

SUMMIT PROCEEDINGS

From Hope to Product – The Brilliant Prospect Nanomedicine and Related Fields

What was Achieved? What are the Future Horizons for Nanomedicine?

THE 35 NPO IDEALISTIC CLINAM SUPPORTERS



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Table of Contents:

INTRODUCTION

CV SPEAKERS

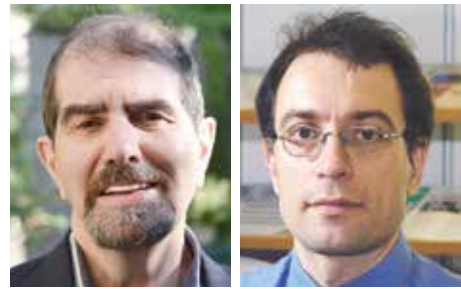
Ahmad Naveed _____	2	Geertsma Robert _____	52	Mühlebach Stefan _____	30	Theuretzbacher Ursula _____	44
Al-Jamal Khuloud T _____	2	Giamarellos-Bourboulis _____		Müller Bert _____	31	Thieringer Florian M. _____	44
Alavizadeh Seyedeh Hoda _____	2	Evangelos _____	17	Nadeau Kari Christine _____	31	Tomalia Donald A. _____	44
Alexiou Christoph _____	3	Gigante Valeria _____	17	Nicoletti Letizia _____	32	Touw Daan _____	45
Aliyazdi Samy _____	3	Gonzalez-Carter Daniel _____	17	Nigo Mutro Maurice _____	32	Tran Natalie _____	45
Årdal Christine _____	3	Govett Anna _____	18	Nisato Riccardo _____	32	Trohopoulos Panagiotis N. _____	46
Ashford Marianne _____	4	Grabbe Stephan _____	18	Nuhn Lutz _____	33	Ucisk Mehmet Hikmet _____	46
Ballerini Laura _____	4	Gu Zhen _____	18	Otterson Kevin _____	33	Van Hest Jan _____	47
Balogh Lajos _____	4	Gunaseelan Nivetha _____	19	O'Reilly Rachel _____	33	Van Hoogevest Peter _____	47
Barenholz Yechezkel _____	5	Haas Heinrich _____	19	Papaluca Amati Marisa _____	34	Varna-Pannerec Mariana _____	47
Bartucci Roberta _____	5	Halbherr Stefan _____	20	Patri Anil _____	34	Vicent Maria _____	48
Barz Matthias _____	6	Harms Alexander _____	20	Peer Dan _____	34	Vogel Viola _____	48
Benn Mario _____	6	Hartmann Ann-Kathrin _____	20	Peitsch Camille _____	35	Wacker Mathias _____	49
Blake Kevin _____	6	Herrmann Inge _____	21	Petrovic Marija _____	35	Wagner Andreas _____	49
Borchard Gerrit _____	7	Hunziker Patrick _____	21	Picaud Serge _____	35	Weichold Frank F. _____	49
Breitkreutz Jörg _____	7	Huwylar Jörg _____	21	Prakash Jai _____	36	Wick Peter _____	50
Brito Luis _____	8	Ibrahim Sarah _____	22	Que Yok-Ai _____	36	Wippermann Marieluise _____	50
Bürki-Thurnherr Tina _____	8	Isles Mike _____	22	Quin Yue _____	36	Witzigmann Dominik _____	50
Carballido José M _____	8	Jiang Wenlei _____	22	Rafael Fernandes de Sousa Diana _____	37	Wykes Robert _____	51
Cavey Andrew _____	8	Jordan Andreas _____	23	_____	37	Yonath Ada _____	51
Cern Ahuva _____	9	Karakoçak Bedia Begum _____	23	Rengstl Benjamin _____	37	Yu Wai Man Cynthia _____	51
Chavakis Triantafyllos _____	9	Keller Michael _____	23	Riebesehl Bernd _____	37	Zagorski Karen _____	52
Cho Nam-Joon _____	10	Khalin Igor _____	23	Rijcken Cristianne _____	37		
Ciechanover Aaron _____	10	Kim Bumjun _____	24	Rocco Paolo _____	38		
Crommelin Daan J.A. _____	10	Kisby Thomas _____	24	Rothen-Rutishauser Barbara _____	38		
Cullis Pieter _____	11	Kostarelos Kostas _____	24	_____	38		
De Vlieger Jon _____	11	Krol Silke _____	25	Rudolph Carsten _____	38		
Decuzzi Paolo _____	11	Lammers Twan _____	25	Salvati Anna _____	39		
Dézi László _____	12	Lecommandoux Sebastian _____	25	Sandvig Kirsten _____	39		
Dobrovolskaia Marina _____	12	Lee Dong Soo _____	26	Satchi-Fainaro Ronit _____	39		
Drescher Simon _____	13	Lehr Claus-Michael _____	26	Schiffelers Raymond _____	40		
Dvir Tal _____	13	Lloyd Jeffs _____	26	Schmid Ruth _____	40		
Eggermont Alexander _____	13	Lovell Jonathan _____	27	Schroeder Avi _____	40		
Ekinci Yasin _____	14	Luxenhofer Robert _____	27	Schubert Stephanie _____	40		
Emanuel Noam _____	14	Löffler Beat _____	27	Schubert Ulrich _____	41		
Engels Boris _____	14	Mailänder Volker _____	28	Schwartz Simo _____	41		
Fahr Alfred _____	14	Marcus-Kalish Mira _____	28	Sedo Kurt _____	42		
Field Anne _____	15	Mäser Pascal _____	29	Sigaeva Alina _____	42		
Formiga Fabio Rocha _____	15	McNeil Scott E. _____	29	Skotland Tore _____	43		
Fuhrmann Gregor _____	15	Merril Carl R. _____	29	Storm Gert _____	43		
Gabizon Alberto _____	16	Moghimi Moien _____	29	Sudbrak Ralf _____	43		
Galon Jérôme _____	16	Mulder Willem _____	30	Szebeni Janos _____	44		
Gazit Ehud _____	16						

CV POSTERS

Agbo Chinazom _____	54
Angelov Borislav _____	54
Arabi Leila _____	54
Bahlool Ahmad _____	55
Baker Rafal _____	55
Brain Danielle _____	55
Deuker Mareike _____	55
Di Francesco Martina _____	55
Fichter Michael _____	56
Fragassi Agnese _____	56
Francia Valentina _____	57
Gaikwad Hanmant _____	57
Gardey Elena _____	57
Gaspar Manuela _____	57
Gatin Eduard _____	58
Han Shunping _____	58
Heiss Bettina _____	58
Kaur Satinderdeep _____	59

Krehan Joshua	59	Drescher Simon	80	Müller Bert	99	Wacker Mathias	120
Kumar Lekshmi G.	59	Dvir Tal	80	Nadeau Kari Christine	99	Wagner Andreas	121
Laprévotte Emilie	60	Dézi László	79	Nicoletti Letizia	99	Wick Peter	121
Liam-Or Revadee	60	Eggermont Alexander	80	Nigo Mutro Maurice	101	Witzigmann Dominik	122
Medina-Montano Carolina	60	Ekinci Yasin	81	Nisato Riccardo	101	Wykes Rob	122
Mzyk Aldona	60	Emanuel Noam	81	Outterson Kevin	102	Yonath Ada	122
Oberländer Jennifer	61	Engels Boris	82	O'Reilly Rachel	102	Yu Wai Man Cynthia	122
Pinto Soraia	61	Fahr Alfred	82	Papaluca Amati Marisa	102	Zagorski Karen	122
Ramachandra Kurup Sasikala		Field Anne	82	Patri Anil	102		
Arathyram	61	Formiga Fabio Rocha	83	Peer Dan	102	POSTER ABSTRACTS	
Rouatbi Nadia	61	Fuhrmann Gregor	83	Peitsch Camille	103	Agbo Chinazom	125
Schunke Jenny	62	Gabizon Alberto	83	Petrovic Marija	103	Angelov Borislav	125
Settanni Giovanni	62	Galon Jérôme	83	Picaud Serge	103	Arabi Leila	126
Sidorenko Valeria	62	Gazit Ehud	84	Prakash Jai	104	Bahlool Ahmad	126
Spadea Alice	63	Geertsma Robert	123	Qin Yue	104	Baker Rafal	127
Spyridopoulou Katerina	63	Giamarellos-Bourboulis		Que Yok-Ai	104	Brain Danielle	128
Telefont Martin	64	Evangelos	84	Rafael Fernandes de Sousa		Deuker Mareike	129
Vogel Theresa	64	Gigante Valeria	84	Diana	105	Di Francesco Martina	130
Wang Shiqi	64	Gonzalez-Carter Daniel	84	Rengstl Benjamin	105	Fichter Michael	131
Zeyn Yanira	65	Govett Anna	85	Rijcken Cristianne	105	Fragassi Agnese	132
		Gu Zhen	85	Rocco Paolo	106	Francia Valentina	132
SPEAKER ABSTRACTS		Gunaseelan Nivetha	87	Rothen-Rutishauser		Gaikwad Hanmant	133
Ahmad Naveed	67	Haas Heinrich	89	Barbara	107	Gardey Elena	134
Al-Jamal Khuloud T.	68	Halbherr Stefan	89	Rudolph Carsten	107	Gaspar Manuela	134
Alavizadeh Seyedeh Hoda	68	Harms Alexander	85	Salvati Anna	108	Gatin Eduard	135
Alexiou Christoph	70	Hartmann Ann-Kathrin	85	Sandvig Kirsten	108	Han Shunping	136
Aliyazdi Samy	70	Herrmann Inge	87	Satchi-Fainaro Ronit	109	Heiss Bettina	137
Årdal Christine	70	Hunziker Patrick	90	Schiffelers Raymond	109	Kaur Satinderdeep	138
Ashford Marianne	71	Huwyler Jörg	90	Schroeder Avi	109	Krehan Joshua	138
Ballerini Laura	71	Ibrahim Sarah	90	Schubert Stephanie	110	Kumar Lekshmi G	139
Balogh Lajos	72	Isles Mike	90	Schubert Ulrich	111	Laprévotte Emilie	139
Barenholz Yechezkel	72	Jiang Wenlei	91	Schwartz Simo	111	Liam-Or Revadee	140
Bartucci Roberta	73	Jordan Andreas	91	Sedo Kurt	111	Medina-Montano Carolina	140
Barz Matthias	73	Karakoçak Bedia Begüm	91	Sigaeva Alina	112	Mzyk Aldona	141
Benn Mario	74	Keller Michael	92	Skotland Tore	113	Nadia Rouatbi	143
Blake Kevin	74	Khalin Igor	92	Storm Gert	113	Oberländer Jennifer	142
Borchard Gerrit	74	Kim Bumjun	93	Sudbrak Ralf	113	Pinto Soraia	142
Breitkreutz Jörg	75	Kisby Thomas	93	Szebeni Janos	114	Ramachandra Kurup Sasikala	
Brito Luis	75	Kostarelos Kostas	94	Theuretzbacher Ursula	114	Arathyram	143
Bürki-Thurnherr Tina	76	Krol Silke	94	Thieringer Florian	114	Rouatbi Nadia	143
Cavey Andrew	77	Lecommandoux Sébastien	95	Tomalia Donald A.	114	Schunke Jenny	144
Cern Ahuva	77	Lee Dong Soo	95	Touw Daan	115	Settanni Giovanni	144
Chavakis Triantafyllos	77	Lehr Claus-Michael	95	Tran Natalie	115	Sidorenko Valeria	145
Chiu Mark	78	Lloyd Jeffs	95	Trohopoulos Panagiotis N.	116	Spadea Alice	146
Cho Nam-Joon	78	Lovell Jonathan	96	Ucisik Mehmet Hikmet	117	Spyridopoulou Katerina	147
Ciechanover Aaron	78	Luxenhofer Robert	96	Van Hest Jan	118	Telefont Martin	147
Crommelin Daan J.A.	78	Marcus-Kalish Mira	96	Van Hoogevest Peter	118	Valeria Sidorenko	145
Cullis Pieter	79	Merril Carl R.	96	Varna Mariana	118	Vogel Theresa	148
Decuzzi Paolo	79	Moghim Moein	97	Vicent Maria	120	Wang Shiqi	148
Dobrovol'skaia Marina	80	Mühlebach Stefan	98	Vogel Viola	120	Zeyn Yanira	149

EDITORIAL



Welcome to the 13th European Summit for Clinical Nanomedicine:

From Hope to Product – The Brilliant Prospect in Nanomedicine and Related Fields

The developments using Nanotechnology in medicine have advanced in the last two years eminently. The mRNA vaccines have elicited potent immunity against infectious disease and have proved to be efficient drugs in the recent pandemic. Lipid Nanotechnology is essential here and in manifold other developments of therapies and drugs.

What has been achieved in what fields? We have shaped a programme that will elucidate the research and development in various medical fields. As a tradition we try every summit to think outside the box and include topics of significant relevance, where Nanomedicine, as cross technology, is of high importance.

This year we, as one example, concentrate in a five hours session on Antibiotic Resistance with Nanomedicine and include worldwide initiatives to learn from each other and to bring together quicker solutions. The summit builds on the principle, that fundamental and applied scientists, developers, clinicians, regulators, and professionals from various nanomedicine related fields can mutually learn to find better solutions for the medicine of the future.

It would have been impossible to have this meeting this May as “real life” meeting. We have members from 31 countries and although Covid-19 seems to be on the path of amelioration, we must guarantee the safety for the entire community. In the CLINAM Virtual debate lounge we have tried to come closer to reality since there verbal communication is possible.

We wish you a fruitful Summit that will be followed in 2024 by hopefully a “real life” Summit again.

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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Individual researchers and research groups from all around the world are therefore **encouraged to submit a research proposal** covering one or more of the research areas mentioned above to apply for funding of research for non-commercial purposes. Especially PhD and Postdoc projects at academic institutes are in focus.

Interested? More information can be found on www.Phospholipid-Research-Center.com/Funding/Research-Proposal or contact info@phospholipid-research-center.com

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CURRICULA VITAE SPEAKERS



Naveed Ahmad

PhD student at Institute of Pharmaceutical Science, Faculty of Life Sciences & Medicine, King's College London, 150 Stamford Street, London SE1 9NH, United Kingdom

Naveed graduated with Pharm.D (2012) and M.Phil (2015) in Pharmacy from Uni-

versity of Malakand, Dir Lower, Pakistan. During his M.Phil studies, he performed pharmacological investigations of novel synthetic flavones in animal models for treatment of epilepsy, anxiety and depression. In another project, he investigated the anti-nociceptive, anti-inflammatory and anti-diabetic activities of synthetic and natural products in animal models. He worked for 2 years as a hospital Pharmacist in a tertiary care hospital in his home country. Currently he is studying PhD under the supervision of Prof. Khuloud Al-Jamal at the Institute of Pharmaceutical Science, Faculty of life science & Medicine, King's College London, United Kingdom. His PhD work is to exploit design of experiment for development of polymeric nanoparticles for intranasal delivery of therapeutic peptides to treat neurological disorders in collaboration with The Institute of Psychiatry, Psychology & Neuroscience of King's College London, United Kingdom.

RECENT PUBLICATIONS

- Hamid, H. A., Khan, S., Shah, S. M. N., Asghar, M., Shahid, M., Hussain, Z., & Ahmad, N. (2021). Piroxicam loaded polymer hybrid microspheres-based tablets with modified release kinetics: Development, characterization and *in vivo* evaluation. *Pakistan Journal of Pharmaceutical Sciences*, 34. doi.org/10.36721/PJPS.2021.34.1.SUP.327-335.1
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- Nabi, G., Ahmad, N., Ali, S., & Ahmad, S. (2015). Irisin: a possibly new therapeutic target for obesity and diabetes mellitus. *World journal of zoology*, 10(3), 205-210. doi.10.5829/idosi.wjz.2015.10.3.9556.



Khuloud T Al-Jamal

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

Khuloud Al-Jamal is a Chair of Drug Delivery & Nanomedicine and Head of Medicines Developments at the Institute of Pharmaceutical Science, King's College London. She has developed an extensive

experience in designing and developing novel nanoscale delivery systems including dendrimers, liposomes, quantum Dots, polymers, viral vectors, chemically functionalised carbon nanotubes and graphene oxide. Her current work involves pre-clinical translation nanomedicines with special interests in brain diseases and cancer. She received several awards including the Maplethorpe Research and Teaching Award, BBSRC New Investigator Award,

Royal Pharmaceutical Society of Great Britain Science Award, the Controlled Release Society Nanomedicine and Nanoscale Delivery Focus Group Young Investigator Award and Wellcome Trust Image Awards. She is a member of the General of Pharmaceutical Council. 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), MedBioMed (Wiley), Scientific Reports (Nature Publisher) and Journal of Drug Targeting. She is a member of the General of Pharmaceutical Council and a Fellow of Royal Society of Chemistry.

RECENT PUBLICATIONS

- Walters AA, Dhadwar B and Al-Jamal KT*. (2021) Modulating expression of inhibitory and stimulatory immune 'checkpoints' using nanoparticulate-assisted nucleic acid delivery. *EBioMedicine*, 73:103624. doi: 10.1016/j.ebiom.2021.103624
- Walters AA*, Santacana-Font G, Li J, Routabi N, Qin Y, Claes N, Bals S, Wang JT and Al-Jamal KT*. (2021) Nanoparticle Mediated in Situ Molecular Reprogramming of Immune Checkpoint Interactions for Cancer Immunotherapy. *ACS Nano*, doi.org/10.1021/acsnano.1c04456
- Li D, Ahmed M, Khan A, Xu L, Walters AA, Ballesteros B, Al Jamal KT*. (2021) Tailoring the Architecture of Cationic Polymer Brush Modified Carbon Nanotubes for Efficient siRNA Delivery in Cancer Immunotherapy. *ACS Appl Mater Interfaces*. 13(26):30284-30294. doi: 10.1021/acscami.1c02627.
- Abdel-Bar HM, Walters AA, Lim Y, Rouatbi N, Qin Y, Gheidari F, Han S, Osman R, Wang J, Al Jamal KT*. (2021) An "Eat me" Combinatory Nano-formulation for Systemic Immunotherapy of Solid Tumours. *Theranostics*. doi:10.7150/thno.56936; doi:10.7150/thno.56936.
- Abdel-Bar H, Walters Adam, Wang JT-W, Al-Jamal KT* (2021) Combinatory Delivery of Etoposide and siCD47 in a Lipid Polymer Hybrid Delays Lung Tumor Growth in an Experimental Melanoma Lung Metastatic Model. *Advanced Healthcare Materials*. doi: 10.1002/adhm.202001853.



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Seyedeh Hoda Alavizadeh was born in Iran on August 14, 1986. She is a Pharm.D (2004-2010), and PhD graduate of Pharmaceutical Nanotechnology (2010-2016), from Pharmacy School, Mashhad University of Medical Sciences (MUMS). She started her professional career as an Assistant Professor at Pharmaceutical Nanotechnology Department in Pharmacy School, Mashhad University of Medical Sciences since 2017. The major research interest she focuses on are the formulation of smart liposomes for delivery of chemotherapeutics to the tumor tissue by exploiting cancer micro-environment features as well as developing gold and iron nanoparticles for theranostics applications and chemo-immunotherapy of cancer. She has published several research papers (H-index:14) including a highly cited paper as reported by Essential Science Indicator of Thompson Reuter ISI in 2016 [Alavizadeh SH, Hosseinzadeh H. *Food Chem Toxicol*. 2014; 64:65-80]. She has presented several of her research work in national and international conferences including CRS, LRD and previous CLINAM summits and honored to get the prize in the area of basic nanoscience. Recently, she served as the scientific vice chair of the prestigious international conference on nanoscience and nanotechnology (ICNN2021) with a great

lineup of international speakers which held virtually in Mashhad University of Medical Sciences. She is currently an active member of Controlled Release Society and is serving as CRS ambassador since 2021.

PEER-REVIEWED PUBLICATION:

- Gheybi F, Alavizadeh SH, Rezayat SM, Hatamipour M, Akhtari J, Majidi R, Badiie A, Jaafari MR* (2021). pH-sensitive PEGylated liposomal silybin: Synthesis, *in vitro* and *in vivo* anti-tumor evaluation. *Journal of Pharmaceutical Sciences*.
- Doagooyan M, Alavizadeh SH, Gheibihayat SM, Gheybi F* (2021). COVID-19 therapy approaches and vaccine development: the role of nanotechnology. *Nanomedicine Journal*. 8(2):89-97.
- Mirhadi E, Mashreghi M, Maleki MF, Alavizadeh SH, Arabi L, Badiie A, Jaafari MR* (2020): Redox-sensitive nanoscale drug delivery systems for cancer treatment. *International Journal of Pharmaceutics*.
- 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.



Christoph Alexiou

Assistant Medical Director, Else Kröner-Fresenius-Foundation-Professorship, Head Section of Experimental Oncology and Nanomedicine (SEON), Universitätsklinikum Erlangen

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 Universitätsklinikum Erlangen. He receives grants from the European Union, German Research Community (DFG), Ministry of Education and Science (BMBF) and Bavarian State Ministry of the Environment 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.

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- Scarletti M, Park H, Wirth J, Englisch S, Eigen A, Drobek D, Vivod D, Friedrich B, Tietze R, Alexiou C, Zahn D, Zubiri B, Spiecker E, Halik M: The remediation of nano-/microplastics from water. *Mat. Today* 48: 38-46, 2021
- Blümler P, Friedrich RP, Pereira J, Baun O, Alexiou C, Mailänder V: Contactless Nanoparticle-Based Guiding of Cells by Controllable Magnetic Fields. *Nanotechnology, Science and Applications* 14: 91-100, 2021

- Mühlberger M, Unterweger H, Band J, Lehmann C, Heger L, Dudziak D, Alexiou C, Lee G, Janko C: Loading of Primary Human T Lymphocytes with Citrate-Coated Superparamagnetic Iron Oxide Nanoparticles Does Not Impair Their Activation after Polyclonal Stimulation. *Cells* 9: 342, 2020
- Janko C, Ratschker T, Nguyen K, Zschiesche L, Tietze R, Lyer S, Alexiou C: Functionalized Superparamagnetic Iron Oxide Nanoparticles (SPIONs) as Platform for the Targeted Multimodal Tumor Therapy. *Frontiers in Oncology* 9: 59, 2019
- Tietze R, Lyer S, Dürr S, Struffert T, Engelhorn T, Schwarz M, Eckert E, Göen T, Vasylyev S, Peukert W, Wiekhorst F, Trahms L, Dörfler A, Alexiou C: Efficient drug-delivery using magnetic nanoparticles — biodistribution and therapeutic effects in tumour bearing rabbits. *Nanomedicine NBM* 9: 961-971, 2013



Samy Aliyazdi

Samy Aliyazdi was born in Saarlouis (Saarland, Germany) 1994. He studied biological sciences at the University of Konstanz and received his bachelor's degree 2016. Afterwards, he moved back to Saarland to study biotechnology. He received his master's degree 2019, where he investigated the application of superparamagnetic nanoparticles. Since April 2019, he has worked as a

PhD student at the Helmholtz-Institute for Pharmaceutical Research Saarland. There he explores the application of 3D bioprinting for *in vitro* systems to investigate nano-antibiotics against bacterial infections.



Christine Årdal

Senior Researcher

Biotext: Christine Årdal MBA PhD has worked for over 20 years on access to medicines through different sectors, including research institutes, governmental development assistance, pharmacy, national health service and insurance. At the Norwegian Institute of Public Health,

her research focuses on the policy aspects of antimicrobial access and innovation. Årdal was previously the co-lead of the research and innovation work package for the European Union's Joint Action on Antimicrobial Resistance and Healthcare-Associated Infections (EU-JAMRAI), which aimed to detail European strategies to implement mechanisms to increase antibiotic and alternative therapeutic innovation. She was also a co-lead in the DRIVE-AB research project which aimed to transform the way policymakers stimulate innovation, the sustainable use, and the equitable availability of novel antibiotics to meet unmet public health needs. She is the co-lead for the Programme Committee of the Oslo Medicines Initiative and active member of the Norwegian assessment of local production for critical antibiotics.

RECENT PUBLICATIONS

- Outtersen K, Orubu ESF, Rex J, Årdal C, Zaman MH. Patient access in fourteen high-income countries to new antibacterials approved by the FDA, EMA, PMDA, or Health Canada, 2010-2020. *Clinical Infectious Diseases*, 2021; ciab612, <https://doi.org/10.1093/cid/ciab612>
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Marianne Ashford

Advanced Drug Delivery,

Marianne Ashford, PhD, is a Senior Principal Scientist in a global role in Advanced Drug Delivery Department within Pharmaceutical Sciences at AstraZeneca. Marianne is responsible for applying drug delivery approaches which enable the progression of innovative medicines and is working to enable novel targets through intracellular delivery of new modalities such as nucleic acid based drugs.

Marianne has been instrumental in introducing nanomedicines to improve therapeutic index into the AstraZeneca Oncology clinical portfolio. She has initiated several collaborations and the building of the internal capability in nanomedicines, drug targeting and intracellular delivery receiving several internal awards for this work. Previously Marianne led a Preformulation and Biopharmaceutics Group which was responsible for influencing candidate drug design from a product perspective and providing support across the portfolio in solid state science and biopharmaceutics.

Marianne has also held project management roles leading pharmaceutical teams and influencing the global product strategy of various AstraZeneca oncology compounds.

Marianne has published over 65 peer reviewed papers and reviews, six book chapters and holds several patents. She has delivered invited talks, keynotes and plenaries in nanomedicine and advanced drug delivery worldwide. Marianne holds Honorary Professor roles at the Universities of Nottingham and Manchester and is a Fellow of the Controlled Release Society. Marianne has served on numerous academic and industrial scientific committees and advisory boards in the field of drug delivery.

Marianne is passionate about using her scientific knowledge and experience to improve therapies for patients and applying drug delivery science to enable medicines of the future.

RECENT PUBLICATION

- Designing Highly Stable Poly(sarcosine)-Based Telodendrimer Micelles with High Drug Content Exemplified with Fulvestrant
- Qing Yu, Richard M. England, Anders Gunnarsson, Robert Luxenhofer, Kevin Treacher, and Marianne B. Ashford, *Macromolecules* 2022 55 (2), 401-412
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Laura Ballerini

Professor of Physiology

Laura Ballerini research focuses on the interactions between neurons and nano-materials or bioactive-nanodevices. Her scientific strategy is the convergence between biophysics, nanotechnology, chemistry and neurophysiology, potentially leading to a new generation of nanomedicine applications in neurology.

After a post doc at UCL, she became associate and then full professor in physiology at the International School for Advanced Studies-SISSA, Trieste, Italy.

RECENT PUBLICATIONS

- Franceschi Biagioni A, Cellot G, Pati E, Lozano N, Ballesteros B, Casani R, Coimbra NC, Kostarelos K, Ballerini L. Graphene oxide prevents lateral amygdala dysfunctional synaptic plasticity and reverts long lasting anxiety behavior in rats. *Biomaterials* 2021 doi: 10.1016/j.biomaterials.2021.120749
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Lajos (Lou) P. Balogh

Editor-in-Chief, Precision Nanomedicine

Dr. Lajos (Lou) P. Balogh, Ph.D. is the Editor-in-Chief of Precision Nanomedicine, the official journal of the European Foundation for Nanomedicine (CLINAM), the former Editor-in-Chief of the Nanomedicine: Nanotechnology, Biology and Medicine (Elsevier) journal for 7 years until 2016

Dr. Balogh published 228 scientific papers, gave >230 invited lectures, and was awarded 12 patents. Lou's publications have been cited over 8800 times (19 papers with more than 100 citations, 10 with more than 200 citations, one is over 1000 times) and is considered to be an international expert in Nanomedicine and scholarly publications. He is one of the five Founders of the American Society for Nanomedicine, serves on the US Technical Advisory Group to ISO TC 229 Nanotechnology, and on the Science Board of a number of international and US national organizations including the External Advisory Board of EuroNanoMed III. Some recent awards include Visiting Professorship for Senior International Scientists at the Chinese Academy of Sciences, Beijing and KOFST Fellowship, Seoul National University, Seoul, Korea. Lou has held faculty positions at Kossuth University Debrecen, Hungary, then in the USA at the University of Massachusetts Lowell, MA, at the Michigan

Molecular Institute, Midland, MI, the University of Michigan, Ann Arbor, MI, and at the Roswell Park Cancer Institute, Buffalo, NY.. Dr. Balogh is the owner and Chief Scientific Advisor of AA Nanomedicine & Nanotechnology (AANMNT), a science consulting firm registered in Essex County, Massachusetts, USA (balogh@aananomedicine.com) and also serves as the Executive Editor of Manuscript Clinic (www.manuscriptclinic.us). For more information see: LinkedIn: <http://www.linkedin.com/in/lajosbalogh> Google Scholar: <https://scholar.google.com/citations?user=jbDlqSwAAAAJ&hl=en>

RECENT PUBLICATIONS

- Balogh, LP, Balancing Interests of Science, Scientists, and the Publishing Business. *Prec. Nanomed.* 2018 Apr; 1(1):5-14. DOI:10.29016/180418.1
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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.



Bartucci Roberta

Roberta Bartucci was born in Rogliano, Cosenza (Italy) on Aug 19th 1989. She attended her secondary education at the Lyceum “B. Telesio” where she finished with 100 out of 100 as final mark. She then pursued the master degree in Pharmaceutical chemistry and technology at the University of Calabria (Italy), and graduated with

a final mark of 106 out of 110. As part of her master, Roberta performed an internship at the University of Calabria, a second internship (although not mandatory) at Utrecht University supported by the Erasmus grant, and a 6-month internship in a pharmacy. Afterwards, she passed the national exam to become officially pharmacist. However, Roberta decided to continue her academic career as PhD student (2015) at the University of Groningen, at the Groningen Research Institute of Pharmacy (GRIP). She graduated with a thesis entitled “Increasing the versatility of an ex vivo model in nanosafety studies and fibrosis” (2020). Roberta assessed Precision-Cut Tissue Slices, a well-established 3D ex vivo model for drug metabolism studies, as an advanced *in vitro* model for nanosafety studies. The potential hazard of different nanomaterials and nanomedicine behavior were tested in murine and human liver, kidney and lung tissue slices. Additionally, longer nanoparticle exposure was tested to investigate potential exacerbation of fibrosis in liver slices, which develop fibrosis spontaneously after 48 h of incubation. In a side project the role of Vanin 1, an enzyme widely expressed in lung, liver, lungs and kidneys, was studied in relation to fibrosis. During these years, Roberta presented her results at national and international conferences, collecting different prizes. She also participated with a video about Nanosafety to the Accomplish impact award, to stimulate societal impact from scientific research. After her PhD, Roberta worked for 1 year as Scientist in a Contract Research Organization (PRA health Sciences), contributing to the success of new drugs developed from national and international pharmaceutical companies. Afterwards, moved from her love for research she decided to continue her career in academia. She is now post-doc researcher in the group of Prof. dr. Anna Salvati, at the University of Groningen where she is working on elucidating the mechanisms of recognition and internalization of nanomedicine by cells.

RECENT PUBLICATIONS

- Bartucci R, C. Åberg, B. N. Melgert, Y. L. Boersma, P. Olinga, and A. Salvati. 2020. Time-Resolved Quantification of Nanoparticle Uptake, Distribution, and Impact in Precision-Cut Liver Slices. *Small*. 1906523. doi:10.1002/smll.201906523.
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Matthias Barz

Professor 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 PUBLICATIONS

- E. J. L. Steen, J. T. Jørgensen, K. Johann, K. Nørregaard, B. Sohr, D. Svatoněk, 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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Mario Benn

Junior Group Leader

Dr. med. vet. Dr. sc. ETH Mario C. Benn is Junior Group Leader in Prof. Viola Vogels Laboratory of Applied Mechanobiology at the Institute of Translational Medicine, ETH Zurich. Mario C. Benn graduated in Veterinary Medicine at Justus-Liebig University Giessen, Germany, and gained specific training in surgical oncology in animal clinics at the University of Tennessee, USA, and Ottawa, Canada. During his doctorate in veterinary medicine (Dr. med. vet.) at the Vetsuisse Faculty, University Zurich, Mario C. Benn conducted pre-clinical research to investigate musculoskeletal regeneration *in vivo*. He integrated *in vitro* tissue engineering approaches and developed a strong interest to understand mechanobiological cues. During his doctorate in science (Dr. sc. ETH) at the Laboratory of Applied Mechanobiology, ETH Zurich, Mario C. Benn further developed a 3D μ Tissue platform that allows the investigation of tissue growth and maturation and how these are regulated by the tensional state, cell phenotypes and extracellular matrix composition at high spatiotemporal resolution. With his research, Mario C. Benn aims to replace, reduce and refine animal experiments, to build interdisciplinary bridges from *in vitro* research towards clinical application, and to contribute to the development of novel strategies for mechanomedicine.

RECENT PUBLICATIONS

- Benn MC, Pot SA, Moeller J, Yamashita T, Fonta CM, Lickert S, Orend G, Kollmannsberger P, Vogel V. Roles of Tenascin-C and tissue transglutaminase in tissue growth and maturation processes. in preparation
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Kevin Blake

Translational Science Senior Specialist

Kevin Blake is the Senior Specialist in Clinical Pharmacology in the Translational Science Office at EMA and has been Scientific Secretariat for the Pharmacokinetics Working Party (PKWP) since 2015. He is also an EMA Scientific Coordinator in the Scientific Advice Office with a focus on procedures relating to generics/hybrids. Prior to joining EMA in 2010 he was a Clinical Assessor at the then Irish Medicines Board (now HPRA) since 2006. Dr. Blake received his primary medical degree (MB, BCh, BAO) at University College Dublin in 1989 and a Ph.D. in Epidemiology at

the University of Western Australia in 2003 as an Australian NHMRC scholar on the topic of fetal growth and cardiovascular disease risk in later life (the 'fetal origins' hypothesis).

While at EMA he has been involved in a number of guidelines including those on post-authorisation efficacy studies (2016), first-in-human clinical trials (2017) and on the reporting of physiologically based pharmacokinetic (PBPK) modelling and simulation (2018). He is also EMA lead in the development of product-specific bioequivalence guidelines with the PKWP. He has over 30 scientific publications including recent overviews of the EMA experience with PBPK models, product specific guidelines and biowaivers. His interests include the regulatory approval of generics, including complex generics; sharing regulator's experience with submitted applications; and the use of modelling and simulation/extrapolation in drug approval.

RECENT PUBLICATIONS

- A Proposed Approach for the Determination of the Bioequivalence Acceptance Range for Narrow Therapeutic Index Drugs in the European Union. Paixão P, Guerreiro RB, Silva N, Blake K, Bonelli M, Morais JAG, Garcia Arieta A, Gouveia LF. *Clin Pharmacol Ther.* 2022 Feb;111(2):470-476. doi: 10.1002/cpt.2451. Epub 2021 Nov 3. PMID: 34657284
- Overview of the European Medicines Agency's Development of Product-Specific Bioequivalence Guidelines. Sullivan JO, Blake K, Berntgen M, Salmonson T, Welink J; Pharmacokinetics Working Party. *Clin Pharmacol Ther.* 2018 Sep;104(3):539-545. doi: 10.1002/cpt.957. Epub 2018 Jan 9. PMID: 29319156
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- Commentary on the EMA Reflection Paper on the use of extrapolation in the development of medicines for paediatrics. Ollivier C, Thomson A, Manolis E, Blake K, Karlsson KE, Knibbe CAJ, Pons G, Hemmings R. *Br J Clin Pharmacol.* 2019 Apr;85(4):659-668. doi: 10.1111/bcp.13883. Epub 2019 Feb 28. PMID: 30707770
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Gerrit Borchard

Professor, Biopharmacy, UNiversity of Geneva, Switzerland. President, Swiss Academy of Pharmaceutical Sciences (SAPHW)

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 (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 160 scientific papers (11442 citations, h-factor 54) 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). Being an enthusiastic long-distance runner, he loves to roam the trails and by-roads of the Swiss mountains on foot and bike.

RECENT PUBLICATIONS

- V. Patrúlea, B.-H. Gan, K. Perron, P. Abdel-Sayed, V. Ducret, E. Sublet, N. Porroche Nerhot, L.A. Applegate, G. Borchard, O. Jor-

dan, J.-L. Reymond, Synergistic activity of antimicrobial peptide dendrimers (AMPDs) coupled to chitosan derivatives to eradicate *Pseudomonas aeruginosa*. *Carbohydrate Polym.* 280 (2022) 119025. doi: 10.1016/j.carbpol.2021.119025.

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Jörg Breikreutz

University Professor, APV President

Jörg Breikreutz is a pharmacist by training and finished his PhD in 1996 at the Institute for Pharmaceutical Technology and Biopharmaceutics in Münster under supervision of Prof. Gröning. From 1996 to 1997 he joined Thiemann Arzneimittel GmbH in Waltrop, Germany, and from 1997 to 2004 the University of Münster to work on his habilitation on paediatric drug formulations. In 2004 he became professor for pharmaceutical technology at the Heinrich-Heine-University in Düsseldorf, Germany. Since 2010 he is the president of the International Association of Pharmaceutical Technology (APV). His research focuses on paediatric drug formulations, orphan drugs, individual medicines and process analytical technologies.

RECENT PUBLICATIONS

- R. Chamberlain, H. Windolf, J. Quodbach, S. Geissler, J. Breikreutz Precise micro-dosing of pramipexole filaments produced via hot-melt extrusion applying various feeding methods, *Pharmaceutics* 14: 216 (2022)
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Luis Brito

VP, Delivery Platform

Luis' is currently VP of the Delivery Platform at Beam Therapeutics. He currently leads a team that is responsible for early research and development of viral and non-viral delivery systems to enable base editing there. Prior to Beam he was at Moderna Therapeutics where he led discovery efforts around novel delivery systems and routes of administration for vaccines and therapeutics. Before Moderna he was at Novartis Vaccines where he developed delivery systems for RNA vaccines and adjuvants. Luis received his PhD in Pharmaceutical Sciences from Northeastern University and has co-authored over 30 peer reviewed papers and is co-inventor on 15 patents.

Moderna Therapeutics where he led discovery efforts around novel delivery systems and routes of administration for vaccines and therapeutics. Before Moderna he was at Novartis Vaccines where he developed delivery systems for RNA vaccines and adjuvants. Luis received his PhD in Pharmaceutical Sciences from Northeastern University and has co-authored over 30 peer reviewed papers and is co-inventor on 15 patents.



Tina Bürki-Thurnherr

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Dr. Tina Buerki-Thurnherr is a biologist with a PhD from ETH Zurich (2006). She continued her scientific career at Empa, where she is currently leading the Particles@Barriers group (since 2015). She has extensive expertise in the assessment of nanosafety and particles-biobarrier interaction studies (placenta, intestine, lung). To achieve results of high predictive value, she develops and employs advanced human *in vitro* and *ex vivo* biobarrier models to establish the groundwork towards the systematic understanding of particle uptake, translocation and biological effects at biological barriers in dependence of material properties. Her research is pivotal for the safe design and use of nanomaterials in industrial, commercial and medical applications and the protection of vulnerable populations. She was investigator and/or co-applicant in several projects founded by the EU (NANOMMUNE, MARINA, NANOSOLUTIONS, GRAPHENE FLAGSHIP) and BMBF (NanoUmwelt). Currently she is principal investigator of a Swiss National Science Foundation (SNSF) project to explore indirect embryo-fetal risks of nanomaterials and a recently SNSF-funded Sino-Swiss Science and Technology Collaboration (Empa- Zhejiang University China) on the development of intelligent single-atom nanozymes for effective and safe therapy of inflammatory diseases in pregnancy.



José M Carballido

Executive Director

José Carballido obtained his M. Sc. degree in Biology at the University of Barcelona (ES) and, after a short training in Immunology at the Pharmaceuticals Research Division of Ciba-Geigy, Basel, he moved to 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 relevance until he became the Head of the Fully Integrated Program of Psoriasis. In 2008, he was appointed Executive Director at the Autoimmunity, Transplantation, and Inflammation Disease Area of the Novartis Institutes for Biomedical Research (NIBR), in Basel (CH) and since 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, he is an active member of several academic societies and faculty of the Eucor (European Campus project: an alliance of five universities based in the Upper Rhine region, namely the Universities of Basel, Freiburg, Haute-Alsace, Strasbourg, and the Karlsruhe Institute of Technology.

José is currently focused on bringing to the clinic immune tolerance approaches, particularly using nanomedicines to ameliorate and cure autoimmune diseases and to prevent humoral and cellular responses to biotherapeutics. In his current role he is also responsible for immunosafety evaluations across the Novartis portfolio and as such, he is advising Novartis teams on drug safety in the context of SARS-CoV-2 infections and COVID-19 vaccination.

RECENT PUBLICATIONS

- Lee JS, Han P, Chaudhury R, Khan S, Bickerton S, McHugh MD, Park HB, Siefert AL, Rea G, Carballido JM, Horwitz DA, Kim D, Fahmy TM. Metabolic and immunomodulatory control of type 1 diabetes via orally delivered bile-acid-polymer nanocarriers of insulin or rapamycin. *Nature Biomed Eng.* 2021, Sep;5(9):983-997. doi: 10.1038/s41551-021-00791-0
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Andrew Cavey

Global Program Head, Prostate Cancer, Novartis

As Global Program Head, Andrew Cavey leads Novartis' activities in prostate cancer, notably across a portfolio of radioligand therapy and imaging agents. Prior to this role, Andrew served in a variety of strategy, commercial and R&D roles at Novartis. He trained as an internal medicine and public health physician in the UK and US, before embarking on a career that spanned humanitarian aid work, a stint in the diplomatic service and time as a management consultant. He is a Member of the United Kingdom's Royal College of Physicians and a Board member of the charity, Parkinson's UK. Andrew holds his Medical and Masters in Physiological Sciences degrees from the University of Oxford, and a Master of Public Health from Harvard University.



Ahuva Cern

Researcher

Dr. Cern is a researcher at the Laboratory of Membrane and Liposome Research at the Hebrew University. Previously, Dr. Cern was an associate director at Pharms Ltd. She then became Director of the Formulation Development Laboratory at Nextar Chempharma Solutions, where she managed many formulation development projects for pharma and biotech companies. Dr. Cern received her Ph.D. from the Hebrew University, working in the laboratory of Prof. Barenholz and Prof. Goldblum in the Department of Biochemistry. Her Ph.D. thesis focused on computational models for the identification of drugs suitable for liposomal delivery.

RECENT PUBLICATIONS

- A.; Bavli, Y.; Hod, A.; Zilbersheid, D.; Mushtaq, S.; Michael-gayego, A.; Barasch, D.; Rotkopf, Y. F.; Moses, A. E.; Livermore, D. M.; Barenholz, Y. Therapeutic Potential of Injectable Nano-Mupirocin Liposomes for Infections Involving Multidrug-Resistant Bacteria. 2021, 1–15.
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Triantafyllos Chavakis

Prof. Dr. T. Chavakis is a clinician scientist. He is specialized in Internal Medicine and Laboratory Medicine. He is director of the Institute for Clinical Chemistry and Laboratory Medicine of the University Hospital at the Technische Universität Dresden (TUD), Dresden Germany since 2017. The Institute for Clinical Chemistry and Laboratory Medicine is responsible for performing the routine laboratory diagnostics of the University Hospital. His group's research focuses on Innate Immunity and Metabolic Inflammation. His group works on the role of innate immune and inflammatory pathways in the context of metabolic-inflammatory pathologies (diabetes, non-alcoholic liver disease), inflammatory bone loss and cancer. In addition, his research focuses on the novel principle of trained innate immunity that represents de facto innate immune memory. His group could show that trained immunity is initiated at the bone marrow and can be leveraged in anti-tumor immunity. Before joining TUD in 2010, he was Principal Investigator and Head of the Inflammation Biology Section at the National Cancer Institute, National Institutes of Health, Bethesda, MD, USA (2005-2010).



Mark Chiu

PhD, President & Chief Scientific Officer

Dr. Mark Chiu is the president and chief scientific officer of Tavotek Biotherapeutics. He has nearly 35 years of experience in new drug discovery at AbbVie/Abbott Laboratories and Janssen, Johnson & Johnson's pharmaceutical business. Prior to joining Tavotek, he was head of process analytical sciences in the biotherapeutics development department and head of antibody engineering in the biologics research department at Janssen. Chiu began his career as a synthetic organic chemist at Microgenics Corp., developing rapid and robust diagnostic assays that currently are utilized by Roche Diagnostics. At AbbVie/Abbott Laboratories, he led an advanced technology team preparing full-length mammalian membrane proteins (GPCRs, ligand gated and voltage gated ion channels, and transporters) for structure-function drug discovery studies.

At Janssen, he led the "developability team" as part of optimization of lead drug candidates. He later transitioned to antibody engineering, where he guided the development of therapeutic bispecific and multispecific antibodies and fusion proteins for areas including oncology, immunology, neuroscience, metabolic diseases and chronic infectious diseases. His leadership resulted in the creation of more than 15 new molecular entities, with six reaching Phase 3 clinical trials. He fostered defense of numerous critical patents covering composition of matter and manufacturing processes. He also led a team responsible for analytical testing to support early- and late-phase development.

