIR light-induced volume expansion and viscosity reduction into biology, endorsed by the finding that most of the mitochondrial water prevails in the form of bound water [8], puts us in the position to cross into the territory of quantum biology. The second intrinsic target of R-NIR photons in LLLT is the NWL confined between the mitochondrial enzymes COX and cytochrome c (CYTc). Shimada *et al.* [9] reported the presence of 3 monolayers of H_2O between the enzymes (Figure 1C). From the predominantly hydrophilic nature of the proximal enzyme sites it inescapably follows that exposure of the enzyme complex to biostimulatory intensities of R-NIR light will cause an instant drop in the viscosity of the NWL [7], complemented by a volume expansion [6]: For electrons crossing between the interacting enzymes this means a longer pathway relative to non-irradiated systems. In accordance with the physical picture we are led to interpret the NWL sandwiched between CYTc and COX in terms of a quantum mechanical barrier. For electrons crossing between the enzymes quantum tunneling recommends itself as the most probable mechanism of transfer. It is instructive to consider electrons of the kinetic energy *E* tunneling across the NWL barrier. The electrons are incident from one side on a potential barrier of height *U-E,* where *E<U*. The transmission probability *T* for an electron to cross a barrier of width *L* is equal to $T = e^{-2kL}$ (1), where $k = \frac{\sqrt{2m(U-E)}}{\hbar}$ (2) with *m* and $\hbar$ standing for the electron mass and Planck constant h divided by 2π, respectively. Clearly, *T* decreases exponentially with the width of the barrier. Translation of the trend described by (1) to the interfacial constellation portrayed by Shimada *et al.* [9] indicates that even a minimal increase in spatial separation between CYTc and COX, caused by the perturbation of the NWL, results in a substantial reduction of the electron flow between them. Alteration of the molecular structure of NWL occurs by collective hydrogen bond excitation [6]. The excitation is different for different wavelengths of light [10]. The working principle of the mitochondrial enzyme system corresponds to that of a field-effect transistor (FET). The elements CYTc (emitter), COX (drain) and NWL (gate) form a perfect biological photo FET (Figure 1C) in which the gate is controlled by photons [4]. The model provides an intuitive explanation to the discovery of the Hüttemann group [11,12]. After a scan from 700 to 1000 nm, 2 frequencies (750 and 950 nm) were found to reduce the activity of COX (via inhibition of the reaction of CYTc and COX) and limited ROS generation. The discovery is clinically relevant, as reflected by the recovery of pigs 13.5 minutes post cardiac arrest, resuscitation, and continuous irradiation of the foreheads for 2 hours with an intensity of $2W/m^2$ distributed on 10 powerful LEDs – 5 operating at 750 and 5 at 950 nm (Figure 1D) – Mike Hüttemann, personal communication. From the postulate claiming that COX is the principal absorber for R-NIR light [2] one would expect a strong absorption of COX at 750 and 950 nm. However, there is virtually no absorption for these wavelengths. Together with the results of Lima *et al.*, who reported that even cells lacking COX proliferated in response to 660 nm laser irradiation [13], this is a final coup de grâce to the COX postulate. What is so special in the wavelengths 750 and 950 nm? The answer is provided by a closer inspection of the solar spectrum (Figure 2).

Figure 1. *A: The classical biostimulatory dose window used in LLLT. B: Rapid wound healing and minimal scar formation – indication for LLLT after cosmetic surgery. C: Principle of the biological FET: Tunneling of electrons across the gate formed by 3 monolayers of H_2O (blue), confined between the CYTc (red) and COX (green), is controlled by R-NIR photons. Image adapted from ref. 9. D: Powerful LEDs used to rescue pigs from terminal brain damage 13,5 minutes post cardiac arrest. Courtesy: Maik Hüttemann.*



MITOCHONDRIAL PHOTOSENSITIVITY: TWO WINDOWS TO ESCAPE THE VICIOUS CIRCLE OF ROS

There are 2 pronounced minima in the R-NIR sector of the spectral solar irradiance, at 750 and 950 nm [14]. During evolution mitochondria (assumed age >1.45 billion years) were not exposed to light except the sun, so the simplest argument accounting for the effect of 750 and 950 nm LED light is that the mitochondrial photo-FET apparatus had no opportunity to adapt to the perturbation of the photo-FET gate, caused by these wavelengths. Considering that NWL dictate protein reactions [15] it is plausible that lack of adaptation of the archetypical photo FET to the action of 750 and 950

nm, e.g., lack of an adequate compensatory mechanism, results in a stronger perturbation of the NWL confined between CYTc and COX, relative to other wavelengths of the solar spectrum. Here, adaptation can be defined as cooperative survival response of the mitochondrial respiratory system to detrimental effects of the solar irradiation, e.g., its inhibitory effect on the tunneling of electrons across the H_2O barrier confined between CYTc and COX [16]. Together with the findings of the Hüttemann group this holds the promise to change the accepted brain death dogma

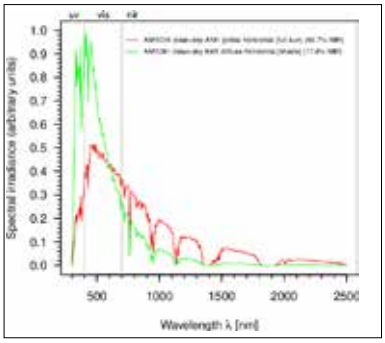


Figure 2. *Solar spectral irradiances incident on a horizontal surface in full sun (red) or shade (green [14]. Courtesy: Ronnen Levinson, Heat Island Group, Lawrence Berkeley National Laboratory.*

HERPES LABIALIS: NO MORE CREAMS

Infection with HSV-1, the virus responsible for herpes labialis, is suspected to be a strong risk factor in the formation of Alzheimer's disease. Untreated symptoms last 1 to 2 weeks. The classical treatment consists in topical application of Acyclovir, which may reduce the duration of the symptoms. There are reports on the use of LLLT to treat herpes labialis. For example, recovery time was shorter by 1 day in subjects who received LLLT compared to the Acyclovir group and by 2 days compared to the placebo group [17]. Effective treatment of herpes labialis needs early intervention. About 2 days before outbreak patients notice symptoms such as itching, burning or tingling. Irradiation of the affected area at the time of the manifestation of the first symptoms with 650 nm laser light (2 W/cm²) for 5 minutes, twice a day for 2 consecutive days, prevented the outbreak (Figure 3). There was no inflammation, no pain, no bleeding, only a mild warm sensation. The experiment was reproducible with identical results. What is the root cause of the antiviral effect of the laser light? Macrophages use ROS to combat bacteria and viruses, and are activated by R-NIR light. However, ROS is for macrophages a red flag. So it can be safely assumed that the virus is kept under control by mitochondrial ROS, triggered by the high laser intensity, making eye protection mandatory. Presumably, the ROS-suppressing wavelengths 750 and 950 nm are not suitable for this protocol. Possible involvement of HSV-1 in the etiology of Alzheimer's disease and rapid recovery from herpes labialis are sufficient motivation for further research efforts.

Figure 3. *A: Herpes labialis treated with Acyclovir, 8 days after manifestation of first symptoms. B: Herpes labialis treated with red laser light, 4 days after manifestation of first symptoms N.B.: The intensity of the laser was ca. 15 times that of the solar constant.*

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POLYMER NANOPARTICLES FOR DELIVERING A MODEL ANTIGEN TO DENDRITIC CELLS

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Delivering antigens to dendritic cells is a powerful approach in vaccine development, since dendritic cells are the most important and efficient antigen presenting cells in the body and are able to induce a strong and long-lasting T cell immunity efficiently protecting us against various pathogens [1].Polymer nanoparticles offer an excellent platform for the antigen delivery to dendritic cells. Due to their size, dendritic cells can easily capture and process them [2]. Additionally, they provide a broad range of possibilities to associate antigens with the nanoparticle, are biocompatible, non-toxic, and known to enhance the immune response towards antigens [3].
Therefore, the goal of this study was to develop a nanoparticulate system capable of delivering the model antigen ovalbumin to dendritic cells and enhance immunogenicity.
We covalently coupled ovalbumin to the surface of PEG-PLGA nanoparticles which were previously modified with a peptide linker. The amount of ovalbumin per nanoparticle was determined with a BCA assay and the absence of adsorbed ovalbumin was verified by SDS PAGE. Particles were further characterized by measuring size, polydispersity index (Fig. 1) and zeta potential. First, we investigated nanoparticle uptake in bone marrow derived dendritic cells (BMDCs). For uptake studies, we fluorescently labeled the nanoparticle core and determined cellular internalization after different incubation times by flow cytometry (Fig. 2).

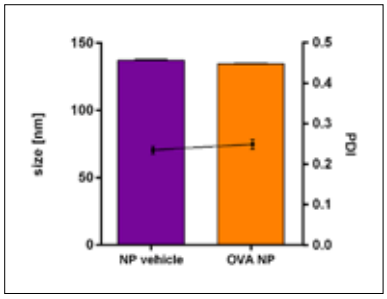

Fig. 1. *Size and polydispersity index (PDI) of nanoparticles with and without anitgen.*

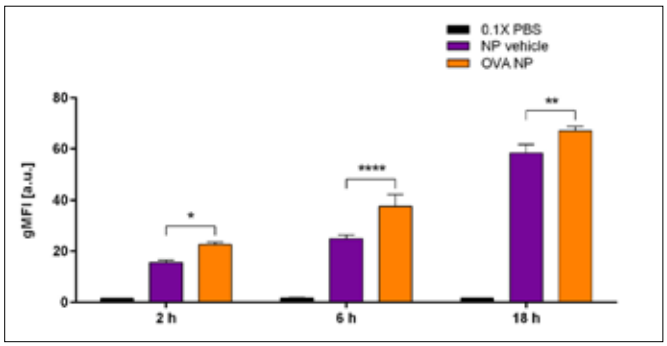

Fig. 2. *Uptake of nanoparticles in BMDCs after different time points.*

The uptake studies revealed that anitgen presenting and control nanoparticles were both internalized by dendritic cells. This is due to the fact that the particle sizes are under 500 nm, which is optimal for uptake by dendritic cells [2]. However, the OVA NP showed a significantly higher uptake at every time point compared to the nanoparticles without antigen, suggesting that the glycoprotein ovalbumin on the surface of nanoparticles enhances the internalization by addressing uptake mediating receptors like mannose receptor CD206 In addition to the uptake studies, we evaluated whether nanoparticles are able to stimulate BMDCs. Therefore, cells were incubated for 18 h with CpG ODN 1826 and OVA NP, soluble ovalbumin or NP vehicle. LPS was used as positive control. Afterwards, stimulation pattern was analyzed by detecting different maturation markers on the surface of dendritic cells using flow cytometry (Fig. 3).

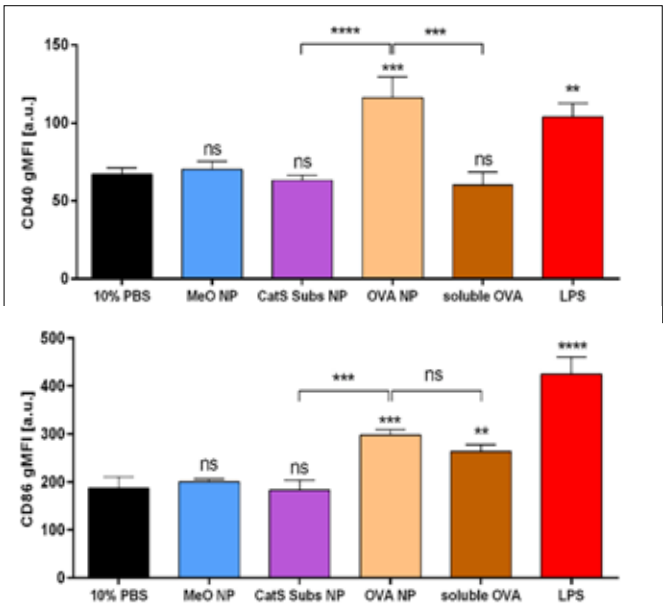

Fig. 3. *Stimulation pattern of BMDCs after treatment with CpG ODN 1826 and different nanoparticle formulations or soluble ovalbumin. LPS was used as positive control.*

The stimulation experiment showed a higher degree of activation for the particle associated ovalbumin compared to the soluble antigen, suggesting that the nanoparticle system enhances the immunogenicity of ovalbumin. In contrast, the antigen-free nanoparticles did not show any activation of BMDCs, indicating that they are pyrogen-free and not immunogenic.Here we show that we successfully developed a nanoparticle platform for delivering the model antigen ovalbumin to dendritic cells. The results confirm that the particle system is taken up by BMDCs and is able to activate them to a higher degree than the antigen in its soluble form. More so, this shows the potential of the technology to be used as vaccine platform.

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CUSTOMIZABLE IMMUNE MODULATING NANO-GEL-PLATFORM FOR CANCER IMMUNOTHERAPY

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Nano-sized delivery systems with stimuli-responsive targeting properties can provide unique opportunities to explore novel frontiers in cancer immunotherapy. However, facile and straightforward access to multifunctional as well as biodegradable nanocarriers remains a key challenge for such therapeutic applications. Self-assembling amphiphilic block copolymers provide access to micellar nanoparticles readily, yet, with limited degree of functionalization, drug load or stability under physiological conditions. We, therefore, developed a polymer-based nanogel platform based on reactive precursor block copolymers, which allow the straightforward covalent attachment of immune modulating molecules into the core. Additional incorporation of ketal-crosslinks exhibits stimuli-responsive particles that decompose into single polymer chains upon exposure to endosomal pH. Those nanogels enable localized immune responses to the tumor microenvironment and its draining lymph nodes after peritumoral injection and, thus, trigger anticancer immune responses towards tumor regression. Furthermore, they can be fabricated with orthogonal reactive groups that are exposed to the carrier surface and, hence, allow the co-delivery of tumor-specific antigens. After intravenous injection those conjugates elicit reduced tumor growth, both in prophylactic and therapeutic therapy approaches. Consequently, this customizable platform can be used to deliver diverse immune modulating cues into the immune system and thus, provides opportunities to resolve further needs in immuno-engineering.

Ref.: [1] J. Stickdorn, L. Nuhn, Reactive-ester derived polymer nanogels for cancer immunotherapy, Eur. Polym. J. 124 (2020) 109481. doi:10.1016/j.eurpolymj.2020.109481.

REDUCED MAGNETIC COUPLING IN ULTRA-SMALL IRON OXIDE T_1 MRI CONTRAST AGENTS

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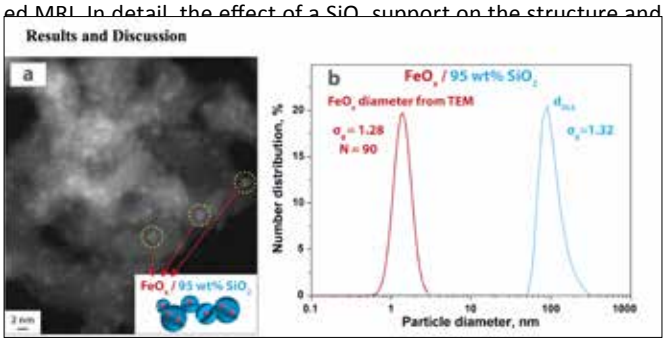
Contrast agents for magnetic resonance imaging (MRI) are essential for evidential visualization of soft tissues pathologies. Contrast-enhanced MRI can be carried out with T_1- and T_2-weighted sequences that require as contrast agents paramagnetic and superparamagnetic materials, respectively. The T_1-weighted imaging is frequently preferred over T_2-, as it induces a bright contrast for sharper image analysis and allows more rapid image acquisition. Commonly used and FDA-approved T_1 contrast agents, however, were shown to be associated with nephrogenic systematic fibrosis due to Gd^{3+} release from the injected complexes.[1] Ultra-small iron oxide nanoparticles

(< 5 nm) are potential alternatives, as they gain the desired paramagnetic properties due to a reduced magnetic volume and increased surface defects.[2]

In this study, flame aerosol technology is used for the synthesis of ultra-small iron oxide nanoclusters as contrast agents for T_1-weighted MRI. In detail, the effect of a SiO_2 support on the structure and



Iron oxide nanoparticles (FeO_x) are co-produced with varying amounts of SiO_2 (0 – 95 wt%) as a support material by flame spray pyrolysis (FSP). Figure 1a shows a z-contrast TEM image of FeO_x / 95 wt% SiO_2. In the inset, the light silica matrix is homogeneously covered by isolated FeO_x clusters appearing as small bright spots. Three of these FeO_x clusters are exemplarily circled by a dotted line. Figure 1b shows the number size distribution of the FeO_x clusters having an average diameter of 1.5 nm with a geometric standard deviation σ_g = 1.28. In comparison, pure FeO_x has an average primary particle diameter, d_{BET}, of 3.9 nm. The small FeO_x size in these particles can be explained by the inhibiting effect of SiO_2 on the gas-phase formation/growth of FeO_x.