Dr. Chiu received his B.S. in Biophysics from University of California at Berkeley and his Ph.D. in Biochemistry from the University of Illinois at Urbana-Champaign. He then completed postdoctoral training at the Laboratory of Physical Chemistry at the Federal Institute of Technology in Zurich, Switzerland, and the Department of Microbiology at the University of Basel Biocentre. He has had comprehensive training and extensive research experience in molecular and cell biology, biochemistry, biophysics and biotechnology. He's authored or co-authored over 20 patents and 100 publications in Science, Nature and other prestigious journals. He has been a member of grant review committees and he is an editor for Current Protocols and Antibodies.

RECENT PUBLICATIONS

- Neijssen, J., Cardoso, R., Chevalier, K. M., Wiegman, L., Valerius, T., Anderson, G. M., Moores, S. L., Schuurman, J., Parren, P., Strohl, W. R., & Chiu, M. L. (2021). Discovery of amivantamab (JNJ-61186372), a bispecific antibody targeting EGFR and MET. *The Journal of biological chemistry*, 296, 100641. <https://doi.org/10.1016/j.jbc.2021.100641>
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- Chiu, M.L., Goulet, D.R., Teplyakov, A., Gilliland, G.L. (2019) "Antibody Structure and Function: The Basis for Engineering Therapeutics", *Special Issue Structure and Function of Antibodies in Antibodies* 8, 55; 1-78 doi:10.3390/antib8040055
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- Kapelski, S., Cleiren, E., Attar, R.M., Philippar, U., Häslar, J., Chiu, M.L. (2019) "Influence of the bispecific antibody IgG subclass on T cell redirection", *MAbs*. 11(6):1012-1024. doi: 10.1080/19420862.2019.1624464



Nam-Joon Cho

School of Materials Science and Engineering, Nanyang Technological University, 50 Nanyang Avenue 639798, Singapore

Viral infections are a leading cause of global morbidity and mortality that urgently need effective therapeutic strategies. While there have been important advances in antiviral drug development over

the past few decades, there remain major challenges associated with the large number of emerging and re-emerging viruses as well as with the rise of drug-resistant virus strains. Developing broad-spectrum antiviral strategies that work against multiple viruses is a high priority to counter emerging viral threats. One promising strategy involves utilizing antiviral agents that target the lipid membrane surrounding a wide range of enveloped viruses such as Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2), Zika (ZIKV), and Dengue (DENV). Unlike other antiviral targets, the lipid envelope is derived from host cell membranes and there is a high barrier to the emergence of drug-resistant virus strains. In this talk, I will present ongoing work to develop a membrane-active peptide that exhibits broad-spectrum antiviral activity against medically important viruses by selectively destabilizing high-curvature viral membranes. By utilizing biophysical assays, we have characterized the mechanism of action of drug candidates down to the single-virus particle level with real-time measurement readouts. Based on these characterization efforts, we have identified a lead peptide drug candidate that exhibits potent, *in vitro* antiviral activity against ZIKV and DENV (all four serotypes) at nanomolar concentrations whereas it is nontoxic to mammalian cells at 1000-fold higher concentrations. The therapeutic efficacy of the peptide was also evaluated in a lethal ZIKV mouse model and treatment started three days after infection. Therapeutic administration of the peptide not only significantly reduced mortality, clinical symptoms, viremia, and inflammation, but also prevented neurodegeneration and brain damage. Furthermore, in a humanized mouse model of DENV infection, peptide treatment reduced viremia levels *in vivo* to nearly undetectable levels. Other arboviruses as well as filoviruses and SARS-CoV-2 have also proven to be susceptible to this targeting strategy. Collectively, our findings support that selective targeting of viral membranes holds great potential for combating emerging viral threats, including SARS-CoV-2 and beyond.



Aaron Ciechanover

Aaron Ciechanover was born in Haifa, Israel in 1947. He is a Distinguished Professor in the Faculty of Medicine at the Technion - Israel Institute of Technology in Haifa, Israel. He received his M.Sc. (1971) and M.D. (1973) from the Hebrew University in Jerusalem. He then completed his national service (1973-1976) as military physician, and

continued his studies to obtain a doctorate in biological sciences in the Faculty of Medicine in the Technion (D.Sc.; 1982). There, as a graduate student with Dr. Avram Hershko and in collaboration with Dr. Irwin A. Rose from the Fox Chase Cancer Center in Philadelphia, USA, they discovered the ubiquitin system for regulated degradation of intracellular proteins. As a post-doctoral fellow with Dr. Harvey Lodish at the M.I.T., he continued his studies on the ubiquitin system and made additional important discoveries. Along the years it has become clear that ubiquitin-mediated proteolysis plays major roles in numerous cellular processes, and aberrations in the system underlie the pathogenetic mechanisms of many diseases, among them certain malignancies and neurodegenerative disorders. Consequently, the system has become an important platform

for drug development. Among the numerous prizes Ciechanover received are the 2000 Albert Lasker Award and the 2004 Nobel Prize (Chemistry; shared with Drs. Hershko and Rose). Among many academies, Ciechanover is member of the Israeli National Academy of Sciences and Humanities, and the National Academies of Sciences (NAS) and Medicine (NAM) of the USA (Foreign Associate).



Daan J.A. Crommelin

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 was adjunct professor at the Department of Pharmaceutics and Pharmaceutical Chemistry at the University of Utah. Crommelin is co-founder of OctoPlus (1995), a Leiden based company specialized in the development of pharmaceutical (mainly protein based) product formulations and advanced drug delivery systems. He published over 360 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 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). His research interests include pharmaceutical technology and physical pharmacy in general and more specifically formulation aspects of small and large molecule drug products. Over the years his interest has focused on: site specific drug delivery with colloidal-nanosized carrier systems/self-assembling systems (in combination with monoclonal antibodies), purification, characterization and stabilization of monoclonal antibodies and other pharmaceutical proteins, receptor-ligand interactions, parenteral systems for (sustained) release of small molecules, proteins and nucleic acids, pharmaceutical aspects of vaccines (adjuvanticity/antigen presentation and delivery), immunogenicity of pharmaceutical proteins.

Over the years his interest has focused on: site specific drug delivery with colloidal-nanosized carrier systems/self-assembling systems (in combination with monoclonal antibodies), purification, characterization and stabilization of monoclonal antibodies and other pharmaceutical proteins, receptor-ligand interactions, parenteral systems for (sustained) release of small molecules, proteins and nucleic acids, pharmaceutical aspects of vaccines (adjuvanticity/antigen presentation and delivery), immunogenicity of pharmaceutical proteins.

RECENT PUBLICATIONS

- Schoenmaker L, Witzigmann D, Kulkarni JA, et al. mRNA-lipid nanoparticle COVID-19 vaccines: structure and stability. *International Journal of Pharmaceutics*. Published online April 2021;120586. doi:10.1016/j.ijpharm.2021.120586
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- Crommelin DJA, Volkin DB, Hoogendoorn KH, Lubiniecki AS, Jiskoot W. The Science is There: Key Considerations for Stabilizing Viral Vector-Based Covid-19 Vaccines. *Journal of Pharmaceutical Sciences*. 2021;110(2):627-634. doi:10.1016/j.xphs.2020.11.015



Pieter Cullis

Pieter R. Cullis, PhD, FRSC, FNAI (USA), OC. Director, Nanomedicines Research Group, Professor, Department of Biochemistry and Molecular Biology, University of British Columbia. Dr. Cullis and co-workers have been responsible for fundamental advances in the development of nanomedicines employing lipid nanoparticle (LNP)

technology for cancer therapies and gene therapies. This work has contributed to five drugs that have received clinical approval. Dr. Cullis has co-founded eleven biotechnology companies that now employ over 300 people, has published over 350 scientific articles and is an inventor on over 60 patents. He has also co-founded three not-for-profit enterprises including the Centre for Drug Research and Development (now AdMare) in 2004 and the NanoMedicines Innovation Network in 2019. Dr. Cullis has received numerous awards including the Order of Canada in 2021 and the VinFuture Prize and the Prince Mahidol Award in 2022. Two recently approved drugs that are enabled by LNP delivery systems devised by Dr. Cullis, members of his UBC laboratory and colleagues in the companies he has co-founded deserve special emphasis. The first is Onpattro which was approved by the US FDA in August 2018 to treat the previously fatal hereditary condition transthyretin-induced amyloidosis (hATTR). Onpattro is the first RNAi drug to receive regulatory approval. The second is BNT162b2 (Comirnaty), the COVID-19 vaccine developed by Pfizer/BioNTech that has received regulatory approval in many jurisdictions including Canada, the USA, the UK and Europe. BNT162b2 is playing a major role in containing the global Covid-19 pandemic with approximately 3B doses administered worldwide in 2021.

RECENT PUBLICATIONS

- Witzgmann, D.; Kulkarni, J. A.; Leung, J.; Chen, S.; Cullis, P. R.; van der Meel, R., Lipid nanoparticle technology for therapeutic gene regulation in the liver. *Advanced drug delivery reviews* 2020, 159, 344-363.



Jon de Vlieger

Director Business Development at Lygature & Coordinator NBCD Working Group
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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 is director of business development at Lygature and a frequent guest lecturer on science & business topics related to public private partnerships. He coordinates several international public private partnerships, such as the European Lead Factory on early drug discovery and the Non Biological Complex Drugs Working Group on regulatory innovation. He serves as a board member at the Federation for Innovative Drug Research Netherlands. He is a co-editor of the book on NBCDs in the AAPS Advances in the Pharmaceutical Sciences Series, co-author on a series of key-papers related to regulatory

challenges for NBCDs and publishes on the value of public-private partnerships in drug discovery and development.

RECENT PUBLICATIONS

- The EU regulatory landscape of non-biological complex drugs (NBCDs) follow-on products: Observations and recommendations. Klein K, Stolk P, De Bruin ML, Leufkens HGM, Crommelin DJA, De Vlieger JSB. *Eur J Pharm Sci.* 2019 May 15;133:228-235. doi: 10.1016/j.ejps.2019.03.029. Epub 2019 Apr 3.
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- Editorial: Public-Private Partnerships as Drivers of Innovation in Healthcare. de Vruh RLA, de Vlieger JSB, Crommelin DJA. *Front Med (Lausanne).* 2019 May 31;6:114. doi: 10.3389/fmed.2019.00114. eCollection 2019.



Paolo Decuzzi

Professor and Senior Scientist

Biotext: I am currently a Senior Scientist and Professor of Biomedical Engineering at the Italian Institute of Technology (IIT) in Genova where, in July 2015, I founded the Laboratory of Nanotechnology for Precision Medicine. I have been serving as an

Associate Professor of Biomedical Engineering at The University of Texas Health Science Center (Houston, TX-USA) from 2007 to 2010 and as a Professor of Biomedical Engineering and Translational Imaging at the Methodist Hospital Research Institute (Houston, TX-USA), until July 2015. My Laboratory in IIT is involved in the rational design of multi-functional nanoconstructs for the treatment and imaging of cancer, cardiovascular and neurodegenerative diseases; the fabrication of microfluidic chips for the rapid screening of novel molecular and nano-based therapeutic agents; the development of multi-scale, hierarchical computational models for predicting the transport and therapeutic efficacy of nanoconstructs; as well as in the organization of dissemination activities at the interface between engineering and biomedical sciences. In this context, I have published over 200 peer-reviewed journal articles and book chapters, generated multiple patents and patent applications. My research activities have been funded by multiple organizations, including NCI, DOD, State of Texas, ESF, ERC, MSCA, and private enterprises, totaling over \$15 million. I have been serving on review panels for the NIH, ERC, ESF and for several national Research Agencies.

RECENT PUBLICATIONS

- Di Mascolo, D., Palange, A.L., Primavera, R., ...Grant, G.A., Decuzzi, P. Conformable hierarchically engineered polymeric micro-meshes enabling combinatorial therapies in brain tumours. *Nature Nanotechnology*, 2021, 16(7), pp. 820–829
- Bedingfield, S.K., Colazo, J.M., Di Francesco, M., ...Decuzzi, P., Duvall, C.L. Top-Down Fabricated microPlates for Prolonged, Intra-articular Matrix Metalloproteinase 13 siRNA Nanocarrier Delivery to Reduce Post-traumatic Osteoarthritis. *ACS Nano*, 2021, 15(9), pp. 14475–14491
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- Primavera, R., Bellotti, E., Di Mascolo, D., ...Thakor, A.S., Decuzzi, P. Insulin Granule-Loaded MicroPlates for Modulating Blood Glucose Levels in Type-1 Diabetes *ACS Applied Materials and Interfaces*, 2021, 13(45), pp. 53618–53629
- Decuzzi, P., Peer, D., Di Mascolo, D., ...Kiessling, F., Lammers, T. Roadmap on nanomedicine. *Nanotechnology*, 2021, 32(1), 012001



László Dézsi

Research Associate Professor

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).

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 was head of laboratory, CRO monitor, research project manager in vascular and safety pharmacology at Gedeon Richter (GR) Pharmaceutical Plc. (1999-2012), and manager of Analgesic Research Laboratory (2006-2012), a joint venture of GR and University of Pécs, Department of Pharmacology. He participated in curriculum development and he had been Secretary of Biomedical Engineering (BE) Course Committee (1994-2000). Now 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 became staff member of Semmelweis University in 2020. He established his own teaching course in 2008 entitled "Cardiorespiratory and neurophysiological measuring techniques" at the Department of Translational Medicine. He participates in postgraduate 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 is member of „EXPERT" project EU's Horizon 2020 research and innovation programme (2019-).

RECENT PUBLICATIONS

- László Dézsi, Tamás Mészáros, Gergely Kozma, Mária H-Velkei, Csaba Zs. Oláh, Miklós Szabó, Zsófia Patkó, Tamás Fülöp, Mark Hennies, Miklós Szebeni, Bálint András Barta, Béla Merkely, Tamás Radovits, János Szebeni (2022) A naturally hypersensitive porcine model may help understand the mechanism of COVID-19 mRNA vaccine-induced rare (pseudo) allergic reactions: complement activation as a possible contributing factor. *GeroScience*, <https://doi.org/10.1007/s11357-021-00495-y>. Published 11 February 2022
- Ákos Pethő, Dorothea Piecha, Tamás Mészáros, Rudolf Urbanics, Christoph Moore, Bernard Canaud, László Rosivall, Tom Eirik Mollnes, Sonja Steppan, Gábor Szénási, János Szebeni, László Dézsi (2021) A porcine model of hemodialyzer reactions: roles of complement activation and rinsing back of extracorporeal blood, *Renal Failure*, 43:1, 1609-1620, DOI: 10.1080/0886022X.2021.2007127
- Gergely Milosevits, Tamás Mészáros, Erik Örfi, Tamás Bakos, Miklós Garami, Gábor Kovács, László Dézsi, Péter Hamar, Balázs Gyórfy, András Szabó, Gábor Szénási, János Szebeni (2021) Complement-mediated hypersensitivity reactions to an amphotericin B-containing lipid complex (Abelcet) in pediatric patients and anesthetized rats: Benefits of slow infusion, *Nanomedicine: Nanotechnology, Biology and Medicine*, 34, 102366, DOI: 10.1016/j.nano.2021.102366



Marina Dobrovolskaia

Director of Operations, Nanotechnology 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

- Shi D, Beasock D, Fessler A, Szebeni J, Ljubimova JY, Afonin KA, Dobrovolskaia MA. To PEGylate or not to PEGylate: Immunological properties of nanomedicine's most popular component, polyethylene glycol and its alternatives. *Adv Drug Deliv Rev*. 2022 Jan;180:114079.
- Dobrovolskaia MA, Afonin KA. Use of human peripheral blood mononuclear cells to define immunological properties of nucleic acid nanoparticles. *Nature Protocols*. 2020 Nov;15(11):3678-3698.
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Simon Drescher

Managing Director at Phospholipid Research Center Heidelberg

After studying pharmacy at the Martin Luther University (MLU) Halle-Wittenberg in Halle, Germany, Dr. Drescher completed 2008 his PhD there in the field of pharmaceutical chemistry and physical chemistry on the synthesis and aggregation behavior

of bipolar phospholipids (bolalipids). He has dedicated himself to this topic at various places of work over 10 years; and finally received the Habilitation and *venia legendi* in pharmaceutical chemistry at MLU in 2017 on the topic of "Artificial phospholipids: syntheses, properties, and applications". After two semesters as deputy professor for pharmaceutical bioanalytics at the University of Greifswald, Germany, he joined the Phospholipid Research Center Heidelberg in December 2019, initially as deputy managing director and, since February 2021, as managing director.

Dr. Drescher's main interests are (i) the synthesis of artificial, i.e. non-naturally occurring, mono- and bipolar phospholipids, including fully synthetic and partial biochemical approaches, (ii) the physicochemical characterization of lipids in 2D and 3D assemblies and their miscibility, mainly using calorimetric methods, infrared spectroscopy, X-ray and neutron scattering techniques, electron microscopy, and mass spectrometry, and (iii) the application of liposomes for (oral) drug delivery.

RECENT PUBLICATIONS

- Van Hoogevest P, Tiemessen H, Metselaar JM, Drescher S, Fahr A: The Use of Phospholipids to make Pharmaceutical Form Line Extensions, *Eur. J. Lipid Sci. Technol.* 2021, 2000297.
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Tal Dvir

Director, Tel Aviv University Center for Nanoscience and Nanotechnology
Director, Sagol Center for Regenerative Biotechnology,
Shmunis School of Biomedicine and Cancer Research, Faculty of Life Science
Department of Biomedical Engineering,
Faculty of Engineering
Tel Aviv University, Tel Aviv, 69978, Israel

Tal Dvir is a Professor at Tel Aviv University, Israel. He obtained his B.Sc. (2003) and Ph.D (2008) degrees from the faculty of Engineering at Ben-Gurion University of the Negev in Israel. His Ph.D research, under the supervision of Prof. Smadar Cohen focused on cardiac tissue engineering and regeneration. Tal continued his postdoctoral studies in the laboratory of Prof. Robert Langer in the Department of Chemical Engineering at MIT. His postdoc re-

search focused on advanced materials for tissue engineering and regeneration. On October 2011 Tal was recruited by the Department of Biotechnology and the Center for Nanotechnology at Tel Aviv University to establish the Laboratory for Tissue Engineering and Regenerative Medicine. On 2013, Tal has also joined the newly established Department of Materials Science and Engineering at Tel Aviv. On 2020 he has also joined the Department of Biomedical Engineering.

Tal's laboratory designs and develops smart bio and nanomaterials and technologies for engineering complex tissues and organs, such as the heart, brain, spinal cord, intestine, eyes and more. During his career Tal has published many high impact papers and received numerous prizes and awards. Tal is also an inventor of numerous patents.

Tal is currently the Director of Tel Aviv University Center for Nanoscience and Nanotechnology and the Founding Director of the Sagol Center for Regenerative Biotechnology.



Alexander Eggermont

CEO: Comprehensive Cancer Center Munich, Germany
CSO: Princess Máxima Center, Utrecht, Netherlands
Prof Immunotherapy, University Medical Center Utrecht, Netherlands

SPECIALTIES & SCIENTIFIC OUTPUT:

Immunotherapy Development, melanoma, sarcoma, general drug development. Scientific Output: >1000 peer-reviewed publications; H-index/citations: HI:110, >60000 (Scopus); HI:127, >83000 citations (Google Scholar)

PROFESSIONAL AWARDS (SELECTION):

Joseph Maisin Chair in Oncology at Catholic University of Louvain in Belgium (2001); ASCO Statesman Award (2010); Michiel Van Vloten Award of the Dutch Surgical Society (2016); John Wayne Award of Clinical Research of Society Surgical Oncology (2018); Doctorate Honoris Causa (University Essen, Germany) (2018); Honorary Chair KAZIOR (Kazakh Institute of Oncology and Radiotherapy)(2019); Deutsche Krebshilfe Cancer Award (2019)

SOCIETAL AWARDS:

Légion d'Honneur, France (2015)

RECENT PUBLICATIONS

- Eggermont et al. Precision Cancer Medicine: Large Studies Indicate Steady Progress. *Cancer Discovery* 2021;11:2677-2678.
- Maio M, ..., & Eggermont A. Neoadjuvant immunotherapy is reshaping cancer management across multiple tumour types: The future is now! *Eur J Cancer* 2021;152:155-164.
- Routy B,... Eggermont A, Zitvogel L. Gut microbiome influences efficacy of PD-1-based immunotherapy against epithelial tumors. *Science* 2018;359:91-97
- Eggermont et al. Adjuvant Pembrolizumab versus Placebo in Resected Stage III Melanoma. *NEJM* 2018;378:1789-1801.
- Eggermont et al. Prolonged Survival in Stage III Melanoma with Ipilimumab Adjuvant Therapy.



Yasin Ekinci

Lab head, LXN, Paul Scherrer Institute

Yasin Ekinci is head of the Laboratory of X-ray Nanoscience and Technologies at PSI. He obtained his PhD in Max-Planck Institute for Dynamics and Self-Organization, Göttingen, Germany in 2003. In 2004, he joined Paul Scherrer Institute as a post-doctoral researcher. Between 2006 and

2012 he worked as a postdoctoral researcher and subsequently as a senior scientist and a lecturer in Department of Materials at ETH Zürich. He is at PSI since 2009 working on various topics of nanoscience and technology, including EUV lithography, resist materials, lensless imaging, plasmonics, semiconductor nanostructures, and nanofluidics. He is author/co-author of more than 230 papers and 8 patent applications. He is a fellow of SPIE.



Noam Emanuel

Dr. Emanuel has vast experience in biotechnology projects, including development of drug delivery systems and immunology. His extensive expertise includes immunotherapy, vaccines, immunodiagnostics, systemic and local drug-delivery, and medical devices. Dr. Emanuel has a number of approved patents in the field of drug delivery and diagnostics. He received

his Ph.D. degree from the Faculty of Medicine at the Hebrew University of Jerusalem.

RECENT PUBLICATIONS

- Ramot Y, Nedvetzki S, Rosenfeld S, Rousselle SD, Nyska A, Emanuel N. D-PLEX100 in an Abdominal Surgery Incision Model in Miniature Swine: Safety Study. *Toxicol Pathol.* 2020 Jul;48(5):677-685. doi:10.1177/0192623320928902. Epub 2020 Jun 11. PMID: 32525456.
- Kachel E, Moshkovitz Y, Sternik L, Sahar G, Grosman-Rimon L, Belotserkovsky O, Reichart M, Stark Y, Emanuel N. Local prolonged release of antibiotic for prevention of sternal wound infections postcardiac surgery-A novel technology. *J Card Surg.* 2020 Oct;35(10):2695-2703. doi: 10.1111/jocs.14890. Epub 2020 Aug 2. PMID: 32743813.
- Emanuel N, Machtei EE, Reichart M, Shapira L. D-PLEX500: a local biodegradable prolonged release doxycycline-formulated bone graft for the treatment for peri-implantitis. A randomized controlled clinical study. *Quintessence Int.* 2020;51(7):546-553. doi: 10.3290/j.qi.a44629. PMID: 32500862.
- Ramot Y, Nedvetzki S, Rosenfeld S, Emanuel N, Nyska A. Toxicity and Safety Study of D-PLEX100 in a Sternal Surgical Defect in New Zealand White Rabbits. *Toxicol Pathol.* 2019 Jun;47(4):504-514. doi: 10.1177/0192623319837887. Epub 2019 Apr 11. PMID: 30975045.
- Emanuel N, Rosenfeld Y, Cohen O, Applbaum YH, Segal D, Baranholz Y. A lipid-and-polymer-based novel local drug delivery system--BonyPid™: from physicochemical aspects to therapy of bacterially infected bones. *J Control Release.* 2012 Jun 10;160(2):353-61. doi: 10.1016/j.jconrel.2012.03.027. Epub 2012 Apr 4. PMID: 22507550.



Boris Engels

Boris Engels, Ph.D., is a cancer immunologist and currently Associate Director in the Cell and Gene Therapy Group of the Immuno-Oncology and Hematology Department at Novartis Institutes for BioMedical Research (NIBR) in Cambridge, MA. Throughout his career, he has focused on adoptive T cell therapy, exploring viral gene-transfer into T cells, T cell biology, as well as the

interplay of T cells with various cells in the tumor microenvironment. At Novartis, Dr. Engels leads multidisciplinary teams progressing chimeric antigen receptor (CAR)-T cells as a new therapy to cure cancer. His experience spans from identifying novel targets and developing next-generation CAR constructs, to early clinical projects, testing novel CAR-T therapies for hematologic and solid cancers. Dr. Engels conducted post-doctoral work studying the interplay of TCR, peptides, and MHC at the University of Chicago. He received his Ph.D. in Biology at the Max-Delbrück Centre for Molecular Medicine and the Freie-Universität, Berlin, Germany, studying retroviral gene transfer into T cells.

PUBLICATIONS

- Singh, N.*, Frey, N.V.*, Engels, B.* ..., Ruella, M. (2021) Antigen-independent activation enhances the efficacy of 4-1BB-costimulated CD22 CAR T cells. *Nat Med.* 27(5):842-850
- Posey, A.D. Jr., Schwab, R.D., Boesteanu, A.C. ..., Engels, B. ..., June, C.H. (2016) Engineered CAR T Cells Targeting the Cancer-Associated Tn-Glycoform of the Membrane Mucin MUC1 Control Adenocarcinoma. *Immunity.* 44(6):1444-1454
- Leisegang, M., Engels, B., Schreiber, K. ..., Schreiber, H. (2016) Eradication of Large Solid Tumors by Gene Therapy with a T-Cell Receptor Targeting a Single Cancer-Specific Point Mutation. *Clin. Cancer Res.* 22(11):2734-2743
- Engels, B., Engelhard, V.H., Sidney, J. ..., Schreiber, H. (2013) Relapse or eradication of cancer is predicted by peptide-major histocompatibility complex affinity. *Cancer Cell.* 15;23(4):516-526
- Engels, B., Chervin, A.S., Sant, A.J. ..., Schreiber, H. (2012) Long-term Persistence of CD4+ but Rapid Disappearance of CD8+ T Cells Expressing an MHC Class I-restricted TCR of Nanomolar Affinity. *Mol Ther.* 20, 652-660



Alfred Fahr

Prof. em.

Alfred Fahr studied Biology and Physics at the Universities of Constance and Oxford. He received his PhD in Biophysics from the University of Constance under the supervision of Peter Läger and Ernst Bamberg. At the Free University of Berlin, he conducted research on phospholipid membranes as a

sub-project leader of a DFG special research area. He worked then at Sandoz (now Novartis) in Basel in the departments Biopharmaceutics, Experimental Therapeutics and Drug Delivery System. At the same time, he habilitated in pharmacy at the University of Basel. He then followed a call to a professorship for pharmaceutical technology at the University of Marburg, from where he later moved to the Chair of Pharmaceutical Technology in Jena. His research focus is in the field of liposomes, where not only the drug delivery aspect, but also the biophysical characteristics and the use of liposomes as membrane models for research on the Origin of Life played an essential role. Alfred Fahr founded three companies in three countries where liposomes are being researched and applied as a drug delivery system. He has published more than 200 scientific papers and wrote two textbooks and is listed as inventor of several patents.



Anne Field

Principal Toxicologist, Scientific Evaluation Branch, Medicines Regulation Division, Therapeutic Goods Administration, Australia

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 at the Therapeutic Goods Administration (TGA) in 1995, and has worked in a number of regulatory areas since then. Her current role is acting Principal Toxicologist in the Scientific Evaluation Branch, Medicines Authorisation Division, where she has responsibility for managing evaluations of nonclinical data submitted to support marketing applications across a wide range of therapies (including haematological disorders and malignancies and endocrine, renal, respiratory, reproductive and ocular disorders). Dr Field is currently co-chair on the IPRP nanomedicines working group. 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 served as a panel member of the Australian Cardiovascular Alliance's Drug Discovery Flagship.



Fabio Rocha Formiga

Research Scientist and Professor, Oswaldo Cruz Foundation and University of Pernambuco

Fabio Rocha Formiga (Natal, 1979) graduated in Pharmaceutical Sciences, obtaining his bachelor's degree in 2004 from the Federal University of Rio Grande do Norte in Brazil, where he also concluded a master's course in Health Sciences. In 2011, he obtained a PhD in pharmaceutical technology from the University of Navarra in Spain, with distinctions, as a MAEC-AECID scholar. Dr. Formiga has worked as a Visiting Professor at the University of Coimbra, Portugal. He has experience in teaching/supervision at the BSc, MSc, PhD and postdoctoral levels. In addition, he has acted as referee for national and foreign funding agencies. He has participated as member of university committees, examining boards and has organized scientific meetings. Dr. Formiga has also been strongly involved in collaborative programs for exchange with European universities (from Portugal, Spain and Denmark). Co-founder and President of the Brazilian Chapter of Controlled Release Society (CRS, 2019- present). He serves editorial board of peer-reviewed qualified journals, including the *Frontiers in Medical Technology* (Frontiers), *Pharmaceutics* (MDPI) and *Drug Delivery and Translational Research* (Springer). He is a member of the Brazilian Association of Pharmaceutical Sciences (ABCF) and the European Foundation for Clinical Nanomedicine (CLINAM). Currently, Dr. Formiga is a research scientist and Head of the Department of Immunology at the Aggeu Magalhães Institute, Oswaldo Cruz Foundation (FIOCRUZ) in Brazil. He also holds a tenured adjunct professor position at the Faculty of Medical Sciences of the University of Pernambuco (FCM/UPE). Dr. Formiga was acknowledged with the Research Award from Spanish Society of Pharmaceutics and Pharmaceutical Technology (SEFIG) in 2013 and the Benjamin Gilbert Award from FIOCRUZ in 2017. His research has focused on pharmaceutics, drug delivery and nanotechnology towards neglected diseases and regenerative medicine.

PUBLICATIONS

- Formiga, Inamuddin and Severino (Eds). Applications of Nanobiotechnology for Neglected Tropical Diseases. 1st Ed. Elsevier, 2021. <https://doi.org/10.1016/C2019-0-01872-9>
- Formiga FR, Leblanc R, de Souza Rebouças J, Farias LP, de Oliveira RN, Pena L. Ivermectin: an award-winning drug with expected antiviral activity against COVID-19. *J Control Release*. 2021;329:758-761. <https://doi.org/10.1016/j.jconrel.2020.10.009>
- Cruz KP, Patricio BFC, Pires VC, Amorim MF, Pinho AGSF, Quadros HC, Dantas DAS, Chaves MHC, Formiga FR, Rocha HVA, Veras PST. Development and Characterization of PLGA Nanoparticles Containing 17-DMAG, an Hsp90 Inhibitor. *Front Chem*. 2021;9:644827. <https://doi.org/10.3389/fchem.2021.644827>
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- Rebouças-Silva J, Tadini MC, Devequi-Nunes D, Mansur AL, S Silveira-Mattos P, I de Oliveira C, R Formiga F, Berretta AA, Marquetele-Oliveira F, Borges VM. Evaluation of *in vitro* and *in vivo* Efficacy of a Novel Amphotericin B-Loaded Nanostructured Lipid Carrier in the Treatment of Leishmania braziliensis Infection. *Int J Nanomedicine* 2020;15:8659-8672. <https://doi.org/10.2147/IJN.S262642>



Gregor Fuhrmann

Professor for Pharmaceutical Biology

Gregor was born in Berlin (Germany) where he studied Pharmacy. He received his PhD in 2013 in Pharmaceutical Sciences from the Swiss Federal Institute of Technology (ETH) Zurich (Switzerland) under the supervision of Prof Jean-Christophe

Leroux. For his dissertation he received both the ETH Silver Medal for an Outstanding Doctoral Thesis and the "Rottendorf Europapreis für Pharmazie" for Excellent Pharmaceutical Research. From 2013-2016 Gregor worked as Postdoctoral Research Associate at the Department of Materials and Department of Bioengineering at Imperial College London (United Kingdom) in the research group of Prof Molly M. Stevens. For this work he received a Marie Curie Intra-European Research Fellowship from the European Commission and a Postdoctoral Fellowship from the German Academic Exchange Service (DAAD). From 2016-2021, Gregor was Head of the independent Junior Research Group „Biogenic Nanotherapeutics“ at the Helmholtz-Institute for Pharmaceutical Research Saarland (Germany). Since 2021, he holds the Chair for Pharmaceutical Biology at the Friedrich-Alexander-University (FAU) Erlangen-Nürnberg. His research is chiefly concerned with novel biogenic avenues against infectious and inflammatory dispositions.

RECENT PUBLICATION

- Herrmann IK, Wood M & Fuhrmann G* (2021) Extracellular vesicles as next generation drug delivery platform. *Nature Nanotechnology* 16:748
- Kuhn T, Koch, M & Fuhrmann G* (2020) Probiomimetics – Novel Lactobacillus-Mimicking Microparticles Show Anti-Inflammatory and Barrier-Protecting Effects in Gastrointestinal Models. *Small* doi.org/10.1002/smll.202003158
- Schulz E, Goes A, Panter F, Koch M, Garcia R, Müller R, Fuhrmann K & Fuhrmann G* (2018) Biocompatible bacteria-derived vesicles show inherent antimicrobial activity. *Journal of Controlled Release* 290:46; Cover story in *Journal of Controlled Release* issue 290

- Fuhrmann G, Chandrawati R, Pamar PA, Keane TJ, Maynard SA, Bertazzo S & Stevens MM (2018) Engineering extracellular vesicles with the tools of enzyme prodrug therapy. *Advanced Materials* 30: 1706616; Selected for the *Advanced Materials* back cover.
- Fuhrmann G*, Serio A, Mazo M, Nair R & Stevens MM (2015), Active loading into extracellular vesicles significantly improves the cellular uptake and photodynamic effect of porphyrins. *Journal of Controlled Release* 205: 35 (**GF co-corresponding author)



Alberto Gabizon

Professor of Medicine and Head of Research Nano-oncology Center, Shaare Zedek MC and Hebrew University of Jerusalem

Alberto Gabizon's inventorship and research contribution played a key role in the development of long-circulating liposomes known as Stealth liposomes and pegylated

liposomal doxorubicin (Doxil/Caelyx™), a unique anticancer formulation extensively used in the clinic with important pharmacologic and safety advantages over conventional chemotherapy. He is founder and director of Lipomedix Pharmaceuticals and LevCo Pharmaceuticals, two start-up companies aimed at advancing his inventions in the field of cancer nanomedicine.

RECENT PUBLICATION

- Grodzinski P, Kircher MF, Goldberg MS, and Gabizon A: "Integrating Nanotechnology into Cancer Care." *ACS Nano*, 13(7):7370–7376, 2019.
- La-Beck NM, Liu X, Shmeeda H, Shudde C, and Gabizon AA: "Repurposing amino-bisphosphonates by liposome formulation for a new role in cancer treatment." *Seminars in Cancer Biology*, 68:175-185, 2021.
- Gabizon AA, TM de Rosales R, and La-Beck NM: "Translational Considerations in Nanomedicine: The Oncology Perspective." *Adv. Drug Deliv. Rev.*, 158:140-157, 2020.
- Gabizon A, Shmeeda H, Tahover E, Kornev G, Patil Y, Amitay Y, Ohana P, Sapir E, and Zalipsky S: "Development of Promitil", a lipidic prodrug of mitomycin c in pegylated liposomes: From bench to bedside." *Adv. Drug Deliv. Rev.*, 154-155:13-26, 2020.
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Jérôme Galon

Director of Research

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-microenviron-

ment 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 a Veracyte company and Chief Scientific Officer Immuno-oncology. 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, and Award from the National Academy of Science, Award from the National Academy of Medicine. He won the prestigious European Inventor Award in Research category in 2019, the Jeantet-Collen Prize in 2020, and the Galien Prize 2021.

RECENT PUBLICATION

- Bruni D, Angell HK, Galon J*. The immune contexture and Immunoscore in cancer prognosis and therapeutic efficacy. *Nat Rev Cancer*. 2020 Nov;20(11):662-680.
- Mascaux C, Angelova M, Vasaturo A, Beane J, Hijazi K, Anthoine G, Buttard B, Rothe F, Willard-Gallo K, Haller A, Ninane V, Burny A, Sculier JP, Spira A, Galon J*; Immune evasion before tumor invasion in early lung squamous carcinogenesis; *Nature*. 2019 Jul;571(7766):570-575
- Galon J* & Bruni D. Approaches to treat hot, altered and cold immune tumours with combination immunotherapies; *Nature Reviews Drug Discovery*. 2019 Mar;18(3):197-218.
- Angelova M, Mlecnik B, Vasaturo A, Bindea G, Fredriksen T, Lafontaine L, Buttard B, Morgand E, Bruni D, Jouret-Mourin A, Hubert C, Kartheuser A, Humblet Y, Ceccarelli M, Syed N, Marincola FM, Bedognetti D, Van den Eynde M, Galon J.* ; Evolution of metastases in space and time under immune selection. *CELL*. 2018 Oct 18;175(3):751-765
- Pagès F, Mlecnik B, Galon J*. Quantifying Immunoscore performance. *Lancet*. 2018 Nov 3;392(10158):1624-1625.



Ehud Gazit

Professor

Ehud Gazit is a Professor and Endowed Chair at the The Shmunis School of Biomedicine and Cancer Research, Faculty of Life Sciences and the Department of Materials Science and Engineering, Faculty of Engineering and a member of the Executive Council of Tel Aviv University. Gazit is

also the founding director of the BLAVATNIK CENTER for Drug Development. From 2014-2019, he was a member of Israel National Council for Research and Development (NCRD). From 2012-2014 he served as the Chief Scientist of the Israeli Ministry of Science and Technology (MOST) and the coordinator of the forum of Chief Scientists of the Israeli ministries. From 2008-2012 Gazit served as Tel Aviv University Vice President for Research and Development and the Chairman of the board of directors of Ramot Ltd., the technology transfer company of Tel Aviv University.

RECENT PUBLICATION

- Laor, D., Sade, D., Shira Shaham-Niv, S., Zaguri, D., Gartner, M., Basavalingappa, V., Raveh, A., Pichinuk, E., Engel, H., Iwasaki, K., Yamamoto, T., Noothalapati, H., & Gazit, E. (2019) Fibril Formation and Therapeutic Targeting of Amyloid-Like Structures in a Yeast Model of Adenine Accumulation. *Nature Commun.* 10, 62.
- Bera, S., Mondal, S., Xue, B., Shimon, L. J. W., Cao, Y., & Gazit, E. (2019) Rigid Helical-like Assemblies from a Self-Aggregating Tripeptide. *Nature Mater.* 18, 503–509.
- Makam, P., Yamijala, S. S. R. K. C., Tao, K., Shimon, L. J. W., Eisenberg, D. S., Sawaya, M. R., Wong, B. M., & Gazit, E. (2019) Non-proteinaceous Hydrolase Comprised of Phenylalanine Metallo-supramolecular Amyloid-Like Structure. *Nature Catal.* 2, 977–985.

- Levin, A., Hakala, T., Schnaider, L., Bernardes, G., Gazit, E. & Knowles, T.P.J. (2020) Biomimetic Peptide Self-Assembly for Functional Materials. *Nature Rev. Chem.* 4, 615-634.
- Sade Yazdi, D., Laor Bar-Yosef, D., Adsi, H., Kreiser, T., Sigal, S., Bera, S., Zaguri, D., Shaham-Niv, S., Oluwatoba, D.S., Levy, D., Gartner, M., Do, T.D., Frenkel, D. & Gazit E. (2021) Homocysteine Fibrillar Assemblies Display Cross-Talk with Alzheimer's Disease β -Amyloid Polypeptide. *Proc. Natl. Acad. Sci. U S A* 118, e2017575118



Evangelos Giamarellos-Bourboulis

Professor of Internal Medicine and Infectious Diseases at the Medical School of the National and Kapodistrian University of Athens

He was trained in the Immunology of Infections at the Department of Internal

Medicine and Infectious Disease of Radboud University in the Netherlands. In 2012 and 2013 he served as guest Professor of the Department of Critical Care Medicine of Jena University Hospital in Germany. His main research contribution is immunomodulation in sepsis and in auto-inflammatory disorders for which he was awarded the Young Investigator Research Award by the European Society of Clinical Microbiology and Infectious Diseases. He has 450 publications in international peer-reviewed journals with more than 23,000 citations and h-index 75. He has contributed in the development of clarithromycin for immunomodulatory treatment of septic shock, the recognition of hidradenitis suppurativa (HS) as an auto-inflammatory disorder and in the licensing of adalimumab for treatment of (HS). He is chairing the Hellenic Sepsis Study Group (www.sepsis.gr). He is the leading investigator of the phase 2 and phase 3 programs ending in the approval of anakinra for COVID-19 pneumonia in adults by the European Medicines Agency.

RECENT PUBLICATION

- Kyriazopoulou E, Poulakou G, Milionis H, Metallidis S, Adamis G, Tsiakos K, Fragkou A, Rapti A, Damoulari C, Fantoni M, Kalomenidis I, Chrysos G, Angheben A, Kainis I, Alexiou Z, Castelli F, Saverio Serino F, Tsilika M, Bakakos P, Nicastrì E, Tzavara V, Kostis E, Dagna L, Koufargyris P, Dimakou K, Savvanis S, Tzatzagou G, Chini M, Cavalli G, Bassetti M, Katrini K, Kotsis V, Tsoukalas G, Selmi C, Bliziotis I, Samarkos M, Doulas M, Ktena S, Masgala A, Papanikolaou IC, Kosmidou M, Myrodi DM, Argyraki A, Cardellino CS, Koliakou K, Katsigianni EI, Rapti V, Giannitsioti E, Cingolani A, Micha S, Akinosoglou K, Liatsis-Douvitsas O, Symbardi S, Gatseelis N, Mouktaroudi M, Ippolito G, Florou E, Kotsaki A, Netea MG, Jesper-Olsen J, Kyprianou M, Panagopoulos P, Dalekos GN, Giamarellos-Bourboulis EJ. Early treatment of COVID-19 with anakinra guided by soluble urokinase plasminogen activator receptor: a double-blind, randomized controlled phase 3 trial. *Nature Medicine* 2021, 27: 1752-1760
- Kyriazopoulou E, Liaskou-Antoniou L, Adamis G, Panagaki A, Melachroinou N, Drakou E, Marousi K, Chrysos G, Spyrou A, Alexiou N, Symbardi S, Alexiou Z, Lagou S, Kolonia V, Gkavogianni G, Kyprianou M, Anagnostopoulos I, Poulakou G, Lada M, Makina A, Roulia E, Koupetori M, Apostolopoulos V, Petrou D, Nitsotolis T, Antoniadou A, Giamarellos-Bourboulis EJ. Procalcitonin to reduce long-term infection-associated adverse events in sepsis. *American Journal of Respiratory and Critical Care Medicine* 2021, 15: 202-210
- Giamarellos-Bourboulis EJ, Tsilika M, Moorlag S, Antonakos A, Kotsaki A, Dominguez-Andrés J, Kyriazopoulou E, Gkavogianni T, Adami ME, Damoraki G, Koufargyris P, Karageorgos A, Bolanou M, Koenen H, van Crevel R, Droggiti DI, Renieris G, Papadopoulos A, Netea MG. ACTIVATE: randomized clinical trial of BCG vaccination against infection in the elderly. *Cell* 2020, 183: 315-323

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- Giamarellos-Bourboulis EJ, Netea MG, Rovina N, Akinosoglou K, Antoniadou A, Antonakos N, Damoraki G, Gkavogianni T, Adami ME, Katsaounou P, Ntaganou M, Kyriakopoulou M, Dimopoulos G, Koutsodimitropoulos I, Velissaris D, Koufargyris P, Karageorgos A, Katrini K, Lekakis V, Lupse M, Kostaki A, Renieris G, Theodoulou D, Panou V, Koukaki E, Koulouris N, Gogos C, Koutsoukou A. Complex immune dysregulation in COVID-19 patients with severe respiratory failure. *Cell Host and Microbe* 2020, 27: 992-1000



Valeria Gigante

Team Lead – One Health Research Priority-Setting & Synergy

Dr. Valeria Gigante is Team Lead at the World Health Organization (WHO) in the AMR Division where she coordinates research and priority-setting.

Dr Gigante worked for the European Medicines Agency, for the Italian Medicines

Agency until joining WHO in 2017.

Dr Gigante holds a Master Degree in Pharmacy with training in Microbiology and Hygiene, a Ph.D. in Pharmacology and Toxicology on available therapies for MDR-TB. She has executive education from INSEAD.

Dr Gigante represents WHO in the Scientific Advisory Committees of GARDP and of the AMR Action Fund as observer.

RECENT PUBLICATIONS

- Butler MS, Gigante V, Sati H, Paulin S, Al-Sulaiman L, Rex JH, Fernandes P, Arias CA, Paul M, Thwaites GE, Czaplowski L, Alm RA, Lienhardt C, Spigelman M, Silver LL, Ohmagari N, Kozlov R, Harbarth S, Beyer P. Analysis of the clinical pipeline of treatments for drug resistant bacterial infections: despite progress, more action is needed. *Antimicrob Agents Chemother.* 2022 Jan 10: AAC0199121. doi: 10.1128/AAC.01991-21. Epub ahead of print. PMID: 35007139.
- Gigante V, Sati H, Beyer P. Recent advances and challenges in antibacterial drug development. *ADMET DMPK.* 2022 Mar 4;10(2):147-151. doi: 10.5599/admet.1271. PMID: 35350117; PMCID: PMC8957241.



Daniel Gonzalez-Carter

Senior Scientist/Junior leader research fellow
Institute for Bioengineering of Catalonia (IBEC), Barcelona, Spain

Dr. Daniel Gonzalez-Carter's research focuses on developing nanotechnology-based strategies to deliver therapies to the brain.

He carried out his Ph.D. studies at the Division of Brain Sciences in Imperial College London (ICL) under a scholarship from the Mexican National Council of Science and Technology (CONACyT), where he focused on investigating pharmacological strategies to modulate microglial activity to combat neuronal death in Parkinson's disease. During his first post-doctoral position at the Department of Materials, ICL, he applied his cellular neuroscience background

to study the interaction of nanomaterials (in particular carbon nanotubes and gold/silver nanoparticles) with the brain, focusing on their ability to cross the blood-brain barrier (BBB) and modulate brain inflammation. After moving to Japan to work at the Innovation Center of Nanomedicine (iCONM) under Prof. Kazunori Kataoka, he won an 'Early Career Scientist' research grant from the Japanese Society for the Promotion of Science (JSPS) to examine the potential of glucose-functionalized polymeric nano-micelles to deliver therapies against Alzheimer's disease across the BBB. He is currently a 'La Caixa' Junior Leader research fellow at the Institute for Bioengineering of Catalonia (IBEC), Barcelona, where he is developing a novel strategy to target nanoparticles to the brain by exploiting the impermeability of the BBB to generate artificial brain targets.

RECENT PUBLICATIONS

- D. Gonzalez-Carter*, X. Liu, T. Tockary et al., (2020) Targeting nanoparticles to the brain by exploiting the blood-brain barrier impermeability to generate brain specific targets. *PNAS*, 117 (32): 19141-19150
- J. Xie, D. Gonzalez-Carter, T. Tockary et al., (2020) Dual-sensitive nanomicelles enhancing systemic delivery of therapeutically active antibodies specifically into the brain. *ACS Nano*, 14 (6): 6729-6742
- D. Gonzalez-Carter*, A. Goode, D. Kiryushko, et al. (2019) Quantification of BBB transport and neuronal toxicity of unlabelled multi-walled carbon nanotubes as a function of surface charge. *Nanoscale*, 11: 22054-22069
- D. Gonzalez-Carter*, Z. Ong*, C. McGilvery, et al. (2019). L-DOPA functionalized, multi-branched gold nanoparticles as brain-targeted nano-vehicles. *Nanomedicine NBM*, 15 (1): 1-11
- D. Gonzalez-Carter*, B. Leo, P. Ruenaroengsak, S. Chen, A. Goode, et al. (2017). Silver nanoparticles reduce brain inflammation and related neurotoxicity through induction of H2S-synthesizing enzymes. *Scientific Reports*, 7:42871.



Anna Govett

Project Director of Future Leaders Against AMR

Anna Govett graduated from the University of Cambridge with a First Class degree in Human, Social, and Political Sciences (specialising in Politics and International Relations) in July 2021. Anna also studied a year of advanced Spanish and French at Cambridge and, during her undergraduate studies, she was extensively involved in networks working on global health issues, overseeing a 'Poverty and Health' working group and organising lectures for students on a range of global and public health matters, including refugee health, epidemiology, and antimicrobial resistance (AMR).

Anna is currently working full-time as the Project Director of 'Future Leaders Against AMR', the first global programme focused on supporting the professional development of the next generation of change agents in the field of AMR. Anna designed the 10-week programme to involve 40 young people from a range of backgrounds, with priority places for those from LMIC and backgrounds outside of the biological sciences. The programme includes 31 lectures and panels from experts, 14 small projects with professional mentorship, soft-skills training, group discussions, and guided independent readings. Due to high demand (320 applications for 40 places), Anna opened one lecture a week to all who applied and other interested parties, acquiring an audience of over 300. The programme runs from January to March 2022.

In her spare time, Anna heads the UK National Working Group on AMR for Students for Global Health UK where she works on political advocacy and youth engagement with AMR in the British context. Further to this, Anna translates documents for Charitable Non Gov-

ernmental Organisations through Translators Without Borders, enjoys travelling, and teaches English to students based in France and China. In 2019, Anna directed a team of Oxbridge English teachers in Hong Kong for 2 months.



Stephan Grabbe

Stephan Grabbe is Director of the Department of Dermatology, University of Mainz Medical Center, Germany. He is also Head of the Skin Cancer Center and of the Research Center for Immunotherapy at University of Mainz. His clinical focus is on skin oncology and immune-mediated skin diseases.

Stephan Grabbe's scientific focus is in cellular immunology and immunotherapy and nanoparticle-mediated immunomodulation. Here, he is speaker of the research cluster SFB1066 ("Nanoparticle-mediated immunotherapy"), and deputy speaker of the research cluster SFB TR156 ("Skin immunology"). He published > 300 papers in peer-reviewed journals, was cited > 16.000 times and has an h-index of 56.

RECENT PUBLICATIONS

- Kappel C, ... Grabbe S. Density of Conjugated Antibody Determines the Extent of Fc Receptor Dependent Capture of Nanoparticles by Liver Sinusoidal Endothelial Cells. *ACS Nano*. 2021;15:15191-15209
- Sahin U, ..., Grabbe S, ..., Türeci Ö. An RNA vaccine drives immunity in checkpoint-inhibitor-treated melanoma. *Nature*. 2020, 585(7823):107-112
- Paßlick D, ..., Grabbe S*, Mailänder V*. Delivering all in one: Antigen-nanocapsule loaded with dual adjuvant yields super-additive effects by DC-directed T cell stimulation. *J Control Release*. 2018, 10;289:23-34. *equal contribution
- Shen L, ..., Grabbe S*, Bros M*. Protein corona-mediated targeting of nanocarriers to B cells allows redirection of allergic immune responses. *J Allergy Clin Immunol*. 2018, 142:1558-1570. *equal contribution
- Sahin U, ..., Grabbe S, ..., Türeci Ö. Personalized RNA mutanome vaccines mobilize poly-specific therapeutic immunity against cancer. *Nature*. 2017, 547(7662):222-226.



Zhen Gu

Distinguished Professor

Dr. Zhen Gu is a Qishi Chair Professor and Dean of College of Pharmaceutical Sciences at Zhejiang University. Dr. Gu received his B.S. degree in Chemistry and M.S. degree in Polymer Chemistry and Physics from Nanjing University. In 2010, he obtained Ph.D. from the Department of Chemical

and Biomolecular Engineering at UCLA. He was a Postdoctoral Associate working with Dr. Robert Langer at MIT and Harvard Medical School during 2010 to 2012. Before he moved to Zhejiang University in 2020, he was a Full Professor in the Department of Bioengineering and Director of the NIH Biotechnology Training in Biomedical Sciences and Engineering Program at UCLA. From 2012 to 2018, he was working in the Joint Department of Biomedical Engineering at the University of North Carolina at Chapel Hill and North Carolina State University, where he had been appointed as a Jackson Family Distinguished Professor. Dr. Gu's group studies controlled drug delivery, biomaterials and cell therapy. He has published over 240 research papers and applied over 150 patents. He is the re-

recipient of the Felix Franks Medal of the Royal Society of Chemistry (2020), Young Investigator Award of Controlled Release Society (2017), Sloan Research Fellowship (2016) and Pathway Award of the American Diabetes Association (2015). MIT Technology Review listed him in 2015 as one of the top innovators under the age of 35. He was elected to the College of Fellows of the American Institute for Medical and Biological Engineering (AIMBE) in 2019 and the International Academy of Medical and Biological Engineering (IAMBE) in 2021. Dr. Gu serves as an Associate Editor for Science Advances, Nano Research and Regenerative Biomaterials .

RECENT PUBLICATIONS

- Zhaowei Chen+, Hongjun Li+, Yijie Bian, Zejun Wang, Guojun Chen, Xudong Zhang, Yimin Miao, Di Wen, Jinqiang Wang, Gang Wan, Yi Zeng, Peter Abdou, Jun Fang, Song Li, Cheng-Jun Sun, and Zhen Gu*, “Bioorthogonal catalytic patch”, Nature Nanotechnology , 2021, 16:933-941
- Quanyin Hu+, Hongjun Li+, Edikan Ogunnaike, Qian Chen, Huitong Ruan, Sarah Ahn, Elena Dukhovlinova, Kang Yang, Di Wen, Gianpietro Dotti*, Zhen Gu*, “Inhibition of post-surgery tumour recurrence via a hydrogel releasing CAR-T cells and anti-PDL1-conjugated platelets”, Nature Biomedical Engineering , 2021, 5: 1038-1047
- Jicheng Yu, Jinqiang Wang, Yuqi Zhang, Guojun Chen, Weiwei Mao, Yanqi Ye, Anna R. Kahkoska, John B. Buse, Robert Langer, and Zhen Gu*, “Glucose-responsive insulin patch for the regulation of blood glucose in mice and minipigs”, Nature Biomedical Engineering, 4(499), 2020
- Qian Chen, Chao Wang, Xudong Zhang, Guojun Chen, Quanyin Hu, Hongjun Li, Jinqiang Wang, Di Wen, Yuqi Zhang, Yifei Lu, Guang Yang, Chen Jiang, Jun Wang, Gianpietro Dotti and Zhen Gu*, “In situ sprayed bioresponsive immunotherapeutic gel for post-surgical cancer treatment”, Nature Nanotechnology, 14(89), 2019
- Chao Wang+, Jinqiang Wang+, Xudong Zhang, Shuangjiang Yu, Di Wen, Quanyin Hu, Yanqi Ye, Hunter Bomba, Xiuli Hu, Zhuang Liu, Gianpietro Dotti, Zhen Gu*, “In Situ Formed Reactive Oxygen Species-Responsive Scaffold with Gemcitabine and Checkpoint Inhibitor for Combination Therapy”, Science Translational Medicine, 10(eaan3682), 2018



Nivetha Gunaseelan

I was born in India and received my undergraduate degree in Electronics and Communication engineering from BITS Pilani Dubai in 2017. Following this, I received my Master's degree in Biomedical engineering from University of Illinois Urbana-Champaign in 2018. I worked as a Reserach and Development engineer for a startup company, InnSight Technology and

fabricated electrochemical sensors for OcuCheck biosensor aimed at point-of-care diagnosis of dry eye disease using target-analyte detection, developed bioassay and immunoassay gold-standard protocols such as ELISA, FRASC, conducted preclinical tests using patient samples to achieve device reproducibility and analyzed experimental data. I also acted as an Entrepreneurial Lead in NSF I-Corps and proved business model hypothesis by spearheading a market research team which conducted customer interviews and helped source development partners which helped transition from in-house to commercially viable processes. I then joined Professor Dipanjan Pan's lab at University of Maryland Baltimore County as a Faculty Research Assistant with a joint affiliation with University of Maryland Baltimore School of Medicine and worked on developing a lab-based biosensing platform and translated it into a commercial device for rapid detection of Sars-Cov-2 to help tackle the current pandemic. I designed 3D CAD models for prototype development and device/software integration for commercialization of sensor kit and performed analytical and functional experiments using patient samples for clinical validation of sensing kit. Following this reward-

ing experience, I started pursuing my PhD in Chemical and Biochemical engineering in May 2021 under Professor Dipanjan Pan at University of Maryland Baltimore County and am currently focused on developing novel k-edge nanoprobes for *in vivo* multiplexed detection of brain diseases namely chronic subdural hematoma and traumatic brain injury using photon counting CT(PCCT) imaging to improve the current imaging modalities and help with early disease diagnosis. All these experiences have provided a strong background in biomedical imaging, electrical and material science with proficiency using *in vivo* and *in vitro* imaging techniques, data analysis, modeling, and visualization tools, experiment design and analysis and scientific computing. I am also interested in developing theranostic approaches using these novel nanoprobes to prevent disease progression such as brain tumors..

RECENT PUBLICATIONS

- Nivetha Gunaseelan, Parikshit Moitra, Dhiraj Gandhi, Uttam K. Bodanapally, Dipanjan Pan, “Molecular Nanoprobe for Multimodal Imaging of Subdural Hematoma in Human Brain Tissues”, Manuscript under preparation
- Nivetha Gunaseelan, Parikshit Moitra, Dhiraj Gandhi, Uttam K. Bodanapally, Dipanjan Pan, “In vivo multiplexed imaging and quantification of angiogenic and mature vessels using photon counting CT for early detection of subdural hematoma”, Manuscript under preparation
- Ketan Dighe, Parikshit Moitra, Maha Alafeef, Nivetha Gunaseelan, Dipanjan Pan, “A rapid RNA extraction-free lateral flow assay for molecular point-of-care detection of SARS-CoV-2 augmented by chemical probes”, Biosensors and Bioelectronics, Volume 200, 2022, 113900, ISSN 0956-5663
- In vivo multiplexed imaging using molecularly targeted multicolor metal nanoprobes labeled exosomes for early and late-stage detection of TBI (Work in Progress)
- Quantification of glycosaminoglycans (GAGs) in articular cartilage extracellular matrix for bone health using praseodymium nanocolloids and photon counting CT (Work in Progress)



Heinrich Haas

VP Formulation and Drug Delivery

Heinrich has his professional focus on development of pharmaceutical products in the field of nanotechnology and drug & RNA delivery. In his Ph.D. in the group of Prof. Dr. Helmuth Möhwald at Johannes-Gutenberg Universität Mainz and academic career in Italy and Brazil he researched lipid membranes and organized bio-molecular systems. Having moved to pharmaceutical industry he developed nanoparticle products with application in cancer, inflammatory diseases autoimmune diseases and other indications. At BioNTech, he is the scientific lead of the formulation and analytics department, which develops RNA therapeutics based on a broad scope of delivery technologies.

RECENT PUBLICATIONS

- Settanni, G., Brill, W., Haas, H. and Schmid, F. (2022), pH-Dependent Behavior of Ionizable Cationic Lipids in mRNA-Carrying Lipoplexes Investigated by Molecular Dynamics Simulations. Macromol. Rapid Commun. 2100683.
- Investigation of pH-Responsiveness inside Lipid Nanoparticles for Parenteral mRNA Application Using Small-Angle X-ray Scattering; L Uebbing, A Ziller, C Siewert, MA Schroer, CE Blanchet, DI Svergun, ... Langmuir 36 (44), 13331-13341
- Polysarcosine-functionalized lipid nanoparticles for therapeutic mRNA delivery; SS Nogueira, A Schlegel, K Maxeiner, B Weber, M Barz, MA Schroer, ... ACS Applied Nano Materials 3 (11), 10634-10645
- Hybrid biopolymer and lipid nanoparticles with improved transfection efficacy for mRNA; CD Siewert, H Haas, V Cornet, SS

Nogueira, T Nawroth, L Uebbing, ... Cells 9 (9), 2034

- An RNA vaccine drives immunity in checkpoint-inhibitor-treated melanoma; U Sahin, P Oehm, E Derhovanessian, RA Jabulowsky, M Vormehr, ... Nature 585 (7823), 107-112



Stefan Halbherr

CEO / Country Manager InnoMedica

Stefan Halbherr studied biochemistry and immunology at the university of Bern in Switzerland. During his PhD, he developed genetically engineered self-amplifying RNA vaccines against influenza A viruses. He played a crucial role in the founding team of InnoMedica and restructured the company into a cutting-edge nanodrug company. Since 2013 he led the R&D department and brought research concepts to a marketable product for patients. In 2019 Stefan Halbherr 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 clinical stage product pipeline. Today, the portfolio of InnoMedica encompasses innovative solutions for oncology with Talidox at the forefront, addressing the fact that many frequently used drugs unfortunately still cause severe adverse effects with yet limited antitumor efficacy. Stefan Halbherr was also the inventor and patent author of Talineuren, a new nanodrug in neurology, currently in phase I testing for treatment of Parkinson's disease. Until to date, Stefan Halbherr has helped InnoMedica to grow into an advanced clinical stage nanopharmaceutical company with >CHF60M of funds raised and 50+ employee strong team.



Alexander Harms

Junior Group Leader (Biozentrum, University of Basel)

Alexander Harms grew up in Germany and studied molecular biology in Basel, Switzerland. In 2015 he obtained his PhD in Microbiology at the University of Basel after which he left Switzerland for postdoctoral research at the University of Copenhagen, Denmark. During this time he worked on dormant, antibiotic-tolerant bacteria that play important roles in relapsing and chronic infections. In 2018 he returned to Switzerland to found a small research group at the Biozentrum of the University of Basel. The research of his team is mostly focused on exploring how special, newly isolated bacteriophages can infect and kill dormant, antibiotic-tolerant bacteria with the aim of developing new treatment options for chronic bacterial infections.

RECENT PUBLICATIONS

Publications ordered by date:

https://scholar.google.com/citations?hl=en&user=LTDcU_kAAAAJ&view_op=list_works&sortBy=pubdate

We will have an important preprint coming out this week. I strongly suggest to list that article as well as the following:

- Enea Maffei, Aislyu Shaidullina, Marco Burkolter, Valentin Druelle, Luc Willi, Fabienne Estermann, Sarah Michaelis, Hubert Hilbi, David S Thaler, Alexander Harms* (2021). Systematic exploration of Escherichia coli phage-host interactions with the BASEL phage collection. PLoS Biology 19(11):e3001424
- Alexander Harms*, Cinzia Fino, Michael A. Sørensen, Szabolcs Semsey, and Kenn Gerdes* (2017). Prophages and growth dynamics confound experimental results with antibiotic-tolerant persister cells. mBio 8(6):e01964-17



Ann-Kathrin Hartmann

Dr. rer. nat. Ann-Kathrin Hartmann
University Medical Center Mainz
a.hartmann@uni-mainz.de

I was born on February 16, 1987 in Siegen. After my school education – in 2006-, I started a dual training program as a chemical laboratory technician which I completed in 2009 (at Wasserverband Siegen Witt-

genstein, drinking water provider). After a part-time further education as a specialist for molecular biology and genetic engineering in 2010, I started my bachelor studies in molecular biology at the Johannes-Gutenberg University Mainz in October 2010.

In my master studies in biology at the same university, I focused on molecular cell biology and immunology. I completed my PhD on “Transcutaneous immunization: mechanisms of intercellular communication in the skin and targeted activation of mast cells” at the Institute of Immunology of the University Medical Center Mainz (supervisor Prof. Dr. Michael Stassen) in 2014 – 2018, where I was subsequently employed as a postdoc until my parental leave in September 2019.

Since September 2020, I am a research associate (postdoc) in the group of Prof. Markus Radsak, 3rd Department of Medicine, University Medical Center Mainz. My research focuses on the establishment of novel transcutaneous immunization methods.

In addition to my research, I am an honorary examiner for the Mainz-Rheinessen Chamber of Commerce and Industry and I was an honorary lecturer in the chemistry department of the University of Mainz and the Fresenius University of Applied Sciences.

RECENT PUBLICATIONS

- Hartmann AK, Aranda Lopez P, Zajac M, et al. 9-Phenanthrol enhances the generation of an CD8+ T cell response following transcutaneous immunization with imiquimod in mice. J Dermatol Sci. 2017;87(3):260-267. doi:10.1016/j.jdermsci.2017.07.018
- Lopez PA, Denny M, Hartmann AK, et al. Transcutaneous immunization with a novel imiquimod nanoemulsion induces superior T cell responses and virus protection. J Dermatol Sci. 2017;87(3):252-259. doi:10.1016/j.jdermsci.2017.06.012
- Stassen M, Hartmann AK, Delgado SJ, Dehmel S, Braun A. Mast cells within cellular networks. J Allergy Clin Immunol. 2019;144(4S):S46-S54. doi:10.1016/j.jaci.2019.01.031
- Bialojan A, Sohl J, Rausch J, Aranda Lopez P, Denny, M, Langguth, P, Hartmann AK, et al. Transcutaneous immunization with CD40 ligation boosts cytotoxic T lymphocyte mediated antitumor immunity independent of CD4 helper cells in mice. Eur J Immunol. 2019;49(11):2083-2094. doi:10.1002/eji.201848039
- Schmiedeke JK, Hartmann AK, Ruckebrod T, Stassen M, Reddehase MJ, Lemmermann NA. The Anti-apoptotic Murine Cytomegalovirus Protein vMIA-m38.5 Induces Mast Cell Degranulation. Front Cell Infect Microbiol. 2020;10:439. doi:10.3389/fcimb.2020.00439



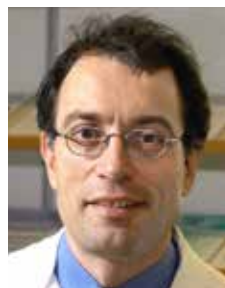
Inge Herrmann

Assistant Professor and Group Leader,
ETH Zurich and Empa

Inge Herrmann is a chemical engineer with additional training in (pre)clinical research. After graduating with a PhD from ETH Zurich, she underwent further training at the University Hospital Zurich (USZ), the University of Illinois (US) and the Imperial College London (UK). Since 2015, she is heading a research group at Empa specialized on nanoscale materials and devices for healthcare. In 2019, Inge Herrmann joined the Department of Mechanical and Process Engineering at ETH Zurich where she is heading the Nanoparticle Systems Engineering Lab. She is an expert in nanoparticle synthesis and characterization, spectromicroscopy and translational nanomedicine. She has spearheaded several translational nanomedicine projects, and serves as a scientific advisor of the spin-off companies hemotune, anavo and veltist commercializing technologies emerging from her lab. Inge Herrmann has won various prestigious awards, including the ETH Dandelion Award 2021, the Bayer Healthcare Award and the Johnson & Johnson Award, the Swiss National Science Foundation Eccellenza Award, the Empa Innovation Award and has been named Emerging Investigator 2021 by the Royal Society of Chemistry. Students under her supervision have won major awards, including the ETH Medal, ETH's best doctoral thesis in the area of materials and processes (MaP), ETH Pioneer Fellowships (2x) and several best presentation awards at international conferences. She is principle investigator (PI) of several national and international projects supported by the Swiss National Science Foundation, the Personalized Health and Related Technologies Initiative (PHRT), the Novartis FreeNovation program and several medical foundations (incl. the Swiss Heart Foundation, Krebsliga and many others).

RECENT PUBLICATIONS

- L.R.H. Gerken, A.L. Neuer, P.M. Gschwend, K. Keevend, A. Gogos, A.H.C. Anthis, S.E. Pratsinis, L. Plasswilm, I.K. Herrmann*, Scalable Synthesis of Ultrasmall Metal Oxide Radioenhancers Outperforming Gold, *Chemistry of Materials* (2021).
- I.K. Herrmann*, M.J.A. Wood, G. Fuhrmann*, Extracellular Vesicles as Next Generation Drug Delivery Platform, *Nature Nanotechnology*, 2021.
- M.T. Matter, M. Doppegieter, A. Gogos, K. Keevend, Q. Ren, I.K. Herrmann*, Inorganic Nanohybrids Combat Antibiotic-resistant Bacteria Hiding within Human Macrophages, *Nanoscale (Emerging Investigator Special Issue)* (2021).
- A.H.C. Anthis, X. Hu, M.T. Matter, A. Neuer, K.C. Wei, A.A. Schlegel, F.H.L. Starsich, I.K. Herrmann*, Chemically stable, strongly adhesive sealant patch for intestinal anastomotic leakage prevention, *Advanced Functional Materials* (2021).
- K. Keevend, R. Krummenacher, E. Truska, L.H.R. Gerken, A. Gogos, M. Stiefel, I.K. Herrmann*, Correlative Cathodoluminescence Electron Microscopy: Immunolabelling using Rare-Earth Element doped Nanoparticles, *Small* (2020).



Patrick Hunziker

Patrick Hunziker has studied Medicine the University of Zurich, Switzerland. He received a doctoral degree 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-founding president of the European Society of Nanomedicine, co-founder 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. In November. He is President of the International Society for Nanomedicine, which is uniting members from all continents in the world.



Jörg Huwyler

Prof. Dr. Jörg Huwyler studied biochemistry at the Biocenter of the University of Basel, Switzerland, where he obtained in 1992 a PhD degree at the Department of Pharmacology. In 1993, he joined the University Hospital of Basel as a postdoctoral fellow, followed by an appointment at the Brain Research Institute, UCLA School of Medicine, Los Angeles, with Prof. Dr. W.M.

Pardridge. From 1999 to 2006 he joined the pharmaceutical industry, where he worked as DMPK project leader for F. Hoffmann-La Roche Ltd. in Switzerland. In 2003, he obtained his habilitation in Pharmacy. He served as Professor of Biopharmacy and Pharmaceutical Technology from 2006 - 2010 at the University of Applied Sciences in Basel. In 2010, he was appointed full professor and head of the Division of Pharmaceutical Technology, Department of Pharmaceutical Sciences, University of Basel. His research interests are in the field of drug delivery and drug targeting using particulate drug carriers. Jörg Huwyler is the author of more than 200 peer-reviewed original research articles and 39 reviews, technical reports and book articles.



Sarah Ibrahim

Associate Director for Global Generic Drug Affairs
Recent PUBLICATIONS

Sarah Ibrahim is the Associate Director for Generic Drug Global Affairs in the Office of Generic Drugs (OGD)/ Center of Drug Evaluation and Research (CDER) at the U.S. Food and Drug Administration (FDA). In this role, Dr. Ibrahim develops OGD strategies to address identified and emerging regulatory challenges in relation to the international nature of the generic drug industry. In collaboration with other CDER and FDA offices, she supports stakeholder engagement concerning issues related to globalization of the generic pharmaceutical supply and harmonization of regulatory approaches for generic drugs. Dr. Ibrahim received her PhD in Biopharmaceutics/Pharmaceutics from the School of Pharmacy, University of Cincinnati and a B.S. in Pharmacy and Pharmaceutical Sciences from Cairo University, Egypt. Dr. Ibrahim started her career at the FDA in 2014 as a scientific reviewer in the Office of Pharmaceutical Quality. Prior to her FDA career, she has years of experience in the US pharmaceutical industry in the area of pharmaceutical development. She is also a co-inventor in a number of patent applications. As an assistant professor, along with the founding faculty, Dr. Ibrahim established the pharmaceutical sciences department for the second school of pharmacy in the state of New Jersey.

RECENT PUBLICATIONS

- Nahid S. Kamal, Alaadin Alayoubi, Khaled H. Elfakhri, Sarah Ibrahim*, Mark Seggel, Muhammad Ashraf, Ahmed Zidan; Effects of formulation variables on the *in vitro* performance of testosterone transdermal gel. *International Journal of Pharmaceutics*: Volume 590, 30 November 2020.
- Loqman A. Mohamed, Nahid Kamal b, Khaled H. Elfakhri, Sarah Ibrahim*, Muhammad Ashraf, Ahmed S. Zidan; Application of synthetic membranes in establishing bio-predictive IVPT for testosterone transdermal gel. *International Journal of Pharmaceutics*: Volume 586, 30 August 2020.
- Amit Mitra & Sarah A. Ibrahim* Development of Topical and Transdermal Dosage Forms: Regulatory Perspective. *Dermal Drug Delivery: From Innovation to Production*. 1st Edition. CRC Press. 2019
- Soo Hyeon Shin, Jukkarin Srivilai, Sarah Ibrahim*, Audra L Stinchcomb. The Sensitivity of In Vitro Permeation Tests to Chemical Penetration Enhancer Concentration Changes in Fentanyl Transdermal Delivery Systems. August 2018. *AAPS PharmSciTech* 19(7) DOI: 10.1208/s12249-018-1130-0
- Ahmed S. Zidan, Nahid Kamal, Alaadin Alayoubi, Mark Seggel, Sarah Ibrahim*, Ziyaur Rahman, Celia N. Cruz, Muhammad Ashraf, Effect of Isopropyl Myristate on Transdermal Permeation of Testosterone From Carbopol Gel. *Journal of Pharmaceutical Sciences* (2017).



Mike Isles

Executive Director EAASM and ASOP EU

Mike is Executive Director of the European Alliance for Access to Safe Medicines (www.eaasm.eu), a pan-European non-profit patient safety organisation. The EAASM is focused on supporting the development of a robust and harmonised EU regulatory framework in the field of

nanomedicines to protect patient safety and guarantee the safety, quality and efficacy of innovative nanomedicines and nanosimilars. Its other key activities include campaigning for the safer use of unlicensed/off-label medicines and compounding medicines, the adoption of medical, pharmacy and nursing practices that aim to eradicate medication errors and the exclusion of falsified and sub-standard medicines from the supply chain.

Mike is also the Executive Director of the Alliance for Safe Online Pharmacy in the EU (www.asop.eu), a non-profit Community Interest Company. With over 35,000 fake pharmacy websites targeting European citizens on any given day, this multisectoral organisation's mission is to enable patients to buy their medicines online safely – where it is legal to do so. ASOP EU collaborates strongly with ASOP Global (www.buysaferx.pharmacy) and its members and observers involve many key internet stakeholders. Its aim is to campaign for new legislation, as well as concrete voluntary actions that will make a real difference and ultimately benefit the health and safety of patients.

RECENT PUBLICATIONS

- Isles MP (2022) Nanomedicines and Nanosimilars—Why a Robust Centralised Regulatory Framework Is Essential to Enhance Patient Safety. *Front. Pharmacol.* 12:787239. doi: 10.3389/fphar.2021.787239



Wenlei Jiang

Dr. Wenlei Jiang is a Senior Biomedical Research and Biomedical Product Assessment Service (SBRBPAS) Expert and currently serves as a Senior Advisor for Innovation and Strategic Outreach in the Office of Research and Standards/Office of Generic Drugs. She is leading complex drug product classification and research, promoting global harmonization of bioequivalence criteria, developing opportunities for scientific outreach, and coordinating post-market generic drug safety investigation. She is current Chair at Product Quality Research Institute (PQRI) Steering Committee and serves at National Cancer Institute (NCI) Nanotechnology Characterization Laboratory (NCL) Scientific Oversight Committee. She also co-chairs IPRP Nanomedicine Working Group, and supports ICH M13, generic drug cluster, and other global regulatory affairs activities. Prior to joining FDA, she was at Novartis Pharmaceutical Corporation where her responsibilities included formulation development of conventional liquid and solid dosage forms, as well as advanced parenteral drug delivery systems. She received her PhD in Pharmaceutics and Pharmaceutical Chemistry from The Ohio State University.

Dr. Wenlei Jiang is a Senior Biomedical Research and Biomedical Product Assessment Service (SBRBPAS) Expert and currently serves as a Senior Advisor for Innovation and Strategic Outreach in the Office of Research and Standards/Office of Generic Drugs. She is leading complex drug product classification and research, promoting global harmonization of bioequivalence criteria, developing opportunities for scientific outreach, and coordinating post-market generic drug safety investigation. She is current Chair at Product Quality Research Institute (PQRI) Steering Committee and serves at National Cancer Institute (NCI) Nanotechnology Characterization Laboratory (NCL) Scientific Oversight Committee. She also co-chairs IPRP Nanomedicine Working Group, and supports ICH M13, generic drug cluster, and other global regulatory affairs activities. Prior to joining FDA, she was at Novartis Pharmaceutical Corporation where her responsibilities included formulation development of conventional liquid and solid dosage forms, as well as advanced parenteral drug delivery systems. She received her PhD in Pharmaceutics and Pharmaceutical Chemistry from The Ohio State University.



Andreas Jordan

Executive Vice President and Managing Director Europe, Founder and Chief Scientific Officer (CSO)

Dr. Andreas Jordan is an internationally recognized academic serial entrepreneur and expert in nanomedicine, thermo-therapy and oncology. Managed and built MagForce AG out of the German Charité

University Clinic spin-off in Berlin through all stages of venture financing to a public Company, listed on the Frankfurt Stock Exchange. Inventor of the NanoTherm® Therapy System (NTTS), which was the first medical device system receiving European approval to treat cancer using nanoparticles. Experienced Chief Executive Officer (CEO) and Chief Scientific Officer (CSO) and Co-founder of MagForce USA Inc.

30 years work experience in R&D, clinical development, regulatory approval and reimbursement strategies as a senior-level scientist, business manager, and marketer, with a creative approach to financing research and development projects, marketing to investors, customers and the public, with an international broadband network of 1,000+ collaborating scientific and industrial organizations. His scientific and clinical results were published in 50+ publications in peer reviewed journals and innovations were protected in a 500+ international patent portfolio.



Karakoçak Bedia Begum

Senior Scientist

Dr. Karakoçak earned her Ph.D. in Energy & Environmental and Chemical Engineering Department of Washington University in St. Louis, School of Engineering in 2018. Upon completion of her thesis on developing engineered nanoparticles for drug delivery and bioimaging, she worked as a

Postdoctoral Research Fellow at Washington University's School of Medicine, Department of Ophthalmology and Visual Sciences, where she developed a biocompatible targeted drug delivery agent for the treatment of retinal dysfunction. In 2019, she won the prestigious Swiss Government Excellence Postdoctoral Scholarship to work with Professors Barbara Rothen-Rutishauser and Alke Petri-Fink at the BioNanomaterials Group to design a bioinspired drug delivery system to target cancer cells selectively.

RECENT PUBLICATIONS

- Laradji A.*, Karakoçak B. B.*, Kolesnikov A. V., Kefalov V. J., Ravi N. Hyaluronic Acid-based Gold Nanoparticles for Topical Delivery of Therapeutics to Retina and the Retinal Pigment Epithelium. *Polymers*. 2021. 28;13(19):3324. <https://doi.org/10.3390/polym13193324>
- Barosova H*, Meldrum K*, Karakoçak B. B., Balog S., Doak S. H., Petri-Fink A., Clift M. J. D., Rothen-Rutishauser B. Inter-laboratory Variability of A549 Epithelial Cells Grown Under Submerged and Air-Liquid Interface Conditions. 2021. *Toxicology In Vitro*. 75, 105178. <https://doi.org/10.1016/j.tiv.2021.105178>
- Laradji A., Kolesnikov A. V., Karakoçak B. B., Kefalov V. J., Ravi N. Redox-Responsive Hyaluronic Acid-based Nanogels for the Topical Delivery of Visual Chromophore to Retinal Photoreceptors. 2021. *ACS Omega*. 6(9): 6172-6184. <https://pubs.acs.org/doi/10.1021/acsomega.0c05535>
- Karakoçak B. B.*, Laradji A.*, Primeau T., Berezin M., Li S., Ravi N. Hyaluronan-conjugated Carbon Quantum Dots for Bioimaging. 2021. *ACS Applied Materials & Interfaces*. 13(1):277-286. <https://doi.org/10.1021/acsaami.0c20088>

- Dijkhoff I. M., Drasler B., Karakoçak B. B., Petri-Fink A., Valacchi G., Eeman M., Rothen-Rutishauser B. Impact of Airborne Particulate Matter on Skin: A Systematic Review From Epidemiology to In Vitro Studies. 2020. *Particle and Fibre Toxicology*. 17(1): 1-28. <https://doi.org/10.1186/s12989-020-00366-y>



Michael Keller

Expert Scientist

Michael Keller obtained his Bachelor in Chemistry & Biochemistry from the ETH Zürich. 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 where he is an Expert Scientist in the Therapeutic Modalities function. He was instrumental in establishing novel nucleic acid delivery principles including the LNP technology at ROCHE, and to advance their evaluation in a variety of exploratory, extrahepatic applications.

RECENT PUBLICATIONS

- Evaluation of bovine milk extracellular vesicles for the delivery of locked nucleic acid antisense oligonucleotides. *European Journal of Pharmaceutics and Biopharmaceutics*; Volume 158, January 2021, Pages 198-210



Igor Khalin

Dr. Khalin Igor has studied Medicine at Kharkiv National Medical University, Ukraine. He received a doctoral decree based on thesis work in chronic irradiated wounds from the Academy of Medical Sciences of Ukraine and did further research in experimental pharmacology at Kharkiv National Medical University.

Then he moved to Malaysia and worked as Associate of Professor and Head of Pharmacology Department at National Defence University of Malaysia. There he switched his interest towards nanomedicine. In 2017 he was awarded to Humboldt Fellowship for experienced researches and started working as Research Fellow at Institute of Stroke and Dementia Research, Ludwig-Maximilian University of Munich, Germany. His research is associated with neurodegeneration, blood-brain barrier and development of drug-delivery systems. In 2018 he was awarded to Marie Curie Individual Fellowship and in 2021 to individual DFG (Germany Research Foundation) grant.

RECENT PUBLICATIONS

- Khalin I, Heimburger D, Melnychuk N, Collot M, Groschup B, Helal F, Reisch A, Plesnila N, and Klymchenko A. Ultrabright fluorescent polymeric nanoparticles with a stealth pluronic shell for live tracking in the mouse brain. *ACS Nano*. 2020, 14, 8, 9755–9770.

- Khalin I, Severi C, Heimburger D, When A, Hella F, Reisch A, Klymchenko A, Plesnila N. Dynamic tracing using ultra-bright labelling and multi-photon microscopy identifies endothelial uptake of poloxamer 188 coated poly(lactic-co-glycolic acid) nano-carriers *in vivo*. *Nanomedicine:NBM*, 13 Dec 2021, 40:102511
- Khalin I., Renad Alyautdin, Tin Wui Wong, Justin Gnanou, Ganna Kocherga & Jörg Kreuter Brain-derived neurotrophic factor delivered to the brain using poly (lactide-co-glycolide) nanoparticles improves neurological and cognitive outcome in mice with traumatic brain injury. // *Drug Deliv.* 2016 Nov;23(9):3520-3528. Epub 2016 Jul 16



Bumjun Kim

Postdoctoral scholar, Chemical and Biological Engineering Department, Princeton University

My research interests lie in designing and developing effective medicines/modalities for the treatment of complex human diseases. My undergraduate research experiences in Dr. Jaebeom Lee's lab and Dr. Robert

Tranquillo's lab opened my eyes to biomaterials and drug delivery. During my PhD, I have designed and performed *in vitro* and *in vivo* studies to prove the efficacy of targeted lipid nanoemulsions that co-deliver chemotherapeutics to breast cancer under the guidance of Dr. Debra Auguste. I have learned to individually design, execute, and evaluate an anti-tumor efficacy experiments in addition to nanoparticle synthesis and characterization skills. Building on my previous training, the current postdoctoral training is focused on the development and application of FlashNanoPrecipitation (FNP) and inverse FlashNanoPrecipitation (iFNP) technology, which enable the scalable encapsulation of small molecules, peptides, oligonucleotides, and proteins at high loading and encapsulation efficiency. Throughout the training, I have generated a foundation in polymer physics and formulation science. During the unexpected research suspension period last year, I have focused on comprehending immunology and autoimmune diseases; this opened a new door for me to conjugate my knowledge in nanomedicine to inflammation and auto-immune diseases. Under the guidance of my sponsor, we have recently earned a grant investigating the role of nanoparticle surface chemistry on immune-modulation in inflammatory bowel diseases. I will continue exploring the application of nanoparticles on answering important biological questions and developing new therapeutic modalities.

RECENT PUBLICATIONS

- Kim B, Liu D, Auguste DT. A lipid targeting, pH responsive nanoemulsion encapsulating a DNA intercalating agent and a HDAC inhibitor reduces TNBC tumor burden. *Advanced Therapeutics*. 2021; 4(3), 2000211.
- McManus S†, Zhang Y†, Kim B, Lee B, ElSayed ME, Prud'homme RK. Co-encapsulation by Flash NanoPrecipitation of Insulin, Trypsin Inhibitor and Caprate Permeabilization Enhancer for Oral Administration. *Precis. Nanomed.* 2020; 3(5): 710-723.
- Letswaart R†, Arat S†, Chen AX†, Farahmand S†, Kim B, DuMouchel W, Armstrong D, Fekete A, Sutherland J, Urban L. Machine Learning Guided Association of Adverse Drug Reactions With *in Vitro* Target-Based Pharmacology. *EBioMedicine*. 2020; 57,102837.
- Kim B, Pena CD, Auguste DT. Targeted lipid nanoemulsions encapsulating epigenetic drugs exhibit selective cytotoxicity on CDH1-/FOXM1+ triple negative breast cancer cells. *Molecular Pharmaceutics*. 2019; 16(5): 1813-1826.
- Tao Y, Li M, Kim B, Auguste DT. Incorporating gold nanoclusters and target-directed liposomes as a synergistic amplified colorimetric sensor for HER2-positive breast cancer cell detection. *Theranostics*. 2017; 7(4):899-911.



Thomas Kisby

Senior Scientist and Leader of NanoTherapeutics Team, Nanomedicine Lab, University of Manchester, Manchester (UK).

Tom obtained a BSc in Medical Sciences at the University of Leeds, UK in 2015. During his studies, he conducted an industrial placement year at MedImmune (Astra Zene-

neca) investigating novel bio-therapies for inflammatory liver disease and fibrosis. On returning to Leeds he completed a research project investigating the application of antioxidant system inhibitors as a therapy for cancers of the brain and nervous system. Tom joined the Nanomedicine Lab as a PhD student funded by the EPSRC & MRC Centre for Doctoral Training in Regenerative Medicine at the University of Manchester. His PhD project investigated *in vivo* cell reprogramming as a strategy to enhance tissue repair and regeneration using adenoviral vectors for gene transfer. In December 2019 he was recruited as a postdoctoral researcher funded by an EPSRC International Centre-to-Centre of Excellence grant between the Universities of Manchester and Harvard to develop novel nanomaterial approaches for drug delivery and immunotherapy. He was appointed as Senior Researcher and Team Leader of the NanoTherapeutics Team in June 2021 to continue to investigate the application of 2D and other nanomaterials for cancer immunotherapy with a particular focus on glioblastoma. To support this work Tom has obtained funding from Advanced Materials in Medicine at Manchester and the Wellcome Trust TPA Projects for Translation (P4T) scheme and is part of the wider EPSRC funded 2D Materials for Next Generation Healthcare Technologies network at the University of Manchester.

RECENT PUBLICATIONS

- Kisby, T., Yilmazer, A., & Kostarelos, K. (2021). Reasons for Success and Lessons Learnt from Nanoscale Vaccines against COVID-19. *Nature Nanotechnology* 16, 843–850 <https://doi.org/10.1038/s41565-021-00946-9>
- Kisby, T., de Lázaro, I., Stylianou, M., Cossu, G., & Kostarelos, K. (2021). Transient Reprogramming of Neonatal Cardiomyocytes to a Proliferative Dedifferentiated State. *PLOS ONE*,16(5): e0251054 <https://doi.org/10.1371/journal.pone.0251054>
- Kisby, T., de Lázaro, I., Fisch, S., Cartwright, E.J., Cossu, G., & Kostarelos, K. (2020). Adenoviral mediated delivery of OSKM factors induces partial reprogramming of mouse cardiac cells *in vivo*. *Advanced Therapeutics* 2000141. <https://doi.org/10.1002/adtp.202000141>
- de Lázaro, I., Sharp, P., Gurcan, C., Ceylan, A., Stylianou, M., Kisby, T., Chen, Y., Vranic, S., Barr, K., Taheri, H., Ozen, A., Bussy, C., Yilmazer, A., Kostarelos, K. (2020). Deep tissue translocation of graphene oxide sheets in human glioblastoma 3D spheroids and an orthotopic xenograft model. *Advanced Therapeutics*. <https://doi.org/10.1002/adtp.202000109>



Kostas Kostarelos

Professor Kostas Kostarelos read Chemistry at the University of Leeds and obtained his Diploma and PhD in Chemical Engineering from Imperial College London. He joined the research staff and faculty of medical schools in the USA (UCSF, CA; Memorial Sloan-Kettering, NY; Weill Medical College of Cornell University, NY) and biomedical research institutions in the UK (Im-

perial College Genetic Therapies Centre, UCL School of Pharmacy). Kostas became the first named Chair of Nanomedicine in the UK (in 2007 at UCL) and was Professorial Fellow, Japanese Society for

the Promotion of Science (JSPS) in 2010. He is a Fellow of the Royal Society of Chemistry (FRSC). He is currently Professor of Nanomedicine at the University of Manchester (www.nanomedicinelab.com) and a Severo Ochoa Distinguished Professor at the Catalan Institute of Nanoscience & Nanotechnology (ICN2) in Barcelona, Spain (www.icn2.cat/nanomedicine).



Silke Krol

Senior Scientist/PI

Since 2018 Silke Krol is with IRCCS Ospedale Specializzato in Gastroenterologia "Saverio de Bellis" developing novel nanoparticle-based therapeutic and diagnostic approaches for inflammatory bowel disease (IBD), and complex *in vitro* models

for colorectal cancer, IBD, and for testing intestinal drug delivery. 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, color-changing drug selective gold nanoparticles, and inorganic topographic surfaces for complex cell culture (spheroids or *in vitro* intestinal models). She is member of the editorial board of the journal "Precision Medicine", and associate editor of "Frontiers in Nanobiotechnology". Recently she co-founded a start-up, Encytos B.V. with the University of Twente.