To stabilize the FeO_x / SiO_2 nanoparticles in liquid dispersion, bovine serum albumin (BSA) was adsorbed onto their surface.[3] Figure 1b shows the hydrodynamic diameter distribution (d_{DLS}) of FeO_x / 95 wt% SiO_2, indicating a stable agglomerate size of approximately 100 nm (σ_g = 1.32).

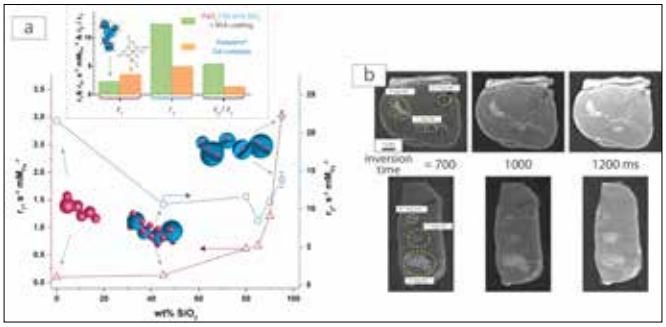


Figure 2: *(a) Relaxivities r_1 (triangles, left axis) and r_2 (circles, right axis) of BSA-coated FeO_x / SiO_2 as a function of SiO_2 content. Inset depicts comparison of BSA-coated FeO_x / 95 wt% SiO_2 with the clinical contrast agent Dotarem (Guerbet). (b) T_1 weighted magnetic resonances images at different inversion times of two different pork skeletal muscle tissues injected with suspensions of FeO_x / 95 wt% SiO_2 at different concentrations.*

Efficient contrast agents for T_1 MRI require high r_1 and low r_2 values as well as low r_2/r_1 ratios.[4] Figure 2a shows T_1 (triangles, left axis) and T_2 (circles, right axis) relaxivities of the as-prepared flame-made FeO_x / SiO_2 with BSA-coating measured as a function of SiO_2 content. The r_1 remains insignificantly low until a SiO_2 content of 45 wt% is reached and thereafter increases up to 3 s^{-1} mM_{Fe}^{-1}. The r_2 for pure FeO_x is r_2 = 21.6 s^1 mM_{Fe}^{-1}. The addition of SiO_2 up to 85 wt% content decreases r_2, before slightly increasing it to 13.8 mM_{Fe}^{1} above that content.

The trend of r_1 can be explained by the reduction of the magnetic domain size. In pure FeO_x the magnetic primary particles are in close contact. This leads to strong magnetic coupling between them, increase in effective magnetic size and low r_1, although the primary particle size (< 4 nm) by itself would imply a good T_1-contrast enhancement. The addition of SiO_2, however, leads to increased spacing between the FeO_x particles and therefore reduced magnetic coupling.[5] This results in a smaller effective magnetic size down to primary particle or crystal FeO_x size. As a result, the r_1 increases, since the FeO_x structures can now act independently and according to their size. This effect is further enhanced by the reduction of FeO_x size with increasing SiO_2 content compared to bare FeO_x.

Changes in MRI relaxivities due to magnetic coupling have been reported previously, however, only for larger nanoparticles.[6] It was found that r_1 is affected only slightly by agglomeration of iron oxide nanoparticles, while r_2 increases for increased particle-particle interactions.[7,8] This is not in agreement with the trends found here, where both relaxivities increase with decreased magnetic coupling. As FeO_x / 95 wt% SiO_2 showed both the highest r_1 and lowest r_2/r_1 of all prepared particles, they were coated with BSA and compared to a clinically applied Gd-complex (Dotarem, Guerbet, France). It can be seen that especially for r_1 (FeO_x / SiO_2: 2.3 s^{-1} mM_{Fe}^{-1}; Dotarem: 3.5 s^{-1} mM_{Fe}^{-1}) both systems perform comparably. The larger difference in r_2 (FeO_x / SiO_2: 12.4 s^{-1} mM_{Fe}^{-1}; Dotarem: 4.9 s^{-1} mM_{Fe}^{1}) can be explained by the partly remaining super-paramagnetism of the FeO_x particles.

As a proof-of-concept the best performing FeO_x / 95 wt% SiO_2 particles were injected into pork skeletal muscle tissues at different concentrations (0.1 – 5 mg mL^1). Figure 2b shows their T_1 weighted magnetic resonance images at different inversion times. The injected suspensions (250 µL) gave the desired bright contrast for all concentrations and inversion times. As expected, 5 mg mL^{-1} resulted in the strongest contrast spot. This simplified clinical scenario verifies the excellent contrast enhancement properties of the prepared particles, even in fatty and therefore hard-to-image muscle tissue.

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NORMALIZATION OF TUMOR BLOOD VESSELS, A REGULATION FACTOR IN TUMOR MICRO-ENVIRONMENT AND IMMUNE SYSTEM FUNCTION

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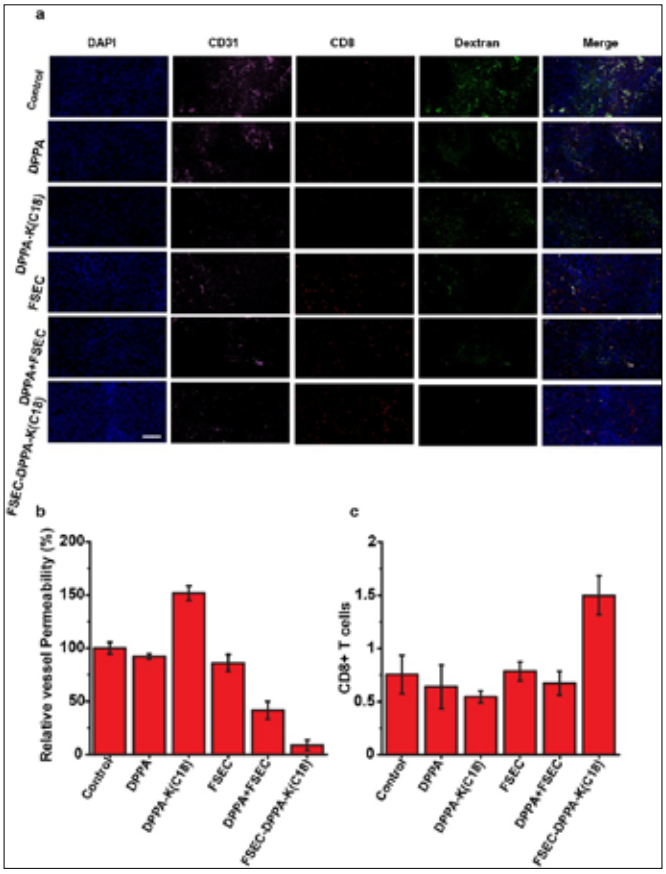
Inhibiting the Vascular Endothelial Growth Factors (VEGF1, VEGF2) and their receptors is an effective way for remodeling the vessels structure. Normalization of vessels in tumor microenvironment have effects on increasing the penetration of nano-medicines and drugs. Another aspect of normalization is enhancing the infiltration of immune cells in the tumor site. Up-regulation of the immunosuppressive pathway by normalization of tumor vessels fascinate the activation of the immune system. Interaction of Programmed Death Ligand (PD-L1) on the tumor cells and its receptor (PD-1) on immune cells is one of the effective types of tumor immune escape pathways. Blocking the combination of PD-L1 and PD-1 by using antagonists, breaks up the immune suppressive pathways and increase the T cells function in tumor side to abolished cancer cells. Preclinical and clinical data have reported the successful application of several antibodies for the blockade of these checkpoints but the newly introduced peptide for targeting and blocking the PD-L1/PD-1 pathways shows promised therapeutic responses *in vitro* and *in vivo*. Dual targeting of tumor microenvironment using a conjugated peptide enables us to inhibit VEGF and PDL-1 simultaneously. These targeting peptides conjugated to each other by three amino acids that are responsive to the specific tumor microenvironment enzyme known as Legumain. The stable shape of nanoparticle after response to the TME which is provided by a long carbonic chain as a hydrophobic structure cusses higher efficiency of PD-L1 blocking. These two smart sequences make a cycle to reproduce each step toward another to enhance and activate the immune system. Figure 1 depicted the infiltration of $CD8^+$ immune cells after normalization and decrease leakage of vessels shown.

Keywords: Immune system, tumor microenvironment, peptide, legumain



Figre 1. Improvement of vessel leakage after normalization and enhance effect on immune cells infiltration. *After two weeks treatment with our different peptide sequences. (a, b) Representative fluorescence images of the tumor tissue perfusion with Dextran–FITC. After normalization of vessels, blood leakage decreased. (a, c) By normalization of vessels, number of $CD8^+$ T-cells infiltration enhanced. (CD31 represent endothelial cells and alpha SMA pericytes)*

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BISALKYLATED POLYSARCOSINE-BASED LIPOPOLYMERS

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Stealth liposomes on the basis of PEG have promoted the development of nanoparticle-based drugs for cancer therapy and chronic diseases. Additionally, multiple PEGylated nanomedicines have advanced into clinical trials. However, immune responses caused by hypersensitivity reactions toward PEG have been reported which in turn reduce the drugs's therapeutic efficiancy substantially. Thus, alternatives to these PEGylated lipids with comparable solution properties are required. In this work, we report the synthesis of polysarcosine-based lipids with bisalkylated amines as initiators. The lipopolymers were obtained by nucleophilic ring opening polymerization of sarcosine NCA. The respective lipopolymers could be synthesised with precise control over chain length, end group integrity and low polymer dispersity (Đ < 1.2) as characterized by size-exclusion chromatography, ^{1}H nuclear magnetic resonance spectroscopy, ^{1}H-DOSY and MALDI-ToF mass spectroscopy.
Sarcosineylated lipids show enhanced biocompatibility that means enlongated blood circulation time in the zebrafish modell and reduction in the macrophage recognition. Additionally, complement activation which is a main compenent in hypersensitivity reactions toward PEGylated liposomes can be significantly reduced by incorporating these lipopolymers into the surface of liposomes.

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SUPRAMOLECULAR PLATFORM FOR THE DESIGN OF MODULAR MULTIFUNCTIONAL GLYCO-CONJUGATE ANTITUMOR VACCINES

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The emerging field of nanomedicine has gained wide attention as the use of molecular toolboxes offers versatile advantages over traditional medicinal approaches. Especially for antitumor vaccines in the context of cancer immunotherapy, there is a big need for sophisticated carrier systems. Taking advantage of the intrinsic ability of peptides to form well-ordered secondary structures enables the design of fully synthetic monomers which undergo self-assembly in aqueous media building up nano-sized supramolecular structures.[1,2] Presentation of immunogens on the surface of those entities renders them pathogen-mimicking, thus constituting potential vaccine candidates. Tumor immunotherapy presents us with the special task of circumventing the self-tolerance mechanisms resulting from the endogenous background of tumor-associated antigens leading to the essential need of co-stimulating B-cells by T-helper cells. Trying to face this challenge, we employ multifunctional supramolecular polymers to co-present different epitopes as well as immunostimulants in a multivalent fashion.

Mucin 1 (MUC1) represents a promising antigenic target structure for such an undertaking as it is known to undergo drastic alterations in its O-glycosylation pattern during tumorigenesis due to a dramatic change in enzyme activity. Fully synthetic, 22 amino acids long MUC1-derived glycopeptides bearing sialylated tumor-associated carbohydrate antigens are thus used as B-cell epitopes.[3,4] T-cell stimulation is achieved by the incorporation of different epitopes, mainly short fragments derived from the extremely immunogenic tetanus toxin (p30). The application of stimulants like an imidazoquinoline as TLR7/8 agonist[5] completes the toolbox of immunoactive substances. Conjugation with peptide amphiphiles results in functional monomers from which potential supramolecular antitumor vaccines of different composition were obtained upon mixing in water and characterized by CD-spectroscopy and TEM. To investigate the immunologic potential, the formulations were administered to C57BL/6 mice intraperitoneally and the antisera were collected after three booster immunizations. Using ELISA, high IgG antibody titers were observed and the high binding affinity of the antibodies to T47D cells could be confirmed by FACS measurements. The results clearly prove the feasibility of this new modular supramolecular approach and encourage further improvement of the system.

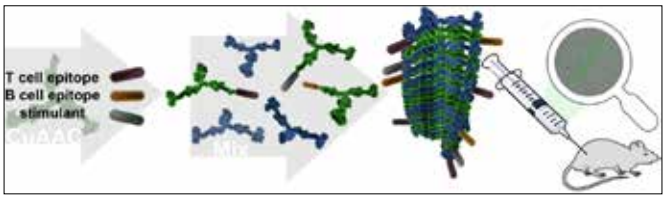

Figure 1: *Schematic illustration of the modular concept of a multifunctional pathogen-mimicking supramolecular antitumor vaccine. Different epitopes (left) can be conjugated to peptide amphiphiles to obtain monomers (middle) capable of self-assembly. Simple mixing of selected components in water leads to tailormade antigen-presenting structures (right).*

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CHARACTERIZATION OF MDA-MB-231 AND MEXICAN BREAST CANCER CELLS BY RAMAN MICROESPECTROSCOPY AND ATOMIC FORCE MICROSCOPY

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Introduction: Breast cancer is of the most common neoplasms around the world (American Cancer Society, 2020). In Mexico, the identification and selection of therapeutic targets drugs for breast cancer is made on African American or Caucasian breast cancer cell lines (Holiday, 2011). Differences in prognosis, aggressiveness and mortality have been observed in regard to ethnicity, this raises the issue on how representative these few cell lines are of the vast majority of the global population (Dai, 2017). Recently, breast cancer cell lines were obtained and partially characterized from tumors removed from Mexican women from the state of Sonora. To complement the cellular characterization and identify potential biochemical and nanoscale topographic biomarkers, Raman spectroscopy and atomic force microscopy (AFM) will be used to analyze Mexican breast cancers cells.

Aim: To obtain the biochemical fingerprint and nano-topographic surface characterization of MDA-MB-231 and Mexican breast cancer cells by Raman Spectroscopy and AFM, respectively.

Methodology: MDA-MB-231 and Mexican breast cancer cells were seeded on sterile CaF_2 substrates. After fixation process, cells were analyzed to obtain the quantitative and qualitative changes of the Raman Spectrum on a RAMAN confocal microscope Alpha 300 RA (WiTec) instrument and using a laser equipment with an excitation of 532 nm. For AFM, we analyzed the cell topography image by scanning area of 50 x 50 μm^2 on the whole cell, 5 x 5 μm^2 on the nucleus and 5 x 5 μm^2 on nucleus periphery and measured the cell surface roughness.

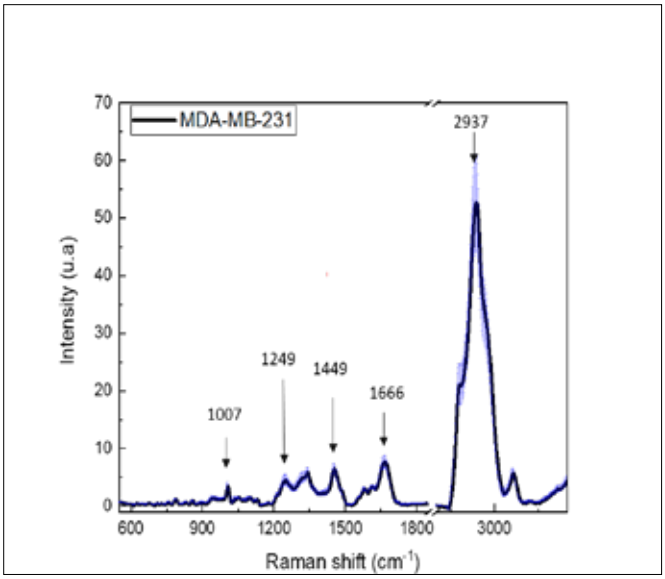

Figure 1. Average Raman spectra of MDA-MB-231 cells. n=150

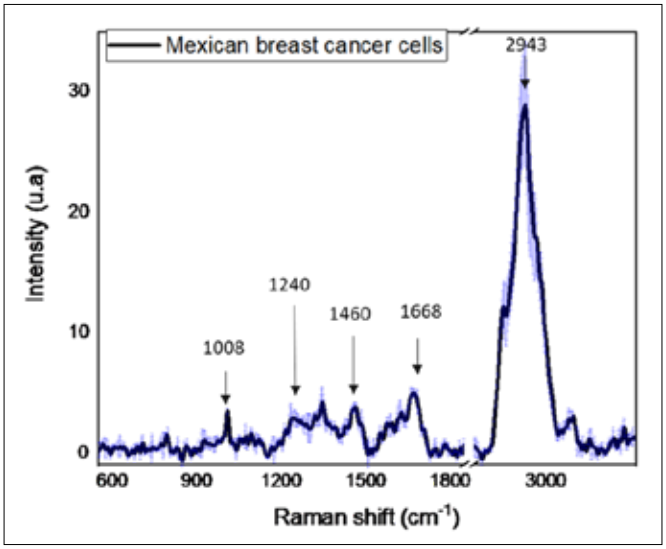

Figure 2. *Average Raman spectra of Mexican breast cancer cells. n=150*

Results: We characterized MDA-MB-231 and Mexican Breast cancer cells with both Raman spectroscopy and atomic force microscopy methods. In the case of Raman characterization, the results showed several Raman bands 1007 cm^{-1} (phenylalanine, protein), 1249 cm^{-1} (amide III; protein), 1449 cm^{-1} (lipid), 1666 cm^{-1} (amide I, protein), 2937 cm^{-1} (acyl chains, lipid). The qualitative characterization of the biochemical composition is in agreement with previous studies (Chaturvedi et al, 2016; Movasaghi et al, 2007). We also measured cell surface roughness and obtained an average of 42.2 nm^2 for MDA-MB-231, in accord with Tellez-Plancarte et al (2018).
Conclusions: It was possible to identified characteristic peaks of biomolecules found in other cancerous and non-cancerous breast cell lines as well as cell surface roughness. These results we can compare similarities and differences between cells at biochemical nanoscale level.