RECENT PUBLICATIONS

- Antonella Zacheo, Jan Hodek, Dariusz Witt, Giuseppe Felice Mangiatordi, Quy K Ong, Ozgun Kocabiyik, Nicoletta Depalo, Elisabetta Fanizza, Valentino Laquintana, Nunzio Denora, Danilo Migoni, Piotr Barski, Francesco Stellacci, Jan Weber, Silke Krol (2020) Multi-sulfonated ligands on gold nanoparticles as virucidal antiviral for Dengue virus Scientific reports 10, 9052. DOI: 10.1038/s41598-020-65892-3
- Silke Krol, Walter Baronti, Piero Marchetti (2020) Nanoencapsulated human pancreatic islets for β -cell replacement in Type 1 diabetes. Nanomedicine 15(18):1735-1738. DOI: 10.2217/nnm-2020-0166
- Dhanik Reshamwala, Sailee Shroff, Olivier Sheik Amamuddy, Valentino Laquintana, Nunzio Denora, Antonella Zacheo, Vili Lampinen, Vesa Hytönen, Özlem Tastan Bishop, Varpu Marjomäki*, Silke Krol* (2021) Polyphenols and polyphenol-coated nanoparticles prevent enterovirus infection through clustering and stabilization of the viruses. Pharmaceutics 13, 1182. DOI: 10.3390/pharmaceutics13081182.* shared
- Francesco Dituri, Matteo Centonze, Erwin J.W. Berenschot, Niels R. Tas, Arturo Susarrey Arce, Silke Krol (2021) Complex Tumor

Spheroid Formation and One-Step Cancer-Associated Fibroblasts Purification From Hepatocellular Carcinoma Tissue Promoted By Inorganic Surface Topography. Nanomaterials 2021, 11(12), 3233; Doi: 10.3390/nano1123233

- Matteo Centonze, Erwin J.W. Berenschot, Simona Serrati, Arturo Susarrey Arce, Silke Krol (2022) The fast track for intestinal tumor cell differentiation and *in vitro* intestinal models by inorganic topographic surfaces. Pharmaceutics 14, 218. DOI:10.3390/pharmaceutics 14010218.



Twan Lammers

Twan Lammers obtained a D.Sc. in Radiation Oncology from Heidelberg University in 2008 and a Ph.D. in Pharmaceutics from Utrecht University in 2009. In the same year, he started the Nanomedicine and Theranostics group at RWTH Aachen University. In 2014, he was promoted to full professor of medicine at RWTH Aachen

University Clinic. His group aims to individualize and improve disease treatment by combining drug targeting with imaging. To this end, image-guided (theranostic) drug delivery systems are being developed, as well as materials and methods to monitor tumor growth, angiogenesis, inflammation, fibrosis and metastasis. He has published over 250 papers (19000 citations, h-index 74), and received multiple scholarships and awards, including a starting and consolidator grant from the European Research Council, the Young Investigator Award of the Controlled Release Society, the Adritelf International Award, and the Belgian Society for Pharmaceutical Science International Award. He is on the editorial board of 10 journals, and serves as a handling editor for the Journal of Controlled Release, Drug Delivery and Translational Research, and Molecular Imaging and Biology. Since 2019, he is included in the Clarivate Analytics list of Highly Cited Researchers.



Sebastian Lecommandoux

Professor

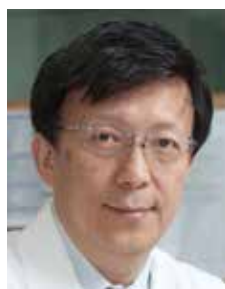
Sébastien Lecommandoux received his Ph.D. (1996) in Physical Chemistry from the University of Bordeaux. After a post-doctoral experience at the University of Illinois (UIUC, USA) in the group of Prof. Samuel I. Stupp, he started his academic career at the Laboratoire de Chimie des

Polymères Organiques as Associate Professor (1998) and was promoted to Full Professor at Bordeaux INP in 2005. He is currently Director of the Laboratoire de Chimie des Polymères Organiques (LCPO-CNRS) and is leading the group "Polymers Self-Assembly and Life Sciences". His research interests include the design of bio-inspired polymers for biomaterials design and tissue engineering, especially based on polypeptide, proteins and polysaccharide-based block copolymers self-assembly, the design of polymersomes for drug-delivery and theranostic, as well as biomimetic approaches toward design of synthetic viruses and artificial cells. He published more than 190 publications in international journal, 6 book chapters and 11 patents (2 being licenced), with over 15000 citations (h-factor 61, Google Scholar). He is also co-director of the joint laboratory LCPO-L'OREAL and co-founder of Emissary Cosmetics. Sébastien Lecommandoux is recipient of the CNRS bronze medal (2004), Institut Universitaire de France Junior Chair (IUF 2007), Fellow of the Royal Society of Chemistry RSC (2017), Seqens Award of the French Academy of Science (2019), Member of the Academia Europaea (2020). He is Editor-in-Chief of Biomacromolecules (ACS) since 2020 after being Associate Editor since 2013. He is also in the

Editorial Advisory Board of several international journals, including Bioconjugate Chemistry (ACS), Polymer Chemistry (RSC) and Biomaterials Science (RSC).

RECENT PUBLICATIONS

- Spatiotemporal Dynamic Assembly/Disassembly of Organelle-Mimics Based on Intrinsically Disordered Protein-Polymer Conjugates; H. ZHAO, E. IBARBOURE, V. IBRAHIMOVA, Y. XIAO, E. GARANGER, S. LECOMMANDOUX *Advanced Science* 2102508 (2021)
- Thermosensitive vesicles from chemically encoded lipid-grafted elastin-like polypeptides V IBRAHIMOVA, H ZHAO, E IBARBOURE, E GARANGER, S LECOMMANDOUX *Angew. Chem. Int. Ed.* 60, 15036-15040 (2021)
- Thermosensitive Hybrid Elastin-like Polypeptide-Based ABC Triblock Hydrogel; M. DAI, G. GOUDOUNET, H. ZHAO, B. GARBAY, E. GARANGER, G. PECASTAINGS, X. SCHULTZE, S. LECOMMANDOUX *Macromolecules* 54, 1, 327–340 (2021)
- Design of thermoresponsive elastin-like glycopolypeptides for selective lectin binding and sorting LMM BRAVO ANAYA, R PETITDEMANGE, M ROSSELIN, E IBARBOURE, B GARBAY, E GARANGER, TJ DEMING, S LECOMMANDOUX; *Biomacromolecules* 22, 1, 76–85 (2021)
- Dynamic Spatial Formation and Distribution of Intrinsically Disordered Protein Droplets in Macromolecularly Crowded Protocell H. ZHAO, V. IBRAHIMOVA, E. GARANGER, S. LECOMMANDOUX; *Angew. Chem. Int. Ed.* 132 (27), 11121-11129 (2020)



Dong Soo Lee

Professor

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 purpose). Brain has recently come to reveal its cell-type or state specific gene expression of all the transcriptomes simultaneously on the space (microscopic slices) using spatial transcriptomics. Novel brain therapeutics and their brain action are now interpreted using functional molecular transcriptomics imaging. This new endeavor spatial transcriptomics imaging is now associated with finding new therapeutics for brain diseases such as dementia.

RECENT PUBLICATIONS

- Bae, Choi, Lee. (2021). Discovery of molecular features underlying the morphological landscape by integrating spatial transcriptomic data with deep features of tissue images. *Nucleic acids research*, 49(10), e55-e55.
- Bae, Lee, Choi, Kim. (2021). CellDART: Cell type inference by domain adaptation of single-cell and spatial transcriptomic data. *bioRxiv*.



Claus-Michael Lehr

Professor & Head of department

Claus-Michael Lehr is Professor at Saarland University as well as cofounder and head of the department “Drug Delivery and Biological Barriers” at the Helmholtz Institute for Pharmaceutical Research Saarland (HIPS). He has also been cofounder of Across Barriers GmbH and PharmBioTec GmbH. Claus-Michael Lehr’s research involves advanced (nano-)carriers as well as human cell culture models of epithelial barriers (lung, gut, skin). According to the strategic goal of the Institute, the focus is on infectious diseases, especially to combat antimicrobial resistance. He is co-editor of the *European Journal of Pharmaceutics and Biopharmaceutics* and has been the initiator of the International Conference on “Biological Barriers”, which takes place biennially at Saarland University. Since 2015, the British magazine “The Medicine Maker” has rated him three years in sequence as one of the top 100 most influencing drug researchers in the world.

RECENT PUBLICATIONS

- Thorn, C. R., et al, Tobramycin Liquid Crystal Nanoparticles Eradicate Cystic Fibrosis-Related *Pseudomonas aeruginosa* Biofilms. *Small* 17, 2100531 (2020).
- Ho, D.-K., et al., Squalenyl Hydrogen Sulfate Nanoparticles for Simultaneous Delivery of Tobramycin and Alkylquinolone Quorum Sensing Inhibitor to Eradicate *P. aeruginosa* Biofilm Infections. *Angewandte Chemie Int Ed* (2020). doi:10.1002/anie.202001407
- Horstmann, J. C., et al. Transferring Microclusters of *P. aeruginosa* Biofilms to the Air–Liquid Interface of Bronchial Epithelial Cells for Repeated Deposition of Aerosolized Tobramycin. *ACS Infect Dis* (2021). doi:10.1021/acscinfecdis.1c00444
- Schütz, C., et al. A New PqsR Inverse Agonist Potentiates Tobramycin Efficacy to Eradicate *Pseudomonas aeruginosa* Biofilms. *Adv Sci* 8, 2004369 (2021).
- Huck, B. C., et al., Nano-in-Microparticles for Aerosol Delivery of Antibiotic-loaded, Fucose-derivatized and Macrophage-targeted Liposomes to Combat Mycobacterial Infections: In Vitro deposition, Pulmonary Barrier Interactions and Targeted Delivery. *Adv Healthc Mater* 2102117 (2022). doi:10.1002/adhm.202102117



Jeffs Lloyd

PhD.

Senior Director of Biopharma Services

Lloyd joined Precision NanoSystems in June 2018. His Biopharma Services team responsible for developing and executing custom programs to meet the clinical manufacturing needs of PNI’s clients. Lloyd 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. Lloyd 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 for key patents in this field.



Beat Löffler

Beat Löffler, MD h.c. MA studied Philosophy, Communication Sciences and Politics at the University the Free University in Berlin, graduating with a Master of Arts. 2007 he absolved the training of the European Center of Pharmaceutical Medicine (ECPM). In 2014 he received an MD h.c. from the University of Basel. He just est-

ablished his thirteenth programme for the CLINAM summit and in the last 15 years he 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. Presently CLINAM is the largest network for clinical nanomedicine debates and has become a meeting place between all stakeholders in Nanomedicine and related fields. The foundation launched the European Journal of Nanomedicine. Löffler co-founded the European Society for Nanomedicine and the International Society for Nanomedicine, which realizes every second year a Nanomedicine Summer school. He was head of dissemination in 5 Projects within the EU-Framework. The European Foundation for Clinical Nanomedicine was established in 2007 together with Patrick Hunziker MD with the aim develop the research of nanomedicine with regard to its use as an innovative technology, better medical care in the future and the establishing an international network in clinical nanomedicine and related fields.



Jonathan Lovell

Associate Professor of Biomedical Engineering

Jonathan F. Lovell is an Empire Innovation Associate Professor of Biomedical Engineering at the State University of New York at Buffalo. He completed his MS in biochemistry at McMaster University and his PhD in biomedical engineering from the

University of Toronto. His work has been recognized with distinctions including the NIH Early Independence Award (2013), the Biomedical Engineering Society Young Investigator Award (2015), an NSF CAREER award (2016), the young investigator award from the Society of Porphyrins and Phthalocyanines (2018), the Basic PDT Research award from the International Photodynamic Association (2019), the ETH Pharmaceutical Science Lectureship (2020) and was named an Avanti Polar Lipids Leader (2021). His research interests center around developing new tools to treat or prevent disease."

RECENT PUBLICATIONS

- An In Vivo Screen to Identify Short Peptide Mimotopes with Enhanced Antitumor Immunogenicity. He et al., Cancer Immunol Res. 2022 doi: 10.1158/2326-6066.CIR-21-0332.
- A liposome-displayed hemagglutinin vaccine platform protects mice and ferrets from heterologous influenza virus challenge. Sia et al., Proc Natl Acad Sci U S A. 2021 118(22):e2025759118.
- A Potent Cancer Vaccine Adjuvant System for Particleization of Short, Synthetic CD8+ T Cell Epitopes. He et al., ACS Nano. 2021 15:4357-4371.
- HPV-Associated Tumor Eradication by Vaccination with Synthetic Short Peptides and Particle-Forming Liposomes. He et al., Small. 2021 (11):e2007165.
- SARS-CoV-2 RBD Neutralizing Antibody Induction is Enhanced by Particulate Vaccination. Huang et al., Adv Mater. 2020 32(50):e2005637.



Robert Luxenhofer

Professor Soft Matter Chemistry

Robert Luxenhofer completed his PhD in 2007 at the TU München in polymer chemistry developing a novel polymer functionalization approach since developed further by Serina Therapeutics to introduce the first-in-human poly(2-oxazoline)-drug conjugates. As a postdoc with Alexander V.

Kabanov at the University of Nebraska Medical Center, he discovered ultra-high loaded drug formulations and investigated structure dependent endocytosis of polymer amphiphiles. Returning to Germany in 2009, he started to investigate polysarcosine and polypeptoids as biomaterials at the TU Dresden. In 2012, he joined the Julius-Maximilians Universität as an Associate Professor, where he continued working on polypeptoids and ultra-high drug formulations, but also started investigating biofabrication and 3D printing using melt electrowriting. In 2019, he joined the University of Helsinki as a Full Professor. He holds 7 patents and is co-founder of two companies focusing on developing novel polymers for medical applications.

RECENT PUBLICATIONS

- C. Hu, T. Ahmad, M. S. Haider, L. Hahn, P. Stahlhut, J. Groll, R. Luxenhofer*, A Thermogelling Organic-Inorganic Hybrid Hydrogel with Excellent Printability, Shape Fidelity and Cytocompatibility for 3D Bioprinting. Biofabrication 2021, in print, DOI: 10.1088/1758-5090/ac40ee.

- S. Hasselmann, L. Hahn, T. Lorson, E. Schätzlein, I. Sébastiani, M. Beudert, Tessa Lühmann, G. Sextl, R. Luxenhofer*, D. Heinrich*, Freeform direct laser writing of versatile topological 3D scaffolds enabled by intrinsic support hydrogel, *Materials Horizons* 2021, 8, 3334-3344. DOI: 10.1039/d1mh00925g.
- M.S. Haider, M. M. Lübtow, S. Endres, S. Forster, V.J. Flegler, B. Böttcher, V. Aseyev, A.-C. Pöppler, R. Luxenhofer*, Think Beyond the Core: The Impact of the Hydrophilic Corona on the Drug Solubilization Using Polymer Micelles. *ACS Appl. Mat. Interfac.* 2020, 12, 24531–24543. DOI: 10.1021/acsami.9b22495.
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Volker Mailänder

Group leader (Univ.-Prof.)

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 BiomaT-ICS (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

- Simon, J., Fichter, M., Kuhn, G., Brückner, M., Kappel, C., Schunke, J., Klaus, T., Grabbe, S., Landfester K., Mailänder, V. Achieving dendritic cell subset-specific targeting *in vivo* by site-directed conjugation of targeting antibodies to nanocarriers. *Nano Today* (2022), 43,2022,101375
- Prawatborisut, M.; Oberländer, J.; Jiang, S.; Graf, R.; Avlasevich, Y.; Morsbach, S.; Crespy, D.; Mailänder, V.; Landfester, K., Temperature-Responsive Nanoparticles Enable Specific Binding of Apolipoproteins from Human Plasma. *Small* (2021), e2103138.

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- J Simon, T Wolf, K Klein, K Landfester, FR Wurm, and V Mailänder: Hydrophilicity Regulates the Stealth Properties of Polyphosphoester-Coated Nanocarriers; *Angewandte Chemie International Edition* (2018) 57(19): 5548-555
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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, data science, simulation modeling and converging technologies.

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, working on defining the AIDS viruses. Dr. Kalish was one of the leaders of the “Matrix of Biological Knowledge” at the Santa Fe Institute. Her B.Sc. is 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 joined later the Weizmann Institute of Science, working with Prof. Ephraim Katzir on protein interactions, specificity and sensitivity definitions. 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 the head of the Enterprise Marketing Department at IBM Israel. She played an active leading 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 work package of the EU Human Brain Flagship Project (HBP), focusing on disease signature identification based on targeted analysis of the micro and macro environmental, clinical & scientific knowledge and data. The newly-developed approach and analytical tools are trying to meet the challenges of big versus small data analysis originated in various data sources, towards reliable, personalized and precise medicine. Other areas of research include rehabilitation of the discrete sensory motor, learning function, drug toxicity, data mining, and, most recently, a broadband initiative of “Healthy Aging”.

Dr. Kalish was one of the founders of the “Dead Sea Research Institute” focusing on “Life in Extreme Conditions” as a lesson from nature. A lesson utilizing this unique area towards broadband translational research for the benefit of the planet and humanities.



Pascal Mäser

Unit Head, Swiss Tropical and Public Health Institute

PUBLICATIONS

- Wittlin S, Mäser P. From magic bullet to magic bomb: reductive bioactivation of antiparasitic agents. *ACS Infect Dis.* 2021; 7:2777
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Carl R. Merrill

MD, Capt USPHS (ret), Chief Scientific Officer, Board Director

Dr. Merrill graduated from the college of William and Mary in 1958 with a BS in chemistry and Georgetown University Medical School in 1962 with an MD degree. In 1963, he completed a clinical residency in Boston, followed by participation in the NIH

Research Associate Program. In 1966 Dr. Merrill attended the Cold Spring Harbor Phage Biology Course. This course helped Dr. Merrill to focus his research efforts on basic phage biology and medical applications, including studies on: interactions of phage and mammalian systems, development of enhanced phage strains for use as antibacterial agents and a concept and principles that would be needed for the development of phage libraries for the treatment of individual clinical infections. In 2003, as multi-drug resistant (MDR) bacteria were starting to be recognized as a serious international health risk, Dr. Merrill, published a pivotal article that outlined limitations antibiotic therapies and of early phage therapy efforts and he suggested concepts that have emerged as the modern approach. Dr. Merrill retired from the NIH, after 43 years of service in 2006 and In 2010, Dr. Merrill's post-doctoral fellows, Dr. Biswas joined the Biological Defense Research Directorate (BDRD) of the US Navy and he helped to facilitate an initiative to explore Dr. Merrill's concepts as a potential way to deal with biodefense threats associated with MDR superbugs. In 2016 this approach achieved a significant milestone with the successful rescue of, a critically ill patient, infected with antibiotic resistant *A. baumannii*. This case was immediately followed by numerous additional patient cases. In response for a need to translate the Navy's phage research into a commercially available therapy, Adaptive Phage Therapeutics (APT) was founded in 2016 by Dr. Merrill and his son, Greg Merrill. The company acquired worldwide exclusive rights to BDRD's phage technology and began efforts to optimize precision phage therapy for rapid, cost effective, clinical adoption. Details concerning the progress of the company's efforts to further develop and use phages to treat antibiotic resistant bacterial infections can be viewed at: www.aphage.com.

PUBLICATIONS

- Merrill, C.R., Biswas, B., Carlton, R., Jensen, N.C., Creed, G.J., Zullo, S., & Adhya, S., Long-Circulating Bacteriophage as Antibacterial Agents, *Proc. Natl. Acad. Sci. U.S.A.*, 93: 3188-3192, 1996.
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Scott E. McNeil

Prof. Dr. Scott McNeil is the Head of the Nanopharmaceutical and Regulatory Science Group within the Department of Pharmaceutical Sciences at the University of Basel. Scientists within his group develop and characterize novel nano-based formulations, to improve the therapeutic index of active pharmaceutical ingredi-

ents. Particular emphasis is given to the encapsulation and delivery of enzymes for lysosomal storage diseases, with the goal of decreasing the anti-drug antibody response. In collaboration with other research institutions, he also identifies and investigates the critical quality attributes (CQAs) of nanopharmaceuticals and nanosimilars, such as mechanisms of action, safety, and practical application issues. Prof McNeil has over 100 publications and has helped transition 12 nanoformulations into clinical trials. Prior to joining the University of Basel, he was the Director of the Nanotechnology Characterization Laboratory (NCL) at the US National Cancer Institute. In addition to his academic career, McNeil served for twenty years in the US Army.



Moein Moghimi

Moein Moghimi is a Professor of Pharmaceutics and Nanomedicine at the School of Pharmacy, and Translational and Clinical Research Institute, Newcastle University (UK), and an Adjoint Professor at the Skaggs School of Pharmacy and Pharmaceutical Sciences, University of Colorado-Denver. He is also co-founder of three

spin-offs in USA and UK, and Associate Editor of *Molecular Therapy* (Cell Press). He graduated with Honors in Biochemistry from the University of Manchester (UK) in 1985 and then completed a PhD in Biochemistry at Charing Cross and Westminster Medical School (Imperial College, London).

Moghimi is widely recognised for his contribution to fundamental and translational research in nanomedicine and drug delivery, especially in mechanistic understanding of nanoparticle-mediated complement activation and infusion reactions, and as an inventor of many nanosystems for tissue-specific targeting. The latter have included "splenotropic" and "lymphotropic" nanoparticles. Among the latest inventions of Moghimi's laboratory are the NanoLigand Carriers. These are induced self-assemblies of phage-derived display peptides that on intravenous injection rapidly target two re-

ceptors on the blood brain-barrier, reaching neurons and microglia. A 2021 study conducted by Stanford University list Moghimi among the top 0.08% of world's leading scientists across in all fields, and rank him at 53 (out of 131,063) in the field of pharmacology in the world and 28 in Europe. As to date, Moghimi's research programme has secured over €25 million funding. He is widely published (>300 research papers, reviews, book chapters, proceedings, etc., ORCID: 0000-0003-0836-926X) and cited (>24,000 citations and h-index of 70, GS as of 2021), and has delivered >400 invited plenaries, keynote speeches and distinguished lectures world-wide. He also serves on editorial board of >10 international journals including *Advanced Drug Delivery Reviews*, *Journal of Controlled Release* and *Nanomedicine (Lond.)*. He is also an active consultant to industry and governmental organisations.

Professor Moghimi's earlier appointments have included: 1) Professor and Chair in Pharmaceutics at the School of Medicine, Pharmacy and Health, Durham University, UK (2016-2017), 2) Full Affiliate Member/Professor at the Methodist Research Institute, Houston Methodist Hospital Systems (an affiliate of Weill-Cornell Medical College, NY), Houston, Texas, USA (2013-2017, 3); Visiting Professor at Università Degli Studi Di Padova, Padova, Italy (2015), 4) Professor of Nanomedicine (at the Department of Pharmacy), Professor of Pharmaceutical Nanotechnology (at the NanoScience Center) and Founder/Director of the Center for Pharmaceutical Nanotechnology and Nanotoxicology (supported by the Danish Agency for Science, Technology and Innovation) at the University of Copenhagen, Denmark (2008-2016), and 5) Honorary Professor of Nanomedicine at the Multidisciplinary Research Center, Shantou University, China (2008-2010).

SELECTED REPRESENTATIVE PUBLICATIONS:

Nature Communications 2021, 12:4858 | Nature Nanotechnology 2019, 14:260 | Nature Communications 2019, 10:4635 | Nature Nanotechnology 2017, 12:387 | Nature Nanotechnology 2017, 12:589



Stefan Mühlebach

Chair Achieving Global Vaccine Equity

Stefan Mühlebach is a trained pharmacist and pharmacologist from the university of Bern (Switzerland). He is a professor emeritus of the Department of Pharmaceutical Sciences and external member at the Division of Clinical Pharmacy & Epidemiology / Hospital Pharmacy at the university of Basel (<https://pharma.unibas.ch/de/personen/stefan-muehlebach/>). He worked as Chief Hospital Pharmacist (1980-2005), Head of the Pharmacopoeia at Swissmedic (2005-2007). From 2008 till to his retirement in 2019 he was Regulatory Science Lead of Non-Biological Complex Drugs with Vifor Pharma Ltd and Chair of the NBCD Working Group (Lygature). He continues teaching and conference activities on nanopharmaceuticals on a national and international level. In 2019 he got a honoris causa doctorate of the Semmelweis University on the 250 years anniversary celebrations. Since 2020 he leads in the Task Force Vaccination of Federal Office of Public Health the group for the national logistics of the procurement and distribution of the COVID vaccines in Switzerland.

RECENT PUBLICATIONS

- Sofia N, Mühlebach S, Musazzi UM, Khatib R, Martinez Sesmero JM, Lipp HP, Surugue J, Di Francesco T, Flühmann B. How Do Hospital Pharmacists Approach Substitution of Nanomedicines? Insights from a Qualitative Pilot Study and a Quantitative Market Research Analysis in Five European Countries. *Pharmaceutics*. 2021 Jul 2;13(7):1010. doi: 10.3390/pharmaceutics13071010
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- Mühlebach S. Regulatory challenges of nanomedicines and their follow-on versions: A generic or similar approach? *Adv Drug Deliv Rev*. 2018 Jun;131:122-131. doi: 10.1016/j.addr.2018.06.024. Epub 2018 Jun 30.



Willem Mulder

Professor of Precision Medicine

Department of Internal Medicine, Radboud Institute of Molecular Life Sciences (RIMLS) and Radboud Center for Infectious Diseases (RCI), Radboud University Nijmegen Medical Center, Netherlands. Laboratory of Chemical Biology, Department of Biomedical Engineering and Institute for

Complex Molecular Systems, Eindhoven University of Technology, Eindhoven, Netherlands. CSO, Trained Therapeutix Discovery.

Prof. Dr. Willem Mulder is a biomedical engineer with a background in chemistry. After acquiring his PhD from the Eindhoven University of Technology in 2006, he moved to the US and founded the Nanomedicine Lab at the Icahn School of Medicine at Mount Sinai in New York. He was awarded several NIH R01 grants and built a successful research program revolving around developing nanomedicine approaches for immune regulation in disease. After a nearly 15-year tenure, he migrated back to The Netherlands in 2021 to establish a new research group, spanning two institutions, the Radboud University Medical Center and the Eindhoven University of Technology. He was previously awarded Vidi and Vici grants from the Dutch Research Council (NWO) and was recently awarded an ERC Advanced Grant. Willem Mulder is co-Founder & Chief Scientific Officer of Trained Therapeutix Discovery and co-Founder & Chief Technology Officer of BIOTRIP.nl

RECENT PUBLICATIONS

- 1. Bram Priem, Mandy M. T. van Leent, Abraham J. P. Teunissen, Alexandros Marios Sofias, Vera P. Mourits, Lisa Willemsen, Emma D. Klein, Roderick S. Oosterwijk, Anu E. Meerwaldt, Jazz Munitz, Geoffrey Prévot, Anna Vera Verschuur, Shequia A. Nauta, Esther M. van Leeuwen, Elizabeth L. Fisher, Karen A.M. de Jong, Yiming Zhao, Yohana C. Toner, Georgios Soutanidis, Claudia Calgano, Paul H.H. Bomans, Heiner Friedrich, Nico A.J.M. Sommerdijk, Thomas Reiner, Raphaël Duivenvoorden, Eva Zupančič, Julie S. Di Martino, Ewelina Kluza, Mohammad Rashidian, Hidde L. Ploegh, Rick M. Dijkhuizen, Sjoerd Hak, Carlos Pérez-Medina, Jose Javier Bravo-Cordero, Menno P. J. de Winther, Leo A. B. Joosten, Andrea van Elsas, Zahi A. Fayad, Alexander Rialdi, Denis Torre, Ernesto Guccione, Jordi Ochando, Mihai G. Netea, Arjan W. Griffioen, Willem J. M. Mulder. Trained immunity-promoting nanobiologic therapy suppresses tumor growth and potentiates checkpoint inhibition. *Cell*. 2020 Oct 29;183(3):786-801.e19.
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Calcagno, Gustav J. Strijkers, Willem J. M. Mulder. Probing myeloid cell dynamics in ischaemic heart disease by nanotracer hot-spot imaging. *Nature Nanotechnology*. 2020 May;15(5):398-405.

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- Marnix Lameijer, Tina Binderup, Mandy M.T. van Leent, Max L. Senders, Francois Fay, Joost Malkus, Brenda L. Sanchez-Gaytan, Abraham J. P. Teunissen, Nicolas Karakatsanis, Philip Robson, Xianxiao Zhou, Yuxiang Ye, Gregory Wojtkiewicz, Jun Tang, Tom T.P. Seijkens, Jeffrey Kroon, Erik S.G. Stroes, Andreas Kjaer, Jordi Ochoa, Thomas Reiner, Carlos Pérez-Medina, Claudia Calcagno, Edward A. Fischer, Bin Zhang, Ryan E. Temel, Filip K. Swirski, Matthias Nahrendorf, Zahi A. Fayad, Esther Lutgens, Willem J. M. Mulder, Raphaël Duivenvoorden.

Efficacy and safety assessment of a TRAF6-targeted nanoimmunotherapy in atherosclerotic mice and non-human primates. *Nature Biomedical Engineering*. 2018 May;2(5):279-292.



Bert Müller

Director Biomaterials Science Center

Bert Müller holds the Thomas Straumann Chair for Materials Science in Medicine at the University of Basel, Switzerland and is founding director of the Biomaterials Science Center. He received his Master degree in Physics from the Dresden University of Technology, Germany, his Ph.D. in Experimental Physics from the University of Hannover, Germany and his Habilitation in Experimental Physics from ETH Zurich, Switzerland. Since April 2001, Bert Müller teaches at the Physics Department of ETH Zurich. His current research interests include hard X-ray imaging down to nanometer scale and physics-based research in medicine and dentistry. He authored more than 400 publications including several patents. More recently, Bert Müller became an entrepreneur, being co-founder and advisor of Bottmedical AG, Acthera Therapeutics AG, and Bottneuro AG, all located in Basel, Switzerland. He is named as the 2022 recipient of the SPIE Biophotonics Technology Innovator Award.

RECENT PUBLICATIONS

- G. Rodgers, C. Tanner, G. Schulz, A. Migga, W. Kuo, C. Bikis, M. Scheel, V. Kurtcuoglu, T. Weitkamp, B. Müller: Virtual histology of an entire mouse brain from formalin fixation to paraffin embedding. Part 2: Volumetric strain fields and local contrast changes, *Journal of Neuroscience Methods* 365 (2022) 109385, DOI:10.1016/j.jneumeth.2021.109385
- G. Rodgers, W. Kuo, G. Schulz, M. Scheel, A. Migga, C. Bikis, C. Tanner, V. Kurtcuoglu, T. Weitkamp, B. Müller: Virtual histology of an entire mouse brain from formalin fixation to paraffin embedding. Part 1: Data acquisition, anatomical feature segmentation, tracking global volume and density changes, *Journal of Neuroscience Methods* 364 (2021) 109354, DOI:10.1016/j.jneumeth.2021.109354

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- G. Schulz, H. Deyhle, C. Bikis, O. Bunk, B. Müller: Imaging the orientation of myelin sheaths in a non-stained histological slice of human brain, *Precision Nanomedicine* 3(4) (2020) 656-665, DOI:10.33218/001c.17211
- B. Osmani, H. Schiff, K. Vogelsang, R. Guzman, P. M. Kristiansen, R. Crockett, A. Chacko, S. Bucher, T. Töpfer, B. Müller, Hierarchically structured polydimethylsiloxane films for ultra-soft neural interfaces, *Micro and Nano Engineering* 7 (2020) 100051, DOI:10.1016/j.mne.2020.100051



Kari Christine Nadeau

Dr. Kari Nadeau is the Naddisy Foundation Endowed Professor of Medicine and Pediatrics and, Director of the Sean N. Parker Center for Allergy and Asthma Research at Stanford University. She is Section Chief in Asthma and Allergy in the Pulmonary, Allergy and Critical Care Division at Stanford. She is the Medical Director of Precision Allergy at Stanford. She is the Sr. Director of Clinical Research for the Division of Hospital Medicine. For more than 30 years, she has devoted herself to understanding how environmental and immune/genetic factors affect allergies, immune tolerance, and asthma. She and her team are focused in areas of vaccine allergies, asthma, global climate change and health by studying air pollution and wildfire exposures, particularly in underserved areas. As one of the globe's foremost experts in adult and pediatric allergy, immunology, and asthma, her research is laying the groundwork for a variety of potential future therapies to prevent and cure allergies and asthma. Dr. Nadeau received her MD and PhD from Harvard Medical School through the NIH MSTP program. She completed a residency in pediatrics at Boston Children's Hospital and a clinical fellowship in allergy, asthma and immunology at Stanford and at University of California, San Francisco. Dr. Nadeau has served as a FDA consultant and a reviewer for NIH Study Sections. Her work has been recognized with numerous grants and awards. She has collaborated with many organizations and institutions. Through NIH, FARE, CoFAR, WHO, the United Nations and other partnerships, she collaborates with colleagues from institutions around the globe.

RECENT PUBLICATIONS

- Arunachalam PS, Scott MKD, Hagan T, Li C, Feng Y, Wimmers F, Grigoryan L, Trisal M, Edara VV, Lai L, Chang SE, Feng A, Dhingra S, Shah M, Lee AS, Chinthrajah S, Sindher SB, Mallajosyula V, Gao F, Sigal N, Kowli S, Gupta S, Pellegrini K, Tharp G, Maysel-Auslender S, Hamilton S, Aoued H, Hrusovsky K, Roskey M, Bosinger SE, Maecker HT, Boyd SD, Davis MM, Utz PJ, Suthar MS, Khatri P, Nadeau KC*, Pulendran B*. Systems vaccinology of the BNT162b2 mRNA vaccine in humans. *Nature*. 2021 Aug;596(7872):410-416. (*-co senior).
- Röltgen K, Nielsen S, et al. Davis MM, Pulendran B, Troxell M, Sigal G, Natkunam Y, Pinsky B, Nadeau KC*, Boyd, SD*. Immune imprinting, breadth of variant recognition and germinal center formation in human SARS-CoV-2 infection and vaccination. *Cell*. Accepted 2022 Jan 21. In print. (*-co senior).
- Sokolowska M, Eiwegger T, Ollert M, Torres MJ, Barber D, Del Giacco S, Jutel M, Nadeau KC, Palomares O, Rabin RL, Riggioni C, Vieths S, Agache I, Shamji MH. EAACI statement on the diagnosis, management and prevention of severe allergic reactions to COVID-19 vaccines. *Allergy*. 2021 Jun;76(6):1629-1639.
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Letizia Nicoletti

Letizia Nicoletti is a PhD student in Bioengineering and Medical-Surgical Sciences at Department of Mechanical and Aerospace Engineering (DIMEAS) of Politecnico di Torino. She works in BIORECAR project (ERC Horizon 2020 grant agreement No 772168, <http://www.biorecar.polito.it>) on the design of new advanced therapies for drug delivery in cardiac regeneration.

She got a Master's degree (April 2019) in Pharmacy at the Department of Drug Science and Technology, University of Turin. She performed her master thesis in collaboration with the Laboratoire d'Automatique et de Génie des Procédés; et Génie Pharmaceutique (LAGEPP, team Gepharm) at University Lyon 1, focused on the formulation and physicochemical characterization of polymeric and lipid nanoparticles tested on human colorectal cell line.

In June 2019, she got a position as a Post-graduate Fellow in BIORECAR project under Prof. Valeria Chiono's supervision at Politecnico di Torino. Her activity was aimed at the design of innovative polymeric and lipid nanoparticles for the direct reprogramming of cardiac fibroblasts into cardiomyocytes. In her work, she acquired progressively more knowledge on tissue engineering and regenerative medicine, in addition to her nanotechnological skills.

During her activity in BIORECAR, Letizia Nicoletti formulated and characterised novel lipoplexes and bioartificial nanoparticles for microRNA encapsulation in collaboration with the Department of Drug Science and Technology at University of Turin. Particularly, she acquired expertise in dynamic light scattering analysis, Qubit fluorimeter/plate reader measurement of encapsulated and released oligonucleotides, Western Blot/electrophoresis analysis of lipoplexes/polypoplexes. Moreover, her research work has included the design and characterization of an injectable hydrogel for the administration of nanoparticles *in vivo*, under Prof. Valeria Chiono and postdoc Elena Marcello's supervision. Results were the subject of 5 poster and 3 oral presentations in national and international congresses. She received 1 Best Poster Presentation Award at NANO-DAY IV edition Conference in December 2019.

During her research activity as Post-graduate fellow and PhD student, Dr. Nicoletti has co-supervised 5 Master's thesis students, and performed lab tutoring activity for the Master Course "Practical formation in biomedical nanotechnologies and advanced therapies" - Biomedical Engineering Faculty at Politecnico di Torino.

She is member of Controlled Release Society (CRS), National Bioengineering Group (GNB), Tissue Engineering and Regenerative Medicine Society (TERMIS) and European Society for Biomaterials (ESB). Letizia Nicoletti has published 1 paper and she is one of the inventors of 1 patent (pending) on nanotechnologies for oligonucleotide release. 1 paper on lipoplexes for oligonucleotide release is under submission.

RECENT PUBLICATIONS

- "Rationally designed hyaluronic acid-based nano-complexes for pentamidine delivery" Flavia Carton, Yves Chevalier, Letizia Nicoletti, Małgorzata Tarnowska, Barbara Stella, Silvia Arpicco, Manuela Malatesta, Lars Petter Jordheim, Stéphanie Briançon, Giovanna Lollo. *International Journal of Pharmaceutics*, Volume 568, 10 September 2019, 118526.
- "Lipoplexes for effective *in vitro* delivery of microRNAs to adult human cardiac fibroblasts for prospective direct cardiac cell reprogramming" Letizia Nicoletti, Camilla Paoletti, Giulia Tarricone, Ilaria Andreana, Barbara Stella, Silvia Arpicco, Carla Divieto, Clara

Mattu, Valeria Chiono, Submitted to *Nanomedicine: Nanotechnology, Biology, and Medicine*, 2022

- "Metodo per la preparazione di nanoparticelle polimeriche ibride per il rilascio di farmaci oligonucleotidici nella terapie farmacologiche a scopo rigenerativo, curativo e preventivo" Valeria Chiono, Clara Mattu, Letizia Nicoletti, Camilla Paoletti, Silvia Arpicco, Barbara Stella, PATENT pending 2021.



Maurice Nigo Mutro

Director

- a) Director of the Higher Institute of Medical Techniques of (Institut Supérieur des Techniques Médicales de) Nyankunde, Bunia city, Democratic Republic of the Congo
- b) Chairman of the Board of the Directors of the CIRCUMFLEX FOUNDATION, Bunia city, Democratic Republic of the Congo

RECENT PUBLICATIONS

- Nigo MM, Odermatt P, Nigo DW, Salieb-Beugelaar GB, Battegay M, Hunziker P. Patients with severe schistosomiasis mansoni in Ituri Province, Democratic Republic of the Congo. *Inf Dis Poverty* 2021; 10, 50-63. <https://doi.org/10.1186/s40249-021-00815-6>
- Nigo MM, Odermatt P, Nigo DW, Salieb-Beugelaar GB, Battegay M and Hunziker P. Morbidity associated with *Schistosoma mansoni* infection in north-eastern Democratic Republic of the Congo. *PLoS Neg Trop Dis*, 2021; 15, e0009375, <https://doi.org/10.1371/journal.pntd.0009375>.
- Nigo MM, Odermatt P, Salieb-Beugelaar GB, Morozov O, Battegay M, Hunziker P. Epidemiology of *Schistosoma mansoni* infection in Ituri Province, north-eastern Democratic Republic of the Congo. *PLoS Neg Trop Dis*, 2021 ;15, e0009486, <https://doi.org/10.1371/journal.pntd.0009486>
- Nigo MM, Odermatt P, Salieb-Beugelaar GB, Battegay M and Hunziker P. Schistosomiasis: from established diagnostic assays to emerging micro/nanotechnology-based rapid field testing for clinical management and epidemiology. *Precision Nanomedicine*, 2020, (3) 1: 439-458.
- Opoku NO, Bakajika DK, Kanza EM, ... Nigo MM ... et al. Single dose moxidectin versus ivermectin for *Onchocerca volvulus* infection in Ghana, Liberia, and the Democratic Republic of the Congo: a randomised, controlled, double-blind phase 3 trial. *The Lancet*, 2018, 392 (10154): 1207-1216.



Riccardo Nisato

Licensing and Grant Associate Manager

With over 20 years of experience in R&D and Business Development in academic, hospital & pharmaceutical industry environments, Dr. Nisato has held top management positions in various start-ups and is currently managing the in and out-licensing activities of antibacterials at the

Swiss-based, biopharmaceutical company Debiopharm. He has published and contributed to over 10 publications. His educational background includes a PhD from the University of Geneva in and an Executive MBA from the EPFL/HEC/McComb's School of Business.



Lutz Nuhn

Chair of Macromolecular Chemistry,
Faculty of Chemistry and Pharmacy,
Julius Maximilian University Würzburg
(DE)

Dr. Lutz Nuhn 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 is a member of the Max Planck Graduate School with the Johannes Gutenberg-University Mainz (MPGC) and received scholarships and awards from the Controlled Release Society (CRS), German National Academic Foundation, the Alexander-von-Humboldt-Foundation, the Research Foundation Flanders ("Fonds Wetenschappelijk Onderzoek Vlaanderen, FWO"), the "Fonds der Chemischen Industrie" (FCI), the "DECHEMA - Gesellschaft für Chemische Technik und Biotechnologie" and the "Gesellschaft Deutscher Chemiker" (GDCh). He is currently also leading two projects in the interdisciplinary Collaborative Research Center "Nano-Sized Polymer Therapeutics for Tumor Immunotherapy" (CRC/SFB 1066).

His research focuses on multi-responsive and degradable polymeric nanocarriers, especially for advanced immunotherapies.

RECENT PUBLICATIONS

- A. Huppertsberg, L. Kaps, Z. Zhong, S. Schmitt, J. Stickdorn, K. Deswarte, F. Combes, C. Czysch, J. De Vrieze, S. Kasmi, N. Choteschovsky, A. Klefenz, C. Medina-Montano, P. Winterwerber, C. Chen, M. Bros, S. Lienenklaus, N. N. Sanders, K. Koynov, D. Schuppan, B. N. Lambrecht, S. David, B. G. De Geest, L. Nuhn – "Squaric Ester-Based, pH-Degradable Nanogels: Modular Nanocarriers for Safe, Systemic Administration of Toll-Like Receptor 7/8 Agonistic Immune Modulators", *J. Am. Chem. Soc.* 2021, 143, 9872-9883.
- E. Bolli, M. Scherger, S. M. Arnouk, A. R. Pombo Antunes, D. Straßburger, M. Urschbach, J. Stickdorn, K. De Vlaminck, K. Movahedi, H. J. Räder, S. Hernot, P. Besenius, J. A. Van Ginderachter, L. Nuhn – "Targeted Repolarization of Tumor-associated Macrophages via Imidazoquinoline-linked Nanobodies", *Adv. Sci.* 2021, 8, 202004574
- M. Scherger, H. J. Räder, L. Nuhn – "Self-immolative RAFT-polymer end group modification", *Macromol. Rap. Commun.* 2021, 42, 202000752.
- J. Kockelmann, J. Stickdorn, S. Kasmi, J. De Vrieze, M. Pieszka, D. Y. W. Ng, S. A. David, B. G. De Geest, L. Nuhn – "Control over Immune Stimulation by pH-Degradable Poly(norbornene) Nanogels", *Biomacromolecules* 2020, 21, 2246-2257.
- L. Lybaert, K. Vermaelen, B. G. De Geest, L. Nuhn – "Immunoengineering through Cancer Vaccines – a Personalized and Multi-step Vaccine Approach towards Precise Cancer Immunity", *J. Controlled Release* 2018, 289, 125-145.



Rachel O'Reilly

Head of School and Professor of
Chemistry, School of Chemistry, University
of Birmingham

Rachel O'Reilly is a Professor of Polymer Science and has been Head of the School of Chemistry at the University of Birmingham in the UK since 2018. She graduated from the University of Cambridge with a

BA in Natural Sciences (1998) and an MSc in Chemistry (1999) and completed her PhD in 2003 at Imperial College London.

Professor O'Reilly has held a number of prestigious fellowships from the EPSRC, Royal Society and Royal Commission for the Exhibition of 1851. Her expertise in novel materials has also encompassed research in California and at the universities of Cambridge and Warwick. Professor O'Reilly was promoted to full Professor at the age of just 34, and has been a Fellow of the Royal Society of Chemistry since 2013.

Working at the biological-materials interface, Professor O'Reilly engineers new polymeric nanostructures with hybrid properties – utilising DNA and polypeptides to create precision materials that mimic nature. Her research, spanning polymer synthesis, self-assembly, catalysis and DNA nanotechnology, has resulted in over 220 publications to date.

Professor O'Reilly has given over 170 invited lectures and was recognised as one of the Royal Society of Chemistry's 175 faces of Chemistry. She was honoured in 2019 as a UK Finalist in the Blavatnik Awards for Young Scientists, and in 2020 she received the Royal Society of Chemistry's Corday-Morgan Prize for her contributions to chemistry and the RSC's Polymer Chemistry Lectureship.

RECENT PUBLICATIONS

- New functional handle for use as a self-reporting contrast and delivery agent in nanomedicine, M.P. Robin, A.B. Mabire, J. Damborsky, E. Thom, U. Winzer-Serhan, J. Raymond and R.K. O'Reilly, *J. Am. Chem. Soc.*, 2013, 135, 9518–9524, DOI: 10.1021/ja403587c
- Precision epitaxy for aqueous 1D and 2D Poly(caprolactone) assemblies, M.C. Arno, M. Inam, Z. Coe, G. Cambridge, L.J. MacDougall, R. Keogh, A.P. Dove and R.K. O'Reilly, *J. Am. Chem. Soc.* 2017, 139, 16980–16985, DOI: 10.1021/jacs.7b10199
- Confinement of Therapeutic Enzymes in Selectively Permeable Polymer Vesicles by Polymerization-Induced Self-Assembly (PISA) Reduces Antibody Binding and Proteolytic Susceptibility; L. D. Blackman, S. Varlas, M. C. Arno, Z. H. Houston, N. L. Fletcher, K. J. Thurecht, M. Hasan, M. I. Gibson and R. K. O'Reilly, *ACS Cent. Sci.*, 2018, 4, 718-723, DOI:10.1021/acscentsci.8b00168.
- Exploiting the role of nanoparticle shape in enhancing hydrogel adhesive and mechanical properties, M. C. Arno, M. Inam, A. C. Weems, Z. Li, A. L. A. Binch, C. I. Platt, S. M. Richardson, J. A. Hoyland, A. P. Dove and R. K. O'Reilly, *Nat. Commun.*, 2020, 11, 1420-1428, DOI: 10.1038/s41467-020-15206-y
- Manipulating the fluorescence lifetime at the sub-cellular scale via photo-switchable barcoding, Y. Xie, M. C. Arno, J. T. Husband, M. Torrent-Sucarrat & R. K. O'Reilly, *Nat. Commun.*, 2020, 11, 2460-2468, DOI: 10.1038/s41467-020-16297-3



Kevin Outtersen

Kevin Outtersen is a Professor of Law at Boston University and Executive Director of CARB-X, a global antibacterial accelerator. His research work focuses on problems with pharmaceutical markets, especially drugs that lose effectiveness over time due to resistance.



Marisa Papaluca Amati

Former EMA Senior Scientific Advisor ,
Visiting professor Imperial College
London, Independent Consultant Health
Products, Chairperson and speaker at
CLINAM 2022

World leading medicines regulatory specialist with interest in evolving science and technologies, biologicals and advanced medicinal products for immunology driven diseases, oncology, cardiometabolic and rare diseases. Retired European Medicines Agency's Senior Scientific Advisor and Honorary Professor at Imperial College London.

PROFESSIONAL PROFILE

September 2021 to date: independent consultant Pharmaceuticals Regulatory Science. Special interest in biologicals and advanced therapies medicinal products for immunology driven diseases, oncology, cardiometabolic and rare diseases. Experience in Pipelines review and innovative health technologies.

September 2019 to date: visiting Professor Imperial College London – Faculty of medicine -Department of Public Health and primary Care

August 2013 - March 2019 EMA Senior Scientific Advisor (SSA) Contributing to the analyses of the scientific working parties operations pilot EMA Regulatory Science Observatory (RSO) leading to the initial draft of EMA regulatory Science Strategy to 2025.

October 1994 – August 2013. EMA Senior Scientist European Medicines Agency. Active role in initiating many areas of activities Medicines EU Marketing Authorisations centralised procedure, European Public Assessment Reports, Biotechnology Working Party. Established “safe harbour” platforms (Innovation Task Force, EMA Business Pipeline), the biomarkers qualification process, the EU-Innovation Network, I have been very instrumental for EMA (international) activities on medical innovations (pharmaceuticals h-r-DNA technologies, gene and cell therapy, nanotechnology, biosimilars, pharmacogenomics, personalised medicine, biomarkers novel clinical designs)

April 1984 – September 1994 – Medical Director at the Pharmaceuticals Department of the Italian Ministry of Health.

Pre-clinical and clinical assessor, as medical director I introduced international-grade processes and standards, including guidelines on assessment reports, electronic reporting to the WHO ADRs monitoring centre, coordination Office for Centralised Community Procedures (OCCP), multinational scientific advice for large clinical trials in EU.

1978 – 1994 – Clinical consultant in internal medicine and metabolic diseases

RECENT PUBLICATIONS

- Assessing the long-term safety and efficacy of COVID-19 vaccines - Majeed, Azeem, Papaluca, Marisa ; Molokhia, Mariam London, England: SAGE Publications; ISSN: 0141-0768; EISSN: 1758-1095; DOI: 10.1177/01410768211013437; PMID: 33945346 Journal of the Royal Society of Medicine, 2021-07, Vol.114 (7), p.337-340
- Time for Change? The Why, What and How of Promoting Innovation to Tackle Rare Diseases – Is It Time to Update the EU's Orphan Regulation? And if so, What Should be Changed? Denis Horgan, Barbara Moss, Stefania Boccia, Maurizio Genuardi, g Maciej Gajewskih Gabriele Capursoi Pierre Fenauj, k Beatrice Gulbisk, I Mariangela Pellegrinik, m Maria del Mar Mañú Pereirak, n Victoria Gutiérrez Valle, Iñaki Gutiérrez Ibarluzeao, p Alastair Kentq Ivana Cattaneor Beata Jagielskas Ivica Belinat Birute Tumienieu Adrian Ward, Marisa Papaluca Biomed Hub 2020;5:509272
- Giuseppe Novelli, Michela Biancolella, Andrea Latini, Aldo Spal-lone, Paola Borgiani and Marisa Papaluca Commentary: Precision Medicine in Non-Communicable Diseases. High-Throughput 2020, 9, 3; doi:10.3390/ht9010003
- Philip Hines, Li Hiu Yu, Richard H Guy, Angela Brand, Marisa Papaluca-Amati Scanning the horizon: a systematic literature re-

view of methodologies. BMJ Open 2019;9:e026764. doi:10.1136/bmjopen-2018-026764

- Philip A. Hines, Richard H. Guy, Anthony J. Humphreys and Marisa Papaluca-Amati The European Medicines Agency's goals for regulatory science to 2025, Nature reviews | Drug Discovery Volume 18 | June 2019 | 403



Anil Patri

Dr. Anil Patri serves as the Director of the Nanocore and Chairs the Nanotechnology Task Force at the US Food and Drug Administration. Nanocore conducts regulatory science research to understand the physico-chemical properties, safety and efficacy of products containing nanomaterial. Dr. Patri serves on behalf of FDA on the Nanoscale Science, Engineering, and Technology Subcommittee (NSET) for inter-agency coordination and collaborations. He serves on ASTM International E56 sub-committee, ISO TC 229 and OECD WPMN to facilitate standards development.



Dan Peer

Vice President for Research, Tel Aviv University; Director, Laboratory of Precision NanoMedicine.

Dan Peer is a Professor and the Director of the Laboratory of Precision NanoMedicine at Tel Aviv University (TAU) funded by the US NIH and the European Union via ERC

grant. Prof. Peer is also the Chair of Tel Aviv University Cancer Biology Research Center; the biggest Cancer Center in Israel that includes 17 affiliated hospitals. In addition, he heads the new SPARK program (Center for 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) for *in vivo* validation of new drug targets within the immune system that has enormous implications in blood cancer and inflammation.

Prof. Peer received more than 30 awards; among them, he was recognized by the Kenneth Rainin Foundation by their Innovator (2010) and Breakthrough (2011 – 2013) Awards for his pioneering work in inflammatory bowel diseases and by the AAAS and the Untold news breakthrough Award for his development of the Gagomers platform for cancer targeted drug delivery. In 2017, he received the 2017 Nanos Award for major contribution to the field of clinical nanomedicine in CLINAM 10th, Basel, Switzerland.

Prof. Peer has more than 90 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. Three of them are in clinical stage companies.

Prof. Peer is a past Present of the Israeli Chapter of the Controlled Release Society, and a Member of the Israel Young Academy of Science.

RECENT PUBLICATIONS

- Kedmi R., Viaga N. Ramishetti S, Goldsmith M, Rosenblum D, Dammes N, Hazan-Halevy I, Nahary L, Leviatan-Ben-Arye S, Harlev M, Behlke M, Benhar I, Lieberman J, and Peer D (2018). A modular platform for targeted RNAi therapeutics. Nature Nanotechnology. 13(3):214-219. A novel universal platform for RNAi therapeutics

- Rosenblum D. et al. (2020). CRISPR/Cas9 Genome Editing using Targeted Lipid Nanoparticles for Cancer Therapy. *SCIENCE Advances*. (Back Cover story).
- Elia U. et al. (2021). Design of SARS-CoV-2 hFc-Conjugated Receptor-Binding Domain mRNA Vaccine Delivered via Lipid Nanoparticles. *ACS Nano*. Jan 22;acs.nano.0c10180. doi: 10.1021/acsnano.0c10180.
- Dammes N. et al. (2021). Conformation Sensitive Targeting of Lipid Nanoparticles for RNA Therapeutics. *Nature Nanotechnology*. June 2021.
- Gutkin A., Rosenblum D. and Peer D (2021). RNA delivery with a human virus-like particle. *Nature Biotechnology*. 2021 Nov 12. doi: 10.1038/s41587-021-01124-xf.



Camille Peitsch

Ph.D., R&D 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 R&D Manager while continuously pursuing her tasks as a Talineuren's product manager.



Marija Petrovic

Marija Petrovic finished her master studies at Faculty on Pharmacy, University of Belgrade and she is currently performing her PhD studies at University of Geneva (expected July '22) focused on oncology, more specifically oligo and dinucleotide nanoparticle formulation. During her education, Marija has taken part in different scientific projects before starting a PhD. Under the supervision of Prof. Lingner at EPFL, she conducted a scientific research for 6 months on "Fragile telomeres in metaphase and factors which lead to this phenotype", where she gained in depth knowledge on siRNA transfection. Her later internship at Dal Peraro's lab "Characterization of aerolysin pore mutants and mimics", lasting for 4 months helped her to better understand how to biotechnologically produce a protein within E.coli named Aerolysin, purify it and finally characterize it by size exclusion chromatography. After those two valuable internships, she started a PhD at Borchard's lab named "Advanced strategies for STING ligands local delivery of immuno-modulating active principles to tumors". Right now she is conducting a proj-

ect on nano-gels for the di (STING ligands) and oligonucleotide's (miRNA-25/93) local delivery to melanoma, glioma and liver cancer. During her PhD she gained experience in drug carrier synthesis, nano-drug formulation, vast orthogonal characterization, targeted oligonucleotide delivery to tumor micro-environment, analytics, *in vitro* and *in vivo*. This project has been completed with great success having a review on STING ligand delivery in *Journal of Controlled Release* and two additional scientific articles (to be published in *Pharmaceutics* and *International Journal of Nanomedicine*) on depth characterization of particles for a gene therapy. This work inspired her to further improve her "nano formulation and characterization" skills and she received a certificate in nano-biotechnology for a professional training in the field, at Joint Research Centre in Ispra.



Serge Picaud

Director of the Paris Vision Institute

Serge PICAUD is the Director of the Paris Vision Institute since January 1st, 2021. After a PhD in Marseille (France), and studies in Frankfurt (Germany) and University of California Berkeley, He returned to Strasbourg and then Paris to launch his own team on retinal information processing enlarging then the focus to neuroprotection. His team, for instance, revealed how an antiepileptic drug is leading to retinal degeneration. More recently, the team has moved to developing strategies for restoring vision in blind patients. The work involved novel materials like Graphene and Diamond for electrodes like Graphene and Diamond or event-based camera for visual information processing. They have validated the photovoltaic and wireless retinal implants *ex vivo* and *in vivo* on the blind primate retina, paving the way for clinical trials by the company Pixium Vision. As an alternative to retinal implants, optogenetic therapy was evaluated on rodents and primate, opening the path toward clinical trials for the company Gensight biologics. The team is now moving toward visual restoration at the level of the visual cortex for patients with optic neuropathies.

RECENT PUBLICATION

- Verschuere A, Boucherit L, Ferrari U, Fouquet S, Nouvel-Jaillard C, Paques M, Picaud S*, Sahel JA* Planar polarity in primate cone photoreceptors: a potential role in Stiles Crawford effect phototropism. *Communication Biology* (2022) 5:89. <https://doi.org/10.1038/s42003-021-02998-y>
- G. Gauvain, H. Akolkar, A. Chaffiol, F. Arcizet, M. Khoei, M. Desrosiers, C. Jaillard, R. Caplette, O. Marre, S. Bertin, C.M. Fovet, J. Demilly, V. Forster, E. Brazhnikova, P. Hantraye, P. Pouget, A. Douar, D. Pruneau, J. Chavas, J.A. Sahel, D. Dalkara, J. Duebel*, R. Benosman*, S. Picaud*, Optogenetic therapy: high spatiotemporal resolution and pattern discrimination compatible with vision restoration in non-human primates. *Commun Biol* (2021) 4, 125. <https://doi.org/10.1038/s42003-020-01594-w>
- P.H. Prevot, K. Gehere, F. Arcizet, H. Akolkar, M.A. Khoei, K. Blaize, O. Oubari, P. Daye, M. Lanoe, M. Valet, S. Dalouz, P. Langlois, E. Esposito, V. Forster, E. Dubus, N. Wattiez, E. Brazhnikova, C. Nouvel-Jaillard, Y. LeMer, J. Demilly, C.M. Fovet, P. Hantraye, M. Weissenburger, H. Lorach, E. Bouillet, M. Deterre, R. Hornig, G. Buc, J.A. Sahel, G. Chenegros, P. Pouget*, R. Benosman*, S. Picaud*. Behavioural responses to a photovoltaic subretinal prosthesis implanted in non-human primates, *Nat Biomed Eng* 4(2) (2020) 172-180.
- K. Blaize, F. Arcizet, M. Gesnik, H. Ahnine, U. Ferrari, T. Deffieux, P. Pouget, F. Chavane, M. Fink, J.A. Sahel, M. Tanter*, S. Picaud*, Functional ultrasound imaging of deep visual cortex in awake non-human primates, *Proceedings of the National Academy of Sciences of the United States of America* 117(25) (2020) 14453-14463. (IF 9,4)
- S. Picaud, D. Dalkara, K. Marazova, O. Goureau, B. Roska, J.A. Sahel, The primate model for understanding and restoring vision, *Proceedings of the National Academy of Sciences of the United States of America* 116(52) (2019) 26280-26287.



Jai Prakash

Jai Prakash is a Professor and Chair of Engineered Therapeutics at the Department of Advanced Organ bioengineering and Therapeutics at the University of Twente in The Netherlands. He is a pharmaceutical and entrepreneurial scientist with a background in developing novel targeted (nano)therapeutics against fibrosis and the tumor microenvironment. His research is highly multi-/inter-disciplinary integrating peptide technology, nanomedicine, as well as bioengineering fields for targeting cancer and fibrosis. He obtained PhD (cum laude) in 2006 from University of Groningen, The Netherlands in the field of drug targeting to treat renal fibrosis. 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. In 2011, he joined Karolinska Institutet in Stockholm (Sweden) as Assistant Professor in the Department of Oncology-Pathology. Later, he was invited to join as a Tenure-Track Professor at University of Twente to set up his new research line on novel therapeutics against fibrosis and cancer. In 2019, he joined the School of Engineering and Applied Sciences at Harvard University as a visiting professor for his sabbaticals. He has published >100 peer-reviewed publications in high impact journals including *Advanced Materials*, *Science Advances*, *Trends in Cancer*, *Advanced Drug Delivery Reviews*, etc. He is also a (co)-inventor on several international patent families. He has an editor and writer of a book on 'Tumor stoma: Biology and Therapeutics'. He is the founder and CSO of ScarTec Therapeutics, a spin-off company, focusing on the development of novel peptide therapeutics against fibrotic diseases and pancreatic cancer. He is serving as the President of Controlled Release Society (CRS) BeNeLux and France Local chapter. He is also the founder and chair of the International Conference on Nanomedicine meets the Tumor Microenvironment (NanoTME) which was organized for the first time in 2021 and a special issue is in progress in the journal *Theranostics*. He is the expert referee and/or panel member on more than 25 grant agencies including ERC, EPSRC, ANR, Inserm, NWO and FWO.

RECENT PUBLICATION

- Pednekar KP, Heinrich MA, van Baarlen J, Prakash J. (2021) Novel 3D μ tissues Mimicking the Fibrotic Stroma in Pancreatic Cancer to Study Cellular Interactions and Stroma-Modulating Therapeutics. *Cancers (Basel)*. 13(19):5006. doi: 10.3390/cancers13195006.
- Heinrich MA, Mostafa AMRH, Morton JP, Hawinkels LJAC, Prakash J. (2021) Translating complexity and heterogeneity of pancreatic tumor: 3D *in vitro* to *in vivo* models. *Adv Drug Deliv Rev*. 2021 Jul;174:265-293. doi: 10.1016/j.addr.2021.04.018. Epub 2021 Apr 23.
- Priwitaningrum DL, Jentsch J, Bansal R, Rahimian S, Storm G, Hennink WE, Prakash J. Apoptosis-inducing peptide loaded in PLGA nanoparticles induces anti-tumor effects *in vivo*. *Int J Pharm*. 2020 Jul 30;585:119535.
- 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, Schifferlers 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.



Yok-Ai Que

Senior Physician, Associate Professor in Intensive Care Medicine

As a board certified physician in both internal and intensive care medicine, I am currently working in a medico-surgical tertiary Intensive Care unit where I am responsible for the management of hard-to-treat infections. The core of my research is to conceive, elaborate and test innovative strategies to combat the rapid spread of antibiotic resistance. I have been a pioneer in the study, development and implementation of phage therapy in Europe and participated as co-principal investigator in the first controlled study that evaluated GMP phage preparations in humans according to GCP criteria (Phagoburn Study). Over the last decade, I developed a comprehensive translational pipeline to systematically evaluate phage bacteria interactions in various settings, including *in silico*, *in vitro*, *ex-vivo*, in experimental models of infection in rodents, and finally in humans.

RECENT PUBLICATIONS

- Prazak J, Valente L, Iten M, Federer L, Grandgirard D, Soto S, Resch G, Leib SL, Jakob SM, Haenggi M, Cameron DR, Que YA. Benefits of aerosolized phages for the treatment of pneumonia due to methicillin-resistant *Staphylococcus aureus* (MRSA): an experimental study in rats. *J Infect Dis*. doi: 10.1093/infdis/jiab112. Online ahead of print. 2021.
- Prazak J, Valente L, Iten M, Grandgirard D, Leib SL, Jakob SM, Haenggi M, Que YA, Cameron DR. Nebulized Bacteriophages for Prophylaxis of Experimental Ventilator-Associated Pneumonia Due to Methicillin-Resistant *Staphylococcus aureus*. *Crit Care Med*. 48(7):1042-1046. 2020.
- Prazak J, Iten M, Cameron DR, Save J, Grandgirard D, Resch G, Goeppfert C, Leib SL, Takala J, Jakob SM, Que YA*, Haenggi M*. Bacteriophages Improve Outcomes in Experimental *Staphylococcus aureus* Ventilator-associated Pneumonia. *Am J Respir Crit Care Med*. 200(9):1126-1133. 2019. *equal contribution.
- Jault P, Leclerc T, Jennes S, Pirnay JP, Que YA, Resch G, Rousseau AF, Ravat F, Carsin H, Le Floch R, Schaal JV, Soler C, Fevre C, Arnaud I, Bretaudeau L, Gabard J. Efficacy and tolerability of a cocktail of bacteriophages to treat burn wounds infected by *Pseudomonas aeruginosa* (PhagoBurn): a randomised, controlled, double-blind phase 1/2 trial. *Lancet Infect Dis*. 19:35-45. 2019.
- Oechslin F, Piccardi P, Mancini S, Gabard J, Moreillon P, Entenza JM, Resch G, Que YA. Synergistic interaction between phage therapy and antibiotics clears *Pseudomonas aeruginosa* infection in endocarditis and reduces virulence. *J Infect Dis*. 215(5):703-712. 2017



Yue Qin

PhD student, King's College London

Miss Yue Qin graduated from Guangxi Medical University (China) in 2017 with Bachelor of Pharmacy and MSc in Pharmacology. She investigated the pro-inflammatory and pro-fibrotic effects of carboxylated single-walled carbon nanotubes (c-SWNTs) administered intravenously to rats. After graduation, she worked as a technician at the Centre for Pharmaceutical Research, Pharmaceutical College, Guangxi Medical University. In 2018, she was awarded the King's-China Scholarship Council to pursue PhD studies to develop stable nucleic acid nanoparticles for combinatory nucleic acid delivery of solid tumours for cancer immunotherapy.

RECENT PUBLICATIONS

- Walters AA*, Santacana-Font G, Li J, Routabi N, Qin Y, Claes N, Bals S, Wang JT and Al-Jamal KT*. (2021) Nanoparticle Mediated in Situ Molecular Reprogramming of Immune Checkpoint Interactions for Cancer Immunotherapy. ACS Nano, doi.org/10.1021/acsnano.1c04456
- Abdel-Bar HM, Walters AA, Lim Y, Rouatbi N, Qin Y, Gheidari F, Han S, Osman R, Wang J, Al Jamal KT*. (2021) An "Eat me" Combinatory Nano-formulation for Systemic Immunotherapy of Solid Tumours. Theranostics. doi:10.7150/thno.56936; doi:10.7150/thno.56936.



Diana Fernandes de Sousa Rafael

Diana Rafael, PharmD, PhD obtained a Master in Pharmaceutical Sciences from the faculty of Pharmacy from the Lisbon University, Portugal in 2010 and in 2017, a PhD in Pharmaceutical Technology from the same University in collaboration with the Ludwig Maximilians University, Munich, the Bellvitge Biomedical Research Institute (IDIBELL), Barcelona and the Vall D'Hebron Research Institute (VHIR), Barcelona. Her work relies on the development of multifunctional delivery systems for personalized medicine namely: (i) design, development, and characterization (physic-chemical and biological) of nanotechnology-based drug delivery systems for the treatment of different diseases including cancer, HIV, hepatic diseases, infectious diseases, and regenerative medicine; (ii) gene therapy; (iii) study and characterization of the biological pathway involved in the development and progression of cancer. Her active participation in more than 15 highly competitive international projects and international collaborations have resulted in the authorship of more than 30 papers in high impact journals, and in 2 patents. Her board knowledge and expertise in biomedical sciences field and an integrative view of different stages of drug development are of utmost importance to the work developed at the Drug Delivery and Targeting Group (VHIR), where she has a position as researcher since 2013.



Benjamin Rengstl

Director Immunoreceptor Therapy & Medical Expert, BioNTech Cell & Gene Therapies, BioNTech SE

Dr. Benjamin Rengstl is a trained physician and biochemist. Since 2009, he is exploring the potential of the immune system to combat cancer and infectious diseases. Between 2014 and 2016, he received his approbation as well as a PhD and a MD degree from the University of Frankfurt, Germany. During his postdoctoral training, 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 cell candidate for treatment of solid tumors. To improve CAR-T cell therapy, his team pioneered an *in vivo* expansion concept based on a liposomally formulated RNA vaccine for systemic delivery of CAR antigen (CAR T-cell Amplifying RNA Vaccine, CARVac). A FIH clinical trial assessing BioNTech's novel CLDN6-specific CAR in combination with CARVac is currently ongoing. Furthermore, he is leading multiple programs to develop next generation cell- and RNA-based immunotherapies and further takes technology agnostic approaches to create new strategies for controlled modulation of the immune system.

RECENT PUBLICATION

- An RNA vaccine drives expansion and efficacy of claudin-CAR-T cells against solid tumors. Reinhard K, Rengstl B, Oehm P, Michel K, Billmeier A, Hayduk N, Klein O, Kuna K, Ouchan Y, Wöll S, Christ E, Weber D, Suchan M, Bukur T, Birtel M, Jahndel V, Mroz K, Hobohm K, Kranz L, Diken M, Kühlcke K, Türeci Ö, Sahin U. Science. 2020 Jan 24;367(6476):446-453. doi: 10.1126/science.aay5967. Epub 2020 Jan 2. PMID: 31896660
- Against solid tumors. Science. 2020 Jan 24;367(6476):446-453. doi: 10.1126/science.aay5967. Epub 2020 Jan 2.



Bernd Riebesehl

Executive Director, TRD TPPM, Novartis AG

At Novartis he is leading the Global Pharmaceutical Innovation Committee and the early technical development of several parenteral and topical 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 Speidel Experimenta AG, Base



Cristianne Rijcken

Founder and CSO of Cristal Therapeutics

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 ~ 45 scientific publications and co-inventor of all patents and patent applications of Cristal Therapeutics. Cristianne was 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).

Cristal Therapeutics is a highly innovative biotech company and applies three distinct and interconnected technologies together with biologic insight to improve the therapeutic profile of our partners' programs in development. Based on over 10 years of real world experience, Cristal's CliCr®, CriPec® and CriVac® technologies provide superior conjugation, enhance target specificity and engender highly selective immune responses, thereby increasing efficacy and reducing toxicity. The company aims to be the partner of choice for overcoming challenges and enabling the full potential of e.g. chemotherapeutic agents, immuno-oncology treatments and vaccines, amongst a broader range of therapeutics, tuned to modality and indication.

RECENT PUBLICATION

- IHC Miedema, GJC Zwezerijnen, M Huisman, E Doeleman, RHJ

Mathijssen, T Lammers, Q Hu, GAMS van Dongen, CJF Rijcken, DJ Vugts, CW Menke-van der Houven van Oordt, Imaging tumor accumulation of a docetaxel-entrapping polymeric nanoparticle: First steps towards patient stratification, submitted

- T Ojhaa, Q Hu, C Colombo, J Wit, M van Geijn, MJ van Steenberg, M Bagheri, H Königs-Werner, EM Buhl, Y Shi, R Bansal, WE Hennink, G Storm, CJF Rijcken, T Lammers, Lyophilization of clinical-stage core-crosslinked polymeric micelles, *Biotechnology Journal* 2020
- I Biancacci, Q Sun, D Möckel, F Gremse, S Rosenhain, F Kiessling, Q Hu, M Thewissen, G Storm, WE Hennink, Y Shi, CJF Rijcken, T Lammers, A Marios Sofias, Optical imaging of the whole-body to cellular biodistribution of clinical-stage PEG-b-pHPMA-based core-crosslinked polymer micelles, *JCR* 2020
- F Atrafi, H Dumez, RHJ Mathijssen, CW Menke - van der Houven van Oordt, J Costermans, CJF Rijcken, R Hanssen, FALM Eskens, P. Schöffski, A phase I dose-escalation and pharmacokinetic study of a nanoparticle with entrapped docetaxel (CPC634) in patients with advanced solid tumours, *Napoly Journal Controlled Release* 2020
- J Weterings, CJF Rijcken, H Veldhuis, T Meulemans, D Hadavi, M Timmers, M Honing, H Ippel, RMJ Liskamp, TMTHSI, a superior 7-membered ring alkyne containing reagent for strain-promoted azide-alkyne cycloaddition reactions, *Chemical Science* 2020



Paolo Rocco

Adjunct Professor

Paolo Rocco is a Researcher at the Department of Pharmaceutical Sciences, Università degli Studi Di Milano, with teaching assignments in the courses “Protein engineering, drug delivery and regulatory aspects” and “Regulatory aspects in toxicology”. He holds a PhD in Pharmaceutical

Chemistry, a Degree in Pharmacy and a Degree in Chemistry. His research mainly focuses on two domains of Regulatory Science: complex drugs, medical devices (MDs) and the structure and properties of biomembranes. Complex drugs encompass biotechnological medicinal products, advanced therapy medicinal products (ATMPs) and non-biological complex drugs (NBCDs). The research on MDs addresses drug-device combinations and MDs based on substances. Biomembranes are investigated from a theoretical point of view, mainly through Molecular Dynamics simulations.

RECENT PUBLICATIONS

- Minghetti, P., Rocco, P., Schellekens, H. The constrained prescription, interchangeability and substitution of biosimilars. *Nat Biotechnol.* 2015; 33(7), pp. 688-689
- Rocco, P., Cilurzo, F., Minghetti, P., Vistoli, G., Pedretti, A. Molecular Dynamics as a tool for in silico screening of skin permeability. *Eur J Pharm Sci.* 2017; 106, pp. 328-335
- Rocco, P., Selletti, S., Minghetti, P. Biosimilar switching and related medical liability. *J Forensic Leg Med.* 2018; 55, pp. 93-94
- Rocco, P., Eberini, I., Musazzi, U.M., Franzè, S., Minghetti, P. Glatiramer acetate: A complex drug beyond biologics. *Eur J Pharm Sci.* 2019; 133, pp. 8-14
- Minghetti P, Musazzi UM, Casiraghi A, Rocco P. Old active ingredients in new medicinal products: is the regulatory path coherent with patients' expectations? *Drug Discov Today.* 2020 Aug;25(8):1337-1347.



Barbara Rothen-Rutishauser

Co-Chair BioNanomaterials

Prof. Dr. Barbara Rothen-Rutishauser received her Ph.D. in 1996 in cell biology at the Swiss Federal Institute of Technology in Zurich (ETHZ). She worked as a postdoc and group leader at ETHZ and University of Bern, Switzerland. She is an expert in the field of cell-nanoparticle interactions, with a particular focus on 3D human lung tissue models. Since 2011, she is the chair in BioNanomaterials at the Adolphe Merkle Institute, University of Fribourg, Switzerland, the position is shared equally with Prof. Alke Fink. Prof. Rothen-Rutishauser has published more than 300 peer-reviewed papers, she is an associate editor of “Particle and Fibre Toxicology”, and currently president of the International Society of Aerosols in Medicine (ISAM).

RECENT PUBLICATION

- Milosevic AM, Haeni L, Ackermann Hirschi L, Vanni S, Campomanes-Ramos P, Rothen-Rutishauser B, Rodriguez-Lorenzo L, Petri-Fink A. The Choice of Nanoparticle Surface-Coupled Fluorescent Dyes Impacts Cellular Interaction. *ChemNanoMat* 8, e20210044 (2022).
- Sousa de Almeida M, Susnik E, Drasler B, Taladriz-Blanco P, Petri-Fink A, Rothen-Rutishauser B. Understanding nanoparticle endocytosis to improve targeting strategies in nanomedicine. *Chem Soc Rev* 50(9):5397-5434 (2021).
- Susnik E, Taladriz-Blanco P, Drasler B, Balog S, Petri-Fink A, Rothen-Rutishauser B. Increased Uptake of Silica Nanoparticles in Inflamed Macrophages but Not upon Co-Exposure to Micron-Sized Particles. *Cells* 9(9):2099 (2020).
- Bourquin J, Septiadi D, Vanhecke D, Balog S, Steinmetz L, Spuch-Calvar M, Taladriz-Blanco P, Petri-Fink A, Rothen-Rutishauser B. Reduction of Nanoparticle Load in Cells by Mitosis but Not Exocytosis. *ACS Nano* 13(7):7759-7770 (2019).
- Milosevic A, Bourquin J, Burnand D, Lemal P, Crippa F, Monnier CA, Rodriguez-Lorenzo L, Petri-Fink A, Rothen-Rutishauser B. Artificial lysosomal platform to study nanoparticle long-term stability. *Chimia* 73(1):55-58 (2019).



Carsten Rudolph

Carsten Rudolph, CEO and president, pharmacist by training, received his PhD from the Department of Pharmacy of the FU Berlin. Since 2003 he is group leader at the Dr. von Haunerschen Kinderspital of the Ludwig Maximilians University, Munich. He is lead inventor of the SNIM® RNA-Technology and co-inventor of numerous drug delivery patent applications. Carsten Rudolph received his post-doctoral lecture qualification at the Department of Pharmacy, FU Berlin in 2009. In 2005 Carsten Rudolph received the prestigious BioFuture Award of the BMBF which is the highest endowed young investigator award in Germany.



Anna Salvati

Associate Professor

Anna Salvati is an associate professor at the Department of Nanomedicine & Drug Targeting of the Groningen Research Institute of Pharmacy of the University of Groningen (the Netherlands). After graduating in biology and obtaining a PhD in physical chemistry from Florence University (Italy, 2007), she worked for 7 years with Prof. Kenneth Dawson in the Centre of Bionano Interactions, University College Dublin (Ireland). In 2014 she was awarded a Rosalind Franklin Fellowship to establish her group in Groningen University. Since then, she has been awarded several grants, including among others an ERC Starting Grant (NanoPaths) where she has investigated the mechanisms of nanoparticle uptake into cells. Her research is focused on characterizing how nano-sized materials interact with and are processed by cells in order to understand how to tune their design for nanomedicine applications. Using methods in cell biology, genetic screening and proteomics, she is studying the early interactions of nanoparticles with cell receptors, the subsequent mechanisms of uptake and intracellular trafficking by cells. This includes also the modifications nanoparticles encounter once applied in biological fluids, such as in blood, where a biomolecule corona adsorbs on their surface. Additionally, she is developing novel methods to study these mechanisms and exploring the use of more complex *in vitro* systems for addressing these questions.



Kirsten Sandvig

Professor

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. The Sandvig group has for many years been interested in 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, and from an EEA grant, a collaboration between Norway and Romania. 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, Nor-

way. Research visits abroad at University of Michigan and at the biological laboratories, Harvard Cambridge, Mass. USA.

SCIENTIFIC ACTIVITY: Sandvig has published 345 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 78.

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.



Ronit Satchi-Fainaro

Director, Cancer Biology Research Center

Prof. Ronit Satchi-Fainaro (Ph.D.) is a Full Professor at Tel Aviv University, where she is head of the Cancer Research & Nanomedicine Laboratory, Director of Tel Aviv University Cancer Biology Research Center, Director of the TAU Kahn 3D BioPrinting Initiative, and holds the Lion Chair in

Nanosciences and Nanotechnologies. She serves on the BoD of Teva Pharmaceutical Industries Ltd. and the BoG of TAU, and is a member of the SAB of several Innovation Centers, Universities, Hospitals, Fellowships Committees, VCs, and editorial boards of scientific journals.

Her multidisciplinary research laboratory focuses on basic research elucidating the mechanisms underlying the switch from cancer dormancy leading to the discovery of new molecular targets to interrupt tumor-host interactions. Her approach is followed by the design of highly selective targeting molecules integrating biology, chemistry, medicine, data science, engineering and nanotechnology to selectively guide drugs into pathological sites. Throughout, she has maintained an interest in understanding the biological rationale for the design of nanomedicines suitable for transfer into clinical testing. She has published more than 130 manuscripts, 13 book chapters, edited two books, is named inventor on 60 patents, some of which were licensed to Pharmaceutical and Biotech companies. She founded three spin-off companies and is actively engaged in translational research with several industry partners and in science outreach.

RECENT PUBLICATIONS

- Gibori H, et al., (Satchi-Fainaro R), Amphiphilic nanocarrier-induced modulation of PLK1 and miR-34a leads to improved therapeutic response in pancreatic cancer, *Nature Communications*, 9(1):16 (2018).
- Yeini E, et al., (Satchi-Fainaro R), P-selectin inhibition alters microglia immunophenotype and blocks glioblastoma progression, *Nature Communications*, 12(1):1912 (2021).
- Florindo, et al. (Satchi-Fainaro), Immune-mediated approaches against COVID-19, *Nature Nanotechnology*, 15(8):630-645 (2020)
- Neufeld, et al. (Satchi-Fainaro), Microengineered perfusable 3D-bioprinted glioblastoma model for *in vivo* mimicry of tumor microenvironment, 7(34):eabi9119, *Science Advances* (2021).
- Coniot J*, Scomparin A*, et al. (Satchi-Fainaro R*, Florindo H*), Immunization with mannoseylated nanovaccines and inhibition of the immune-suppressing microenvironment sensitizes melanoma to immune checkpoint modulators, *Nature Nanotechnology*, 14(9):891-901 (2019) *Corresponding authors.



Raymond Schiffelers

Professor of nanomedicine
University Medical Center Utrecht
Utrecht, The Netherlands
Group website:
Nanomedicinelab.eu

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 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. Since 2021 he also works part-time for Nanocell Therapeutics as VP Preclinical R&D and is president of the ETPN.

(Chair 2019-2021) and the EARTO working group "Emerging Technologies for Healthcare" (2016-present). Dr. Schmid is author/co-author of 50 scientific publications, 25 patents and patent applications and 95 oral/poster presentations and 16 webinar, mass media and popular science publications.

RECENT PUBLICATIONS

- M. Germain, F. Caputo, S. Metcalfe, G. Tosi, K. Spring, AKO Åslund, A. Pottier, R. Schiffelers, A. Ceccaldi & R. Schmid, Journal of Controlled Release, 326, 164-171 (2020). "Delivering the power of nanomedicine to patients today."
- JP. Martins, J. das Neves, María. de la Fuente, C. Celia, H. Florindo, N. Günday-Türeli, A. Papat, JL. Santos, F. Sousa, R. Schmid, J. Wolfram, B. Sarmento & HA. Santos, Drug Delivery and Translational Research, 10, 726-729 (2020); <https://doi.org/10.1007/s13346-020-00743-2>. "The solid progress of nanomedicine."
- A. Prina-Mello, R. Schmid, P. Wick, F. Caputo, P. Boisseau, R.M. Crist & S. McNeil, Correspondence in Nature Nanotechnology, 14, 626-635 (2019). "On the issue of transparency and reproducibility in nanomedicine."
- S. Snipstad, E. Sulheim, C. de Lange Davies, C. Moonen, G. Storm, F. Kiessling, R. Schmid & T. Lammers, Expert Opinion on Drug Delivery, <https://doi.org/10.1080/17425247.2018.1547279> (2018). "Sonopermeation to Improve Drug Delivery to Tumors: From Fundamental Understanding to Clinical Translation."
- E. Sulheim, T.G. Iversen, V.T. Nakstad, G. Klinkenberg, H. Sletta, R. Schmid, A.R. Hatletveit, A.M. Wågbø, A. Sundan, T. Skotland, K. Sandvig & Y. Mørch, Int. J. Mol. Sci.18 (11), 2454 (2017); doi: 10.3390/ijms18112454. "Cytotoxicity of Poly(Alkyl Cyanoacrylate) Nanoparticles."



Ruth Schmid

Vice President Marketing

Dr. Schmid is Vice President Marketing at SINTEF Industry in Trondheim, Norway with special responsibility for the area of medical technology, including nanomedicine. SINTEF is one of Europe's largest independent non-profit research institute.

Her present research activities include the preparation and characterisation of micro- and nanoparticles by various technologies and from a wide variety of materials (including biodegradable polymers and hybrid materials), as well as the surface modification of polymers and polymer particles by wet-chemistry, to introduce tailor-made properties. Lately, focus has been on the encapsulation and immobilisation of liquids and solids from emulsions, for protection and controlled release. Examples of encapsulated substances are liquid crystals, magnetic iron oxides, insect repellents and fragrances. Another focus has been on coating of biomaterials by self-assembling methods and covalent attachment with biocompatible, biomimetic and functional coatings, e.g. for introduction of antimicrobial properties, for increased osseointegration or for immobilisation of biological molecules. There is special focus on applied research and product orientated solutions. Fields of special interest are the emerging fields of nanomedicine, targeted drug delivery and release, nanotechnology-based diagnostics and regenerative medicine, with special focus on applications based on particle technology and surface modification. Application of encapsulation technologies and controlled release in various industrial segments, e.g. medicine, animal health, cosmetics, house-hold and body care products, food and beverages, agriculture, etc. is another field of interest.

Dr. Schmid is a Swiss citizen living in Norway since 1979. She gained her Diploma (1975) and PhD (1979) in Natural Sciences (physical organic chemistry) at ETH Zürich, Switzerland. She is a member of ACS, CRS (member of the board of directors (2009-2018), President (2016-2017), the European Technology Platform in Nanomedicine



Avi Schroeder

Avi Schroeder is a tenured 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 above 50 research papers inventor of 19 patents and co-founder of multiple startup companies based on these discoveries.

Schroeder is a member of Israel Young National Academy of Sciences, Israel's National Council for Civilian Research and Development, and the President of the Israel Institute of Chemical Engineers.



Stephanie Schubert

Dr. Stephanie Schubert studied chemistry at the Friedrich-Schiller-University Jena, Germany, and received her diploma in 2005. The PhD studies at the FSU Jena with Prof. Thomas Heinze were complemented by research stays at Virginia Tech, USA, and Sophia Antipolis, France. Stephanie joined the group of Prof. Jean M.J. Fréchet for a postdoc after her PhD defense in 2008.

Since 2010, she was working in the Pharmaceutical Technology department at the FSU Jena with Prof. Dagmar Fischer and in the Jena

Center for Soft Matter. Her main research interests are the synthesis of natural and synthetic polymers and their formulation into functional nanoparticles for controlled drug delivery applications.

RECENT PUBLICATIONS

- C. Kretzer, B. Shkodra, P. Klemm, P. M. Jordan, D. Schröder, G. Cinar, A. Vollrath, S. Schubert, I. Nischang, S. Hoepfner, S. Stumpf, E. Banoglu, F. Gladigau, R. Bilancia, A. Rossi, C. Eggeling, U. Neugebauer, U. S. Schubert, O. Werz, "Ethoxy Acetalated Dextran-based Nanocarriers Accomplish Efficient Inhibition of Leukotriene Formation by a Novel FLAP Antagonist in Human Leukocytes and Blood", *Cell. Mol. Life Sci.* 2022, 79, 40.
- P. Klemm, M. Behnke, J. I. Solomon, C. Bonduelle, S. Lecommandoux, A. Träger, S. Schubert, "Self-assembled PEGylated Amphiphilic Polypeptides for Gene Delivery", *J. Mater. Chem. B* 2021, 9, 8224.
- P. Klemm, S. Huschke, M. Rodewald, N. Ehteshamzad, M. Behnke, X. Wang, G. Cinar, I. Nischang, S. Hoepfner, C. Weber, A. T. Press, C. Hoepfner, V. Deckert, M. Schmitt, J. Popp, M. Bauer, S. Schubert, "Characterization of a Library of Vitamin A Functionalized Polymethacrylate-Based Nanoparticles for siRNA Delivery", *Polym. Chem.* 2021, 12, 911–925.
- S. Rahimi, S. Stumpf, O. Grimm, F. H. Schacher, U. S. Schubert, S. Schubert, "Dual Photo and pH-Responsive Spirooxazine-Functionalized Dextran Nanoparticles", *Biomacromolecules* 2020, 21, 3620–3630.
- B. Shkodra-Pula, C. Kretzer, P. M. Jordan, P. Klemm, A. Koeberle, D. Pretzel, E. Banoglu, S. Lorkowski, M. Wallert, S. Höppner, S. Stumpf, A. Vollrath, S. Schubert, O. Werz, U. S. Schubert, "Encapsulation of the Dual FLAP/mPEGS-1 Inhibitor BRP-187 into Acetalated Dextran and PLGA Nanoparticles Improves Its Cellular Bioactivity", *J. Nanobiotechnology* 2020, 18, 73.



Ulrich Schubert

Professor, Chair of Organic and Macromolecular Chemistry

Ulrich S. Schubert performed his Ph.D. studies at the Universities of Bayreuth/Germany and South Florida/USA. After a post-doctoral training position with Prof. Lehn at the University of Strasbourg/France, he moved to the TU Munich/Germany and obtained his Habilitation in 1999. In 1999–2000 he was professor at the University of Munich/Germany, and during 2000 and 2007 full professor at the TU Eindhoven/The Netherlands. Since 2007, he has been a full professor for organic and macromolecular chemistry at the Friedrich Schiller University Jena/Germany. He is the founding director of the Center for Energy and Environmental Chemistry Jena (CEEC Jena) and the Jena Center for Soft Matter (JCSM) as well as the coordinator of the EU ETN POLYSTORAGE, of the DFG priority program "Polymer-Based Batteries" (SPP 2248) and spokesman of the DFG collaborative research center 1278 "PolyTarget".