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TGF-β INHIBITION COMBINED WITH CYTOTOXIC NANOMEDICINE NORMALIZES TRIPLE NEGATIVE BREAST CANCER MICROENVIRONMENT TOWARDS ANTI-TUMOR IMMUNITY

CHRYSOVALANTIS VOUTOURI, Myrofora Panagi, Fotios Mpekris, John D Martin, Andreas Stylianou, Maria Louca, Kazunori Kataoka, Horacio Cabral, Triantafyllos Stylianopoulos

Advances in cancer nanomedicine have led to the development of several new systemically administered nanoparticles to treat various tumor types, including breast and pancreatic cancers (1). The enhanced permeability and retention (EPR) effect has served as a key rationale for using nanoparticles to target cancer cells or improve their pharmacokinetic properties for the treatment of solid tumors. As a result, clinically approved nanomedicines have succeeded in reducing adverse effects - owing to the EPR effect and preferential accumulation to the tumor - but survival benefits are modest in most cases [1, 2].
Inadequate perfusion in tumors is a major barrier to the efficacy of cancer nanomedicines. In many tumor types, blood vessels are hyper-permeable, which is the basis of the EPR effect, owing to the increased expression of pro-angiogenic factors (e.g., vascular endothelial growth factor, VEGF) that drive tumor-induced angiogenesis [3, 4]. Vessel hyper-permeability causes fluid loss from the vascular to the interstitial space of the tumor, reducing tumor blood flow (i.e., perfusion) [5]. Additionally, the dense tumor extracellular matrix and the rapid proliferation of cancer cells in the confined space surrounding the tumor result in the development of intratumoral mechanical forces that can strain components of the TME, including blood vessels, thus causing vessel compression [6-8]. Both vessel hyper-permeability and compression can reduce tumor blood flow, rendering tumors hypo-perfused and hypoxic. Impaired blood supply and hypoxia not only hinder nanoparticle delivery to the tumor but also help cancer cells evade the immune system and increase their invasive and metastatic potential [9-11].
Tumor normalization strategies aim to improve tumor blood vessel functionality (i.e., perfusion) by reducing the hyper-permeability of tumor vessels or restoring compressed vessels. Despite progress in strategies to normalize the tumor microenvironment (TME), their combinatorial antitumor effects with nanomedicine and immunotherapy remain unexplored. Vascular normalization and normalization of the tumor stroma are two strategies that have been clinically used separately, to improve perfusion and efficacy of chemotherapy [5, 12, 13]. However, there is little evidence that combinatorial use of a normalization agent and nanomedicine can improve TME normalization, enhance antitumor immune responses and improve overall survival [14]. The recent development of micellar particles loaded with valsartan, an angiotensin receptor blocker with TME normalization properties, is an example of such an approach [15]. To this end, we employed two syngeneic triple negative murine breast tumor models (4T1 and E0771) and used the transforming growth factor (TGF)-β inhibitor tranilast (Rizaben), a clinically approved antihistamine and anti-fibrotic drug, as the normalization agent alone or in combination with doxorubicin or its nanoparticle formulation Doxil (~100 nm, PEGylated liposomal doxorubicin). In previous work, we found that tranilast suppresses the expression of TGF-β target genes involved in collagen (e.g., COL1A1 and COL3A1) and hyaluronan synthesis (e.g., HAS2 and HAS3), as well as, other components of the extracellular matrix (ECM) such as connective tissue growth factor (CTGF) and lysyl oxidase (LOX) gene expression in breast tumors [16]. The aim of the study was to investigate whether the normalization effects of tranilst can be optimized with use of low doses of a cytotoxic drug (i.e., doses that cannot lead to primary tumor regression). We demonstrate that combination of tranilast with Doxil nanomedicine, significantly improved blood vessel functionality and oxygenation and delayed tumor growth compared to the other treatments employed. Based on these data, we conducted a second study using the 4T1 tumor model to evaluate the therapeutic potential of immune checkpoint blockade under normalized TME conditions. Again, we observed that the combinatorial tranilast-Doxil treatment significantly improved the efficacy of the immune checkpoint blocking anti-CTLA-4 and anti-PD-1 cocktail. Our findings strongly suggest that the effect of TGF-β inhibition is drastically increased when combined with cytotoxic nanomedicine or immunotherapy, proposing a new treatment strategy.

RESULTS

TME normalization improves the efficacy of both chemo- and nanomedicine

We found that tranilast, doxorubicin or Doxil monotherapy did not induce any significant delay in tumor growth compared to the untreated group, as indicated by the tumor-doubling time in both tumor models. This confirmed our aim to administer low doses of the

two drugs. In contrast, combination of tranilast with doxorubicin caused a 2-fold increase in doubling time of both 4T1 and E0771 tumors, whereas tranilast-Doxil combination produced a more than 3-fold increase in doubling time (Figure 1A, B). Furthermore, tranilast and doxorubicin alone had no effect on animal survival, whereas overall survival was modestly improved after Doxil monotherapy and tranilast–doxorubicin combinatorial therapy compared to controls. Importantly, the survival benefit was significantly improved following tranilast-Doxil combinatorial treatment compared to the rest of the groups (Figure 1C, D). These data demonstrate that the effect of tranilast is necessary for chemotherapy and nanomedicine to exert their anticancer effects and prolong overall survival.

Enhanced tumor normalization by Doxil nanomedicine improves efficacy of immunotherapy

To test our hypothesis that improved tumor perfusion, oxygenation and immunostimulation caused by the combined tranilast-Doxil treatment can enhance the efficacy of immunotherapy, we performed a tumor growth study employing a cocktail of immune checkpoint blockers (ICBs). Specifically, we used an immunotherapy cocktail comprising the programmed cell death-1 (PD-1) and cytotoxic T-lymphocyte-associated antigen-4 (CTLA-4) antibodies. Interestingly, immunotherapy cocktail alone did not affect tumor growth while Doxil monotherapy displayed a modest decrease in tumor size (Figure S10B). Combination of immunotherapy with Doxil nanomedicine reduced tumor growth by 40%, while its combination with the tranilast caused a reduction greater than 50% compared to the untreated group. Consistent with our previous results, tranilast-Doxil combinatorial therapy significantly reduced tumor growth by 60%. However, the combination of ICBs with tranilast-Doxil treatment let to the most significant reduction in tumor volume in both mammary tumor models, 4T1 and E0771 (Figure 2A-B). Therefore, our data highlight the use of TME normalization strategies for immunotherapy, in accordance with other recent studies [15, 17] and suggest that TGF-β inhibition could be combined with cytotoxic nanomedicine as a potential therapeutic strategy to improve anti-tumor immunity of highly metastatic and immunotherapy-resistant tumors.

Figure 1. *TME normalization increases the efficacy of both chemo- and nanotherapy. Quantification of tumor growth rate, based on the time to reach double the initial volume, for orthotopic 4T1 (**A**) and E0771 (**B**) murine breast tumors implanted in female BALB/c and C57BL/6 mice, respectively. Kaplan-Meier survival curves for 4T1 (**C**) and E0771 (**D**) tumor models treated as indicated (arrows). Statistical analyses were performed by comparing the treated groups with the control * and the tranilast-Doxil groups with all other treatment groups **, p≤0.05 (n=8-10).*

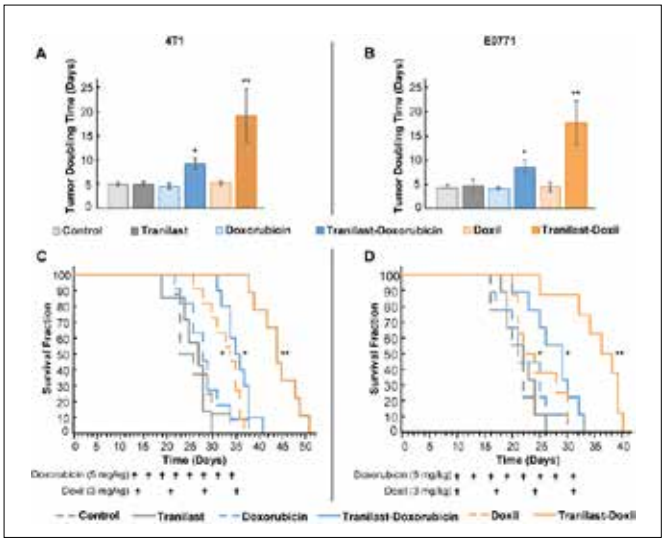
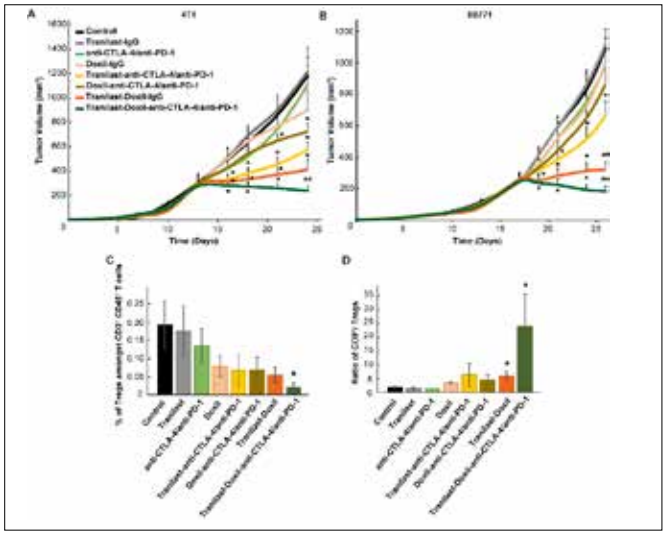


Figure 2. *(**A**) Tumor volume curves of Balb/c mice bearing 4T1 tumors and (**B**) C57BL/6 mice bearing E0771 tumors.Statistical analyses were performed by comparing the treated groups with the control * and the tranilast-Doxil- anti-CTLA4/anti-PD-1 immunotherapy cocktail groups with all other treatment groups **, p≤0.05 (n=8-10).*



CONCLUSION

Our findings identify vessel compression as a main mechanism of resistance to nanoformulations of chemotherapy, which could explain to some extent why nanomedicines have not been successful in drastically increasing overall survival. This is in accordance to our recent findings that metastatic cells can co-opt and eventually compress blood vessels [18]. It also provides new insights for the use of nanomedicine and the development of new nanoparticle formulations. We suggest that nanomedicine efficacy can be significantly improved in combination with agents that normalize the TME. Given the fact that the normalization agent (tranilast), the nanomedicine (Doxil) and the immune checkpoint blockers that we employed in our study, are already clinically approved, the findings of our research could be directly translated in clinical trials. As far as the design of new nanoparticle formulations is concerned, current research in nanoparticle development for cancer treatment aims to the design of multifunctional nanoparticle formulations that incorporate several stimuli-responsive or cancer cell targeting features. Increased sophistication, however, most often leads to increased nanoparticle size, which further hinders delivery. Here, we propose the possibility of nanoparticles to carry normalization agents, which will assist even large particles (larger than Doxil, >100nm) to enter and penetrate deeper into the tumor.

Finally, while tranilast was employed as a normalizing agent in this study, other similar agents have also been successfully tested by our group and others (e.g. losartan, pirfenidone, vismodegib, metformin, fasudil, dexamethasone) [19-24]. In principle, any agent that can induce normalization effects to the TME could be combined with nanomedicine but its degree of efficacy remains to be tested.

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THE DEVELOPMENT OF A NOVEL DUAL TARGETING NUCLEIC ACID-BASED IMMUNOTHERAPY FOR TREATMENT OF TUMOURS

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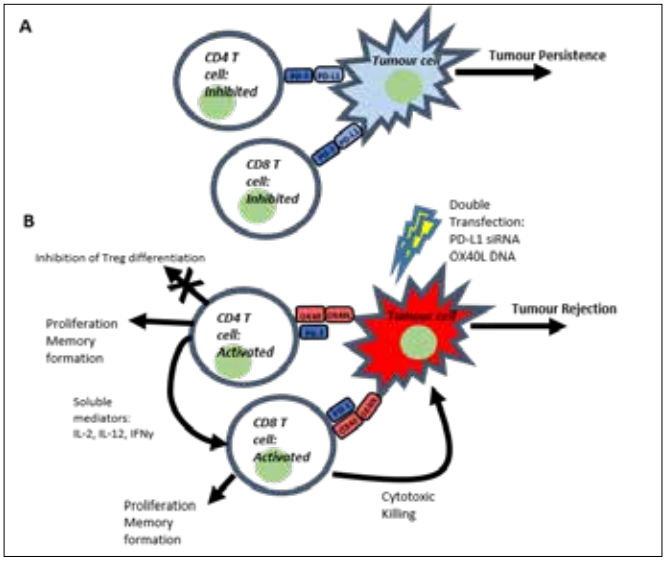
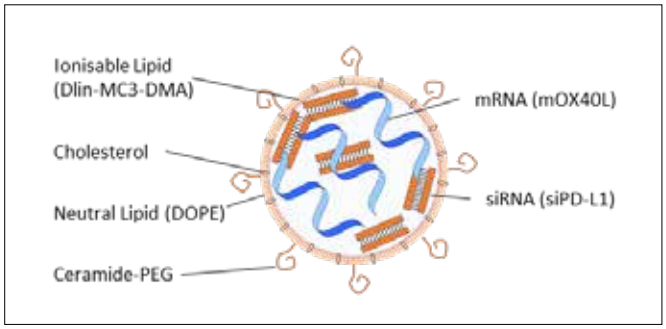
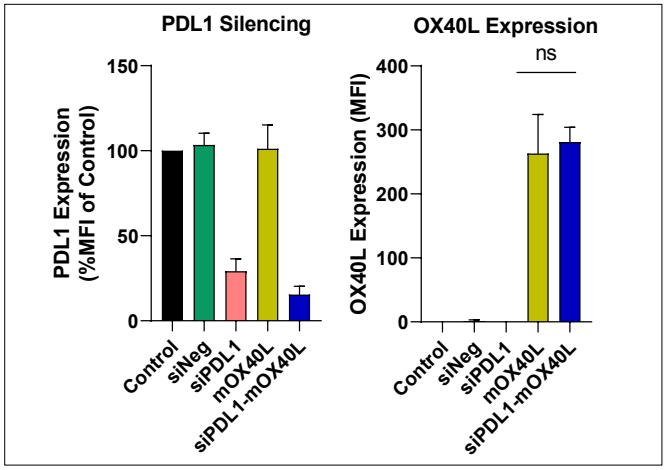
Immunotherapy is a revolutionary approach in the treatment of cancer that, over the past decades, has transformed the landscape of cancer care. Immunotherapy is based on the premise that it is possible to mobilise the patient's own immune system to destroy cancerous cells. Clinically, while the success has been miraculous and durable in positive responders, there are many cases where checkpoint blockade has failed leaving significant room for improvement. For instance, rationally targeting multiple check points, both stimulatory and inhibitory, within the tumour microenvironment may improve on the current response rate [1]. However, the use of monoclonal antibodies precludes or seriously limits this possibility as they cannot be specifically targeted to the tumour and as such are linked to significant off target effects [2]. This project seeks to develop a rationally formulated 'genetic immunotherapy' regime based on the co- delivery of siRNA to remove inhibitory check point molecules (siPD-L1) while simultaneously delivering mRNA expressing stimulatory checkpoint molecules (mOX40L) to the tumour environment.