Ulrich S. Schubert is co-author of 1.100 scientific publications. They received 59.000 citations (Google Scholar 76.000); his h-index is 108 (Google Scholar 123). Ulrich S. Schubert is listed as "highly cited researcher" since 2014. In addition, he is an external scientific member of the Max Planck Society (MPG/Germany), elected member of acatech (National Academy of Science and Engineering/Germany), elected fellow of the National Academy of Inventors/USA and fellow of the Royal Society of Chemistry/UK. Ulrich S. Schubert was awarded with the Federal Cross of Merit/Germany and named University Professor of the Year 2019/Germany

Ulrich S. Schubert is co-author of 1.100 scientific publications. They received 59.000 citations (Google Scholar 76.000); his h-index is 108 (Google Scholar 123). Ulrich S. Schubert is listed as "highly cited researcher" since 2014. In addition, he is an external scientific member of the Max Planck Society (MPG/Germany), elected member of acatech (National Academy of Science and Engineering/Germany), elected fellow of the National Academy of Inventors/USA and fellow of the Royal Society of Chemistry/UK. Ulrich S. Schubert was awarded with the Federal Cross of Merit/Germany and named University Professor of the Year 2019/Germany

RECENT PUBLICATIONS

- D. Bandelli, I. Muljajew, K. Scheuer, J. B. Max, C. Weber, F. H. Schacher, K. D. Jandt, U. S. Schubert, *Macromolecules* 2020, 53, 5208–5217.

- Muljajew, A. Erlebach, C. Weber, J. R. Buchheim, M. Sierka, U. S. Schubert, *Polym. Chem.* 2020, 11, 112–124.
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- M. Dirauf, D. Bandelli, C. Weber, H. Görls, M. Gottschaldt, U. S. Schubert, *Macromol. Rapid. Commun.* 2018, 39, 1800433.
- M. Dirauf, A. Erlebach, C. Weber, S. Hoepfner, J. R. Buchheim, M. Sierka, U. S. Schubert, *Macromolecules* 2020, 53, 3580–3590.



Simo Schwartz

Senior Scientist. CIBBIM-Nanomedicine. Drug Delivery and Targeting

Dr Simó Schwartz Jr (1967th, Barcelona). Currently appointed as President of the European Society of Nanomedicine and Executive Board member of the International Society of Nanomedicine. He acts as Senior Scientific Advisor and former director and

board member of CIBBIM-Nanomedicine at the Vall d'Hebron Research Institute (VHIR). A center fostering research on new biomedical advanced therapies and nanotechnology-based applications for clinical practice. In particular, advanced cell therapies, new biomaterials and drug delivery systems in the areas of oncology and rare diseases. Dr Schwartz is also former General Director of the Banc de Sang i Teixits (Blood and Tissue Bank) of Catalonia (www.bancsang.net). A leading centre in the field of immunodiagnostics and advanced therapy development. Former Director Assistant of Translational Research at VHIR, and member of the Science Advisory Board of the European Nanomedicine Characterization Laboratory (EU-NCL). He holds 17 patents, most transferred to leading companies of the biotech and pharma sectors and coauthors more than 100 papers in high impact factor journals. Dr Schwartz Jr has been coordinator and collaborator of several research projects directly related with the obtention and validation of therapeutic drug delivery systems. Among them are international and EU projects involving SMEs and big biotech and pharma industry. Dr Schwartz Jr is also a member of the Nanomedicine Spanish Platform (NanomedSpain) and of the "European Platform for Nanomedicine". Dr Schwartz Jr was also member of the Steering Committee and Nanomedicine Coordinador of the "CIBER de Bioingeniería, Biomateriales y Nanomedicina" (CIBER-BBN) of the Spanish Health Institute CarlosIII (ISCIII) which gathers 45 groups of national excellence in the field of nanotechnology and nanomedicine at the national level. Later appointed as Deputy Director and technology transfer coordinator. Dr Schwartz Jr was Co-founder and Science Advisor of ARGON Pharma Ltd (2008–2015), a Spin-Off company at the Barcelona Science Park with the mission to develop innovative therapies for unmet medical needs in oncology, and of BSure Medical Ltd. (created in 2020), a Spin-Off company from VHIR that commercializes tests to prevent severe immune adverse reactions to implanted materials. Dr Schwartz Jr is also member of the editorial Board of Precision Nanomedicine, *J. Nanotechnology* and of *J. of Nanotheranostics*. Former editorial Board member of *Eur. J. Nanomedicine and Nanomedicine-NBM* and Science Advisor and Consultant of SOM BIOTECH, U-Cell Therapeutics and CELGENE. Former member of the Advisory Board of The Lundbeck Foundation Center of Excellence AnvoCAN (Nanomedicine Research Center for Cancer Stem Cell Targeting Therapeutics), Southern Denmark Univ.

Keywords: Nanomedicine, Clinical nanomedicine, drug delivery systems, preclinical studies, oncology, rare diseases.

Scientific interests: Research on new biomedical advanced therapies and nanotechnology-based applications for clinical practice. In particular, advanced cell therapies, new biomaterials and drug delivery systems, image based diagnostic systems and preclinical validation of therapeutic conjugates, mainly in the areas of oncology and rare diseases.

RECENT PUBLICATIONS

- Fernández Y, Movellan J, Foradada L, Giménez V, García-Aranda N, Mancilla S, Armiñán A, Burgos SE, Hyldbakk A, Bogdanska A, Gobbo OL, Prina-Mello A, Ponti J, Calzolari L, Zagorodko O, Gallon E, Niño-Pariente A, Paul A, Schwartz S Jr, Abasolo I, Vicent MJ. In Vivo Antitumor and Antimetastatic Efficacy of a Polyacetal-Based Paclitaxel Conjugate for Prostate Cancer Therapy. *Adv Healthc Mater.* 2021 Oct 27:e2101544. doi: 10.1002/adhm.202101544.
- Seras-Franzoso J, Díaz-Riascos ZV, Corchero JL, González P, García-Aranda N, Mandaña M, Riera R, Boullosa A, Mancilla S, Grayston A, Moltó-Abad M, García-Fruitós E, Mendoza R, Pintos-Morell G, Albertazzi L, Rosell A, Casas J, Villaverde A, Schwartz S Jr*, Abasolo I*. Improved Enzymatic Activity and In Vivo Efficacy of Recombinant Proteins Using Extracellular Vesicles in Lysosomal Storage Disorder (*co-corresponding authors). *Journal of Extracellular Vesicles*, 2021 Mar;10(5):e12058. Epub 2021 Mar 12.
- Carreño G, Pereira A, Ávila-Salas F, Marican A, Andrade F, Roca-Melendres MM, Valdés O, Vijayakumar S, Schwartz S Jr, Abasolo I, Rafael D, Durán-Lara EF. Development of “on-demand” thermo-responsive hydrogels for anti-cancer drugs sustained release: Rational design, in silico prediction and *in vitro* validation in colon cancer models. *Mater Sci Eng C Mater Biol Appl.* 2021 Dec;131:112483. doi: 10.1016/j.msec.2021.112483. Epub 2021 Oct 13.
- Abu-Serie MM, Andrade F, Cámara-Sánchez P, Seras-Franzoso J, Rafael D, Díaz-Riascos V, Gener P, Abasolo I, Schwartz S Jr. Pluronic F127-micelles improve the stability and enhance the anti-cancer stem cell efficacy of citral in breast cancer. *Nanomedicine*, 2021 Jul;16(17):1471-1485
- Andrade F*, Rafael D, Vilar-Hernández M, Montero S, Martínez-Trucharte F, Seras-Franzoso J, Díaz-Riascos V, Boullosa A, García-Aranda N, Cámara-Sánchez P, Arango D, Nestor M, Abasolo I, Sarmiento B*, Schwartz S Jr*. Polymeric Micelles targeted against CD44v6 receptor increase Niclosamide efficacy against Colorectal Cancer Stem Cells and reduce Circulating Tumor Cells *in vivo*. (*co-corresponding authors). *Journal Controlled Release*, 2021 Mar 10;331:198-212.



Kurt Sedo

Vice President Operations, PharmaCircle LLC USA

Kurt is Vice President of Operations at PharmaCircle, a leading provider of authoritative information, global insight, and expert analysis on the pharmaceutical, biotech, drug delivery technology and device, and animal health industries. Kurt received his B.S. in Chemistry and Mathematics from the University of Wisconsin Stevens Point in 1992. Prior to joining PharmaCircle in 2003, Kurt held various positions within Pharmaceutical Sciences and Analytical Development at Searle/Pharmacia.

RECENT PUBLICATIONS

Most important publications in the field of nanoparticle research:

- 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.
- Skotland T and Sandvig K: Transport of nanoparticles across the endothelial cell layer. *Nano Today* 36 (2021) 101029.



Alina Sigaeva

I was born in 1993 in Moscow, Russia. After finishing school, I have entered Lomonosov Moscow State University, the Faculty of Biology. During my undergraduate studies, I specialized in cell biology and histology. Back then, my research was focused on the effects of the blue LED illumination on the physiology of the eye – more specifically,

of the ocular blood vessels. After obtaining my Master's degree, I have moved to the Netherlands for my PhD. I have been living and working here since 2017.

During my PhD, I have been developing a new approach to detecting free radicals in live cells. The idea of the project comes from quantum physics, where the optical properties of certain defects in nanodiamonds are used to sense external magnetic fields with nanometer spatial resolution. My task, as a cell biologist, has been to implement and validate the existing sensing protocols in cells – bring diamond nanoparticles inside the cells, characterize their distribution, target them to the region of interest. I have developed protocols improving the uptake and intracellular targeting, designed and performed the proof-of-concept measurements in several models, including primary cells and live organ slices. We are currently working in close collaboration with the medical professionals to bring our method into the (pre-)clinical research.

Apart from the research activities, I have been involved in teaching and mentoring of the undergraduate students, from giving lectures and tutorials on image processing to supervising practical courses and a Master's thesis. I have also been the head of the organizing committee of the Symposium for Biology Students in Europe (SymbioSE-2020) – an annual event made for students by students, which aims to connect the international community of future biologists from all fields of life sciences.

I am passionate about interdisciplinary research and collaboration. Thus, I have been striving to bring together the worlds of quantum physics, nanotechnology and biology to improve the quality of diagnosis and treatment of patients.

RECENT PUBLICATIONS

- Sharmin R., Hamoh T., Sigaeva A., Mzyk A., Damle V. G., Morita A., Vedelaar T., Schirhagl R. Fluorescence nano-diamonds for detecting free radical generation in real time during shear stress in human umbilical vein endothelial cells // *ACS Sensors*, 2021, Nov 19
- Sigaeva A., Perona Martinez F., Nusantara A. C., Mougios N., Chippaux M., Schirhagl R. Diamond based nanoMRI for quantum sensing of free radical production in cells // 2021, under revision in *Small*
- Zhang Y., Sharmin R., Sigaeva A., Klijn C. W. M., Mzyk A., Schirhagl R. Not all cells are created equal – endosomal escape in fluorescent nanodiamonds in different cells // *Nanoscale*, 2021, 13, 13294-13300
- Sigaeva A., Morita A., Hemelaar S. R., Schirhagl R. Nanodiamond uptake in colon cancer cells: the influence of direction and trypsin-EDTA treatment // *Nanoscale*, 2019, 11, 17357-17367.
- Sigaeva A., Ong Y., Damle V. G., Morita A., van der Laan K. J., Schirhagl R. Optical Detection of Intracellular Quantities Using Nanoscale Technologies // *ACS Accounts of Chemical Research*, 2019, 52, 7, 1739–1749.



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 market (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 approx. 140 publications (H-index: 40) and is used as referee for many journals in the field of bioanalysis, metabolism, biochemistry, nanomedicine, extracellular vesicles 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.

RECENT PUBLICATIONS

Most important publications in the field of nanoparticle research:

- 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.
- Skotland T and Sandvig K: Transport of nanoparticles across the endothelial cell layer. *Nano Today* 36 (2021) 101029.



Gert Storm

Professor Pharmaceutics

Gert Storm is a (bio)pharmaceutical scientist and professor (Nanomedicine/Targeted Drug Delivery) at the Department of Pharmaceutics of the Utrecht University (UU), The Netherlands. He keeps also professor positions (Targeted Therapeutics) at

the Department Advanced Organ Bioengineering and Therapeutics of the University of Twente (UT), Enschede and the Department of Surgery at the National University of Singapore (NUS). He is the (co-)author of > 600 publications (H-index 112, Google Scholar, January 2022), and since 2014 every year in the Highly Cited Researcher lists of Clarivate Analytics (Researcher ID: O-8696-2016).



Ralf Sudbrak

Senior Scientific Programme Officer

Dr. Ralf Sudbrak (male) is Senior Scientific Programme Officer at the Global AMR R&D Hub in Berlin. The main goal of the HUB is to promote high-level coordination among governments and upstream funders from different world regions, in order to better align national and international R&D efforts

in the fight against AMR. The central deliverable of the GLOBAL AMR R&D HUB will be a close to real-time Dynamic Dashboard providing information and analysis at a high level on current initiatives, funding flows and activities in the field of AMR R&D. He is a biologist by training and specialist in genetic and genomics as well as personalised medicine. From 2014 to 2018 he was involved as an independent researcher in tenders of the European Commission about the assessment of European Added Value and the economic impact of the EU framework programmes FP7 and Horizon2020 and the evaluation of personalised medicine and medical technology projects of the FP7 framework programme. From 2013 to 2014 he worked in a young Berlin-based SME that has been specialised in the development of novel processes and technologies in personalised medicine in cancer patients. Until 2013 he used to work at the Max Planck Institute for Molecular Genetics in Berlin, Germany for over 15 years. He was involved in the Human Genome project and later was project leader in the 1000 Genome project. He participated as principal investigator, workpackage leader and coordinator in several EU and national funded projects. He was head of the coordination and communication office of the EC FET (Future & Emerging Technologies) Flagship IT Future of Medicine pilot project. He is used to coordinate and organise large research consortia. He published his research findings in over 50 publications in peer-reviewed journals including, *Nature*, *Nature Genetics* and *Science*. He worked at a Postdoctoral research fellow in the laboratory of Anthony Monaco at the Wellcome Trust Centre for Human Genetics of the University of Oxford.

RECENT PUBLICATIONS

- Zheng-Bradley X, Streeter I, Fairley S, Richardson D, Clarke L, Flicek P; 1000 Genomes Project Consortium (2017) Alignment of 1000 Genomes Project Reads to Reference Assembly GRCh38. *Gigascience* doi: 10.1093/gigascience/gix038.
- Ferreira PG, Oti M, Barann M, Wieland T, Ezquina S, Friedländer MR, Rivas MA, Esteve-Codina A; GEUVADIS Consortium., Rosentstiel P, Strom TM, Lappalainen T, Guigó R, Sammeth M. (2016) Sequence variation between 462 human individuals fine-tunes functional sites of RNA processing. *Sci Rep* 12;6:32406. doi: 10.1038/srep32406.
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 - 1000 Genomes Project Consortium, Auton A, Brooks LD, Durbin RM, Garrison EP, Kang HM, Korbel JO, Marchini JL, McCarthy S, McVean GA, Abecasis GR (2015). A global reference for human genetic variation *Nature* 526: 68-74.

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 - Critical analysis of antibacterial agents in clinical development. Theuretzbacher U, Bush K, Harbarth S, Paul M, Rex JH, Tacconelli E, Thwaites GE. *Nat Rev Microbiol.* 2020 May;18(5):286-298. doi: 10.1038/s41579-020-0340-0.
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 - Non-traditional Antibacterial Therapeutic Options and Challenges. Theuretzbacher U, Piddock LJV. *Cell Host Microbe.* 2019 Jul 10;26(1):61-72. doi: 10.1016/j.chom.2019.06.004.



Janos Szebeni

Janos Szebeni, M.D., Ph.D., D.Sc., Med. Habil., immunologist, professor at Semmelweis and Miskolc universities. He is also founder and CEO of a contract research company, SeroScience Ltd. During his 44-years professional career he lived and worked some 22 years outside of Hungary, mostly in the United States, but also

in Switzerland, New Zealand and England for shorter periods. In the USA he worked at prestigious institutions, such as the National Institutes of Health, National Cancer Institute, Harvard University, Walter Reed Army Institute of Research. His studies on various themes in hematology, membrane biology and immunology has resulted in over 260 publications, 2 textbooks, 2 granted patents, and many other types of communications, referred to in >11,000 citations, H index: 54. His original work at the Walter Reed Army Institute of Research in the late 1990s led to the "CARPA" concept, i.e., that complement activation underlies numerous drug-induced pseudo-allergic infusion reactions.



Ursula Theuretzbacher

Independent antibiotic expert

Ursula Theuretzbacher is an independent expert for antibacterial drug research, discovery/development strategies and policies based on clinical and public health needs. Her broad area of expertise includes public and philanthropic funding strategies for antibacterial drug R&D and initiatives

to recover the global pipeline, evaluation and comparative assessment of antibacterial drugs, and optimization of antibacterial therapy concepts. She was member of the coordinating group of the WHO project Priority Pathogen List for R&D and leading scientist for the Clinical and Preclinical Pipeline analysis, and development of Target Product Profiles at WHO. Previously, she collaborated in several large EU funded projects focused on antibacterial drug R&D and reviving of old antibiotics. Additionally, she served as President of the International Society for Anti-Infective Pharmacology (ISAP) and as Executive Committee member of the International Society for Infectious Diseases (ISID). She holds a PhD in Microbiology from the University of Vienna and the University of Innsbruck in Austria and lectured at the University of Vienna for 10 years.

RECENT PUBLICATIONS

- Role of new antibiotics for KPC-producing *Klebsiella pneumoniae*. Theuretzbacher U, Carrara E, Conti M, Tacconelli E. *J Antimicrob*



Florian M. Thieringer

Sen. Consultant, Cranio-Maxillofacial Surgery, University Hospital Basel
Head of Medical Additive Manufacturing Research Group DBE/DKF (Swiss MAM)
Co-Director 3D Print Lab, University Hospital Basel, and Core Facility 3D Print Lab, Unibas
PD Dr. Florian M. Thieringer, MHBA is an internationally trained, board certified oral-

and cranio-maxillo-facial surgeon and a medical 3D expert, with focus on tumor-, trauma-, reconstructive- and orthognathic surgery. He is located at the University Hospital Basel and University of Basel, Switzerland. FMT is currently senior surgeon and Ass. Professor for OCMF Surgery at the University Hospital Basel (USB). He is the Head of the Medical Additive Manufacturing Research Group (Swiss MAM) at the University of Basel's Department of Biomedical Engineering (UNIBAS DBE). He is an internationally recognized expert for computer assisted surgery (CAS) and medical additive manufacturing, extensively exploring, and promoting the integration of virtual surgical planning, 3D printing and other innovative (regenerative) technologies at the point-of-care.

Since 2016 he is Co-Director of the multidisciplinary 3D Print Lab at the University Hospital Basel. Since 2020 he is Co-Principal Investigator of the innovative MIRACLE II project (Minimally Invasive Robot-Assisted Computer-guided Laserosteotomy). The aim of this UNIBAS flagship research project is to develop a robotic endoscope for non-contact bone surgery using laser light. Precisely fitting patient-specific implants will be designed in AR/VR and manufactured by intra- and extracorporeal 3D printing. Since 2021 Thieringer is Steering Committee and Executive Board Member of the USB's Innovation Focus "Regenerative Surgery".



Donald A. Tomalia

CEO/Founder
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Dr. Tomalia is the CEO/Founder of NanoSynthons LLC and the National Dendrimer & Nanotechnology Center, Adjunct Professor (Chemistry) University of Pennsylvania, PA and Affiliate Professor (Physics) Virginia Commonwealth University, VA. He received his B.A. in Chemistry from the University of Michigan and Ph.D. in Physical-Organic Chemistry from Michigan State University while working at The Dow Chemical Company. He has founded three dendrimer-based nanotechnology companies; namely, NanoSyn-

thons LLC (2010-present), Dendritic Nanotechnologies, Inc. (2001-2006) (acquired by Starpharma, Melbourne AU) and Dendritech, Inc. (1992-98) (acquired by Dow Chemical, Midland MI). Other positions currently held by Tomalia include: Advisory Board CLINAM, European Foundation for Clinical Nanomedicine; Faculty Member, Faculty Opinions, Biology; Associate Editor, Journal of Nanoparticle Research (Nature/Springer); Editorial Advisory Board, Nanomedicine (Elsevier), Biomolecules (MPDI) and Biomedicines (MDPI).

Tomalia is the pioneering scientist/inventor credited with the discovery of living cationic polymerizations of 2-oxazolines leading to poly(oxazolines) (Industrial Research-100 Awards in 1978 & 1986) and the first synthesis of dendrimers. His 1979 discovery of poly(amidoamine) (PAMAM) dendrimers (dendritic polymer architecture) led to a third R&D-100 Award in 1991 and the Leonardo da Vinci Award (Paris, France) in 1996. He received the International Award of The Society of Polymer Science Japan (SPSJ) (2003) which recognized his discovery of the fourth major macromolecular architectural class; namely, dendritic polymers. Tomalia has been granted >155 patents, authored over 275 peer-reviewed publications with more than >50,700 citations and an h-index=101 (Google Scholar, 1-12-22), he was inducted into the Thomson Reuters Hall of Citation Laureates in Chemistry (2011) (i.e., top 40 most highly cited scientists in the field of chemistry) and inducted as a Fellow (2016) in the AAAS (American Association for Advancement of Science).

RECENT PUBLICATIONS

- D.A. Tomalia, W.A. Goddard, Starburst Dendrimers: Molecular Level Control of Size, Shape, Surface Chemistry, Topology and Flexibility from Atoms to Macroscopic Matter, *Angew. Chem. Int. Ed. Engl.*, 1990, 29(2), 138.
- D.A. Tomalia, S.N. Khanna, A Systematic Framework and Nanoperiodic Concept for Unifying Nanoscience: Hard/Soft Nanoelements, Superatoms, Meta-Atoms, Periodic Property Patterns, and Predictive Mendeleev-like Nanoperiodic Tables, *Chem. Rev.*, 2016, 16, 2705
- D.A. Tomalia, et al, Non-traditional Intrinsic Luminescence: Inexplicable Blue Fluorescence Observed for Dendrimers, Macromolecules and Small Molecular Structures Lacking Traditional/Conventional Luminophores, *Prog. Polym. Sci.*, 2019, 90, 35.
- D.A. Tomalia, L.S. Nixon, The Role of Branch Cell Symmetry and Other Critical Nanoscale Design Parameters in Determining Dendrimer Encapsulation Properties, *Biomolecules*, 2020, 10, 642

2000 he went back to The Hague, Haga Hospital. From 2013 he is head of the laboratory of the Department of Clinical Pharmacy and Pharmacology in the University Medical Center Groningen and is full professor at the University of Groningen at the Faculty of Medicine and the Faculty of Pharmacy. He supervises 10 PhD students and (co)authored >400 papers and book chapters, see <https://www.rug.nl/staff/d.j.touw/research>.

RECENT PUBLICATIONS

- Visser M, Olgers TJ, Postema HE, van den Boomgaard MA, Bouma HR, Touw DJ. Met de handen in het haar [Clinical applications of hair analysis in the Netherlands]. *Ned Tijdschr Geneesk.* 2022 Jan 6;165:D5625. Dutch. PMID: 35129898.
- Sotomayor CG, Giubergia F, Groothof D, Ferreccio C, Nolte IM, Navis GJ, Gomes-Neto AW, Kremer D, Knobbe TJ, Eisenga MF, Rodrigo R, Touw DJ, Bakker SJL; TransplantLines Investigators. Plasma Lead Concentration and Risk of Late Kidney Allograft Failure: Findings From the TransplantLines Biobank and Cohort Studies. *Am J Kidney Dis.* 2021 Dec 3:S0272-6386(21)01014-3. doi: 10.1053/ajkd.2021.10.009. Epub ahead of print. PMID: 34871698.
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- Zijp TR, Izzah Z, Åberg C, Gan CT, Bakker SJL, Touw DJ, van Boven JFM. Clinical Value of Emerging Bioanalytical Methods for Drug Measurements: A Scoping Review of Their Applicability for Medication Adherence and Therapeutic Drug Monitoring. *Drugs.* 2021 Nov;81(17):1983-2002. doi: 10.1007/s40265-021-01618-7. Epub 2021 Nov 1. PMID: 34724175; PMCID: PMC8559140. 10.1038/s41579-020-0340-0.



Daan Touw

Hospital Pharmacist, clinical pharmacologist/toxicologist ERT. Head Laboratory Department of Clinical Pharmacy and Pharmacology, Professor of Bioanalysis, Therapeutic Drug Monitoring and Clinical Toxicology, University Medical Center Groningen, The Netherlands

Daniel Johannes Touw (born in The Hague 1958) studied Pharmaceutical Sciences in Leiden from 1977 to 1984. After his military service, he did his traineeship as hospital pharmacist in the Apotheek Haagse Ziekenhuizen in The Hague from 1986 to 1989. He worked as hospital pharmacist in the Bronovo Hospital and next to his job he gained interest in Cystic Fibrosis (CF). He studied the pharmacokinetics of intravenously administered tobramycin in CF and started to study the inhalation of tobramycin to fight the pulmonary infection more specifically. This resulted in the development of an inhalation formulation of tobramycin in cooperation with the faculty of Pharmacy from the University of Groningen. In 1996 he defended his PhD thesis 'Optimisation of tobramycin in cystic fibrosis, a pharmacokinetic approach' in the University of Leiden. Next, he was trained as clinical pharmacologist and European Registered Toxicologist (ERT). In 1995 he went to the University Hospital of the Free University Amsterdam and in



Natalie Tran

Natalie Lan Linh Tran is a second-year PhD candidate, enrolled in a cotutelle doctoral programme between University Paris Saclay in France and University of Namur in Belgium. She graduated from Goethe University Frankfurt, Germany, in 2018 with the German "Staatsexamen" (equivalent to a Master degree) in pharmacy and subsequently undertook a research placement at Monash University in Melbourne, Australia in drug development and disposition. In 2020, she completed the process to become a licensed pharmacist in Germany. Afterwards, she joined the University Paris Saclay as part of UMR CNRS 8612 for her first two years of the PhD programme and will work as part of the research team at University of Namur by September 2022 to complete the second half of her cotutelle PhD. She is working on the development of innovative nanomedicines for cardiovascular diseases, with a focus on multi-drug formulations.

RECENT PUBLICATIONS

- Amandine Gendron, Natalie Lan Linh Tran, Julie Laloy, Romain Brusini, Aurélie Rachet, Frédéric Gobeaux, Valérie Nicolas, Pierre Chaminade, Sonia Abreu, Didier Desmaele and Mariana Varna (2021) New Nanoparticle Formulation for Cyclosporin A: In Vitro Assessment. *Pharmaceutics*, 13(1), 91; <https://doi.org/10.3390/pharmaceutics13010091> 03/2020

- Liang Jin, Yijun Pan, Natalie Lan Linh Tran, Leon N. Polychronopoulos, Aparna Warriar, Kim L. R. Brouwer, and Joseph A. Nicolazzo (2020) Intestinal permeability and oral absorption of selected drugs are reduced in a mouse model of familial Alzheimer's disease. *Mol. Pharmaceutics* 2020, 17, 5, 1527–1537

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Panagiotis N. Trohopoulos

Cardiologist
 Founder / Owner / Managing Director of
 CosmoPHOS Ltd
 Thessaloniki, Greece (Ellas)

Dr med Panagiotis (Panos) N. Trohopoulos is a distinction of excellence Greek (Ellin) medical doctor for more than 28 years graduated from the Medical School of the

Aristotle University of Thessaloniki, Greece (Ellas), he is a specialist cardiologist for more than 17 years, and he is based in Thessaloniki, Greece (Ellas). Dr med Trohopoulos is also the Founder / Owner / Managing Director of CosmoPHOS Ltd which is a radically innovative translational nanomedicine SME (small-medium enterprise) founded by Dr med Trohopoulos 10 years ago in 2012, and based in Thessaloniki, Greece (Ellas) continuously since 2012. CosmoPHOS Ltd is focused on the translational research and development and the clinical trials of disruptive nanotechnology-enabled / combination / borderline medical products for the early diagnosis, targeted therapy (not only treatment), and therapy monitoring mainly for cardiovascular diseases, particularly for coronary artery disease (CAD) that causes myocardial infarctions (heart attacks), carotid artery disease that causes strokes (brain attacks), peripheral artery disease that causes intermittent claudication and gangrene of the limbs, and for wider atherosclerotic cardiovascular diseases, as well as for other diseases such as, but not limited to, cancer, autoimmune / inflammatory diseases, diabetes mellitus, neurodegenerative diseases, and infectious diseases. Dr med Trohopoulos was also the Founder (since 2004) and the Scientific / Exploitation / Strategic Coordinator and the driving force of the entire CosmoPHOS-nano Project, which was a large-scale 5-year (2013 – 2018) EU FP7 NMP funded translational nanomedicine R&D project for cardiovascular diseases and particularly for CAD. More specifically, the CosmoPHOS-nano Project was co-funded by the European Union under the FP7 Programme / NMP Theme (Nanosciences, Nanotechnologies, Materials and New Production Technologies) with 8.5 million euros, having a total project budget of 13 million euros. The multi-disciplinary CosmoPHOS-nano Project consortium consisted of 22 world-class participants, recruited by Dr med Trohopoulos, and included 15 Universities and Research Foundations and 7 Companies from 11 European countries, Japan, and USA, with a wide variety of cutting-edge scientific, technological and manufacturing expertise. The CosmoPHOS-nano Project was one of the world's largest R&D projects of nanomedicine in cardiology and cardiovascular medicine aiming to develop a disruptive nanotechnology-enabled system, the CosmoPHOS System which was entirely conceived-and-designed exclusively by Dr med Panagiotis (Panos) N. Trohopoulos, for the early diagnosis, therapy, and therapy monitoring of the vulnerable atherosclerotic plaques applicable to CAD and wider atherosclerotic cardiovascular diseases that are by far the leading cause of human death and morbidity worldwide. The CosmoPHOS System is anticipated to substantially reduce the number of deaths and the morbidity caused by CAD and wider atherosclerotic cardiovascular diseases. This is forecast to result in a significant reduction of the global healthcare costs caused by CAD and wider atherosclerotic cardiovascular diseases, and intensively alleviate the global society from a huge disease-and-economic-and-societal burden. Dr med Trohopoulos is also founding member of the International Society for Nanomedicine; member of the European Society for Nanomedicine; former member of the Advisory Board

of the European Foundation for Clinical Nanomedicine (CLINAM); former member of the Executive Board and former vice-chair of the Working Group Business of the European Technology Platform for Nanomedicine (ETPN); member of the Hellenic Cardiological Society; and member of the European Society of Cardiology.

Dr med Trohopoulos is also an inventor on 2 granted patents and on 2 pending patents.

RECENT PUBLICATIONS

- Nature Communications, 2021, 12(1): 4858. Dendrimer end-terminal motif-dependent evasion of human complement and complement activation through IgM hitchhiking. Lin-Ping Wu, Mario Ficker, Jørn B. Christensen, Dmitri Simberg, Panagiotis N. Trohopoulos, Seyed M. Moghimi.
- Nature Portfolio Health Community, August 11, 2021. (<https://go.nature.com/2WBHaGo>). Dendrimers escape sensing by the human complement defence system. Seyed M. Moghimi, Panagiotis N. Trohopoulos.
- Journal of Controlled Release, 2017, 246: 88–97. Poly-(amidoamine) dendrimers with a precisely core positioned sulforhodamine B molecule for comparative biological tracing and profiling. Lin-Ping Wu, Mario Ficker, Søren L. Mejlisøe, Arnaldur Hall, Valentina Paolucci, Jørn B. Christensen, Panagiotis N. Trohopoulos, Seyed M. Moghimi.
- Bioconjugate Chemistry, 2015, 26(7): 1198–1211. Dendrimers in Medicine: Therapeutic Concepts and Pharmaceutical Challenges. Lin-Ping Wu, Mario Ficker, Jørn B. Christensen, Panagiotis N. Trohopoulos, Seyed M. Moghimi.



Mehmet Hikmet Ucisik

Assistant Professor / Faculty Member

Mehmet H. Ucisik studied chemical engineering at Boğaziçi University, Istanbul, Turkey. Following his MSc study in Biotechnology at TU Delft, the Netherlands, he pursued doctoral degree in nanobiotechnology in the University of Natural

Resources and Life Sciences, Vienna, Austria. His main research interest involves nanomedicine including lipid- and DNA-based drug delivery systems and exosomes. As a faculty member at Genetics and Bioengineering Department of Yeditepe University, he run collaborative research on various projects on cancer, neurodegenerative and parasitic disease models. Ucisik has written 11 papers and 2 book chapters, owns 5 international patents and has 1 on-going patent application.

RECENT PUBLICATIONS

- Islek, Z., Ucisik, M. H.*, Keskin, E., Sucu, B. O., Gomes-Alves, A. G., Tomás, A. M., ... & Sahin, F. (2022). Antileishmanial Activity of BNIPDaact-and BNIPDanon-loaded Emulsomes on.
- Islek, Z., Bozkurt, B. T., Ucisik, M. H., & Sahin*, F. (2022). The Role of Extracellular Vesicles in Immunosuppression and Pathogenesis of Leishmania and Other Protozoan Infections.
- Islek, Z., Ucisik, M. H., & Sahin*, F. (2021). Novel dual-fluorescent flow cytometric approach for quantification of macrophages infected with Leishmania infantum parasites. *Parasitology*, 1-7.
- Bolat, Z. B., Islek, Z., Demir, B. N., Yilmaz, E. N., Sahin, F., & Ucisik, M. H.* (2020). Curcumin-and piperine-loaded emulsomes as combinational treatment approach enhance the anticancer activity of curcumin on HCT116 colorectal cancer model. *Frontiers in bioengineering and biotechnology*, 8, 50.
- Yilmaz, E. N., Bay, S., Ozturk, G., & Ucisik*, M. H. (2020). Neuroprotective effects of curcumin-loaded emulsomes in a laser axotomy-induced CNS injury model. *International Journal of Nanomedicine*, 15, 9211.



Jan Van Hest

Professor of Bio-organic Chemistry,
Eindhoven University of Technology

Jan van Hest obtained his PhD from Eindhoven University of Technology in 1996 in macro-organic chemistry with prof E.W. Meijer. He worked as a postdoc with prof D.A. Tirrell on protein engineering. In 1997

he joined the chemical company DSM in the Netherlands. In 2000 he was appointed full professor in Bio-organic chemistry at Radboud University Nijmegen. As of September 2016 he holds the chair of Bio-organic Chemistry at Eindhoven University of Technology. Since May 2017 he is the scientific director of the Institute for Complex Molecular Systems (ICMS). The group's focus is to develop well-defined compartments for nanomedicine and artificial cell research. Using a combination of techniques from polymer science to protein engineering, well-defined carriers and scaffolds are developed for application in e.g. cancer treatment, immunology and ophthalmology.

Van Hest was elected member of the "Jonge Akademie", (The Young Academy of the Royal Netherlands Academy of Arts and Sciences) in 2005. He has won a TOP grant (2007) and a VICI grant (2010), the highest personal career grant obtainable through open competition in the Netherlands. In 2012 he was one of the main applicants of the 10-year gravitation program on functional molecular systems (26.9 M€, with the universities of Eindhoven, Groningen and Nijmegen). In 2016 he was awarded an ERC Advanced grant. Van Hest is associate editor of *Bioconjugate Chemistry*. He is furthermore an advisory board member of *Macromolecular Bioscience*, *Biomacromolecules*, *Journal of Materials Chemistry*, *Chemical Science* and *ACS Central Science*. He has been elected member of the Academy of the Royal Netherlands Academy of Arts and Sciences in 2019, and was awarded the Spinoza premium in 2020, the highest scientific distinction in the Netherlands. He has published around 400 papers. He is also cofounder of four start-up companies (Encapson, FutureChemistry, Noviosense and Noviotech).

RECENT PUBLICATIONS

- L. K. E. A. Abdelmohsen., D.S. Williams, J. Pille, S.G. Ozel, R.S.M. Rikken, D.A. Wilson, J.C.M. van Hest, Formation of Well-Defined, Functional Nanotubes via Osmotically Induced Shape Transformation of Biodegradable Polymersomes. *Journal of the American Chemical Society* 2016 138, 9353-9356.
- A.F. Mason, B.C. Buddingh, D.S. Williams, J.C.M. van Hest, Hierarchical Self-Assembly of a Copolymer-Stabilized Coacervate Protocell. *Journal of the American Chemical Society* 2017, 139, 17309-17312.
- J. Shao, M. Abdelghani, G. Shen, S. Cao, D.S. Williams, J.C.M. van Hest, Erythrocyte Membrane Modified Janus Polymeric Motors for Thrombus Therapy. *ACS Nano* 2018, 12, 4877-4885.
- S.P. Cao, J.X. Shao, H.L. Wu, S.D. Song, M.T. de Martino, I.A.B. Pijpers, J.H. Friedrich, L.K.E.A. Abdelmohsen, D.S. Williams, J.C.M. van Hest, Photoactivated nanomotors via aggregation induced emission for enhanced phototherapy. *Nature Comm.* 2021, 12, 2077
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Peter van Hoogevest

Consultant, CEO and Owner at PHARMANOVIATION, Rheinfelden (Baden), 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 Privatdozent (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 (Muttens, CH) and became CEO of this company and was member of the Board of Directors. In 2000 he joined Phares Drug Delivery AG (Muttens, 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. From 2012 till 2021 he was 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). He runs from 2021 on his own consulting business PHARMANOVIATION, based in Rheinfelden (Baden), Germany.

His drug delivery expertise, especially in the (phospho)lipid research and development area, is underscored by 78 scientific publications, including 8 book chapters, 33 symposium posters, co-promotion of 48 PhD Theses, 13 patents and 45 patent applications.

RECENT PUBLICATIONS

- Otto, F., van Hoogevest, P., Syrowatka, F., Neubert, R.H.H., Assessment of the applicability of HLB values for natural phospholipid emulsifiers for preparation of stable emulsions, *Die Pharmazie - An International Journal of Pharmaceutical Sciences*, 75: 365-370 (2020).
- Van Hoogevest, P., Review-Non-Aqueous Phospholipid Concentrates for Increasing the Bioavailability of Poorly Soluble Compounds, DOI: 10.1002/ejlt.201900411.



Mariana Varna-Pannerec

Associate Professor

Dr. VARNA Mariana graduated in Molecular Genetics from University Pierre et Marie Curie (UPMC, Paris 6) and got her PhD in Molecular Basis of Oncogenesis from University Denis Diderot (Paris 7, Paris). After a first Postdoctoral Fellowship, during which she worked on the stem cells and tumor stem cells, she moved to Superior School of Physics and Industrial Chemistry (ESPCI Paris Tech), Langevin Institute, where she was hired as a junior researcher. During this time, she collaborates with Nanyang Technological University (NTU, Singapore), and she worked on gold nanoparticles applications as imaging tools or as therapeutic tools through hyperthermia. In 2014, she won a Postdoctoral Fellowship from Institut Universitaire de France, and thus she joined Inserm U1148 research Units, where she started her work on atherosclerosis. In 2015, she joined Patrick Couvreur's team at Institut Galien, Paris Saclay University as Associate Professor. Dr. Varna's research activities focus

on developing nanomedicines for therapy and imaging in cardiovascular diseases.

She has published over 50 peer-reviewed articles in prestigious international journals and book chapters (H-index 23, i-index 35).

Dr. Varna is reviewer for different international journals, including Journal of Drug Targeting, Pharmaceutics, Clinical Cancer Research, Journal of Controlled release.

RECENT PUBLICATIONS

- A Gendron, NLL Tran, J Laloy, R Brusini, A Rachet, F Gobeaux, V Nicolas, P Chaminade, S Abreu, D Desmaële and M Varna*, New Nanoparticle Formulation for Cyclosporin A: In Vitro Assessment, *Pharmaceutics* 2021, 13, 91.
- Brusini R, Varna M, Couvreur P., Advanced nanomedicines for the treatment of inflammatory diseases, *Adv Drug Deliv Rev.* 2020 Jul 19:S0169-409X(20)30094-6. doi: 10.1016/j.addr.2020.07.010.
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- Brusini R, Dormont F, Nicolas V, Cailleau C, Peramo A, Varna M, Couvreur P, Squalene-based nanoparticles for the targeting of atherosclerotic lesions, *International Journal of Pharmaceutics*, May 15;581:119282. doi: 10.1016/j.ijpharm.2020.119282
- Dormont F, Rouquette M, Mahatsekake C, Gobeaux F, Peramo A, Brusini R, Calet S, Testard F, Lepetre-Mouelhi S, Desmaële D, M Varna, P Couvreur, Translation of nanomedicines from lab to industrial scale synthesis: The case of squalene-adenosine nanoparticles, *Journal of Controlled Release*, Volume 307, 10 August 2019, Pages 302-314, <https://doi.org/10.1016/j.jconrel.2019.06.040>

Therapeutic Solutions S.L.' (Valencia, Spain) in 2012. María was the President of the SPLC-CRS up to 2013, is currently vicepresident of the specialised Chemical Biology Section of the Spanish Royal Society of Chemistry (RSEQ) 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 in Valencia. María is also the executive editor of *Adv. Drug Deliv Rev*, the associate editor of *DDTR*, and a member of the editorial boards of key journals in the field.

RECENT PUBLICATIONS

- E. Giraldo, V. Nebot, O. Zagorodko, Raquel Requejo-Aguilar, A. Alastrue-Agudo, A. Armiñán, B. Martínez-Rojas, Pablo Bonilla-Villamil, S Dordevic, M.J. Vicent*, V. Moreno-Manzano* A rationally designed self-immolative linker enhances the synergism between a polymer-rock inhibitor conjugate and neural progenitor cells in the treatment of spinal cord injury. *Biomaterials* 2021, 276, 121052
- A. Duro-Castano, C. Borrás, V. Herranz-Pérez, M.C. Blanco-Gandía, I. Conejos-Sánchez, A. Armiñán, C. Mas-Bargues, M. Inglés, J. Miñarro, M. Rodríguez-Arias, J. M. García-Verdugo, J. Viña, M.J. Vicent* Targeting Alzheimer's disease with Multimodal Polypeptide-based Nanoconjugates. *Science Advances* 2021, 7, eabf9180.
- Arroyo-Crespo, J.J., Armiñán, A., Charbonnier, D., Balzano-Nogueira, L., Huertas-López, F., Martí, C., Tarazona, S., Forteza, J., Conesa, A., Vicent, M.J.* Tumor microenvironment-targeted poly-L-glutamic acid-based combination conjugate for enhanced triple negative breast cancer treatment. *Biomaterials*, 2018. 186: p8-21.
- Arroyo-Crespo, J.J., Deladriere, C., Nebot, V.J., Charbonnier, D., Masiá, E., Paul, A., James, C., Armiñán, A.*, Vicent, M.J.*, Anticancer Activity Driven by Drug Linker Modification in a Polyglutamic Acid-Based Combination-Drug Conjugate. *Advanced Functional Materials*, 2018. 28(22): p. 1800931
- Duro-Castaño, A., Nebot, V. J.*, Niño-Pariente, A., Armiñán, A., Arroyo-Crespo, J. J., Paul, A., Feiner-Gracia, N., Albertazzi, L. and Vicent, M.J.* Capturing Extraordinary Soft-Assembled Charge-Like Polypeptides as a Strategy for Nanocarrier Design. *Advanced Materials*, 2017. 29(39): p. 1702888-n/a.



Maria Vicent

Head of the Polymer Therapeutics Lab and Coordinator of the Advanced Therapies Area at Prince Felipe Research Center.

Dr. María J. Vicent received her Ph.D. degree in 2001 in chemistry after her research on solid supports from the Univ. 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 Prince Felipe Research Center (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. She is part of the Strategic Committee of the Valencian Agency of Innovation (AVI) and it is Director at Large of the CRS. María's research group (<http://www.VicentResearchLab.com>) 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. She is fellow of the American Institute for Medical and Biological Engineering (AIMBE) College of Fellows 2019 and Controlled Release Society College of fellows 2021. María has co-authored ≈135 peer-reviewed papers and 11 patents. Three patents have been licensed to the pharmaceutical industry, one being used for co-founding the spin-off company 'Polypeptide



Viola Vogel

Professor
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 in 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), serves 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) since 2018 and of the National Academy of Sciences USA (NAS) since 2020, 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.

RECENT PUBLICATIONS

- V. Vogel, Unraveling the mechanobiology of extracellular matrix, *Annual Review Physiology*, 80 (2018) 353-387.
- C. M. Fonta, S. Arnoldini, D. Jaramillo, A. Moscaroli, A. Oxenius, M. Behe, V. Vogel, Fibronectin fibers are highly tensed in healthy organs in contrast to tumors and virus-infected lymph nodes, *Matrix Biology Plus* 8 (2020) 100046.
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- S. Lickert, K. Selcuk, M. Kenny, J. L. Mehl, S. M. Früh, M. A. Burkhardt, J.-G. Studt, I. Schoen, V. Vogel, Platelets exploit fibrillar adhesions to assemble fibronectin matrix revealing new force-regulated thrombus remodeling mechanisms, *Sciences Advances*, in press. <https://doi.org/10.1101/2020.04.20.050708>
- N. Jain, V. Vogel, Downsizing the inflammatory response of macrophages by spatial confinement, *Nature Materials*, 17, (2018) 1134-1144.



Mathias Wacker

Associate Professor

Matthias G. Wacker studied Pharmacy at Goethe University in Frankfurt (Germany) where he obtained his Ph.D. in pharmaceutical technology. As a principal investigator, he has joined Jennifer Dressman and Jörg Kreuter in the Institute of Pharmaceutical Technology, Goethe University (Germany). There he accomplished his habilitation exploring the rational design of nanocarriers and was awarded the Venia legendi in pharmaceutical technology. Before joining NUS, he headed the Department of Pharmaceutical Technology and Nanosciences of the Fraunhofer IME in Frankfurt (Germany). Currently, he serves the *European Journal of Pharmaceutics and Biopharmaceutics* and the *Journal of Pharmacy and Pharmacology* as an editorial board member. Furthermore, he is a scientific advisor to the *Journal of Pharmaceutical Sciences* editors and was guest editor for the *Beilstein Journal of Nanotechnology*, *Frontiers in Chemistry*, and *Frontiers in Digital Health*. In recognition of his research excellence, he was honored with the Phoenix Pharmaceutics Science Award 2017. From 2020-2025, he is a member of the General Chapters – Dosage Forms Expert Committee and the Expert Panel on New Advancements in In-Vitro Product Performance Testing of the United States Pharmacopeia. His research emphasizes the formulation and characterization of drug delivery systems in the nanoscale following a quality-by-design approach. Also, his team develops biopredictive methodologies for complex oral and injectable dosage forms.

RECENT PUBLICATIONS

- Mast MP, Modh H, Knoll J, Fecioru E, Wacker MG (2021); An Update to Dialysis-based Drug Release Testing – Data Analysis and Validation using the Pharma Test Dispersion Releaser. *Pharmaceutics* (10.3390/pharmaceutics13122007).
- Mast MP, Modh H, Champanhac C, Wang JWW, Storm G, Krämer J, Mailänder V, Pastorin G, Wacker MG (2021); Nanomedicine at the crossroads – A quick guide for IVIVC, *Adv. Drug Del. Rev.* (10.1016/j.addr.2021.113829)
- Gao GF, Ashtikar M, Kojima R, Yoshida T, Kaihara M, Tajiri T, Shanesazzadeh S, Modh H, Wacker MG (2021); Predicting drug release and degradation kinetics of long-acting microsphere formulations of tacrolimus for subcutaneous injection, *J. Control. Release* (10.1016/j.jconrel.2020.11.055)
- Jung F, Thurn M, Krollik K, Gao GF, Hering I, Eilbrecht E, Emará Y, Weiler M, Günday-Türelí N, Türelí E, Parnham MJ, Wacker MG (2020); Predicting the environmental emissions arising from conventional and nanotechnology-related pharmaceutical drug products, *Environ. Res.* (10.1016/j.envres.2020.110219)
- 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. Release* (10.1016/j.jconrel.2019.06.029)



Andreas Wagner

Head Liposome Technology

Dr Andreas Wagner is currently the Head, Liposome Technology at Polymun Scientific GmbH. He has significant expertise in incorporation and optimization of hydrophilic, lipophilic and amphipatic substances into liposomes and LNPs and development for clinical use. He studied Biotechnology in Vienna, Austria and earned his Master and Ph.D. degrees in the field of biopharmaceutical technology/ liposomology at the Institute of Applied Microbiology supervised by Prof. Hermann Katinger. Dr Andreas Wagner is listed as inventor on several patents, like the liposome technology and some product patents of liposomal formulations. Furthermore, he has published several peer reviewed articles dealing with liposomes, the technology, products thereof and their application in preclinical and clinical studies.



Frank F. Weichold

Dr. Weichold is Senior Science Advisor in ORSI, the office of the Chief Scientist and the Office of the Commissioner for 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. While he was the director for Critical Path and Regulatory Science Initiatives 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 represented 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 standardization, interoperability and liberation, as well 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.



Peter Wick

Head of Particles-Biology Interaction, Empa

Peter Wick heads since 2010 the research laboratory for Particles-Biology Interactions at the Federal Laboratories on Materials Science and Technologies Empa in St. Gallen. He got his PhD degree in 2002 at the University of Fribourg, Switzerland. His general research interest is to study the interactions of nanomaterials with human tissues including barrier tissue *in vitro* and *ex vivo* with the purpose to obtain detailed mechanistic understanding about their uptake, accumulation, biotransformation, transport and effect on different types of cells or entire tissue. He has published more than 150 papers, was member of the advisory board of the Swiss Action Plan on Nanomaterials, member of the EDQM working group for NBCs, Editorial Board Member of Nanotoxicology, associated editor of the Journal NanolImpact, member of the Swiss National COVID-19 Science Task Force and coordinator of the Swiss National Contactpointnano.ch.

RECENT PUBLICATIONS

- Iranpour Anaraki N, Liebi M, Ong Q, Blanchet C, Maurya AK, Stellacci F, Salenting S, Wick P, Neels A, (2021) In-situ Investigations on Gold Nanoparticles Stabilization Mechanisms in Biological Environments Containing HSA, *Adv Funct Materials* 2110253
- Iranpour Anaraki N, Sadeghpour A, Iranshahi K, Ong Khac Q, Cendrowska U, Toncelli C, Dommann A, Wick P, Neels A, (2020) A new approach for time-resolved and dynamic investigations on nanoparticles agglomeration, *Nanoresearch* 13(10)2847-2856
- Nikraves N, Borchard G, Hofmann H, Philip E, Flühmann B, Wick P, (2020) Factors Influencing safety and efficacy of intravenous iron-carbohydrate nanomedicines: from production to clinical practice, *Nanomed: Nanotechnol, Biol Med* 26, 102178
- Milosevic A, Romeo D, Wick P, (2020) Predictive nanotoxicology: How nanomaterial biotransformation remains an unmet challenge, *SMALL* 16(36) 1907650
- Romero D, Saleri B, Hischer R, Nowack B, Wick P, (2020) An integrated pathway based on *in vitro* data for the human hazard assessment of nanomaterials *Environ Intern* 137;105505



Marieluise Wippermann

CEO, TECOmedical AG, Sissach, Switzerland
E-mail: wippermann@tecomedical.com

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



Dominik Witzigmann

CEO & Co-Founder NanoVation Therapeutics

Dominik obtained his Ph.D. in Pharmaceutical Technology from the University of Basel (Switzerland), and he has a proven track record in nanomedicines enabling tissue and cell specific drug and gene delivery.

Dominik had leadership roles within the NanoMedicines Innovation Network (NMN), a Canadian Excellence Network, he co-founded and led NMN's NanoCore to support >30 projects with advanced nucleic acid delivery technologies, and he served as a Board Member of the CRS "Gene Delivery and Genome Editing" Focus Group. To translate next-generation lipid nanoparticle (LNP) technologies into the clinic, Dominik co-founded and leads the LNP-nucleic acid company NanoVation Therapeutics. (<https://ca.linkedin.com/in/dominikwitzigmann>)

RECENT PUBLICATIONS

- Pattipeiluhu et al., Anionic Lipid Nanoparticles Preferentially Deliver mRNA to the Hepatic Reticuloendothelial System, *Advanced Materials* 2022
- Kulkarni & Witzigmann et al., The Current Landscape of Nucleic Acid Therapeutics, *Nature Nanotechnology* 2021
- Rothgangl et al., In vivo adenine base editing of PCSK9 in macaques reduces LDL cholesterol levels, *Nature Biotechnology* 2021
- Schoenmaker et al., mRNA-lipid nanoparticle COVID-19 vaccines: Structure and stability, *International Journal of Pharmaceutics* 2021
- Francia et al., The biomolecular corona of lipid nanoparticles for gene therapy, *Bioconjugate Chemistry* 2020



Robert Wykes

Group leader at the UCL Queen Square Institute of Neurology, UK & NanoNeuroTeam leader, Nanomedicine Lab, University of Manchester, UK.

I lead 2 research labs, one in London, and the other in Manchester. My research focuses on Paroxysmal CNS disorders with a particular focus on epilepsy, and can be

broadly broken down into 2 research categories:

1. Gene therapy approaches to treat drug-refractory forms of epilepsy.
2. Development and application of novel imaging and electrophysiological approaches to detect seizure activity and spreading depolarisations in preclinical models of epilepsy, migraine, glioblastoma and stroke. A recent focus is application of implantable Graphene-based transistor arrays to map pathological brain activity. CSD and sudden unexplained death in epilepsy (SUDEP).

RECENT PUBLICATIONS

- Bonaccini Calia A, Masvidal-Codina M, Smith TM, Schäfer N, Rathore D, Rodríguez-Lucas E, Illa X, De la Cruz JM, Del Corro E, Prats-Alfonso E, Viana D, Bousquet J, Hébert C, Martínez-Aguilar J, Sperling JR, Drummond M, Halder A, Dodd A, Barr K, Savage S, Fornell J, Sort J, Guger C, Villa R, Kostarelos, Wykes RC*, Guimerà-Brunet A*, & Garrido JA*. (2021). Full bandwidth electrophysiology of seizures and epileptiform activity enabled by flexible graphene micro-transistor depth neural probes. *Nature Nanotechnology*. DOI: 10.1038/s41565-021-01041-9. * Corresponding authors.
- Jafarian A, Zeidman P, Wykes RC, Walker MC & Friston K. (2021). "Adiabatic dynamic causal modelling". *Neuroimage*. PMID 34116151
- Masvidal-Codina E, Smith TM, Rathore D, Gao Y, Illa X, Prats-Alfonso E, Del Corro E, Bonaccini Calia A, Rius G, Martín-Fernandez I, Guger C, Reitner P, Villa R, Garrido JA, Guimerà-Brunet A*, Wykes RC*. (2021). Characterization of optogenetically-induced cortical spreading depression in awake mice using graphene micro-transistor arrays. *Journal of Neural Engineering* PMID: 33690187. *Corresponding authors.
- Tosh JL, Rhymes E, Mumford P, Whittaker H, Pulford LJ, Noy SJ, Cleverley K, Schorge S, Walker MC, Tybulewicz VLJ, Wykes RC, Fisher EMC, Wiseman FK. (2021) Genetic dissection of Down syndrome-associated alterations in APP/amyloid- β biology using mouse models. *Scientific Reports* PMID 33707583.
- A. Guimerà-Brunet, E. Masvidal-Codina, X. Illa, M. Dasilva, A. Bonaccini-Calia, E. Prats-Alfonso, J. Martínez-Aguilar, J.M. De la Cruz, R. Garcia-Cortadella, N. Schaefer, A. Barbero, P. Godignon, G. Rius, E. Del Corro, J. Bousquet, C. Hébert, R. Wykes, M.V. Sanchez-Vives, R. Villa and J.A. Garrido. (2020). "Neural interfaces based on flexible graphene transistors: A new tool for electrophysiology". 2019 IEEE International Electron Devices Meeting (IEDM). DOI: 10.1109/IEDM19573.2019.8993433



Ada Yonath

Ada Yonath is focusing on the translation of the genetic code to proteins by ribosomes, on antibiotics hampering this process, on human diseases associated with ribosomal mutations and on origin of life. She initiated these studies despite persistent global skepticism, and introduced forefront key scientific developments, which became

common routines. She is the director of the Kimmelman Center for Biomolecular Structures at Weizmann Institute (WIS), where she got her PhD and is currently a Professor. She postdoced at CMU,

MIT and U. Chicago. In the seventies, she established at WIS a structural-biology lab, which was the only one in Israel for almost a decade. During 1986-2004, she headed Max-Planck-Research-Unit for Ribosome-Structure in Hamburg, in parallel to her WIS activities.

Among others, she is a member of US-National Academy; British Royal Society; Israel Academy; German Leopoldina; Pontificia Accademia-delle-Scienze (Vatican); Japanese, Korean, Italian & European Academies, and holds honorary doctorates from over 45 Universities worldwide. Her awards include Louisa-Gross-Horwitz, Wolf, Harvey & Israel prizes; Linus-Pauling Gold Medal; UNESCO/L'Oréal Prize; Albert-Einstein Award for Excellence; and the 2009 Nobel Prize.

RECENT PUBLICATIONS

- Halfon, Y., Jimenez-Fernandez, A., La Rosa, R., Espinosa Portero, R., Krogh Johansen, H., Matzov, D., Eyal, Z., Bashan, A., Zimmerman, E., Belousoff, M., Molin, S. and Yonath, A. (2019). Structure of *Pseudomonas aeruginosa* ribosomes from an aminoglycoside-resistant clinical isolate. *Proc Natl Acad Sci U S A*, 116, 22275-22281; PMID: 31611393.
- Halfon, Y., Matzov, D., Eyal, Z., Bashan, A., Zimmerman, E., Kjeldgaard, J., Ingmer, H., and Yonath, A. (2019). Exit tunnel modulation as resistance mechanism of *S. aureus* erythromycin resistant mutant. *Sci Rep* 9, 11460; PMID: 31391518
- Yonath, A. (2019). Could a bright outlook for antibiotics usage emerge from colossal health issue? In *Bacterial resistance to antibiotics: from molecules to man*, B.B. Bonev, and N.M. Brown, eds. (UK: John Wiley & Sons).
- Matzov D., Eyal Z., Benhamou R.I., Shalev-Benami M., Halfon Y., Krupkin M., Zimmerman E., Rozenberg H., Bashan A., Fridman M. and Yonath, A. (2017). Structural insights of lincosamides targeting the ribosome of *Staphylococcus aureus*. *Nucleic Acids Res*, 45, 10284-10292; PMID: 28973455
- Belousoff, M.J., Eyal, Z., Radjainia, M., Ahmed, T., Bamert, R.S., Matzov, D., Bashan, A., Zimmerman, E., Mishra, S., Cameron, D., Elmlund, H., Peleg, A. Y., Bhushan, S., Lithgow, T., and Yonath, A. (2017). Structural basis for linezolid binding site rearrangement in the *Staphylococcus aureus* ribosome. *mBio* 8 (3); PMID: 28487427



Cynthia Yu Wai Man

Assistant Professor in Ophthalmology and MRC Clinician Scientist

Dr Cynthia Yu-Wai-Man is an MRC Clinician Scientist, Assistant Professor in Ophthalmology and Consultant Ophthalmic Surgeon. Dr Yu-Wai-Man leads the Glaucoma and Therapeutics Lab at King's College

London. She has set up a project licence and animal models for the preclinical testing of new therapeutics and devices in glaucoma. She has also set up a UK Glaucoma BioResource of RNA, DNA and tissue samples from glaucoma patients, linked to detailed longitudinal phenotyping and imaging modalities. Her group is focused on translational research, multi-omics research in glaucoma and personalised medicine.

Dr Yu-Wai-Man has been awarded over £2.1 millions in research funding over the last 3 years. She has received several prestigious awards as PI, namely the MRC Clinician Scientist Fellowship (2020), King's Prize Fellowship (2019), NIHR CRN Greenhoots funding (2019), MRC Confidence-in-Concept Grant (2016), and EUREKA Translational Medicine Fellowship (2015).

Dr Yu-Wai-Man is a Chief Investigator of advanced therapeutics and nanomedicine in glaucoma surgery. Her research goal is to bring new targeted therapeutics to patients. Nanotechnology enables the design of multifunctional and targeted therapies, thereby increasing the therapeutic efficacy and reducing the side effects for patients. Dr Yu-Wai-Man has published several peer-reviewed pub-

lications and has shown that targeted siRNA therapeutics and novel therapeutic nanocomplexes increase bleb survival and prevent conjunctival fibrosis after glaucoma surgery.

RECENT PUBLICATIONS

- Fernando O, Tagalakakis AD, Awwad S, Brocchini S, Khaw PT, Hart SL, Yu-Wai-Man C. Development of targeted siRNA nanocomplexes to prevent fibrosis in experimental glaucoma filtration surgery. *Mol Ther*. 2018 Dec 5; 26(12): 2812-2822.
- Sanghani A, Kafetzis KN, Sato Y, Elborai S, Fajardo-Sanchez J, Harashima H, Tagalakakis AD, Yu-Wai-Man C. Novel PEGylated Lipid Nanoparticles Have a High Encapsulation Efficiency and Effectively Deliver MRTF-B siRNA in Conjunctival Fibroblasts. *Pharmaceutics*. 2021; 13(3): 382.
- Gupta A, Kafetzis KN, Tagalakakis AD, Yu-Wai-Man C. RNA therapeutics in ophthalmology - translation to clinical trials. *Exp Eye Res*. 2021; 205: 108482.
- Tagalakakis AD, Madaam S, Larsen SD, Neubig RR, Khaw PT, Rodrigues IA, Goyal S, Lim KS, Yu-Wai-Man C. In vitro and *in vivo* delivery of a sustained release nanocarrier-based formulation of an MRTF/SRF inhibitor in conjunctival fibrosis. *J Nanobiotechnology*. 2018 Nov 27; 16(1): 97.
- Sanghani A, Andriesei P, Kafetzis KN, Tagalakakis AD, Yu-Wai-Man C. Advances in exosome therapies in ophthalmology-From bench to clinical trial. *Acta Ophthalmol*. 2021 Jun 11; doi: 10.1111/aos.14932.



Karen Zagorski

PhD student

Karen Zagorski has graduated from Russian-Armenian (Slavonic) University in Yerevan, Armenia with an MD/MS in medical biochemistry. His primary research is focused on vaccines for neurodegenerative diseases, as well as development of modular vaccines for personalized medicine. He has been involved in development of several protein and nucleic acid based vaccines for Alzheimer's and Parkinson's diseases. Currently he is working towards a PhD in pharmaceutical sciences in University of Nebraska Medical Center. His most recent work is related to use of nucleic acids as a scaffold for controlled nanoassembly of modular vaccines.



Robert Geertsma

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 twentyfive 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 the recently finalised 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.

RECENT PUBLICATIONS

- 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.

CLINAM

European Foundation for Clinical Nanomedicine

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Basel, May 2-4

CURRICULA VITAE POSTERS



Chinazom Agbo

Chinazom Precious Agbo, is an early career researcher with interest in exploiting nanotechnology for the design and characterization of novel delivery systems for administration through the nasal route. These formulations are to serve as alternatives for drugs administered parenterally. Her goal is to achieve safer and more convenient therapies in order to realize better

treatment outcomes.

Chinazom holds a Bachelor of Pharmacy honours degree from the University of Nigeria, Nsukka obtained in 2010 with distinctions. She also made a First Class in her Master of Pharmacy (MPharm.) degree programme in 2013 from the same University. In 2020, Chinazom obtained a Ph.D. in Pharmaceutics from the same University. As part of her Ph.D. programme, she was a Visiting Research Student in the School of Pharmacy, University of Birmingham, United Kingdom from 2017 to 2018. Chinazom is currently a lecturer in the Department of Pharmaceutics, University of Nigeria, Nsukka. In the course of her career, she has won other scholarships (DAAD), travel grants (Global Health Travel grant) and research grants (TET Fund). Chinazom has worked on a number of projects involving the formulation of novel drug delivery devices to tackle challenges encountered in the treatment of tropical and neglected diseases. She has expertise in the formulation of nanoparticulate systems such as nanostructured lipid carriers, ethosomes, and other lipid-based drug delivery systems. She has also worked on designing novel carrier systems for some African herbal extracts.

Her PhD research project was on the use of nanotechnology for the design, characterization and comparison of lipid- and polymer-based delivery systems for intranasal administration of antimalarials for the treatment of severe and cerebral malaria, as a safer alternative to parenteral formulations. These intranasal formulations would be of immense benefit to rural dwellers in sub-Saharan Africa disadvantaged by limited access to medical personnel with the skills to administer these drugs parenterally. Some of her recent publications are listed below:

RECENT PUBLICATION

- Ogbonna, J. D. N, Echezona, A. C. ... Agbo C. P. et al. Formulation, In vitro and In vivo evaluation of sustained released artemether-lumefantrine-loaded microstructured solid lipid microparticles (SLMs). *Trop J Nat Prod Res.* 2021; 5(8):1460-1469.
- Onugwu, A. L., Agbo, C. P., Nwagwu, C. S., et al. Development of lipid-based microsuspensions for improved ophthalmic delivery of gentamicin sulphate. (2021). [Dio.10.4155/tde-2021-0039](https://doi.org/10.4155/tde-2021-0039).
- Agbo, C.P., Ugwuanyi, T.C., Ugwuoke, W.I. et al. Intranasal artesunate-loaded nanostructured lipid carriers: A convenient alternative to parenteral formulations for the treatment of severe and cerebral malaria. *Journal of Controlled Release.* 2021 334, 224–236. <https://doi.org/10.1016/j.jconrel.2021.04.020>.
- Momoh M. A., Franklin, K. C., Agbo, C. P. et al. Microemulsion-based approach for oral delivery of insulin: formulation design and characterization. *Heliyon.* 2020; 6: 1–8.
- Echezona A.C, Momoh M. ...Agbo, C. P. et al. Effect of molecular interaction on the antiplasmodial efficacy of lumefantrine in amorphous polymethacrylate-urea solid solution. *Journal of Drug Delivery and Therapeutics.* 2020;10(5):56–69.



Borislav Angelov

Borislav Angelov received a Ph.D. degree in Biophysics from the Institute of Biophysics at the Bulgarian Academy of Sciences where he was appointed a Research Associate in 2002. He was a fellow of the Marie Curie host site Laboratoire de Physique de Solides (LPS Orsay), Paris-Sud University. After a postdoctoral stay at the GKSS Research Centre (Geesthacht, Germany) and

Aarhus University (Denmark), he continued X-ray structural investigations of drug delivery nanoparticles at the Institute of Macromolecular Chemistry (Prague). Currently he is a senior scientist at ELI Beamlines (Institute of Physics, Czech Academy of Sciences, Prague). His research interests are in the field of time resolved studies of biomolecular structural dynamics.

RECENT PUBLICATION

- Angelov, B.; Angelova, A.; Ollivon, M.; Bourgaux, C.; Campitelli, A. *J. Am. Chem. Soc.* 2003, 125, 7188-7189. ;
- Angelov, B.; Angelova, A.; Papahadjopoulos-Sternberg, B.; Lesieur, S.; Sadoc, J.-F.; Ollivon, M.; Couvreur, P. *J. Am. Chem. Soc.* 2006, 128, 5813.
- Angelov, B.; Angelova, A.; Garamus, V. M.; Lebas, G.; Lesieur, S.; Ollivon, M.; Funari, S. S.; Willumeit, R.; Couvreur, P. *J. Am. Chem. Soc.* 2007, 129, 13474.
- Angelova, A.; Angelov, B.; Mutafchieva, R.; Lesieur, S.; Couvreur, P. Self-Assembled Multicompartment Liquid Crystalline Lipid Carriers for Protein, Peptide, and Nucleic Acid Drug Delivery. *Accounts of Chemical Research* 2011, 44(2), 147-156 (including cover page.);
- Angelov B., Angelova, A., Filippov, S.K., Drechsler, M., Štěpánek, P., Lesieur, S., Multicompartment lipid cubic nanoparticles with high protein upload: millisecond dynamics of formation, *ACS Nano*, 2014, 8(5), 5216-5226.



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.



Ahmad Bahlool

Ahmad Z. Bahlool is a pharmacist and a 3rd year PhD student at the Royal College of Surgeons in Ireland (RCSI). During his PhD, he is working on developing inhaled host-directed immunotherapy for tuberculosis under a supervisory team from RCSI, St. James' hospital Trinity College Dublin and Aerogen Ltd.

Ahmad received his Erasmus mundus joint master's degree in nanomedicine for drug delivery from 4 European universities jointly (University of Paris, University of Angers (France), University of Pavia (Italy) and University of Patras (Greece)). During His masters, Ahmad has undertaken two internships; First, at the translational micro and nanomedicine lab (MiNT) at university of Angers in France, where he worked on developing Ferrocifen loaded lipidic nanocapsules for ovarian cancer in collaboration with institute Curie and Ferrosan company. Second, he did a 6 months internship (master thesis) at Queen's university Belfast UK, working on his thesis to develop engineered targeted nanomedicine for advanced prostate cancer. During his undergrad pharmacy degree at the University of Jordan, he worked on developing silver nanoparticles integrated with hydrogen peroxide to treat drug resistant bacteria.

Ahmad had many prestigious awards such as the strategic academic recruitment (StAR) PhD scholarship from RCSI, the Erasmus mundus joint masters degree scholarship to do his masters in Europe and the RCSI secondment travel award to go and spend up to 3 months at Imperial college London to do *in vivo* work for his PhD thesis.

RECENT PUBLICATION

- O'Leary, S., Bahlool, A. Z., O'Connor, G., Cryan, S. A., Keane, J. M., O'Sullivan, M. P. An Automated Culture System for Use in Preclinical Testing of Host-Directed Therapies for Tuberculosis. *Vis. Exp.* (174), e62838, doi:10.3791/62838 (2021).
- Alkawareek MY, Bahlool A, Abulateefeh SR, Alkilany AM (2019) Synergistic antibacterial activity of silver nanoparticles and hydrogen peroxide. *PLOS ONE* 14(8): e0220575. <https://doi.org/10.1371/journal.pone.0220575>



Rafal Baker

Rafal obtained her B.Sc. Degree in Pharmacy from the University of Petra – Jordan in 2017. She then completed her MSc. Degree in Drug Discovery and Pharmaceutical Sciences at the University of Nottingham in 2019. Rafal joined Al-Jamal's team right after to pursue her PhD degree at the Institute of Pharmaceutical Science, King's College London and is currently working

on developing a novel nanoparticulate delivery system to deliver treatments for Amyotrophic Lateral Sclerosis (ALS).



Danielle Brain

Danielle was born in Stratford-upon-Avon, UK and has a background in pharmacology and immunology. She graduated with a first class B.Sc (Hons) degree in Pharmacology at the University of Liverpool and is currently in the 4th year of her PhD at the University of Liverpool. Her research is focused on investigating biocompatibility and immunological safety of Long-

acting formulations. She is particularly interested in exploring the role of the inflammasome in mediating immunological responses to these long-acting formulations and also the role it plays in COVID-19 infection. She has been a research associate at the University of Liverpool since September, developing *in vitro* and *ex vivo* assays of immune sites. Selected publications: Brain D, Plant-Hately A, Heaton B, Arshad U, David C, Hedrich C, Owen A, Liptrott NJ. "Drug delivery systems as immunomodulators for therapy of infectious disease: Relevance to COVID-19." *Adv Drug Deliv Rev.* 2021 Nov;178:113848.



Mareike Deuker

After graduating high school in 2013, Mareike Deuker studied biomedical chemistry at the Johannes Gutenberg University of Mainz. During her studies, she spent four months at Durham University (UK) for a research stay in the group of Prof. Liam Hutchings. After completing her Master in 2019, with a master project on the degradation of polyphosphoesters, she started

to pursue her Ph.D in chemistry at the Max Planck Institute for Polymer Research in the group of Prof. Katharina Landfester. Her thesis focuses on the interaction of nanocarriers with anti-PEG antibodies.



Martina Di Francesco

Post-doc

Martina Di Francesco is a Post Doc in the Nanotechnology for Precision Medicine research line at Istituto Italiano di Tecnologia since May 2017. Her research activities focus on the design and the development of polymeric micro- and nano-constructs for delivering of different drugs (anti-inflammatory drugs and/or growth factors), in order to combine therapeutic activity and regenerative medicine.

Dr Di Francesco achieved her MSc Degree in Chemistry and Pharmacology Technology in October 2013 at the "G. d'Annunzio" University of Chieti – Pescara discussing the thesis "Implantable nanodevices for sustained zero-order release of liposomal nano-drugs". In this work, she demonstrated that nanochannels can be exploited to passively control the sustained release of intact liposomes from subcutaneously implantable reservoirs, designed for systemic delivery. Following that, she collaborated with a Pharmacy preparing and checking drugs following pharmacopoeia. In January 2014, she started her PhD in Life Sciences at the University of Catanzaro under the supervision of Prof. Massimo Fresta. Whilst completing her PhD thesis she joined IIT for an internship leading her to her current position. Her PhD project "Supramolecular vesicular aggregates (SVAs) as innovative nanomedicine for the treat-

ment of solid tumors” was the synthesis of and physical-chemical characterization of innovative supramolecular vesicular aggregates (SVAs) made up from different combinations of hydrophilic and hydrophobic surfactants or lipids for anticancer treatments.

RECENT PUBLICATION

- Primavera R, Bellotti E, Di Mascolo D, Di Francesco M, Wang J, Kevadiya BD, De Pascale A, Thakor AS, Decuzzi P, Insulin Granule-Loaded MicroPlates for Modulating Blood Glucose Levels in Type-1 Diabetes”, ACS Appl. Mater. Interfaces 2021, 13, 45, 53618-53629, doi: 10.1021/acsami.1c16768.
- Bedingfield SK*, Colazo JM*, Di Francesco M*, Yu F, Liu DD, Di Francesco V, Himmel LE, Gupta MK, Cho H, Hasty KA, Decuzzi P, Duvall CL, “Top-Down Fabricated microPlates for Prolonged, Intra-articular Matrix Metalloproteinase 13 siRNA Nanocarrier Delivery to Reduce Post-traumatic Osteoarthritis”, ACS Nano 2021, doi: 10.1021/acsnano.1c04005.
- Palomba R, Di Francesco M, Di Francesco V, Piccardi F, Catelani T, Ferreira M, Palange A L, Decuzzi P, “Boosting nanomedicine performance by conditioning macrophages with methyl palmitate nanoparticles”, Materials Horizons, 2021, doi: 10.1039/d1mh00937k.
- Di Francesco M*, Bedingfield SK*, Di Francesco V, Colazo JM, Yu F, Ceseracciu L, Bellotti E, Di Mascolo D, Ferreira M, Himmel LE, Duvall C, Decuzzi P, “Shape-Defined microPlates for the Sustained Intra-articular Release of Dexamethasone in the Management of Overload-Induced Osteoarthritis”, ACS Appl. Mater. Interfaces 2021, 13, 31379–31392, doi.org/10.1021/acsami.1c02082.
- Di Francesco M*, Primavera R*, Summa M, Pannuzzo M, Di Francesco V, Di Mascolo D, Bertorelli R, Decuzzi P, “Engineering shape-defined PLGA microPlates for the sustained release of anti-inflammatory molecules”, J Control Release, 2019; 319:201-212. doi: 10.1016/j.jconrel.2019.12.039



Michael Fichter

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Michael Fichter received his Bachelor’s and Master’s degree in Applied Life Sciences at the University of Applied Sciences in Kaiserslautern in 2009 and 2011, respectively. For his Bachelor’s thesis he evaluated the influences of breast milk components on the growth and differentiation of enteric neurons in the group of Prof. Karl-Herbert Schäfer in Zweibrücken. For his Master’s thesis he characterized enteropancreatic interactions and the neuronal regulation of the islets of Langerhans with Sanofi in Frankfurt.

Subsequently, Michael Fichter joined Prof. Stephan Gehring’s lab at the University Medical Center Mainz as a PhD students focusing on the modulation of intrahepatic immune responses through nanoparticle-mediated delivery of drugs, adjuvants, and antigens. After completing his PhD in 2016, he received a fellowship grant by the German Research Foundation and joined Prof. Darrell Irvine’s lab at the Massachusetts Institute of Technology as a postdoctoral researcher. His main research focus was the development of cytokine-based nanogel formulations for the T cell-mediated treatment of liver tumors.

In 2020 he joined Prof. Volker Mailänder’s group where is currently working on the antibody- and nanobody-based targeting of nanoparticles to dendritic cell subtypes as well as on the therapeutic use of adjuvant- and antigen-loaded nanocapsules for the treatment of melanoma within the SFB1066 in Mainz.

RECENT PUBLICATION

- Simon, J.*, Fichter, M.*, Kuhn, G.*, Brückner, M., Kappel, C., Schunke, J., Klaus, T., Grabbe, S., Landfester, K., and Mailänder, V., Achieving dendritic cell subset-specific targeting *in vivo* by site-directed conjugation of targeting antibodies to nanocarriers. Nano Today, 2022. 43: p. 101375. *shared first authors
- 2. Ma, L., Dichwalkar, T., Chang, J.Y.H., Cossette, B., Garafola, D., Zhang, A.Q., Fichter, M., Wang, C., Liang, S., Silva, M., Kumari, S., Mehta, N.K., Abraham, W., Thai, N., Li, N., Wittrup, K.D., and Irvine, D.J., Enhanced CAR-T cell activity against solid tumors by vaccine boosting through the chimeric receptor. Science, 2019. 365(6449): p. 162-168.
- 3. Fichter, M., Piradashvili, K., Pietrzak-Nguyen, A., Pretsch, L., Kuhn, G., Strand, S., Knuf, M., Zepp, F., Wurm, F.R., Mailänder, V., Landfester, K., and Gehring, S., Polymeric hepatitis C virus non-structural protein 5A nanocapsules induce intrahepatic antigen-specific immune responses. Biomaterials, 2016. 108: p. 1-12.
- 4. Fichter, M.*, Dedters, M.*, Pietrzak-Nguyen, A., Pretsch, L., Meyer, C.U., Strand, S., Zepp, F., Baier, G., Landfester, K., and Gehring, S., Monophosphoryl lipid A coating of hydroxyethyl starch nanocapsules drastically increases uptake and maturation by dendritic cells while minimizing the adjuvant dosage. Vaccine, 2015. 33(7): p. 838-46. *shared first authors
- 5. Fichter, M., Baier, G., Dedters, M., Pretsch, L., Pietrzak-Nguyen, A., Landfester, K., and Gehring, S., Nanocapsules generated out of a polymeric dexamethasone shell suppress the inflammatory response of liver macrophages. Nanomedicine, 2013. 9(8): p. 1223-34.



Agnese Fragassi

PhD student

Agnese Fragassi is a PhD student in the Laboratory of Nanotechnology for Precision Medicine at Istituto Italiano di Tecnologia (IIT), directed by Prof. Paolo Decuzzi. Her research activities focus on the development of polymeric micro- and nano-particles for delivering different drugs (anti-

inflammatory drugs and/or growth factors), in order to combine therapeutic activity and regenerative medicine.

She joined IIT in March 2019 as Master Student for doing her thesis work and she achieved her MSc Degree in Pharmacy in November 2019 at the “G. d’Annunzio” University of Chieti – Pescara discussing the thesis “ Spherical Polymeric nanoconstructs loaded with Curcumin and Docetaxel for the treatment of Neuroblastoma”. In this work, she demonstrated that by combining Nanomedicine with combination therapy it’s possible to modulate Neuroblastoma progression with a significant increase in overall survival. After graduating, she started an internship in IIT during which she continued to work on the same research line. In November 2020, she started her PhD in Sciences and Technologies of Chemistry and Materials at University of Genova under the supervision of Prof. Paolo Decuzzi.

RECENT PUBLICATION

- Di Francesco*, M., Fragassi, A. *, Pannuzzo, M., Ferreira, M., Brahmachari, S., & Decuzzi, P. (2022). Management of osteoarthritis: From drug molecules to nano/micromedicines. Wiley Interdisciplinary Reviews: Nanomedicine and Nanobiotechnology, e1780



Valentina Francia

Valentina graduated in molecular biology from the University of Milan, Italy, with a thesis on the cellular mechanisms of resistance to cancer drugs. She defended her PhD in 2018 at the Groningen Research Institute of Pharmacy, the Netherlands, where she investigated the endocytosis of nanomaterials. She was then selected for a joint postdoctoral fellowship between the

laboratory of Prof. Cullis at the University of British Columbia, Canada, and the lab of Prof. Schiffelers at the University Medical Center Utrecht, Netherlands. Her project was focused on unraveling the biomolecular corona of lipid nanoparticles for nucleic acid delivery. Currently, she is a postdoctoral fellow at the University of Basel, in the Laboratory of Nanopharmaceutical & Regulatory Science of Prof. McNeil, working on developing liposomal formulations for lysosomal storage diseases. Valentina is also board member of the Gene Delivery and Gene Editing Focus group of the Controlled Release Society.

RECENT PUBLICATION

- The biomolecular corona of lipid nanoparticles for gene therapy V Francia, RM Schiffelers, PR Cullis, D Witzigmann; *Bioconjugate Chemistry* 31 (9), 2046-2059
- Corona composition can affect the mechanisms cells use to internalize nanoparticles; V Francia, K Yang, S Deville, C Reker-Smit, I Nelissen, A Salvati; *ACS nano* 13 (10), 11107-1112



Hanmant Gaikwad

I am a highly motivated individual with a broad background in Organic Chemistry, with specific experience in synthesis of small molecules, nanomaterial conjugates for biomedical imaging, diagnostics, and therapy. The main focus of research is to develop translational nanoparticles for the imaging and treatment of cancers. As a postdoctoral fellow at Skaggs School of

Pharmacy, University of Colorado, Denver, I synthesized indocyanine lipid conjugates of anticancer drugs. In addition, I synthesized iron oxide nanoparticle conjugates with cleavable linkers for targeted drug delivery.

RECENT PUBLICATION

- Gaikwad, H., Li, Y., Gifford, G., Groman, E., Banda, N. K., Saba, L., Scheinman, R., Wang, G., Dmitri Simberg, D. Complement Inhibitors Block Complement C3 Opsonization and Improve Targeting Selectivity of Nanoparticles in Blood. *Bioconjugate Chemistry* (2020), 31, 1844-1856.
- Wang, G., Gaikwad, H., McCarthy, M. K., Gonzalez-Juarrero, M., Li, Y., Armstrong, M., Reisdorph, N., Morrison, T. E., Simberg, D. Lipid nanoparticle formulation of niclosamide (nano NCM) effectively inhibits SARS-CoV-2 replication *in vitro*. *Precis. Nanomed.* (2021), 4, 724-737.
- Gaikwad, H., Tsvirkun, D., Merquiol, E., Popovtzer, R., Blum, G. (2018) Molecular Imaging of Cancer Using X-ray Computed Tomography with Protease Targeted Iodinated Activity-Based Probes. *Nano Letters*, (18), 1582-1891.
- Gaikwad, H., Wang, G., Smith, W. J., Alexander, K. L., D'Alessandro, A., Zhang, W., Purev, E., Simberg, D. Clickable Methyltetrazine-Indocarbocyanine Lipids: A Multicolor Tool Kit for Efficient Modifications of Cell Membranes. *Bioconjugate chemistry* (2019), 30, 2106-2114.



Elena Gardey

Elena Gardey is a Research Assistant at Department of Internal Medicine IV, Division of Gastroenterology, Hepatology and Infectiology in Jena University Hospital with seven years of experience working as a Senior Microbiologist at Pharmaceutical Company "Belmedpreparaty" in Minsk. She submitted her PhD thesis "Translocation of polymeric nanoparticles through

the gastrointestinal barrier and their uptake by immune cells in inflammatory bowel disease" to the Medical Faculty of Friedrich Schiller University Jena in November 2021. She is interested in targetable nanoparticles for efficient translocation across gastrointestinal barriers. She graduated from Belarusian State University in 2010 with a 5-year Diploma in Biochemistry. During her study she was working as a Research Assistant in Laboratory of Toxicology in Institute of Pharmacology and Biochemistry of the National Academy of Sciences of Belarus. She was born in Minsk, Belarus.

RECENT PUBLICATION

- E. Gardey, F.H. Sobotta, S. Quickert, T. Bruns, J. C. Brendel, A. Stallmach, ROS-Sensitive Polymer Micelles for Selective Degradation in Primary Human Monocytes from Patients with Active IBD. *Macromol Biosci.* (2022), e2100482. doi: 10.1002/mabi.202100482
- E. Gardey, F.H. Sobotta, D. Haziri, P.C. Grunert, M.T. Kuchenbrod, F.V. Gruschwitz, S. Hoepfener, M. Schumann, N. Gaßler, A. Stallmach, J.C. Brendel, Selective uptake into inflamed human intestinal tissue and immune cell targeting by wormlike polymer micelles, (2021). *bioRxiv preprint*; doi: 10.1101/2021.01.26.428316
- E. Gardey, F.H. Sobotta, S. Hoepfener, T. Bruns, A. Stallmach, J.C. Brendel, Influence of Core Cross-Linking and Shell Composition of Polymeric Micelles on Immune Response and Their Interaction with Human Monocytes, *Biomacromolecules* 21(4) (2020) 1393-1406.
- N. Kose-Vogel, S. Stengel, E. Gardey, T. Kirchberger-Tolstik, P.A. Reuken, A. Stallmach, T. Bruns, Transcriptional Suppression of the NLRP3 Inflammasome and Cytokine Release in Primary Macrophages by Low-Dose Anthracyclines, *Cells* 9(1), 79 (2019).



Manuela Gaspar

Maria Manuela de Jesus Guilherme Gaspar is a Researcher at the Faculty of Pharmacy, Universidade de Lisboa and member of the Research Institute for Medicines, iMed.Ulisboa, since January 2009. She completed the graduation in Chemical Engineering in 1991, at Instituto Superior de Engenharia de Lisboa. She obtained her PhD degree in 2005 in Pharmacy, Pharmaceutical Technology, Universidade de Lisboa. Between october 2007 - october 2008 she was a post-doc at School of Pharmacy and Pharmaceutical Sciences, Trinity College, University Dublin, Ireland.

The area of research has been focused on design, development and biological evaluation of drug delivery systems for improving the therapeutic index of incorporated molecules for the treatment of infectious, inflammatory and cancer diseases. Design and characterization of appropriated delivery systems, of lipid and polymeric nature, for different routes of administration namely pulmonary, intranasal and parenteral. *In vitro* and *in vivo* evaluation of low and high molecular weight molecules namely antibiotics (rifabutin, paramomycin) and enzymes (L-Asparaginase, Superoxide Dismutase). *In vitro* and *in vivo* screening of new synthesized molecules and metal-based complexes with antitumor activity. Establishment of infectious and cancer murine models, such as M.

avium, M. tuberculosis, leishmania, MRSA, melanoma, colon cancer, for evaluating the therapeutic effect of developed nanoformulations. She has participated in more than 30 research projects as team member or as PI. She has been supervising PhD, master and undergraduate students. She is co-author of more than 75 articles in journals (Indexed Scopus), book chapters (6) and patents (h-index 25). She is responsible for the animal facilities and member of the welfare body of the Faculty of Pharmacy since 2011. In addition, she also belongs to governing bodies of the Portuguese Society for Laboratory Animal Science.

RECENT PUBLICATION

- M.M. Gaspar, S. Calado, J. Pereira, H. Ferronha, I. Correia, H. Castro, A.M. Tomás, M.E.M. Cruz, Targeted delivery of paromomycin in murine infectious diseases through association to nano lipid systems. *Nanomedicine: Nanotechnology, Biology and Medicine*, 2015, 11 (7), 1851-1860. <https://doi.org/10.1016/j.nano.2015.06.008>.
- J.O. Pinho, I.V. da Silva, J.D. Amaral, C.M.P. Rodrigues, A. Casini, G. Soveral, M.M. Gaspar, Therapeutic potential of a copper complex loaded in pH-sensitive long circulating liposomes for colon cancer management. *Int. J. Pharm.* 2021, 599. <https://doi.org/10.1016/j.ijpharm.2021.120463>
- A. Grenha, A.D. Alves, F. Guerreiro, J. Pinho, S. Simões, A.J. Almeida., M.M. Gaspar, Inhalable locust bean gum microparticles co-associating isoniazid and rifabutin: Therapeutic assessment in a murine model of tuberculosis infection. *Eur. J. Pharm. Biopharm.* 2020, 147, 38-44. <https://doi.org/10.1016/j.ejpb.2019.11.009>.
- J.O. Pinho, J.D. Amaral, R.E. Castro, C.M.P. Rodrigues, A. Casini, G. Soveral, M.M. Gaspar, Copper complex nanoformulations featuring highly promising therapeutic potential in murine melanoma models, *Nanomedicine* 2019, 14 (7), 835-850. <https://doi.org/10.2217/nmm-2018-0388>
- M. Ferreira; S.N. Pinto; F. Aires-da-Silva; A. Bettencourt; S.I. Aguiar; M.M. Gaspar, Liposomes as a Nanoplatfrom to Improve the Delivery of Antibiotics into Staphylococcus aureus Biofilms. *Pharmaceutics*, 2021, 13 (3), 321-321. <https://doi.org/10.3390/pharmaceutics13030321>.



Eduard Gatin

Lecturer, Ph.D
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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 – Biophysics 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 succeeded 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.

RECENT PUBLICATION

- 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* 26 (2) 2020;
- 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.
- E.Gatin, P. Nagy, SM Iordache, AM Iordache, C. Luculescu „Raman Spectroscopy: In vivo Application for Bone Evaluation in Oral Reconstructive (Regenerative) Surgery”, *Diagnostics* 2022, in press.



Shunping Han

PhD student, King’s College London

Shunping is a PhD student in King’s College London. She graduated from Zhejiang Chinese Medical University (China) with a bachelor degree of pharmacy in 2014 and then continued her first MRes study in pharmaceuticals from 2014 to 2017, under the supervision of Prof. Fanzhu Li. During

this 3-year MRes study, she worked on the construction of targeting PAMAM dendrimers and mesoporous silica nanoparticles for glioma therapy. She undertook a second MRes in drug discovery and development at the Chemistry Department, Imperial College London. Her research then focussed on metal organic frameworks (MOFs) synthesis and modification as drug vehicles under the guidance from Dr. Rob Davies, Prof. Paul Lickiss and Prof. Jane Mitchell. Following the graduation with distinction, she was awarded the King’s-China Scholarship Council to undertake her PhD studies under the supervision of Professor Khuloud Al-Jamal, The Institute of Pharmaceutical Science, KCL. Her current project involves the investigation of the biodistribution of functionalized gold nanostructures for brain targeting.