Scheme 1. Proposed mechanism under investigation in this study. *(**A**) Under diseased conditions the tumour presents an immunosuppressive microenvironment through, though not exclusively by, expression of PD-L1 which limits T cell activation and proliferation. This leads to tumour persistence. (**B**) Using the formulation proposed within this study the PD-L1 mediated immunosuppression will be removed using siRNA. The tumour will also be induced to express positive check point molecule OX40L using mRNA. These molecules will serve to amplify the immune response through a number of potential mechanisms such as cytotoxic killing and inhibition of Treg differentiation. The ultimate outcome should be tumour rejection and the establishment of long-term immunological memory.*

Methods: Plasmid DNA encoding OX40L was transcribed into mRNA using commercial kit, siRNA against PD-L1 was obtained commercially. The nucleic acid constructs were formulated into SNALPs using a rapid mixing technique. The transfection of the constructs individually or in combination was tested *in vitro* in brief: mouse tumour cell lines were incubated with constructs at a range of concentrations over a time course of 24-72 h and surface stained with appropriate monoclonal antibody. The optimal formulation was then tested in relevant mouse model. Mice bearing established B16F10 melanomas were treated with nucleic acid containing SNALPs and tumour growth was monitored. At a predetermined endpoint mice were culled and leukocyte populations were assessed using flow cytometry.





Development of a mRNA:siRNA dual encapsulating SNALP suitable for delivery of combination nucleic acid immunotherapy.
This project describes the development of a SNALP system for co-encapsulation of mRNA and siRNA. The proposed formulation will be composed of ionizable lipid, cholesterol, neutral lipid and PEG ceramide surrounding the relevant nucleic acid pay load. A graphical representation is shown in (A). B16F10 cells were cultured until 90% confluent before being pulsed with SNALP formulations (0.75 µg of each type of RNA) for 48h at 37°C. Cells were harvested and double stained with fluorescently labelled anti mouse OX40L and PDL1 monoclonal antibodies. The conditions are as follows: Untransfected (Control), siPDL1, mOX40L, mOX40L-siPDL1 (co-formulation). The values obtained for PDL1 silencing, expressed as MFI percentage of control normalized to 100%, is shown in (B). OX40L expression (MFI) is shown in (C). For all the graphs, error bars correspond to standard error of the mean (SEM), significance was examined with One-way ANOVA multiple comparison test (Tukey's). n=3-8 repeats for each SNALP formulation

Results: SNALP RNA constructs were able to successfully encapsulate both siRNA and mRNA with minimal steric hindrance between

the two molecules. Furthermore, it was demonstrated *in vitro*, that SNALPs were able to deliver double check point blockade simultaneously knocking down expression of PD-L1 and upregulating OX40L on tumour cell surface. In murine models the double check point blockade formulation was shown to significantly slow tumour growth. A number of immunological parameters were found to be altered dependant on construct.

Conclusion: This is the first example of a multi target nucleic acid-based immunotherapy targeting both positive and negative regulators of the immune system.

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THROMBOLYTIC THERAPY BASED ON P-SELECTIN TARGETED POLYSACCHARIDE NANOPARTICLES

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KEYWORDS: Polysaccharide nanoparticles; Fucoidan; P-Selectin; Thrombolytic therapy.

The rapid recanalization of the vascular occlusion is of the utmost importance for the patients suffering from acute thrombotic diseases such as ischemic stroke and myocardial infarction. Current thrombolytic therapy, the intravenous injection of recombinant tissue plasminogen activator (rtPA), is yet limited by a narrow therapeutic window, rapid drug elimination, and risks of *cerebral* hemorrhagic complications[1],[2]. Hence, there is an unmet medical need for nanomedicine-based innovative approaches for safe thrombus targeting thrombolytic therapy. The objective is to construct safe, biocompatible, and biodegradable nanocarrier which is functionalized with a targeting agent and suitable for the targeted treatment of thrombotic diseases. P-selectin is an inflammatory adhesion molecule that is overexpressed by activated platelets and activated endothelium and can serve as a molecular marker of thrombosis [3],[4]. Fucoidan (Fuco), an abundant and cost-effective anionic polysaccharide extracted from brown algae, shows a strong affinity for P-selectin and holds thrombus targeting properties [5],[6].
Herein, we develop fucoidan-functionalized polysaccharide nanoparticles (NPs) targeting P-selectin. After extensive NPs formulation & characterization and validation of their targeting properties in the *in vitro* flow assays, a clinically available rtPA (Actilyse®, Boehringer Ingelheim) is loaded onto these NPs, and their thrombolytic activity is tested *in vitro* and *in vivo*.

EXPERIMENTAL METHODS

NPs were elaborated by a simple and reproducible water-in-oil (w/o) emulsification protocol based on the use of the vegetable oil combined with a crosslinking of hydrophilic natural polysaccharides: dextran and fucoidan with Sodium TriMetaPhosphate (STMP). NPs were characterized by hydrodynamic size and surface electrical charge (dynamic and electrophoretic light scattering, DLS/ELS). The morphology of the NPs was assessed by Transmission Electron Microscopy (TEM) and Environmental Scanning Electron Microscopy (E-SEM). The presence of the fucoidan was confirmed by the quantification of the Sulphur content (elemental analyzer-mass spectrophotometer). *In vitro* cytocompatibility experiment was carried out with confluent HUVECs, and cell metabolic activity (Resazurin conversion) was analyzed after 24h. Hemolysis assay was performed on washed isolated human erythrocytes after 4h exposure to the NPs.
rtPA was loaded onto NPs by adsorption (1 mg NPs, 100 µg rtPA); free or poorly adsorbed rtPA was removed by 3 cycles of ultracentrifugation. The loading efficiency of rtPA on the NPs was quantified by BCA (bicinchoninic acid) protein assay. Flow cytometry monitored a rtPA release from NPs in 0.9% NaCl. The enzymatic activity of rtPA-loaded NPs was assessed using fluorogenic substrate PefaFlour® rtPA. The experiment of the fibrinolytic activity of the rtPA-loaded NPs was performed on the *in vitro* fibrin agarose plate. The targeting efficacy of Fuco-NPs (unloaded or rtPA-loaded) was validated with microfluidic experiments *in vitro* on recombinant human P-Selectin and activated platelet aggregates in arterial and venous shear stress conditions. Adhesion of fluorescent Fuco-NPs or Control-NPs was visualized and quantified in real-time under fluorescence microscopy. Biodistribution of NPs was evaluated by histological analysis in healthy rats. Thrombolytic efficacy was estimated in the *in vivo* $FeCl_3$ mouse model of venous thrombosis.

RESULTS AND DISCUSSION

Functionalized targeted polysaccharide NPs were obtained by a green chemistry method using fully biodegradable and biocompatible compounds, all of them with the U.S. Food and Drug Administration (FDA) approval. Spherical and homogeneous fucoidan-functionalized NPs exhibited a size 662 ± 27 nm and a zeta potential -30.3 ± 0.1 mV with favorable biodistribution *in vivo*. Fucoidan presence constituted 9% w/w. The obtained NPs preserved their integrity in a physiological solution of 0.9% NaCl and PBS. They are able to swell in an aqueous medium; hence they can be referred to as nanogels. The functionalized NPs remained stable for 30 days at 4°C storage. NPs were cyto- and hemocompatible at concentrations ranging from 0.1 to 1.5 mg/ml. The nanogel nature of the NPs allowed reaching the encapsulation efficiency of the rtPA equal to 70% with a classical rtPA release profile from the NPs. The enzymatic and fibrinolytic activities of rtPA-loaded NPs were maintained *in vitro*. Fuco-NPs bounded significantly more to P-selectin coating than Control-NPs in a dose-dependence manner as regards to the coating concentration. Moreover, Fuco-NPs (unloaded or rtPA-loaded) accumulated at the surface of platelet aggregates in arterial and venous conditions, validating the targeting strategy. Preliminary *in vivo* thrombolytic efficacy experiment demonstrated that Fuco-NPs were more effective to induce thrombolysis than free rtPA.

CONCLUSION

To conclude, novel fucoidan-functionalized polysaccharide NPs were loaded with rtPA, and their thrombolytic activity was tested in preclinical models of thrombosis. This proof of concept study demonstrates the potential of biomaterial-based targeted nanomedicine for the personalized treatment of acute thrombotic pathologies.

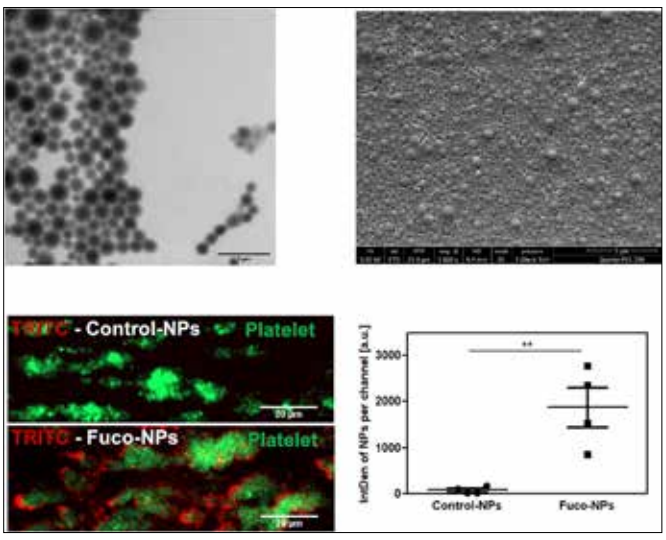

Figure 1. Morphology *by Transmission Electron Microscopy (TEM)* (**A**) *and Environmental Scanning Electron Microscopy (E-SEM)* (**B**). ***In vitro flow assay of rtPA-loaded NPs.*** *Platelets are labeled in green with DIOC6, and TRITC-NPs are red. Human whole blood is first passed at 67.5 dyn/cm². Once aggregates are formed, NPs are passed at the same shear stress* (***C***). *After 5 min, the Integrated Density (IntDen) is normalized over the platelet IntDen per channel (**p<0.01, n=4)* (***D***).

ACKNOWLEDGMENT

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CONOTOXIN-DERIVED BIOMIMETIC PEPTIDES FOR ACTIVE TARGETING OF NEUROBLASTOMA CELLS

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Neuroblastoma is an extremely aggressive metastatic type of cancer and also the most lethal tumor in children below 5 years of age. Treatment via chemotherapy and/or radiotherapy is often ineffective and problematic because it leads to high toxicity for non-malignant cells and the development of chemoresistance of cancer cells.[1, 2]

Recently, a new way of neuroblastoma treatment has been targeted therapy. Specifically, active targeting of neuroblastoma cells using suitable small molecule or peptide ligands attached to the surface of nanocarriers with encapsulated chemotherapeutic drug. This allows for enhanced and specific delivery of encapsulated drug to target neuroblastoma cells, thanks to the high affinity between the ligand and receptor or protein situated on the membrane of target cancer cells. Peptides as targeting ligands provide with ideal properties for their application in neuroblastoma nanomedicine, mainly because of their low toxicity, good biocompatibility, ease of synthesis, small size, speed of modification and good potency. The use of these targeting ligands allows the supply of drug-encapsulated nanocarriers to uniquely identifiable cancer cells, which would lead to reducing negative side effects on off-target non-malignant cells and increased efficiency of cancer therapy.[3, 4, 5]

In case of this project, biomimetic peptides were designed by in silico computational chemistry and biology and derived from conotoxin (neurotoxic peptide from Conus marmoreus), exhibiting a specific affinity to hNET (human norepinephrine transporter), to obtain the peptides with a high targeting efficiency. On the basis of preliminary analyses, we synthesized five peptides with similar sequences composed mainly of 3 amino acids (tyrosine, lysine, and leucine). To enhance the flexibility of the peptides, in the aim of increasing the binding to hNET, glycine, and alanine were introduced to some of the peptides.

As a compound for testing the active targeting of neuroblastoma cells by conotoxin-derived peptides, potential chemotherapeutic drug ellipticine (Elli) was chosen mainly because of its disposition to defuse growth of certain types of cancer cells, such as neuroblastoma.6 In order to decrease the negative side effects and also increasing the biocompatibility and specificity of this drug in patient's organism, it was encapsulated into the organic biocompatible nanocarrier called apoferritin (EcLHFRT) isolated from equine spleen and formed by 22 light (19-kDa) subunits and 2 heavy (21 kDa) subunits. The EcLHFRT is a hollow cage of 460 kDa in size with internal diameter of 7-8 nm and external diameter of 12-13 nm. The main advantage is that dissociation and re-association of EcLHFRT structure is influenced by the pH of environment. This property was utilized for the process of encapsulation of Elli, which was optimized by our research in Research Group for Molecular Biology and Nanomedicine, in order to minimize mutagenic and genotoxic effects of Elli on healthy non-malignant cells.[7, 8] According to results in Fig. 1A, obtained from dynamic light scattering (size), Doppler microelectrophoresis (ζ-potential) and spectrophotometry (encapsulation efficiency of Elli), it is obvious that the peptide surface modifications did not induce too large structural changes of EcLHFRT. Before process of encapsulation of Elli, size of EcLHFRT was 13.5 nm, process of encapsulation enhanced it to 28.2 nm or 37.8 nm. However, for nanomedicine, the largest recorded size (37.8 nm) is also still satisfactory. Values of ζ-potential of prepared nanocarriers were very similar and average encapsulation efficiency of Elli in nanocarriers with unmodified or modified surface was 79%. Putative structure of non-targeted and targeted apoferritin nano-

carrier is shown in Fig. 1B. As shown in Fig. 1C, the degree of re-association of non-targeted and targeted EcLHFRT was the same like the degree of re-association of empty EcLHFRT. Prepared nanocarriers with unmodified or modified surface caused increased uptake of Elli to target neuroblastoma cells (UKF-NB-4), but only targeted nanocarrier with YKL-6 peptide induced increased uptake of Elli to target cells, while decreasing its uptake to off-target cells (HBL-100), compared to the nanocarrier with the unmodified surface (Fig. 1D). Thanks to viability assay shown in Fig. 1E, we found that targeted nanocarriers exhibit a direct cytotoxicity in target UKF-NB-4 cells. In off-target cells HBL-100, the viability after treatment with these nanocarriers was much higher.

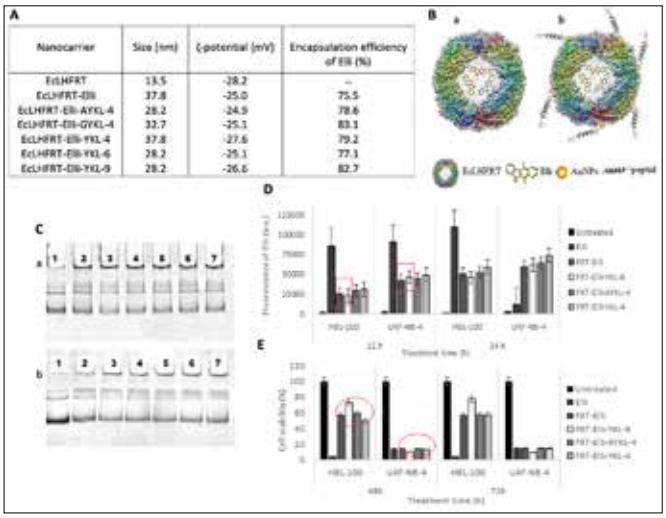

Fig. 1: *Characterization of prepared nanocarriers, cellular uptake and viability assay: A) Results of average size, ζ-potential and encapsulation efficiency of Elli into nanocarriers; B) Structure of non-targeted (a) and targeted (b) nanocarriers C) Native (a) and SDS (b) PAGE: 1 - EcLHFRT, 2 - EcLHFRT-Elli, 3 - EcLHFRT-Elli-AYKL-4, 4 -EcLHFRT-Elli-GYKL-4, 5 - EcLHFRT-Elli-YKL-4, 6 - EcLHFRT-Elli-YKL-6, 7 - EcLHFRT-Elli-YKL-9; D) Comparison of uptake of free and encapsulated Elli in off-target (HBL-100) and target (UKF-NB-4) cells, concentration of free and encapsulated Elli was 20 μM; E) Comparison of viability of off-target and target cells after treatment via non-targeted and targeted nanocarriers, concentration of free and encapsulated Elli was 20 μM.*

The process of Elli encapsulation into non-targeted and also targeted nanocarriers led to reduction of hemolytic ability of Elli, as shown in Fig. 2B and 2C because only free Elli (Fig. 2A) had hemolytic effect on fresh human blood. As shown in Fig. 2D and 2E, no FBS proteins or plasma proteins were found on our prepared non-targeted and also targeted nanocarriers.

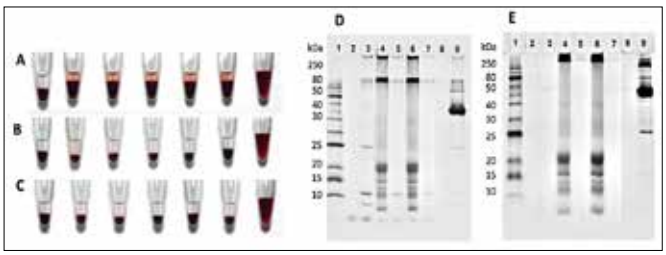

Fig. 2: *Evaluation of biocompatibility (A, B, C) and formation of protein coronas (D, E) on the prepared nanocarriers. A) Hemolytic assay of free Elli; samples from left to right: negative control (PBS), 80, 40, 20 10, 5 μM Elli; positive control (Triton X-100). B) Hemolytic assay of encapsulated Elli in non-targeted nanocarrier (EcLHFRT-Elli); samples from left to right: negative control (PBS), 80, 40, 20 10, 5 μM Elli; positive control (Triton X-100). C) Hemolytic assay of encapsulated Elli in targeted nanocarrier with YKL-6 peptide (EcLHFRT-Elli-YKL-6); samples from left to right: negative control (PBS), 80, 40, 20 10, 5 μM Elli; positive control (Triton X-100). D) SDS-PAGE: 1 - NEB Protein Ladder 10-250 kDa, 2 – Elli, 3 – Elli + FBS, 4 –FRT-Elli, 5 – FRT-Elli + FBS, 6 – FRT-Elli-YKL-6, 7 – FRT-Elli-YKL-6 + FBS, 8 – FBS + PBS (1 : 1), 9 – FBS + PBS (1 : 100). E) SDS-PAGE: 1 - NEB Protein Ladder 10-250 kDa, 2 – Elli, 3 – Elli + plasma, 4 –FRT-Elli, 5 – FRT-Elli + plasma, 6 – FRT-Elli-YKL-6, 7 – FRT-Elli-YKL-6 + plasma, 8 – plasma + PBS (1 : 1), 9 – plasma + PBS (1 : 100).*

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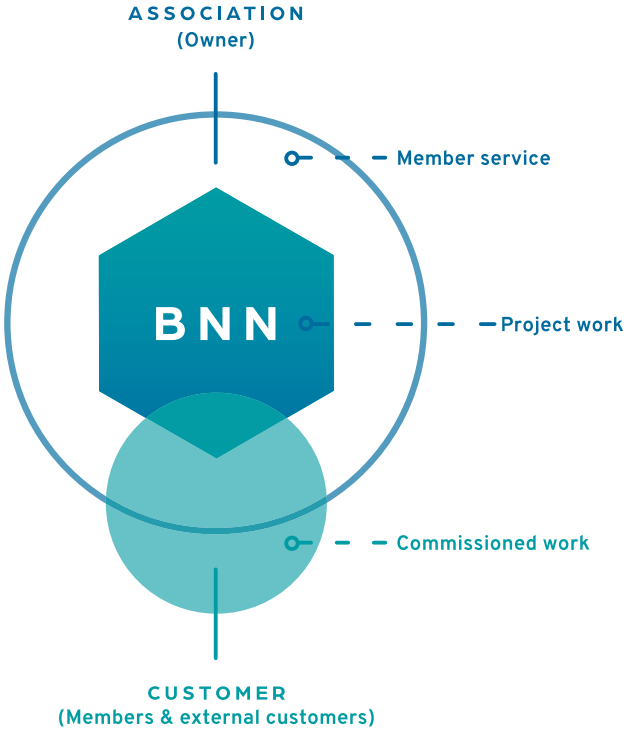
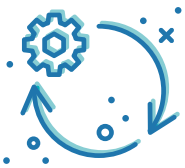


OUR SERVICES



Design for Technology Development

We support you from the start by addressing safety, quality and sustainability aspects during the development phase of a new technology.

Innovation Support

By sharing our knowledge within the technological and business field, we help you visualizing your concept and getting the fastest way for your idea to become reality.

Alliances & Clustering

We connect you with strategic partners to share and join new initiatives. Through the right clustering of resources and competences, we lead the change of Europe together.

Complementary Business Support

We help you to build and manage small to large scale consortia, and to promote and coordinate dissemination and exploitation activities of your projects.

ciber-bbn
Biomedical Research Networking Center
Bioengineering, Biomaterials, Nanomedicine

About

CIBER-BBN was officially established in 2006, with its head office in Zaragoza. The CIBER-BBN, consortium created under the leadership of the Carlos III Health Institute (ISCIII) of the Spanish Ministries of Economy and Health, to promote research excellence and build a critical mass of researchers in the field of Biomedicine and Health Sciences, is a multidisciplinary and multi-institutional translational research center integrating basic, clinical and technological research. As of January 1st, 2014 the CIBER-BBN merged with other seven existing CIBERs in Spain in a single management unit with headquarters in Madrid, which involves more than 5.000 Spanish researchers in biomedicine. The organizational structure of CIBER is based on the research groups that compose it and which have been selected on a basis of scientific excellence in the three areas of research of the cluster.

Currently, its center of Bioengineering, Biomaterials and Nanomedicine, CIBER-BBN (www.ciber-bbn.es/en), employs 130 researchers directly hired by the CIBER and conduct their research at any of the 46 participating groups, as well as more than 350 associate researchers, who although having a contract with the corresponding consortium institution they are considered as attached-to-CIBER staff.

Vision

"To become a centre of reference ***in research and innovation both at a national and international level*** *achieving a* ***leading position in technological advances and their transfer to clinical practice****."*

Mission

"To perform a ***research of excellence*** *aimed at industrial* ***transfer*** *and* ***clinical translation*** *through the develop*

ent of the scientific areas of ***bioengineering, biomaterials and nanomedicine****."*

The CIBER- BBN Research Program is organized in three different scientific areas and eight strategic lines. The area of Bioengineering consists of the strategic lines of Multimodal Diagnosis and Intelligent Devices, the area of Biomaterials of the lines of Cell and Gen Therapy, Tissue Engineering and Prostheses and Implants, while the area of Nanomedicine includes the strategic lines of Nanodiagnoses, Therapeutic Nanosystems and Nanobiotechnology.

CIBER -BBN research groups are continuously evaluated under the light of scientific excellence by using markers such as their number of publications (632, only during 2013; 426 in the 1st quartile), but also for their activities in obtaining financing in competitive calls, the transfer of technology and their relations with the private sector. During its rather short life, CIBER-BBN was awarded over 100 grants and raised over € 170 millions in competitive calls (HEALTH, NMP, PEOPLE, ERA- NET EuroNanoMed and ERC Starting and Advanced Grants) and in the contracts with companies, having signed more 200 contracts and supporting more than 65 patents in 201

About

The Spanish Strategy for Science, Technology and Innovation 2013-2020, adopted by the Council of Ministers on February 1st, 2013, includes the update of the "National Scientific and Technological Infrastructure Map (ICTS)" for the period 2013-2016 and 2016-2020. The so-called singular scientific and technical infrastructures (ICTS) are large installations, resources, facilities and services, unique in its kind, that are dedicated to cutting edge and high quality research and technological development, as well as to promote exchange, transmission and preservation of knowledge, technology transfer and innovation. These are facilities of different scientific areas ranging from the life sciences to astrophysics or engineering, distributed throughout the Spanish territory. NANBIOSIS has been recognized by the Spanish Government as one of these 29 UNIQUE existing ICTS, which are OPEN to competitive access to users of the whole research community in the public and private sector.

Due to the current pandemic outbreak of COVID-19, caused by the SARS-CoV-2 virus, knowledge about the virus characteristics and the development of its possible diagnostic, treatment and prophylactic devices and systems are high priority in all research institutions worldwide. NANBIOSIS assigns high priority to the projects related to the SARS-CoV-2 virus offering preferential access.

Information on CIBER and Nanbiosis

Dr. Nerea Argarate Madariaga
R&D project manager, International projects and Initiatives
Coordinator of Nanbiosis Research Infrastructure ICTS
Biomedical Research Networking Center (CIBER)
Bioengineering, Biomaterials & Nanomedicine Research Area (CIBER-BBN)
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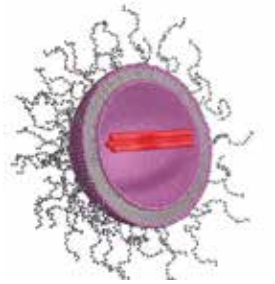
Innovative Nanomedicine for Targeted Therapy

Innovative nanomedicine start-up develops novel cancer therapy and Parkinson's medication

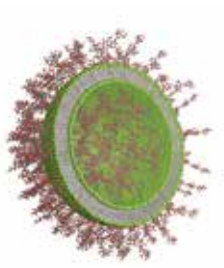
Despite major advances in medicine, cancer, cardiovascular disease and central nervous system disorders remain among the leading causes of death. Although potent drugs are available, they often do not reach the target in the body in sufficient quantities and thus cannot work effectively. In chemotherapy, often less than one percent of the drug reaches the tumor. The challenge for the development of successful therapeutic approaches therefore is not necessarily a lack of potent drugs, but rather their biological distribution in the body.

InnoMedica has successfully developed a means of transport for active agents that mimics the natural transport system of the body. The active agents are packaged in a coat of fat, a so-called liposome. The nanometer-sized liposomes are manufactured in such a way that they reach the diseased tissue in the body with biological precision. This makes it easier to treat a disease while unwanted side effects can be reduced.

InnoMedica has chosen oncology as the first area of application for its liposomal nanotechnology and with its product Talidox targets a chemotherapeutic agent to the tumor. A second therapeutic area, neurology, has proven to be even more promising. Talineuren is on its way to enter clinical trials, soon treating patients with various neurodegenerative diseases such as Parkinson's and Huntington's disease as well as Amyotrophic Lateral Sclerosis (ALS).



Talidox liposome: Doxorubicin is packaged into a liposome, thereby shielding the drug from premature reactivity in the blood stream and enabling transport to and uptake into tumor tissue.



Talineuren liposome: GM1 is spiked into the membrane of a liposome, thereby GM1 is protected from excessive early degradation and is transported to the brain, even after oral intake. GM1 plays an important role in the regulation and function of nerve cells.

In-house production – Swiss made, GMP-certified and increasingly automated

InnoMedica operates a production facility with a GMP-certified clean room in the Marly Innovation Center near Fribourg where it produces Talidox and Talineuren. The new larger clean room could be completed in summer 2020. The additional production facility allows InnoMedica to supply medicine even for a potentially rapidly growing number of patients. The technically challenging scale-up has been accomplished by InnoMedica and now paves the way for capacity expansion and increasingly automated production.

Talidox – Chemotherapy intelligently packaged

Chemotherapy remains the most common medical treatment for cancer. Although it often inhibits tumor growth effectively, it causes severe side effects. InnoMedica's nanotechnology optimizes the efficacy/side effect profile of doxorubicin with the liposomal transport system. Doxorubicin is an extremely potent chemotherapeutic drug that has been used in cancer treatment for decades. Talidox packages doxorubicin into a liposome. Shielded this way, the drug circulates in the bloodstream and unfolds its effect specifically in the tumor. The preclinical studies with Talidox showed higher drug concentration in the tumor, a strong tumor-inhibiting effect, fewer side effects and the possibility of longer treatment cycles.

Clinical study with Talidox

The first clinical trial phase I study was conducted in five Swiss hospitals together with the Swiss Association for Clinical Cancer Research (SAKK) as a partner. The study provides information on the maximum tolerated dose and on side effects. Starting with a low dose in the first patient, the treatment is escalated with increasing doses for each new patient. In accordance with the protocol, a total of 21 patients have been treated with Talidox in the Phase I clinical trial. The results show that Talidox allows a higher dosage than other liposomal doxorubicin formulations, but is gentler on the patient. After setting the maximum dose at 45 mg/m^2, the Phase I study could be amended to 9 additional patients which are currently recruited. Further clinical studies in cooperation with SAKK are already in planning in order to examine

the pharmacokinetics (distribution in the blood) and to prove the efficacy in comparison to the standard of care (e.g. Doxil).

Since Talidox uses doxorubicin, an already approved substance in a new liposomal formulation, Swissmedic categorized the drug as known substance with innovation. This fact speeds up the clinical study, simplifies registration and significantly reduces the translational risk. With Talidox, InnoMedica wants to launch a drug on the market which, unlike immune therapies, is not a cost-intensive solution for only a few patients, but enables a more effective and gentle treatment of a large number of patients. The reduced stress on the body favors longer treatment cycles, which increases the prospect of successful treatment for the patient.

Talineuren – a Regenerative Therapy Approach for Parkinson's Disease

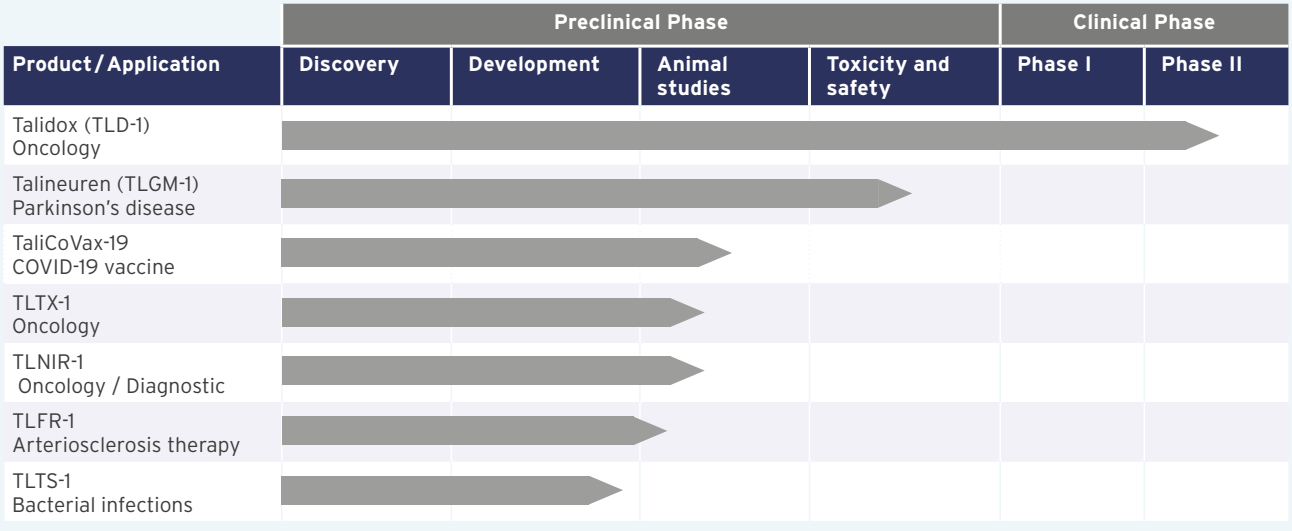
To improve the current treatment options for Parkinson's patients, a medication is needed that addresses the disease as close to its cause as possible and that in the long term supports the body's own production of dopamine, otherwise limited by Parkinson's disease. Current research on Parkinson's shows that the brain component GM1 is able to promote the survival and regeneration of damaged nerve cells. In a human study with Parkinson's patients, the active substance GM1 achieved a strong, immediate and long-lasting effect. However, the drug had to be injected under the skin twice a day in order to accumulate sufficient amounts of GM1 in the brain for a therapeutic effect. These injections caused severe side effects.

InnoMedicas Talineuren combines the potential of GM1 in Parkinson's therapy with the advantages of liposomal technology. Contrary to the widespread scientific assumption that liposomes cannot penetrate into the brain, InnoMedica was able to determine a specific composition of the liposome, which allows a crossing of the blood-brain barrier and thus brings drugs directly into the brain. The preclinical data of InnoMedicas Talineuren showed major advantages. The active substance GM1 has a regenerative effect in the brain, no side effects have been observed in preclinical studies with Talineuren and the drug can be administered intravenously or even orally.

Following initial positive preclinical study data in Parkinson's models, Talineuren has now also been used in preclinical studies on Amyotrophic Lateral Sclerosis (ALS). This indication opens the way for Talineuren to apply for orphan drug status, which will facilitate faster drug approval. In the first clinical trial with Talineuren, patients with Parkinson's disease, Huntington's disease and ALS will be included.

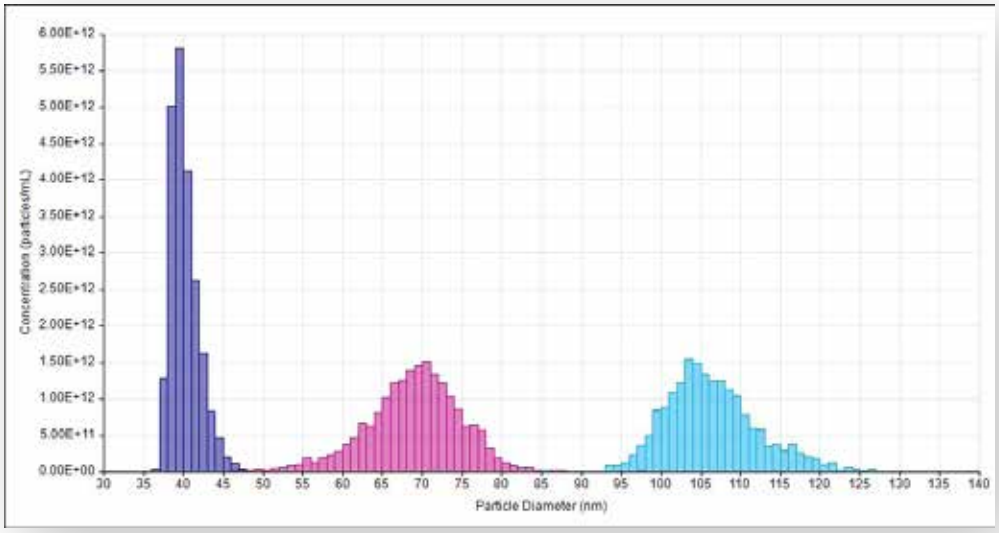
Pipeline

Based on the liposomal technology platform, InnoMedica has built a broadly diversified pipeline. All pipeline products have in common that no new molecules are used, but substances with known efficacy profiles. This approach is interesting from a medical point of view as well as from entrepreneurial risk and return considerations. The therapeutic effect of these substances is substantially improved by the combination with the liposomal nanocontainer and the biological fine-tuning. With this approach, drugs can be designed that have a high chance of success in clinical translation and are expected to have great therapeutic benefits. In addition to Talidox and Talineuren, InnoMedica is currently developing another drug against cancer, a drug against arteriosclerosis as well as a contrast agent for the identification of tumors for more precise surgical removal. In a joint project with the University of Bern, InnoMedica is also looking for a medical solution to fight resistant bacterial strains. In view of the current COVID-19 pandemic, InnoMedica launched the TaliCoVax-19 vaccination project aiming to get proof of concept that the proprietary liposomal technology can also be used for vaccines.



TUNABLE RESISTIVE PULSE SENSING

The most accurate and precise nanoparticle measurement system available is getting a big update.



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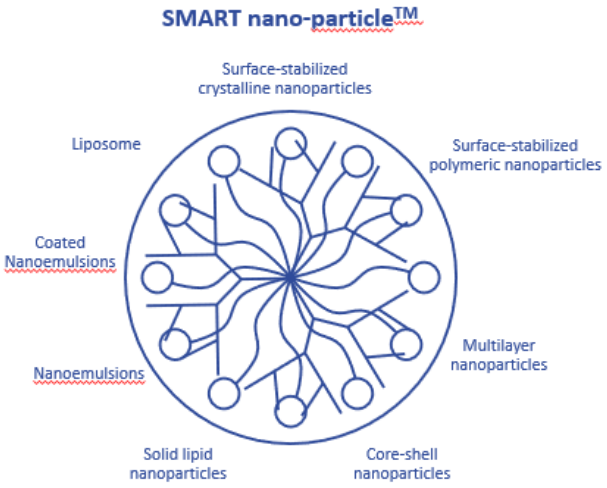
Creating formulations that are truly effective means finding an answer to the question of solubility – and that answer can be found in leon's unique approach to nano. For New Chemical Entities and generics alike, our intelligence in nanotechnology provides a clear solution for addressing poor solubility and smoothing the path for downstream processing to answer unmet patient needs and unlock new value from APIs for pharmaceutical partners.
It is based on patented technology to deliver a classic bottom-up approach to the synthesis of SMART nanoparticles or microparticles.

Our technology: High-precision particle synthesis.



Our definition of SMART

Stable crystalline or polymeric particles
Measurable tailored size: 10-1000nm & 1-70µm
Achievable easy scale-up: bench to commercial
Relevant for all application forms
Timebound: fast continuous precipitation
< 0.1ms easily integrated into downstream processes

Scalability is built into our development pathway, with easy scale-up facilitating transfer between the process used to test prototype nano formulations and the process to support clinical and commercial availability. With us, new formulations can be developed faster and the process successfully validated sooner, so that positive impacts can be felt by patients more quickly.
leon can quickly and reliably produce a wide range of nano particles by tailoring to the API and modifying process conditions. These include surface-stabilized nanocrystals, micelles, liposomes, nanoemulsions, polymer nanoparticles and even solid lipid nanoparticles. Our proprietary approach shares the same fundamental process for small-scale testing as it does for full-scale production, providing a swift and cost-effective route to commercial development.

Our service: Helping you realise new potential through nano.

We work collaboratively with our pharmaceutical partners on a contract or co-development basis, and the engagement process typically follows five key stages:
1. Evaluation→2. Testing →3. Pre-Production →4. Pilot & clinical trial supplies →5. Scaling to commercial production within GMP facilities. Market Release
At every stage, communication is a crucial aspect of our partnership approach. We ensure there is open dialogue, insight sharing and transparency at every stage, providing a platform to smooth and speed the path to market and keep costs to a minimum.

Exciting new possibilities exist at the scale of nano. Opportunities to accelerate drug development. Opportunities for collaboration between global pharma, biotech and network partners to amplify the effectiveness of New Chemical Entities, generate novel generics and super generics, revitalize forgotten formulations and negate the attrition of molecules.

That is why at leon, we are enabling nano now. Join us.

The nano revolution is here.

@leon-nanodrugs @leon

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ENABLING NANO NOW

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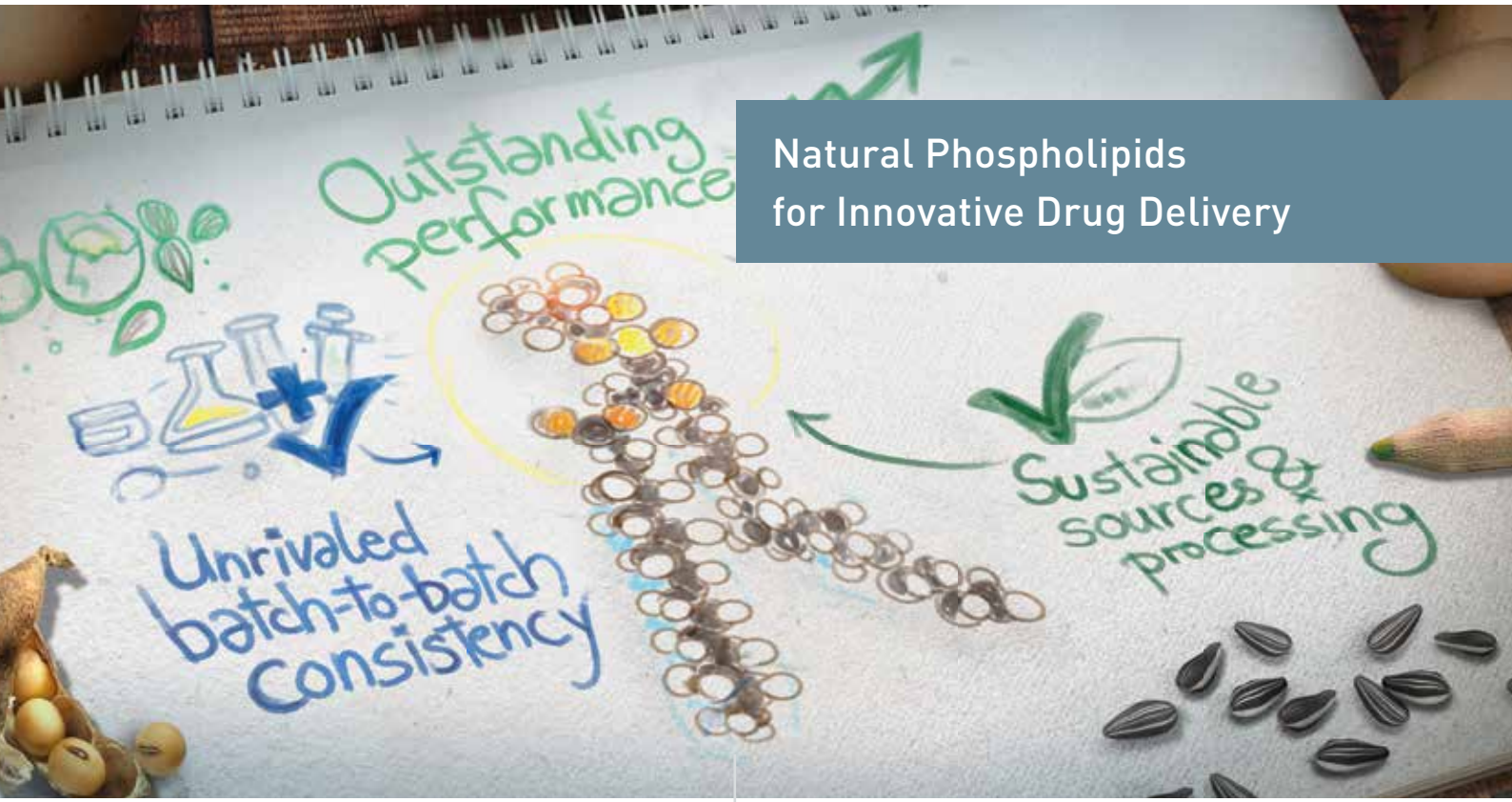


Natural Phospholipids for Innovative Drug Delivery

- The full spectrum of phospholipids at pharmaceutical quality in large scale
- Unique multifunctional excipients with unmatched safety profile

We Invest in Quality.

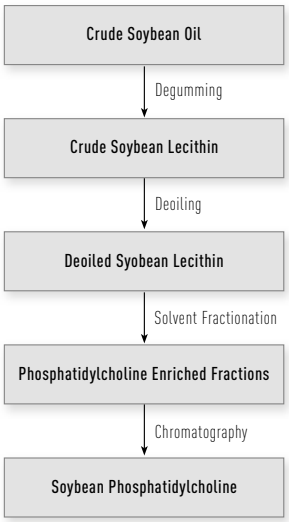
The Use of Natural Phospholipids as Pharmaceutical Excipients

Dr. Peter van Hoogevest
Phospholipid Research Center Heidelberg, Im Neuenheimer Feld 515, D-69120 Heidelberg, Germany
email: pvanhoogevest@phospholipid-institute.com

Phospholipids are used in many types of formulations such as fat emulsions, mixed micelles, suspensions, and liposomal preparations for any administration route [1-3]. Phospholipids are surface-active, comprising a polar headgroup and a lipophilic tail. They are used as emulsifiers, wetting agents, solubilizers, and liposome formers. The phospholipid molecule comprises a glycerol backbone esterified in positions 1 and 2 with fatty acids and in position 3 with phosphate, the latter being further esterified with an alcohol. The most common phospholipid is phosphatidylcholine (PC), which is the main component of lecithin as described in the United States Pharmacopoeia (USP). Phospholipids are essential components of cell membranes, have digestion/metabolic functions [4] as a lipoprotein component, and are a source for the biosynthesis of acetylcholine (in the case of PC) and (essential) fatty acids and energy [5].

Natural phospholipids are isolated from natural sources such as soybean, rape- and sunflowerseed, and animal material (e.g., egg yolk, milk, and krill). These raw materials are produced worldwide at very large scale. The phospholipid compositions of the lecithins are dependent on the raw material sources. In all cases, PC is the main phospholipid component. Higher quality pharmaceutical-grade phospholipids are obtained with excellent inter-batch reproducibility by validated extraction and chromatography procedures using non-toxic solvents (**Figure 1**).

Figure 1. Flow chart of the isolation process steps of soybean phosphatidylcholine from crude soybean oil.



Control of the raw material quality and use of a validated purification method ensure the quality of the phospholipid excipients Egg phospholipids, isolated from hen egg yolk with similar methods as for soybean lecithin, play an important role as excipients as well.

The natural phospholipids can be further modified to saturated phospholipids by hydrogenation [6] and use of enzymes to make from soy PC e.g., soy phosphatidylglycerol (PG) (**Figure 2**). Besides natural phospholipids, synthetic phospholipids are also being used in pharmaceutical products.

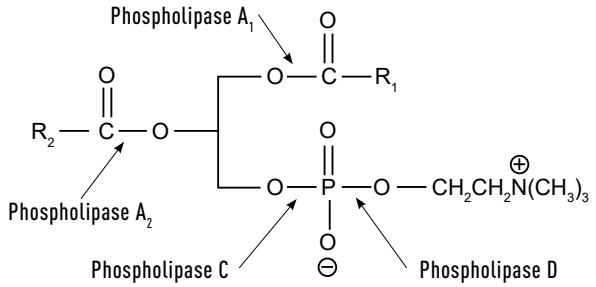
In pharmaceutical products for oral and dermal administration, mainly soybean phospholipids are used. For dermal use, hydrogenated soybean phospholipids are also applied. In parenteral products, natural phospholipids are prevalent in addition to synthetic phospholipids, as described in the FDA's Inactive Ingredient (excipient) list [8].

Egg phospholipids serve as emulsifiers in parenteral nutrition products (e.g., Intralipid®) [9]. These emulsions can also be used as carriers for oil-soluble drug substances such as diazepam (Diazemuls®) and propofol (Diprivan®) [10, 11].

Parenteral mixed micellar formulations, comprising soybean phospholipids and cholate salts, are either suitable as solubilizers for poorly water-soluble compounds such as vitamin K or soybean phospholipids intended as APIs for the treatment of liver disorders [12, 13]. These products underscore the safe intravenous use of soybean phospholipids.

Considering pulmonary products comprising phospholipids, natural as well as synthetic phospholipids can be used. For instance, natural phospholipids extracted from bovine or calf lung have been used to treat Respiratory Distress Syndrome, a disease in infants characterized by immature lung epithelium [14]. The inhalation product for systemic treatment with levodopa (Inbrija®) comprises 1,2-dipalmitoyl-*sn*-glycero-3-phosphocholine (DPPC).

Figure 2. Enzymatic conversion possibilities of phosphatidylcholine [7].



Natural phospholipids are well known to regulatory authorities and described in many pharmacopoeias [15]. Regarding the toxicity of phospholipids, the World Health Organization, US FDA, and EU place no limit on the oral intake of lecithin as a food additive [16-18]. The safe intravenous use of soybean and egg phospholipids is well documented [19].

In conclusion, natural phospholipids are derived from renewable sources, are produced with ecologically friendly processes, and are available in large scale at relatively low costs. They comply with all requirements from major regulatory authorities and are safe for any administration route. For parenteral application, egg, soybean-, and hydrogenated soybean phospholipids are used, besides synthetic phospholipids. In oral administration soybean phospholipids prevail, whereas for dermal administration to the skin soybean phospholipids and their hydrogenated versions are popular. Inhalation products contain natural phospholipid extracts and synthetic phospholipids

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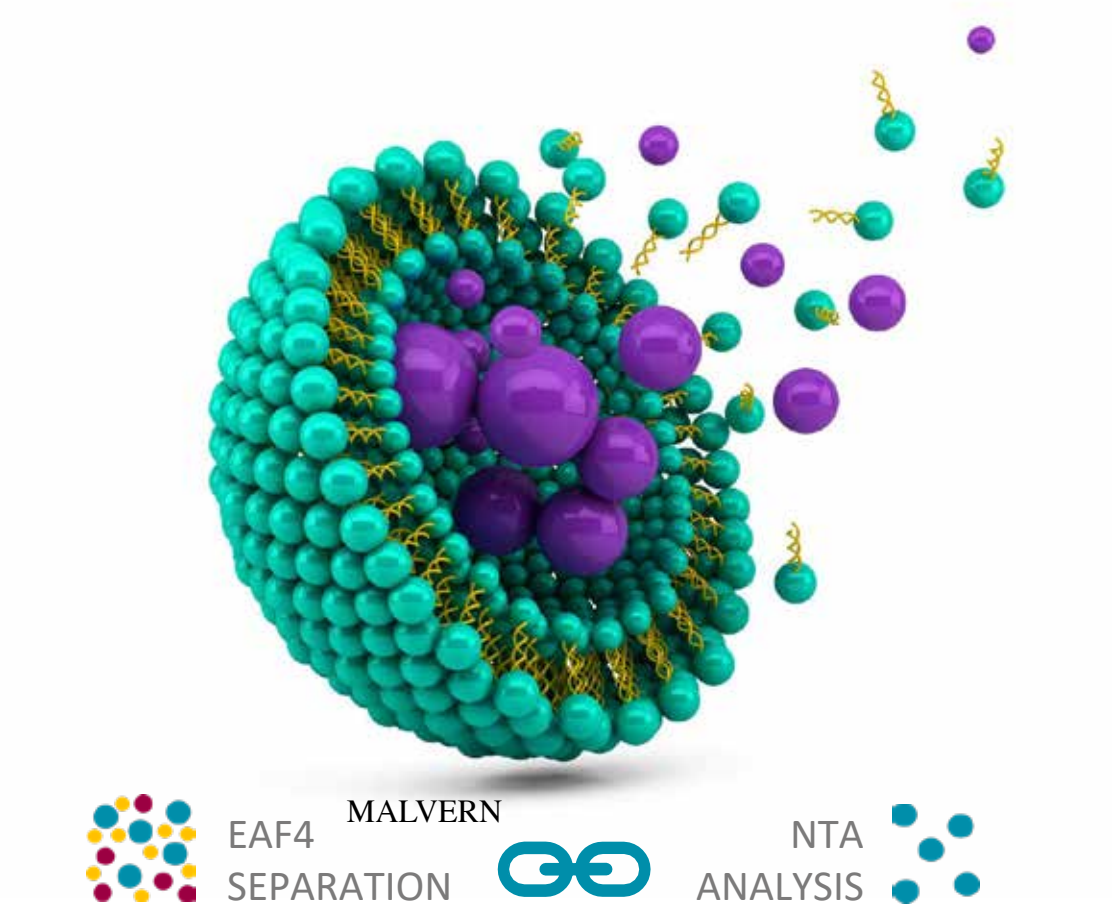

EAF4 SEPARATION

MALVERN

NTA ANALYSIS

SIZE – ZETA – CONCENTRATION

Physio-chemical characterization of exosomes and liposomes in biological media

For more information please visit our virtual Booths: Postnova and Malvern Panalytical

Join; **Dr. Florian Meier**, *Group Leader Research, Postnova Analytics GmbH, Research & Development, Landsberg (D)*

Wednesday - October 28 - 16:10
Stream 2, The Plethora of Nanomedicine Aspects Session 2:

Hyphenation of Electrical Asymmetrical Field-Flow Fractionation with Multi-Angle Light Scattering and Nanoparticle Tracking Analysis for Multi-dimensional Characterization of Liposomes and Exosomes in Complex Biological media

We look forward to meeting you after the talk.

POSTNOVA

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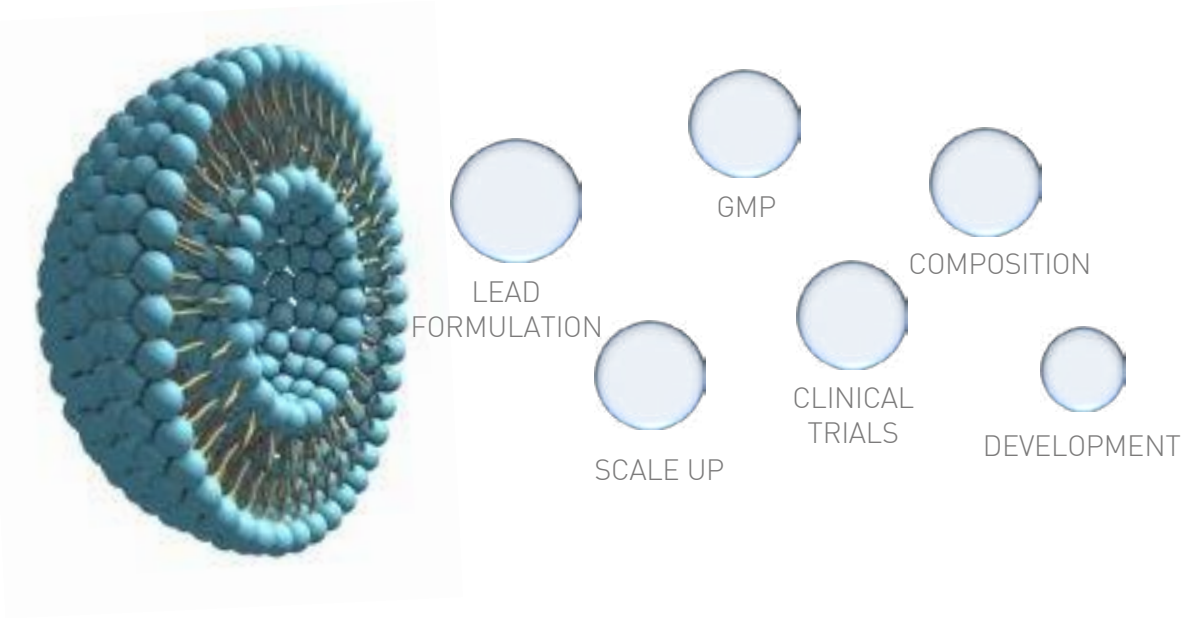
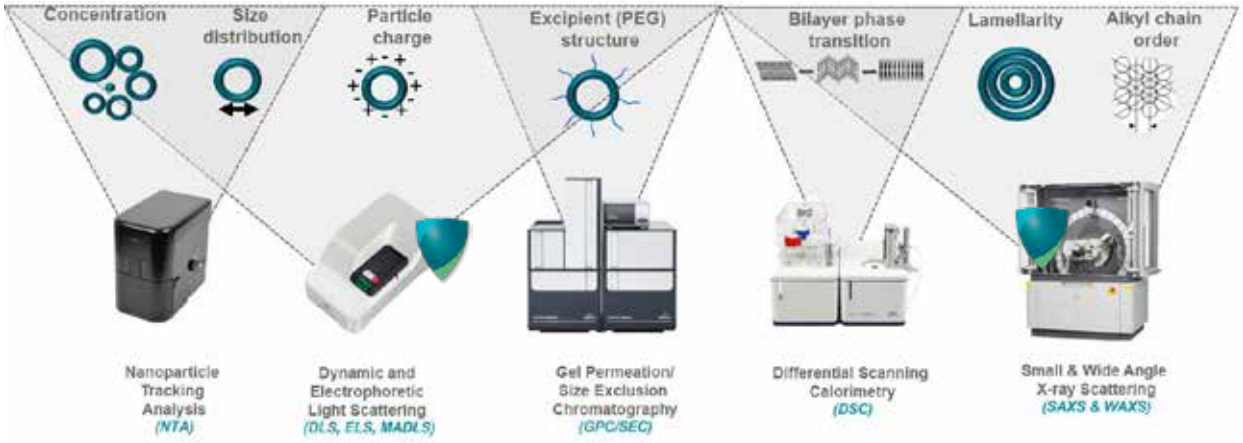


INSTRUMENTS FOR EVERY PHASE OF THE FORMULATION PROCESS



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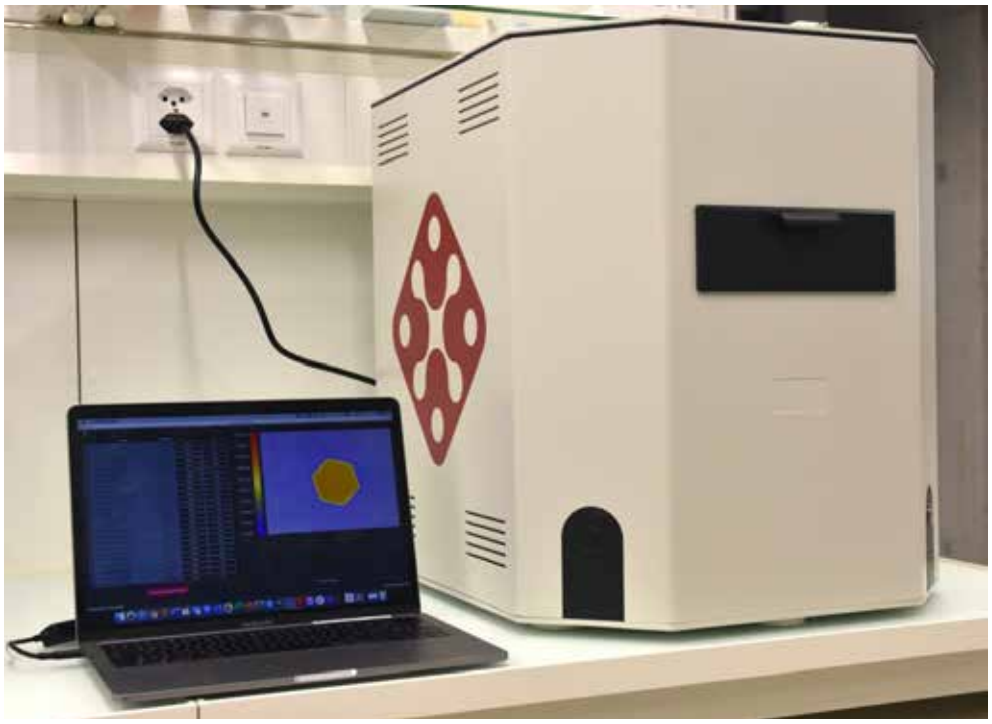
NanoLockin
ADVANCED THERMAL IMAGING

About

NanoLockin is developing instruments for the simple and fast analysis of nanoparticles. The company was founded in early 2018 as a spin-off of the Adolphe Merkle Institute (Switzerland) and the Zurich University of Applied Sciences in Zurich (Switzerland). The young start-up is based in Fribourg in the premises of FriUp.

The Calorsito VIS-NIR

The instrument uses light of different wavelengths (standard: 400 – 950 nm) to stimulate nanoparticles that results in the production of heat. Using proprietary thermal analysis, this resulting heat is analyzed and quantified to give valuable insights about the nanoparticles like their spatial distribution, amount, or their aggregation state.
The Calorsito VIS-NIR can for example be used as a powerful screening tool for nanoparticle cell uptake over a large number of samples without complex sample preparation or pre-labelling. 15 samples can be easily run in less than one hour with the Calorsito VIS-NIR instrument saving time and reducing costs.



Separation and Sizing of a Virus Mixture Using Asymmetrical Flow Field-Flow Fractionation Coupled to Multi-Angle Light Scattering

George Bou-Assaf[a], Andy Blum[a], Omar Matalka[a], Ruth Frenkel[a], Robert Reed[b], Soheyl Tadjiki[b]
(a) Analytical Development, Biogen, Cambridge, MA; (b) Postnova Analytics Inc, Salt Lake City, UT

General Information

ID0018

Application	Viruses, Biopharmaceuticals
Technology	AF4-MALS-RI
Info	Postnova AF2000, PN3621 MALS, PN3150 RI
Keywords	Asymmetrical Flow Field-Flow Fractionation, Virus, Biopharmaceuticals, Multi-Angle Light Scattering

Introduction

Viruses are increasingly used as gene therapy delivery vehicles due to their versatility and safety. They can be loaded with DNA or RNA and delivered to a specific location in the body to treat or cure a disease [1]. One of the biggest challenges for manufacturing a homogeneous virus sample is the presence of viral aggregates, which negatively affect transduction efficiency, biodistribution, and immunogenicity [2]. Due to their relatively large size, often over 50 nm in diameter, virus aggregates are challenging to separate and characterize by column-based chromatography techniques such as size exclusion chromatography (SEC). In this Application Note, we present data on separation of a virus mixture using Asymmetric Flow Field-Flow fractionation (AF4) and measurement of their radius of gyration (R_g).

A schematic for the AF4 channel is shown in Figure 1. The combination of cross flow and channel flow enable size separation over the course of the analysis: small particles elute and reach the connected detectors before larger particles, including aggregates.

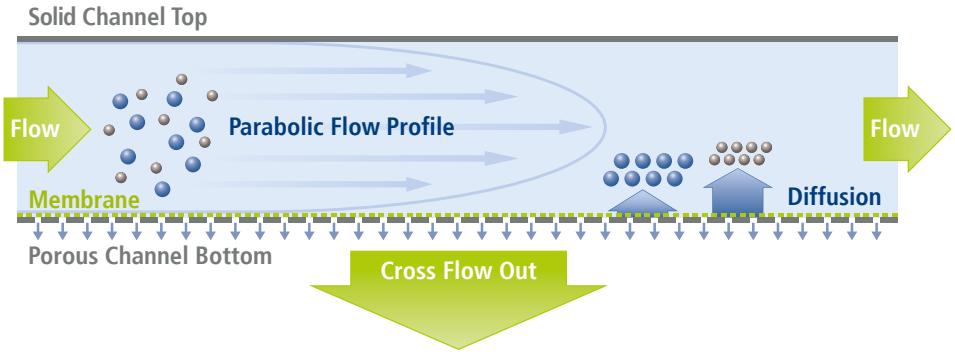


Figure 1: Schematic of the AF4 separation principle.

Experimental Details and Results

A virus mixture sample was created by combining smaller adeno-associated viruses (AAV) with larger adenovirus type 5 (Ad5) in solution to simulate a sample with virus monomer and aggregates. To separate the viruses by size, an AF4 (Postnova AF2000) was used, coupled to a Postnova 21-angle multi-angle light scattering (MALS, PN3621) detector for measuring the R_g. Both the AAV-only and virus mixture samples were analyzed by AF4-MALS to highlight the differences between the samples. The carrier solution was phosphate buffered saline (PBS). The AF4 membrane used was 10 kDa regenerated cellulose.

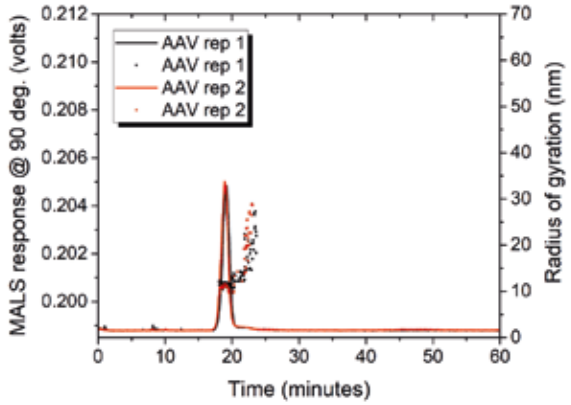


Figure 2. Analysis of AAV-only sample. The curves are replicate AF4-MALS fractograms, overlaid with R_g values (black and red dots).

The MALS response for the AAV-only sample is shown in Figure 2. The main peak corresponds to the AAV monomer eluting between 17 and 20 min. The R_g measured for the monomer is ~ 12.5 nm, very consistent with an expected diameter of 25 nm. There is a small shoulder to the right of the monomer peak, and the increasing R_g for this shoulder peak indicates the presence of a small amount of dimer/trimer/small aggregates in the sample. Fully separating the monomer from any aggregate species was beyond the scope of this work. Further method optimization is required to achieve this goal.

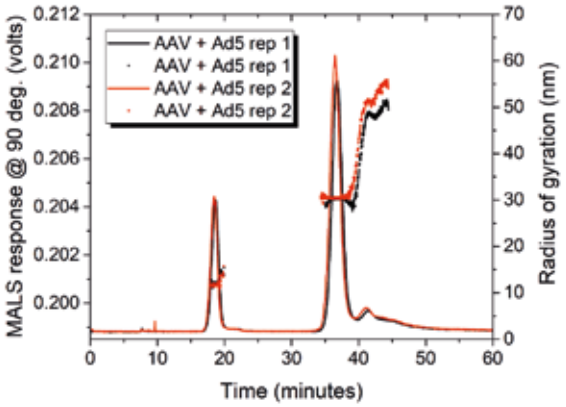


Figure 3. Analysis of AAV and Ad5 virus mixture sample. The curves are replicate AF4-MALS fractograms, overlaid with R_g values (black and red dots).

In Figure 3, the virus mixture is separated into multiple peaks, with the R_g plotted as black and red dots. The monomers have measured R_g values consistent with AAVs, at about 12.5 nm, with the slight shoulder (aggregates) still observed as in the AAV-only sample. A second peak between 33 and 40 min corresponds to the Ad5 virus, whereas a third peak which elutes between 40 and 50 minutes are aggregates of the Ad5 virus. The Ad5 virus and its aggregates have radii in the range of 30-55 nm, most likely too large to be successfully separated by SEC.

Conclusion

The data presented here demonstrates that AF4-MALS is a powerful tool in the separation of virus particles. It can separate viruses and aggregates from a few nm up to >100 nm. This means that AF4-MALS can easily separate and size multi-modal virus samples, including the larger Ad5 virus and its aggregates with high resolution and precision.

References

[1] M.F. Naso, B. Tomkowicz, W.L. Perry III & W.R. Strohl, BioDrugs, 2017, 31(4), 317–334.
[2] J.F. Wright, T. Le, J. Prado, J. Bahr-Davidson, P.H. Smith, Z. Zhen, J.M. Sommer, G.F. Pierce, G. Qu, Molecular Therapy, 2005, 12(1), 171-178.

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These are the funded projects:

BioSMAMPs – Polyitaconic Acid-Based Polymers as Antimicrobial Agents and Coatings for Medical Devices

InGel-NxG – Next Generation Injectable, Adaptive Hydrogels

InnoPoly – Innovative Polymer Composites for the Patient-Specific Additive Production of Biodegradable Jaw Implants

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Next-PEG – PEG-Alternative Polymers for Bioconjugation to Proteins

Poly-IMPLANT-Druck – Research into an Industrial Laser-Based Nano 3D Printing Process for Hierarchically Structured Cartilage-Bone Implants Based on Polymers

PolyAiD – Polymer-Assisted Administration of Autoantigens for the Curative Treatment of Autoimmune Diseases

PolyAntiBak – Research into Spatially Separated "On Demand" Multifunctional Polymer Compartments for Applications in the Field of Antibacterial and Healing- Promoting Implant Coatings

PolyKARD – Synthesis of a Biomimetic Pericardial Polymer for Cardiac Applications

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ProMatLeben-WIN – ProMatLeben Knowledge Transfer: Innovative and Sustainable

Planned Events

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October 26, 2020 • 1:00 pm - 2:00 pm: https://www.gotomeet.me/technik4/clinam-day-1

October 27, 2020 • 1:00 pm - 2:00 pm: https://www.gotomeet.me/technik4/clinam-day-2

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Research supported by TECOmedical Ltd.

Complement inhibition against cytokine storm in a whole blood assay: a pilot study with Implications for COVID-19

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Abstract

Uncontrolled release of inflammatory cytokines, called cytokine storm, is known to be a key contributor to fatal outcomes in severe COVID-19. Our study provides evidence that inhibition of complement (C) activation in peripheral blood mononuclear cell (PBMC) cultures supplemented with intact C significantly reduces the release of cytokines (IL-1β, IL-6, TNFα) induced by C activator liposomes, zymosan or lipopolysaccharide (LPS). C was inhibited achieved by EDTA, heat inactivation or mini-factor H, a short recombinant version of fH in plasma. These data are consistent with the known accelerating role of C activation to cytokine storm, as well as with clinical reports on attenuation of COVID-19 by different C inhibitors. In addition to confirming the benefits of C inhibition and drawing attention to fH and its derivatives as possibly useful medications against COVID-19, our data highlight the utility of the PBMC-based, C-intact in vitro test to screen drugs against cytokine storm, in general, and COVID-19, in particular. The test utilizes multiplex assaying for C activation byproducts and inflammatory cytokines.

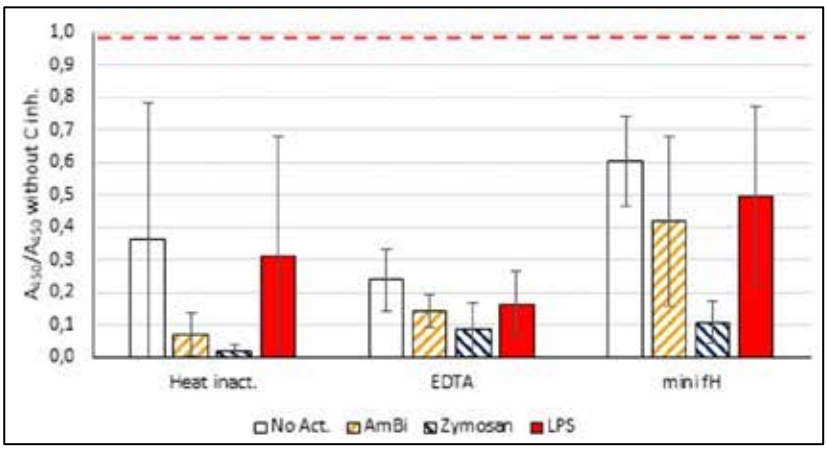
Methods

PBMC was separated from the blood of healthy donors on Ficoll and cultured with normal or heat-inactivated autologous serum, the immune activators AmBisome, Zymosan and LPS, with and without EDTA or recombinant mini-fH, consisting of the first 4 short consensus repeats (SCRs) of fH. C activation was assessed at 45 min after starting the incubation, by measuring sC5b-9 by ELISA. The levels of 16 cytokines (IL-1α, IL-1β, IL-2, IL-4, IL-5, IL-6, IL-8, IL-10, IL-12, IL-13, IL-15, IL-17, IL-23, IFNγ, TNFα and TNFβ in the cell culture supernatant was measured by a multiplexed, chemiluminescence-based ELISA platform (16-Plex Human Cytokine kit at 6 and 18 h (TECO*Medical Inc)*.

Results

Fig 1 shows preliminary data on the inhibitory effects of heat inactivation, EDTA and mini-fH on C activations by AmBisome, Zymosan and LPS in PBMC cultures, using sC5b-9 as endpoint. The

bars show the inhibition in relative terms, i.e., the ratio of inhibited to non-inhibited control values, which enables the comparisons of different treatments. All three inhibition methods caused



significant reduction of sC5b-9.

Fig. 1. Inhibition of C activation by AmBisome, zymosan and LPS (columns with different colors) by heat inactivation of serum at 56oC, EDTA (20 mM) and mfH (1 mg/mL). The OD readings in the sC5b-9 ELISA at 45 min were related to the control (cGM). Bars show the mean ± SD (n=3). Essentially similar inhibitions were obtained for Bb and C5a (not shown).

Fig. 2 shows the clinically relevant results obtained in our study, attesting to significant inhibition of AmBisome, Zymosan and LPS-induced IL-6 liberation in PBMC culture by EDTA and heat inactivation (Heat) of serum. Mini-fH (mfH) also caused significant inhibition of Zymosan-induced IL-6 release, along with a trend for inhibition in the case of AmBisome and LPS. More or less similar observations were made for other cytokines[1].

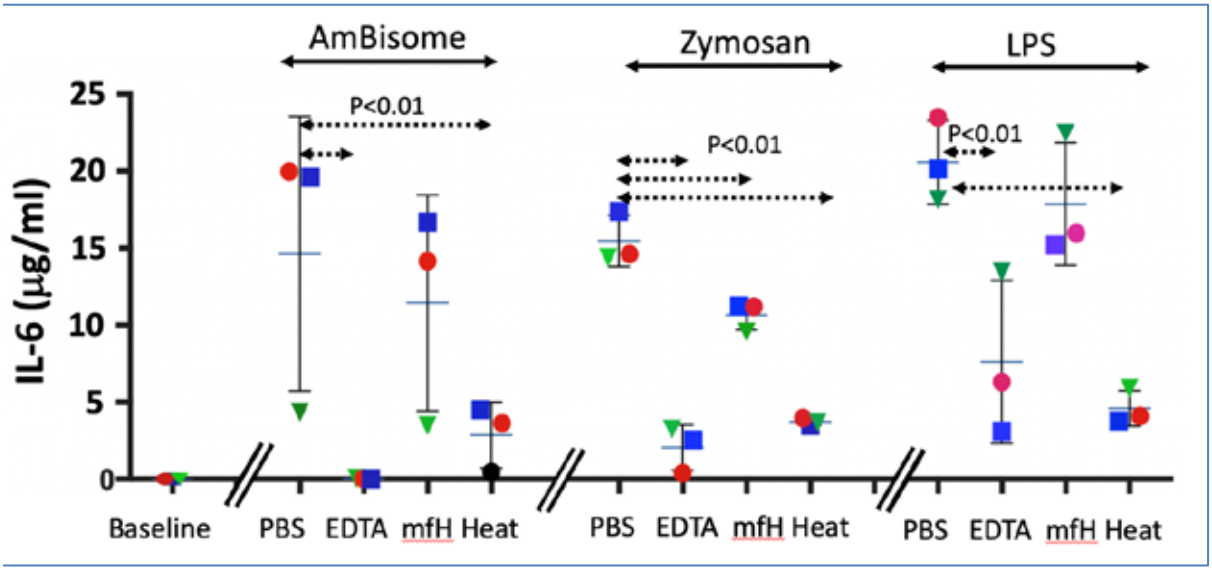


Fig. 2. IL-6 levels in PBMC culture supernatants after 18 hours activation with AmBisome, Zymosan or LPS. The different activators are specified above the data groups, and the differently colored spheres, triangles and rectangles specify 3 different blood donors. PBS, EDTA, mfH and Heat mean culturing without C inhibitor (PBS), or in the presence of 20 mM EDTA, 1,3 µM mini-

fH or in serum inactivated at 56°C. The statistically significant differences among the treatments are shown by the dotted lines.

Discussion

Cytokine storm, involving fever and other systemic symptoms of severe acute inflammation, is one of the most frightened adverse effects of cell therapies, certain biopharmaceuticals and some nanomedicines. Recently, it has come to worldwide attention because it has been shown to play a key role in the lethal outcome of COVID-19. Thus, testing for cytokine induction and its pharmacological inhibition is gaining increasing importance. The activation of the other arm of innate immunity, the C system, is also known to contribute to cytokine storm, and, hence, to the aggravation of COVID-19. However, the currently used PBMC-based cytokine storm assays lack intact C, and are therefore insensitive to detect the contribution of C activation to cytokine storm. By supplementing the PBMC assay medium with autologous serum, our assay filled this gap. We observed significant, sometimes total inhibition of cytokine release by EDTA and heat inactivation of serum and partial inhibition by mini-fH, which are known methods to inhibit C activation. These observations are consistent with the increasing recognition of the efficacy of clinically applicable C inhibitors against COVID-19. Considering that 1) IL-6 is one of the cytokines highly elevated in severe COVID-19, and 2) mini-fH is a druggable natural inhibitor of C that has, to our knowledge, not got much attention, our findings may have clinical relevance in the fight against the coronavirus pandemic[1].

[1]Complement dependent cytokine release from peripheral blood mononuclear cells: a basic cytokine storm model with implications for COVID-19 and beyond (under publication). Same authors and affiliations.

Enabling biotech breakthroughs with polymer technology

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Main advantages

- Biocompatible polymers
- Ultra high quality, purity*
- Unparalleled Mwt definition*
- Versatile functionalization*
- Tunable properties*

* Exclusive Ultroxa® technology

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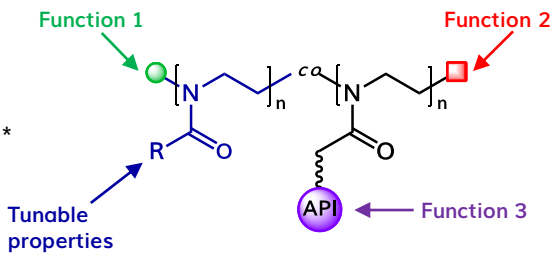

The polyoxazoline polymer platform exhibits fundamental advantages for its use pharma and biotech.

Currently **9 patented technologies** developed at Ghent University have been exclusively licensed to our company, including key polymer structures in the field of polyoxazoline and polyethyleneimines.

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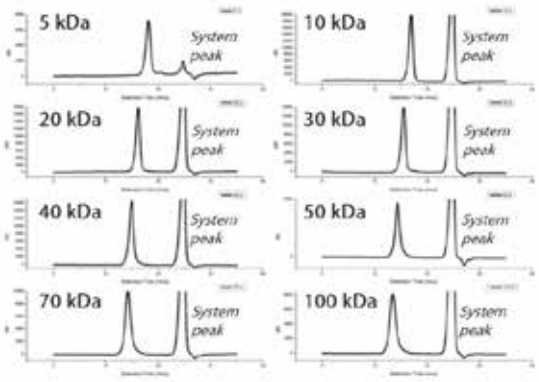
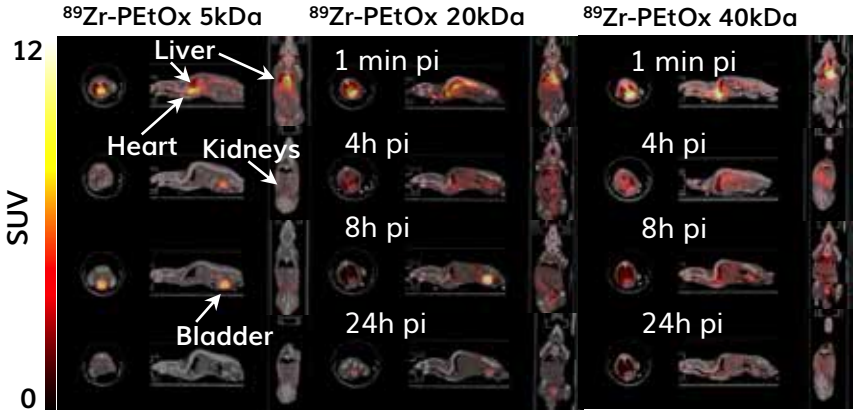
Our ability to synthesize polymers with unparalleled molar mass definition ensures full control over polymer residence time and performance.

Unparalleled Molar mass definition



Size Exclusion Chromatography eluograms of (unpurified) PEtOx

Residence time controlled by chain length



In vivo μPET imaging. *J. Control. Release* 2016, 235, 63-71

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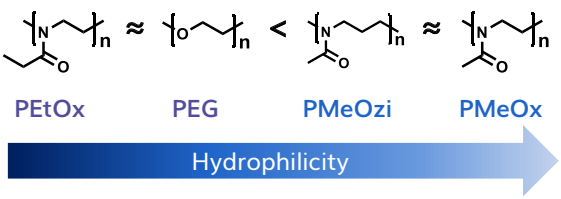

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- Long-term stability remarkably higher than PEG (*Macromol. Rapid Commun.* 2012, 33, 1663–1676)

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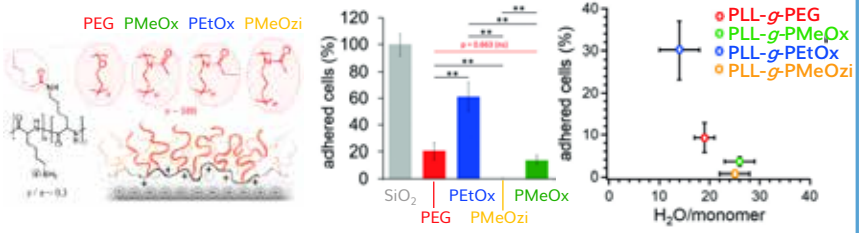

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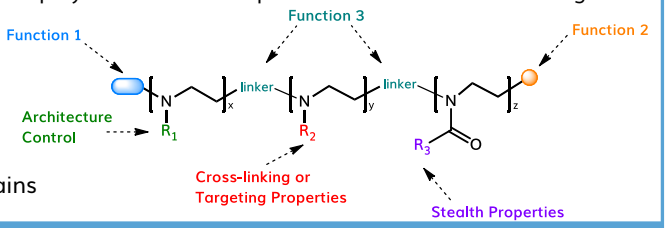

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Our unique know-how on synthesis and structure-property relationships allows us to design and deliver a tailored material with the optimal properties for the customer's application. In us, our customers find a partner that fulfills a fundamental gap in their product development while advantaging from the inherently beneficial properties of our Ultroxa polymer platform. Both our technology and collaborative approach situate our company in a unique market position.

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- **Unparalleled hydrophilicity**: superior hydrophilicity and antifouling properties compared to the state-of-the-art. This enables new products such as diagnostic devices with outstanding sensitivity through minimal unspecific biomolecule interaction.
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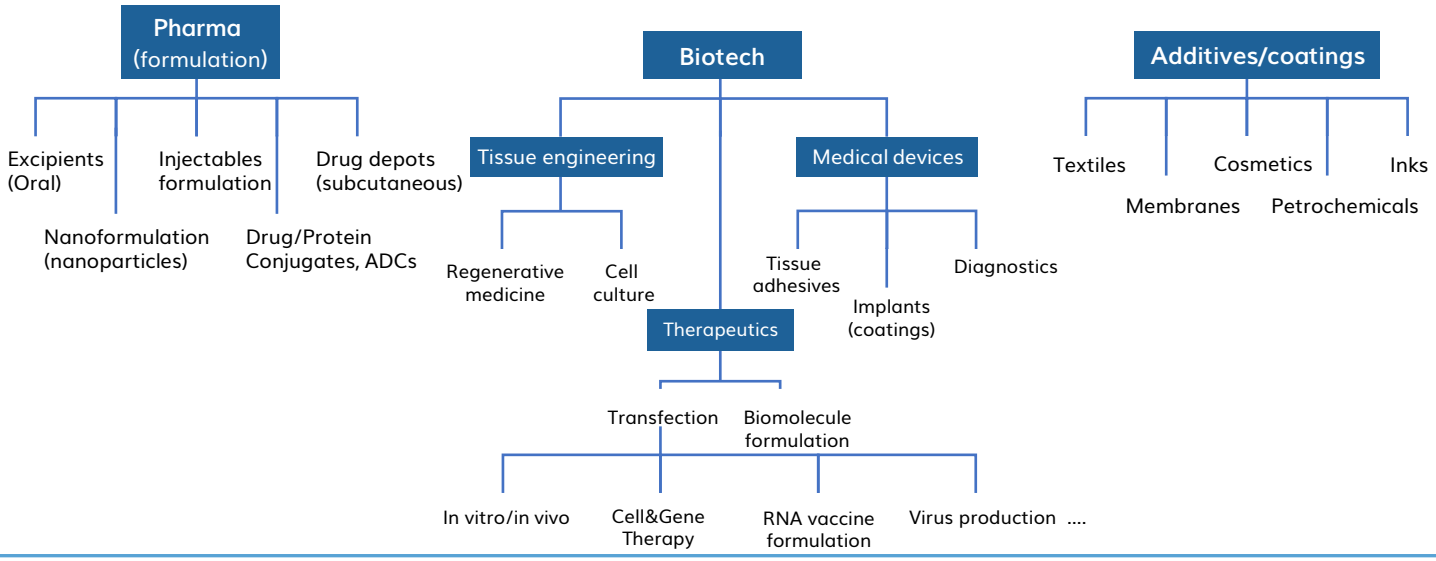
Ultroxa Polymer Technology covers a wide range of Applications



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- setting nanomedicine into the broad context of related medical procedures, technologies and therapeutic trends
- promoting and supporting the global transfer of knowledge
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