RECENT PUBLICATION

- Helal D O, Rouatbi N, Han S, et al. A natural protein based platform for the delivery of Temozolomide acid to glioma cells[J]. *European Journal of Pharmaceutics and Biopharmaceutics*, 2021, 169: 297-308.
- Abdel-Bar H M, Walters A A, Lim Y, Rouatbi N, Qin Y, Gheidari F, Han S, et al. An “eat me” combinatory nano-formulation for systemic immunotherapy of solid tumors[J]. *Theranostics*, 2021, 11(18): 8738.
- Giram P S, Wang J T W, Walters A A, Rade P P, Akhtar M, Han S, et al. Green synthesis of methoxy-poly (ethylene glycol)-block-poly (L-lactide-co-glycolide) copolymer using zinc proline as a biocompatible initiator for irinotecan delivery to colon cancer *in vivo*[J]. *Biomaterials Science*, 2021, 9(3): 795-806.



Bettina Heiss

I was born on 27.02.1991 in Gräfelting, Germany. After going to Primary School in Ohlstadt, I continued my education at the Werdenfels Gymnasium in Garmisch-Partenkirchen. After having spent the 10th grade abroad at Aspen High School in Colorado, USA, from 2007 to 2008, I gained my A levels (Allgemeine Hochschulreife, Grade

2.1) in 2010. From 2010 to 2014 I studied Biology at the University of Regensburg, finalized by getting a Bachelor of Science degree (Grade 2.0). From July to September 2014, I was engaging in a 10-week internship at InGeneron in Houston, Texas, USA, focusing on stem cells research (harvest, cell culture, histology) and getting an insight on clinical application. Afterwards I changed profession and enrolled in veterinary-studies program at Justus-Liebig Universität of Gießen. I achieved my veterinarian diploma (Tierärztliche Approbation, Grade 1.9) in March 2019. During my vet-studies I have enrolled in several internships as well as worked in the intensive care unit of the clinic of small animals (Famulatur, JLU Gießen) to enhance my practical knowledge. Since April 2019, I am working on my doctoral thesis in the AG Liposomen of Prof. Lars Lindner at the Klinikum Großhadern (Ludwig-Maximilians-University, Munich), where I engage in the research of thermosensitive Liposomes (TSL) and heat-induced drug release. Since August 2019 I am also specializing in laboratory animals (Fachtierarzt für Versuchstierkunde) under the external supervision of Dr. Susanne Sauer.



Satinderdeep Kaur

My name is Satinderdeep Kaur. I am originally from India which is culturally very rich and diverse country. Being interested in Science, I took admission in B.Sc Biotechnology (2009) in Punjab University, Chandigarh, India. After my under graduation, I was more keen to do Masters in Microbiology to study in more depth about Virus, Plant, Animal Microbiology as well as Mycology.

Fortunately, I got admission in Guru Nanak Dev University (2012) located in my dream city Amritsar which is considered as a holy city in my country. I enjoyed my 2 years of study there and realized my interests are more in research. After finishing my Masters, I started to apply for Research fellow positions. I got the opportunity to join Dr. Jagmohan Singh's group in CSIR-IMTECH, Chandigarh. I worked on very interesting project 'A Universal Expression Platform for production of low cost biotherapeutic proteins in fission yeast'. We developed strong expression vectors by using *Schizosaccharomyces pombe* to make it best model for basic research which would be useful for recombinant gene expression and protein production. I worked with fission yeast system for almost 2 years and then I thought to work with baker's yeast, *S.cerevisiae* as well. In same Institute, I joined Dr. Deepak Sharma's lab who were more focused on Chaperone Biology and Protein Biochemistry of *S.cerevisiae*. I 'validated potential biomolecules against Parkinson's disease'. It was lovely to work with Dr. Sharma's group who were celebrating birthdays, student's PhD or Post-Doc success parties or big Indian festivals after work. Till that time, I had lost my close one due to stroke which deep-down was motivating and provoking me to work on stroke. Coincidentally, I saw the PhD position advertised by Dr. Zahraa Al-Ahmady about therapeutic approaches for post-stroke infections. In 2019, I got selected for PhD position by Dr. Zahraa in Nottingham Trent University. In NTU, I am working on 'Nanomedicine-Based Approaches to Prevent Post-Stroke Infections' the project brought me here across the oceans. It's blessing to work with Dr. Zahraa who never realize me that I am away from my family and friends, especially during this pandemic. I am very excited to attend this Clinam Summit to know more about this emerging nanomedicine field.

RECENT PUBLICATION

- Sensitive detection of *E. coli* by long period grating based optical sensor (AIP proceedings 1724, 02011 2016). Siddharth Kaushik, Umesh Tiwari, Satinderdeep Kaur, Rajesh, A.K Paul
- Expression vectors with altered backbone giving enhanced expression of heterologous proteins in fission yeast. (Indian Appl. No. 201811000716, Appl. No PCT/IN2019/050014, Filing date for PCT 8 Jan, 2019) Suchita Srivastava, Satinderdeep Kaur, Suman Saini, Jagmohan Singh

- Reciprocal relation between reporter gene transcription and translation efficiency in fission yeast (doi: 10.1016/j.plasmid.2021.102557. Epub 2021 Feb 2) Suchita Srivastava, Satinderdeep Kaur, Hemant K Verma, Suman Saini, Manish Thakur, Swat Haldar, Jagmohan Singh
- The Yeast Hsp70 Cochaperone Ydj1 Regulates Functional Distinction of Ssa Hsp70s in the Hsp90 Chaperoning Pathway (doi: 10.1534/genetics.120.303190. Epub 2020 Apr 16) Deepika Gaur, Prashant Singh, Jyoti Guleria, Arpit Gupta, Satinderdeep Kaur, Deepak Sharma
- Lipid Nanoparticles Offer a Selective Targeting Strategy for Peripheral Inflammation Following Brain Injury (under communication) Zahraa S. Al-Ahmady, Laura McCulloch, Satinderdeep Kaur, Mohammed Abdulmaksoud, Dhifaf Jasim, Barry McColl, Stuart M. Allan, Kostas Kostarelos
- Re-directing Nanomedicines to the Spleen: A Potential Technology for Peripheral Immunomodulation (under communication) Satinderdeep Kaur, Stuart M. Allan, Zahraa S. Al-Ahmady



Joshua Krehan

Joshua Krehan completed his Masters and Bachelors degree in chemistry at the Johannes Gutenberg University Mainz. After completing his master thesis at the Max Planck Institute for Polymer Research with a focus on polymeric micelles for drug delivery applications, he joined the group of Prof. Dr. Andreas Walther as a PhD student in March 2021. His current research interests

include the development of smart carrier systems with encapsulated pH-effects and pH-modulation systems for cancer immunotherapy. He is part of the collaborative research center 1066.



Lekshmi G. Kumar

Phd Student Amrita Centre for Nanosciences and Molecular medicine, Amrita Vishwa vidyapeetham University

Research description: Presently working in the development of an oral nanoformulation of small molecule inhibitors with enhanced kinetics and reduced toxicity for cancer therapy. The research includes

preparation and characterization of protein based nanoformulation using GMP compliant process and further testing is done in animals. Animal testing comprises of pharmacokinetic analysis, tissue distribution analysis, toxicological analysis and efficacy studies in tumor models.

Purpose of work

- Clinical translation, bench side to bed side, of developed oral nanoformulation by establishing cGMP compliant scalable process methods.
- The technology can be utilized as a platform to improve the oral bioavailability of poorly soluble drugs
- The study was funded by Department of Biotechnology, Govt. of India and the second phase has been approved for regulatory toxicity studies prior to Phase I clinical trials (funded by Department of Biotechnology, Govt. of India).

Area of Expertise

- Preparation of different protein, polymer and lipid based nanoformulations.
- Various characterization techniques like DLS, SEM, TEM, FESEM, XRD for nanoformulation.
- Invitro assays such as drug screening cytotoxicity assays and syn-

- ergistic studies, cell uptake and cell death studies.
- Imaging techniques like fluorescent microscopy, confocal microscopy.
- Animal handling, conducting different studies in animals like pharmacokinetics studies, toxicity studies as per Schedule Y
- Development of tumor models; subcutaneous and orthotopic models.
- Development and validation of HPLC protocols for various molecules.

RECENT PUBLICATION

- S. Maya, L. G. Kumar, B. Sarmento, N. Sanoj Rejinold, D. Menon, S. V. Nair and R. Jayakumar, Carbohydrate Polymers, 2013, 93, 661– 669 (MTech Thesis work)
- Enhanced Oral Bioavailability and Antitumor Therapeutic Efficacy of Sorafenib - current status: manuscript submitted



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 enzen[®]-therapeutic enzymes. Emilie is now Chief Development Officer at Perseo pharma.



Revadee Liam-Or

Final year PhD candidate

Revadee Liam-Or graduated from Mahidol University, Thailand, with a Doctor of Pharmacy degree. She worked as a pharmacist for the Government Pharmaceutical Organisation (Thailand) to maintain and improve Quality Standards of pharmaceutical products. She then continued studying for

a master's degree in Pharmaceutical Technology at King's College London (KCL). During her master's, she joined Al-Jamal's lab working on exosomes to determine factors controlling their uptake in cells as drug delivery systems. Upon completing her master's, she was awarded the PGR International Scholarship to pursue her PhD studies in Pharmaceutical Science at KCL (success rate 1%). She has been working with exosomes more than 4 years. Her current project involves exploration of mesenchymal stem cell derived exosomes for drug delivery systems and regenerative medicine.

RECENT PUBLICATION

- Faruqu FN, Liam-Or R, Zhou S, Nip R, Al-Jamal KT. (2021) Defined serum-free three-dimensional culture of umbilical cord-derived mesenchymal stem cells yields exosomes that promote fibroblast proliferation and migration *in vitro*. FASEB J. 35(1):e21206. doi: 10.1096/fj.202001768RR.
- Xu L, Faruqu FN, Lim YM, Lim KY, Liam-Or R, Walters AA, Lavender P, Fear D, Wells CM, Tzu-Wen Wang J, Al-Jamal KT. (2020) Exosome-mediated RNAi of PAK4 prolongs survival of pancreatic cancer mouse model after loco-regional treatment. Biomaterials. 264:120369. doi: 10.1016/j.biomaterials.2020.120369.

- Xu L, Faruqu FN, Liam-Or R, Abu Abed O, Li D, Venner K, Errington RJ, Summers H, Wang JT, Al-Jamal KT. (2020) Design of experiment (DoE)-driven *in vitro* and *in vivo* uptake studies of exosomes for pancreatic cancer delivery enabled by copper-free click chemistry-based labelling. J Extracell Vesicles. 9(1):1779458. doi: 10.1080/20013078.2020.1779458.



Medina-Montano Carolina

Ms. Medina-Montano has graduated in Microbiology and Bio-analysis at the University of Antioquia in Medellin, Colombia. Ms. Medina-Montano's carrier started in Colombia by working in clinical laboratories of different hospitals. Back then she discovered her passion and interest for biomedical research, and therefore decid-

ed to move to Germany to pursue her studies. Ms. Medina-Montano came to Germany 9 years ago to continue her studies in Biomedicine at the Johannes Gutenberg University in Mainz. After she finished her Master degree, she worked at the University Medical Centers in Mainz and in Frankfurt on several topics, including tropical medicine, translational immunology and hemato-oncology.

In December 2019, she started her PhD in Nano-medicine in the research laboratory of Professor Grabbe in the Department of Dermatology of the University Medical Center in Mainz. She is working on several nanoparticle-relateds projects, which include collaborations with other 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 speak Spanish as mother tongue, English and German.



Aldona Mzyk

I am an early career researcher with a background in biotechnology and materials science. I enjoy working on fundamental scientific questions, but the real fun starts for me when it comes to bringing science into the industrial and clinical reality. Recently I've realized that I became pretty pragmatic over the years in academia. When I started my romance with science,

it was more about 'tell me all about how it works'. However, there is limited amount of money for 'how it works' so I had to switch towards 'make it work' and this is how I have been awarded with two polish 3-year scholarships to pursue my PhD thesis. In consequence, I have developed biomimetic coatings, which is the subject of my ongoing collaboration with the Foundation for Cardiac Surgery Development on functionalization of the polish Religa Heart[®] assistant devices.

After my PhD I was hired as an assistant professor in the Institute of Metallurgy and Materials Science Polish Academy of Sciences (IMMS PAS, Kraków in Poland), where I have been working on biomimetic materials and surface engineering methods to be applied in cardiovascular implants and devices. I was lucky to be awarded with a personal grant from the Polish National Science Centre to pursue fundamental research on cardiac progenitor cells response to biomaterials. The biomaterial part of my project found interest of the 3D printing company – ATMAT Ltd. We have secured funding from the Polish National Centre for Research & Development, which gives me the opportunity to work part-time for the company. In September 2019, I have joined prof. Romana Schirhagl group in the BME department at the UMCG in Groningen, The Netherlands

as postdoctoral researcher to work on perfectly fundamental topics with imperfect nanodiamonds. However, it has turned out that my drive to see things in action, built my appetite in running more and more projects that have clinical relevance. Currently, I am running a few clinical projects focused on monitoring drug efficacy with nanodiamond magnetometry.



Jennifer Oberländer

Jennifer Oberländer is a Ph.D. candidate at the Max Planck Institute for Polymer Research in the Department of Physical Chemistry of Polymers under the supervision of Prof. Dr. Katharina Landfester and Prof. Dr. Volker Mailänder. She studied biomedical chemistry at the Johannes Gutenberg-University Mainz and received her M.Sc. in 2019. After her Master thesis

at the Institute for Pharmacology at the University Medicine Center Mainz she joined the group of Prof. Landfester in August 2019. Her research is focused on the formation of the protein corona on nanocarrier.



Soraia Pinto

Soraia Pinto got her Master Degree in Pharmaceutical Sciences at Faculty of Pharmacy, University of Porto, in 2018. From 2014 to 2017, she was volunteer researcher at Department of Applied Chemistry, Faculty of Pharmacy, University of Porto, where she had the opportunity to learn different methodologies and techniques related with the production and

characterization of drug-loaded nanosystems. In 2017, she had the chance to integrate the curricular unit of Project I, at Department of Applied Chemistry, Faculty of Pharmacy, University of Porto, where she developed an experimental work in the project entitled "Nanostructured lipid carriers as a promising drug delivery system for streptomycin". In 2019, she was congratulated with a master scholarship in a group specialized in the production and characterization of nanosystems, Nanomedicines and Translational Drug Delivery (NTDD) Group, at Instituto Nacional de Engenharia Biomédica (INEB)/Instituto de Investigação e Inovação em Saúde (i3S). In 2020, Soraia becomes a PhD student in NTDD group at i3S/INEB under the supervision of Professor Bruno Sarmento. Her research interests focused on the nanotechnology field, specifically in the development of peptide-loaded nanosystems, surface functionalized with molecules that target an intestinal receptor, the neonatal Fc receptor (FcRn), for the treatment of diabetes mellitus. Since 2019, Soraia was involved in the written of three review papers entitled "In vivo, ex vivo and *in vitro* assessment of buccal permeation of drugs from delivery systems", "Prevention of diabetes-associated fibrosis: Strategies in FcRn-targeted nanosystems for oral drug delivery" and "Mucus-producing 3D cell culture models". Furthermore, Soraia was part of a team with more three researchers from NTDD group that won the Spin Your Thesis 2020 Program Award from European Space Agency, due to the submission of a project with the aim to assess the effect of hypergravity in the intestinal permeability of nanosystems. This project resulted in one publication entitled "The effect of hypergravity in the intestinal permeability of nanoformulations and molecules". Likewise, Soraia is a member of the Controlled Release Society and Sociedade Portuguesa de Diabetologia, and she was involved in the organizing committee of the PhD students' seminars and in the PhDay conference of i3S (Porto, Portugal).



Arathyram Ramachandra Kurup Sasikala

Arathyram Ramachandra Kurup Sasikala is a Marie Curie Fellow at the School of Pharmacy, University of Birmingham. She has completed a master's degree in Physics and pressed ahead an interdisciplinary research career in Nanomedicine. She received her PhD from the Department of Bionanosystem Engineering, Chonbuk National University, South Korea in 2016 with the dissertation entitled 'Controlled Synthesis and Surface Tailoring of Iron Oxide Nanoparticle-Based Nanocomposites for Cancer Theranostics'.

During her PhD training, she gained experience in innovative biomaterial synthesis methods, cutting edge characterisation techniques, developed new protocols to physically tailor biomaterials for cancer theranostics and regenerative application. After completing her PhD, she joined as an Assistant Research Professor at Chonbuk National University as she was awarded a 3-year funded project from National Research Foundation (NRF) of Korea, on the 'Development of Blood Flow Driven Piezoelectric Nanogenerator for *in vivo* Powered Smart Stent'. The main objective of this research was the development of a piezoelectric nanogenerator built-in drug eluting stent for the controlled local release of anti-proliferative agents along with real-time monitoring of patients' health status.

Currently, as a Marie Curie research fellow, she is addressing the unmet need of delivering drugs to the brain by developing new generation multiferroic nanomaterials to cross the blood-brain barrier (BBB), to systemically treat aggressive brain tumours. Due to the ability of multiferroic nanomaterials to convert mechanical energy into electricity, they act as remotely controlled nano transducers under low-intensity ultrasound (US) and convert US signals to electrical signals to create temporary pores in the cell membranes of the BBB, via a mechanism termed "Nano-electroporation". As part of the project, she is developing transwell co-culture BBB models and multicellular spheroidal BBB models

She has obtained ~£350K in Principal Investigator research income to date with 18 publications, including 10 first author and 3 senior author (h-index 12; 748 citations). A recent highlight includes a senior author publication in *Nanoenergy* (July 2021). She has edited a book entitled 'Biomimetic Nanoengineered Materials for Advanced Drug Delivery', Elsevier (2019) and contributed to 4 book chapters. She has communicated my findings to the international scientific community through several oral presentations and gained considerable teaching and outreach experiences. She is a STEM ambassador of West Midlands, UK, and actively take part in public engagement. She presented at the 'Curiosity Carousel in European Researchers' Night hosted by the University of Bristol and took part in the British Science week celebration.



Nadia Rouatbi

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

Nadia obtained her BSc in Biomedical Engineering from the University of Genoa, Italy. She undertook a two-year MSc in Bioengineering (Professors Laura Pastorino and Orietta Monticelli). Her MSc project focussed on the development of multi-compartment hydrogels for the local delivery of chemotherapeutic drugs for the treatment of Glioblastoma. She spent 9 months as part of an ERASMUS+ internship at Professor Al Jamal's group, Institute of Pharmaceutical Science, King's College London during her MSc degree. Nadia's PhD project focusses on the development of

lipid-based nanoparticles for the *in vivo* gene-editing for brain cancer therapy.

RECENT PUBLICATION

- Rouatbi, N., Tasneem McGlynn & Al-Jamal, K. T. (2021). Pre-Clinical Non-Viral Vectors Exploited for In Vivo CRISPR/Cas9 Gene Editing: An Overview. *Biomaterial Science*. Accepted.
- Walters AA*, Santacana-Font G, Li J, Routabi N, Qin Y, Claes N, Bals S, Wang JT and Al-Jamal KT*. (2021) Nanoparticle Mediated in Situ Molecular Reprogramming of Immune Checkpoint Interactions for Cancer Immunotherapy. *ACS Nano*, doi.org/10.1021/acsnano.1c04456 (IF 15.88)
- Helal DO, Rouatbi N, Han S, Tzu-Wen Wang J, Walters AA, Abdel-Mottaleb MMA, Kamel AO, Geneidi AS, Awad GAS, Al-Jamal KT*. (2021). A Natural Protein Based Platform for the Delivery of Temozolomide Acid to Glioma Cells. *Eur J Pharm Biopharm*. S0939-6411(21)00262-9. doi: 10.1016/j.ejpb.2021.10.007. (IF 5.57)
- Abdel-Bar HM, Walters AA, Lim Y, Rouatbi N, Qin Y, Gheidari F, Han S, Osman R, Wang J, Al Jamal KT*. (2021) An "Eat me" Combinatory Nano-formulation for Systemic Immunotherapy of Solid Tumours. *Theranostics*. doi:10.7150/thno.56936; doi:10.7150/thno.56936. (IF 11.55).
- Rouatbi N, Lim YM, Grant V, Pollard S, Wang J and Al-Jamal KT*. (2019) CRISPR/Cas9 gene editing of brain cancer stem cells using lipid-based nano-delivery. *Neuro-Oncology*, Oxford University Press.21 (supplement 4): iv3-iv3. (IF 12.3)



Jenny Schunke

After successfully completing my bachelor's degree in Molecular Biology (2017), I started to study Biomedical Sciences. During my master thesis (2019), I focused on the investigation of treatment resistances in penile carcinoma. Since October 2019, I am a Doctoral candidate of the AG Mailänder at the University Medical Center in Mainz. My PhD project is settled in the field of nanoparticle-based immunotherapies. Specifically, I am targeting dendritic cells (professional antigen presenting cells) with adjuvant-loaded protein-based nanocapsules for antigen-specific activation of T cells to generate an effective antitumor response in melanoma mouse models.



Giovanni Settanni

Giovanni Settanni was born in Italy in 1973 and grew up in Turin. He graduated with full marks "cum Laude" in Physics in 1997 from the University of Turin, Italy with a master thesis in computational Neuroscience supervised by Dr. Alessandro Treves from the International School of Advanced Studies (SISSA) in Trieste. Just after his master in 1997, he was awarded a PhD fellowship at SISSA, Trieste, Italy, under the supervision of Prof. Amos Maritan. He got his PhD in 2001 with full marks "cum Laude" with a thesis about "The role of native state topology in protein folding and dynamics". After the PhD, Giovanni worked as a Postdoc (2001-2004) in the Biochemistry department of the University of Zurich in Switzerland under the supervision of Prof. Amedeo Caffisch. There he met Prof. Andreas Plueckthun, with whom he coauthored several high impact publications on the folding properties of consensus-designed ankyrin repeat proteins. After Zurich, Giovanni moved to Cambridge, UK, with a fellowship to work with Prof. Sir Alan R. Fersht at the MRC-Centre for Protein

Engineering (2005-2010), where he continued his research on protein folding and dynamics using computational methods. In 2010 Giovanni moved to the Physics department of the University of Mainz, Germany, where he has been leading a small group working on computational biophysics with a particular focus on the interactions between biomolecules and nanomaterials, collaborating with Prof. Dr. Friederike Schmid. In Mainz, Giovanni has been working within the SFB1066 since its first funding period, collaborating with Prof. Dr. Katharina Landfester and Prof. Dr. med. Volker Mailänder from the MPI for Polymer research, Prof. Dr. Matthias Barz from the Chemistry department of the University of Mainz, and, more recently, with Dr. Heinrich Haas and Dr. Wolfgang Brill from BioNTech.

RECENT PUBLICATION

- G Settanni, W Brill, H Haas, F Schmid, pH-Dependent Behavior of Ionizable Cationic Lipids in mRNA-Carrying Lipoplexes Investigated by Molecular Dynamics Simulations, *Macromolecular Rapid Communications*, 2021, 2100683
- MR Bauer, A Krämer, G Settanni, RN Jones, X Ni, R Khan Tareque, et al., Targeting cavity-creating p53 cancer mutations with small-molecule stabilizers: The Y220X paradigm, 2020, *ACS chemical biology* 15 (3), 657-668
- G Settanni, T Schäfer, C Muhl, M Barz, F Schmid, Poly-sarcosine and poly (ethylene-glycol) interactions with proteins investigated using molecular dynamics simulations, 2018, *Computational and Structural Biotechnology Journal* 16, 543-550
- G Settanni, J Zhou, T Suo, S Schöttler, K Landfester, F Schmid, et al., Protein corona composition of poly (ethylene glycol)-and poly (phosphoester)-coated nanoparticles correlates strongly with the amino acid composition of the protein surface, 2017, *Nanoscale* 9 (6), 2138-2144
- S Köhler, F Schmid, G Settanni, The internal dynamics of fibrinogen and its implications for coagulation and adsorption, 2015, *PLoS computational biology* 11 (9), e1004346



Valeria Sidorenko

Ph.D. student

After finishing my bachelor's studies in the field of microbiology and genetic engineering (2017), I started my master's in the field of biomedicine and joined the Laboratory of Precision and Nanomedicine (www.cancerbiology.ee) at the Institute of Biomedicine and Translational Medicine, University of Tartu. During the studies, I gained experience in the synthesis and characterization of polymeric nanoparticles, nano-encapsulation of drugs, 2D and 3D cell cultures, confocal and electron microscopy, and mice work. In my master thesis, I optimized the encapsulation of anticancer drugs inside polymersomes (PS) and the density of receptor-specific ligands on the surface of PS for specific targeting and killing of tumor cells. The results showed that our tumor penetrating peptide-targeted polymersomes can be used for tumor detection, imaging, and treatment, providing higher antitumor efficacy and fewer adverse effects than the current chemotherapy. The thesis received First Prize in the Estonian National Contest for University Students, in the field of Health Research amongst master theses (2019). Inspired by the gathered results, I decided to continue my studies by undertaking a Ph.D. in Prof. Teesalu's group. The goal of my doctoral studies is to create innovative drug candidates used in targeted cancer therapy, with the potential clinical application, using a novel anthracycline developed by Toxinvent LLC, which possesses a potent anticancer effect. The drug candidate utorubicin (UTO) is protected by a patent (WO2017207540A1) and a patent application for nanoformulated UTO has also been filed (TD40725). My role in this project is to develop, optimize, and preclinically validate

the UTO nanoplatform in cancer cells (*in vitro*) and cancer-bearing mice (*in vivo*). As a result, we have developed a cancer-specific UTO-nanoplatform that shows therapeutic activity in peritoneal carcinomatosis-bearing mice.

RECENT PUBLICATION

- Simón-Gracia, L., Loisel, S., Sidorenko, V., Scodeller, P., Parizot, C., Savier, E., Haute, T., Teesalu, T., & Rebollo, A. (2022). Preclinical Validation of Tumor-Penetrating and Interfering Peptides against Chronic Lymphocytic Leukemia. *Molecular Pharmaceutics*.
- Simón-Gracia, L., Sidorenko, V., Uustare, A., Ogibalov, I., Tasa, A., Tshubrik, O., & Teesalu, T. (2021). Novel Anthracycline Utorubicin for Cancer Therapy. *Angewandte Chemie - International Edition*, 60(31).
- Tobí, A., Willmore, A. A., Kilk, K., Sidorenko, V., Braun, G. B., Soomets, U., Sugahara, K. N., Ruoslahti, E., & Teesalu, T. (2021). Silver Nanocarriers Targeted with a CendR Peptide Potentiate the Cytotoxic Activity of an Anticancer Drug. *Advanced Therapeutics*, 4, 2000097.
- Lepland, A., Ascitutto, E. K., Malfanti, A., Simón-Gracia, L., Sidorenko, V., Vicent, M. J., Teesalu, T., & Scodeller, P. (2020). Targeting Pro-Tumoral Macrophages in Early Primary and Metastatic Breast Tumors with the CD206-Binding mUNO Peptide. *Molecular Pharmaceutics*, 17(7), 2518–2531.
- Simón-Gracia, L., Scodeller, P. D., Fuentes, S. S. S., Vallejo, V. G. V. G., Ríos, X., Sebastián, E. S., Sidorenko, V., di Silvio, D., Suck, M., de Lorenzi, F., Rizzo, L. Y. L. Y., Stillfried, S., Kilk, K., Lammers, T., Moya, S. E. S. E., Teesalu, T., San Sebastián, E., Sidorenko, V., di Silvio, D., ... Teesalu, T. (2018). Application of polymersomes engineered to target p32 protein for detection of small breast tumors in mice. *Oncotarget*, 9(27), 18682–18697.



Alice Spadea

Alice's highly multidisciplinary research focuses on nanomedicine and drug delivery. She obtained Bachelor degree in Biology (2010) and Master degree in Molecular Biomedical Science (2012) at the University of Perugia in Italy. Her experimental theses were on PLGA nanoparticles as drug delivery systems for enzymatic replacement therapy for genetic diseases. Afterwards, she was awarded a scholarship to spend at the University-Hospital of Udine (Italy), where she worked on brain cancer (2013). Then, she was selected over 70 candidates for a PhD scholarship funded by Astrazeneca and Cancer Research UK, so she moved to the University of Manchester to work on Chitosan/Hyaluronic acid nanoparticles (CS/HA NPs) for cancer hypoxia targeting (2014-18). Part of her PhD work was published in 2019 (Spadea A. et al., Evaluating the Efficiency of Hyaluronic Acid for Tumor Targeting via CD44. *Mol Pharm.* 2019 Jun 3;16(6):2481-2493) and presented at conferences, as selected speaker, including the British Society of Nanomedicine (BSNM) Early Career Researcher Meeting (ECRM) at the Crick Institute in London in 2018. In 2020 she was awarded £5000 from Advanced Materials in Medicine at Manchester (AMM) - Early Career Researcher Funding Scheme – after she applied to complete an *in vivo* study from her PhD work on CS/HA NPs, which was successfully completed.

Alice is currently a postdoctoral researcher at the North West Centre of Advanced Drug Delivery (NoWCADD), a joint venture between the University of Manchester and AstraZeneca (2018 to present). She has worked on different projects including preparation of RGD-based polymeric nanoparticles using microfluidic systems for tumour targeting. While this work was being published (Rios De La Rosa JM, Spadea A. et al., Microfluidic-assisted preparation of RGD-decorated nanoparticles: exploring integrin-facilitated uptake in cancer cell lines. *Sci Rep.* 2020 10, 14505) and presented to national conferences as selected speaker, including the BSNM

ECR virtual meeting in 2020, she also worked on studying biological systems with Raman, Infrared and Photothermal spectroscopy, producing another important scientific output (Spadea A, et al. Analysis of Fixed and Live Single Cells Using Optical Photothermal Infrared with Concomitant Raman Spectroscopy, *Analytical Chemistry* 2021 93 (8), 3938-3950). Her current project is on studying interactions between lipid nanoparticles (LNPs) and model membranes to get novel insights on endosomal escape. This work that uses for the first time reflectometry techniques to understand physical-chemical interactions between lipids occurring during this crucial step in RNA delivery, has been presented at the prestigious Controlled Release Society (CRS) Virtual Annual Meeting in 2021 and has been submitted to a high impact journal (*ACS Applied Materials and Interfaces*).

During her post-doctoral years, Alice contributed to the organisation of conferences, as the NoWCADD conference in Manchester entitled "Advanced Drug Delivery: crossing the interdisciplinary divide" (2019), outreach activities (University of Manchester FLS Community Open Days, Manchester Pharmacy School, Cancer Treatment: from bench to bedside a pathway to a cure, Science Spectacular), welcoming events and career events for students (Undergraduate Research day). She has also been part of the Pharmacy Postgraduate Committee and she is currently the Research staff rep for her division, which allowed her to create a link between researchers and academics, sharing experiences, concerns, suggestions and, mainly, playing an active role in the development of the division and the staff organising research events.

Beside research, teaching is the other Alice's main interest. She started as demonstrator, supervising undergrad Pharmacy students during lab practicals (2015/16). As a postdoc, she enhanced her teaching experience leading group based learning (GBL) tutorials for small groups of students (2019-20), lab practicals for MRes students (2020), final year MSci Journal clubs (2021).



Katerina Spyridopoulou

Katerina Spyridopoulou is a molecular biologist and a postdoctoral researcher working as a Research Fellow for the EU co-funded project, 'InTechThrace: Integrated Technologies in biomedical research: multilevel biomarker analysis in Thrace' (Regional Excellence Action) at the Department of Molecular Biology and Genetics of the Democritus University of Thrace in

Greece. She graduated in 2011 from the same department, while in 2013 she completed her Master's degree in Clinical Pharmacology and Therapeutics, in the Department of Medicine of the same university. She obtained her doctorate in 2018 under the supervision of Dr. Katerina Chlichlia (Prof. of Molecular Immunology) from the Department of Molecular Biology and Genetics of DUTH, where she has since been conducting postdoctoral research. Among her scientific interests are the biomedical applications of magnetic and selenium nanoparticles in cancer prevention and therapy. Her work focused mainly on the development of novel therapeutic approaches against colon cancer, based on externally stimulated magnetic nanoparticles. More specifically, she studied the effect of magneto-mechanical stress applied via the actuation of endocytosed magnetic nanoparticles in cancer cells. Furthermore, she developed a protocol for improving the transplantable syngeneic cancer model in mice based on the magnetic manipulation of implanted cells employing magnetic nanoparticles. Moreover, by employing green synthesis, she has developed a protocol for the production and purification of biogenic selenium nanoparticles with antitumor and immunomodulatory activities, using *Lactobacillus casei*. In parallel, she served as a Biochemistry lecturer at the Aristotle University of Thessaloniki (AUTH) and has given several talks on Nanotechnology and Nanomedicine for university courses and workshops. Dr. Spyridopoulou is co-author of fourteen manuscripts

published in peer-reviewed international scientific journals and she has presented her work in several International Conferences.

RECENT PUBLICATION

- Katerina Spyridopoulou, Georgios Aindelis, Aglaia Pappa, Katerina Chlichlia. "Anticancer Activity of Biogenic Selenium Nanoparticles: Apoptotic and Immunogenic Cell Death Markers in Colon Cancer Cells." *Cancers* (Basel), 13(21), 5335 (2021).
- Katerina Spyridopoulou, Eleni Tryfonopoulou, Georgios Aindelis, Petros Ypsilantis, Charalampos Sarafidis, Orestis Kalogirou, Katerina Chlichlia. "Biogenic selenium nanoparticles produced by *Lactobacillus casei* ATCC 393 inhibit colon cancer cell growth *in vitro* and *in vivo*." *Nanoscale Adv* 3(9), 2516–28 (2021).
- Georgios Aindelis, Angeliki Tiptiri-Kourpeti, Evangeliki Lampri, Katerina Spyridopoulou, Eleftheria Lamprianidou, Ioannis Kotsianidis, Petros Ypsilantis, Aglaia Pappa, Katerina Chlichlia. "Immune Responses Raised in an Experimental Colon Carcinoma Model Following Oral Administration of *Lactobacillus casei*." *Cancers* 12(2), 368 (2020).
- Katerina Spyridopoulou, Georgios Aindelis, Evangeliki Lampri, Maria Giorgalli, Eleftheria Lamprianidou, Ioannis Kotsianidis, Anastasia Tsingotjidou, Aglaia Pappa, Orestis Kalogirou, Katerina Chlichlia. "Improving the subcutaneous mouse tumor model by effective manipulation of magnetic nanoparticles-treated implanted cancer cells." *Annals of Biomedical Engineering* 46(12), 1975-1987 (2018).
- Katerina Spyridopoulou, Antonios Makridis, Nikolaos Maniotis, Nikoleta Karypidou, Eirini Myrovali, Theodoros Samaras, Makis Angelakeris, Katerina Chlichlia, Orestis Kalogirou. "Effect of low frequency magnetic fields on the growth of MNPs-treated HT29 colon cancer cells." *Nanotechnology* 29(17), 175101 (2018).



Martin Telefont

Martin Telefont completed his academic education with a PhD in Neuroscience using technics ranging from molecular biology to animal behavior. During his time as researcher for the EPFL Blue Brain Project (2010 – 2015) he supported development and adoption of best practices in neuroinformatics and data curation. Between 2015 and 2021 he working in the EPFL unit

tasked with improving coordination of the Human Brain Project. Since the summer of 2021 he continues this work in EBRAINS to advance the development of the European Research Infrastructure for Brain Research.



Theresa Vogel

I was born on 26th August 1995 in Würzburg. I am a chemist currently employed at the University Clinic in Würzburg as a PhD student.

I am part of the research group of Prof. Dr. Jürgen Groll at the University of Würzburg where I work in the nano-biotechnology subgroup as a PhD student under the supervision of Dr. Krystyna Albrecht. With

my collaboration partners from the workgroups of Prof. Andreas Beilhack, MD (Würzburg University Hospital) and Prof. Dr. med. Oliver Kurzai (Würzburg University Hospital) I am contributing to research in the field of using polymer-based nanoparticles to treat fungal infections. Within these collaborations and my own research, I am able to apply skills and knowledge that I learned during the chemistry studies. Currently, I am continuously gaining new expertise and knowledge in the field of nanomedicine.

After finishing high school, I started the bachelor's degree in chemistry at TU Dresden. Here I first learned about the broad field of polymer chemistry and its wide range of applications. During my research and writing of my bachelor thesis with the topic "Cationic poly(2-oxazoline)s for application in gene therapy" under the supervision of Prof. Rainer Jordan I first came in contact with nanomedicine and further deepened my knowledge about polymers. In the master's program at TU Dresden, I gained further expertise by taking various modules with a focus on macromolecular chemistry and biochemistry. Especially through an associated internship, I expanded my practical skills in polymer synthesis and polymer analysis using NMR spectroscopy and gel permeation chromatography. During my master studies, I completed an internship in the workgroup of Prof. PhD Helena Florindo at Universidade de Lisboa. For this research work I synthesized different biocompatible copolymers and performed various reactions in order to functionalize the polymer end groups. These polymers were further used for coating PLGA nanoparticles for anti-cancer vaccination. Through this collaboration, I also gained skills and knowledge in synthesis and loading of polymeric nanoparticles with low polydispersity indices. The analysis of the nanoparticles was performed by dynamic light scattering and atomic force microscopy. Based on the research in Lisbon the topic of my master thesis "Coating of loaded PLGA nanoparticles with poly(2-oxazoline)s" emerged. I completed the master's degree with a grade of "very good".



Shiqi Wang

Dr. Shiqi Wang received her Bachelor and Master degrees from Tsinghua University China in 2012 and 2014 respectively. Then she moved to Imperial College London as a Marie Curie Early Stage Researcher in the department of chemical engineering, where she got her Ph.D. degree in 2018. Afterwards, she joined University of Helsinki as a postdoctoral researcher in Prof.

Santos group. Dr. Wang's research interests include developing pH-responsive polymeric materials for intracellular drug delivery applications. She is a co-author of 50 peer-reviewed papers, with 2860 citations in total (h-index=22). She has also received many awards and grants, including Weinberg Prize of Imperial College London for research of outstanding ingenuity, originality and elegance during PhD, Finnish Pharmaceutical Society most outstanding research article award, and Academy of Finland Postdoctoral research grant.

RECENT PUBLICATION

- Guedes, G., Wang, S.* et al. (2021). Dual-Crosslinked Dynamic Hydrogel Incorporating {Mo154} with PH and NIR Responsiveness for Chemo-Photothermal Therapy. *Advanced Materials*, 2007761. <https://doi.org/10.1002/adma.202007761>
- Wang, S. et al. (2020). Intracellular Delivery of Budesonide and Polydopamine Co-Loaded in Endosomolytic Poly(butyl methacrylate-co-methacrylic acid) Grafted Acetalated Dextran for Macrophage Phenotype Switch from M1 to M2. *Advanced Therapeutics*. <https://onlinelibrary.wiley.com/doi/full/10.1002/adtp.202000058>
- Wang, S. et al. (2020). Superfast and Controllable Microfluidic Inking of Anti-inflammatory Melanin-like Nanoparticles Inspired by Cephalopods. *Materials Horizons*. <https://pubs.rsc.org/ko/content/articlehtml/2020/mh/d0mh00014k>
- Wannasari, S., Wang, S.* et al. (2019). A Virus-Mimicking pH-Responsive Acetalated Dextran-Based Membrane-Active Polymeric Nanoparticle for Intracellular Delivery of Antitumor Therapeutics. *Advanced Functional Materials*, 29, 1905352. <https://onlinelibrary.wiley.com/doi/full/10.1002/adfm.201905352>
- Wang, S. et al. (2018). A New Strategy for Intestinal Drug Delivery via pH-Responsive and Membrane-Active Nanogels. *ACS Applied Materials & Interfaces*, 10 (43), 36622–36627. <https://pubs.acs.org/doi/abs/10.1021/acsami.8b15661>



Yanira Zeyn

Ms. Zeyn was born on July 15th, 1994 in Offenbach am Main, Germany, where she graduated from secondary school qualifying for university admission in July 2013.

In the same year, she started her bachelor studies in Biomedical Chemistry at the Johannes Gutenberg University Mainz and finished 2017 with her bachelor's thesis at the Institute of Toxicology of the University

Medical Center Mainz. There she first encountered application-based cell biological experiments, whereof the results were published successfully in a peer-reviewed journal.

In October 2017, Ms. Zeyn started her masters studies in Biomedical Chemistry at the Johannes Gutenberg University Mainz where she was also able to work on medicinal topics, e.g. during her master's thesis at the Max Planck Institute for Polymer Research in Mainz. There she focused on exploring bioactive peptides for promoting angiogenesis in artificial matrix mimicking materials. Besides, she spent a semester abroad in Trieste, Italy during her master's, where she focused on self-assembling tripeptides and the effects of chirality changes. After her MSc graduation in April 2020, Ms. Zeyn was further able to gain useful working experience as research associate at the Institute of Toxicology of the University Medical Center Mainz for 13 months. The topics she worked on included cell biological investigation of novel histone deacetylase inhibitors. Her results were published in three articles of different peer-reviewed journals.

In July 2021, Ms. Zeyn started her PhD in the group of Prof. Grabbe and PD Bros at the Department of Dermatology in the University Medical Center Mainz, where she focusses on nanovaccines loaded with antigen-coding mRNA / nucleic acid-based adjuvants to activate dendritic cells for tumor therapy.

CLINAM

European Foundation for Clinical Nanomedicine

13/2022
Basel, May 2-4

ABSTRACTS SPEAKERS

EXPLOITING DESIGN OF EXPERIMENT FOR DEVELOPMENT OF POLYMERIC NANOPARTICLES FOR INTRANASAL DELIVERY OF THERAPEUTIC PEPTIDES TO TREAT NEUROLOGICAL DISORDERS

NAVEED AHMAD, Shunping Han, Sukhvinder Bansal, Julie Tzu-Wen Wang and Khuloud T. Al-Jamal
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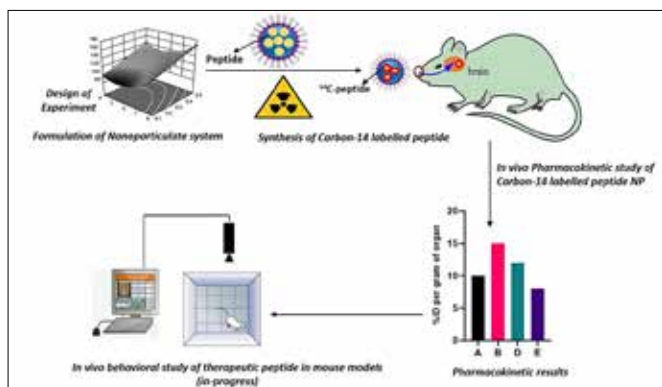
INTRODUCTION

The development of potential therapeutic peptides for CNS is currently hampered due to inability of these drugs to cross the blood-brain barrier (BBB) in therapeutic concentrations. The capillary endothelium of the blood-brain barrier exhibits tight junctions with high resistance between the cells which forms a central barrier, preventing peptide drugs to enter in significant concentration to brain tissues and susceptibility to degradation in the gut after oral administration. Polymeric nanocarriers of natural or synthetic biodegradable polymers have recently attracted significant research attention for Nose to Brain (NTB) delivery of peptide drugs due to their enhanced stability, ability to protect and control the release of therapeutic payload and multiple possibilities for surface modification. We hypothesise that encapsulation of therapeutic peptide (confidential) in the FDA approved, biocompatible and biodegradable poly(lactic-co-glycolic acid) (PLGA) will offer protection from enzymatic degradation, prolong its half-life and increase its brain concentration *via* direct NTB delivery.

METHODS

A quality nanoparticulate system was formulated for encapsulation of peptide in the FDA approved, biocompatible and biodegradable poly (lactic-co-glycolic acid) nanoparticles (NP) by nanoprecipitation method with the aid of 3³ factorial design. Entrapment efficiency was determined using Reverse-phase High Performance Liquid Chromatography (RP-HPLC). The formulation was further characterized for physicochemical properties including shelf-life stability studies by dynamic light scattering. NPs were innocuous to the epithelial RPMI 2650 cell line up to 24 and 72 h of incubation. PEGylated peptide NP was formulated to improve NTB delivery of peptide. *In vitro* diffusion of fluorescent labelled peptide NP and PEGylated peptide NP through artificial nasal simulated mucus models was conducted using Transwell® Inserts. Carbon-14 labelled peptide (¹⁴C-peptide) was synthesized using Solid Phase Peptide Synthesis. Characterization of the synthesized ¹⁴C-peptide was carried out using RP-HPLC and LC/MS. Biodistribution studies, including brain uptake was conducted using liquid scintillation counting of free ¹⁴C-peptide and ¹⁴C-peptide PEGylated NP at different time intervals post intranasal and intravenous dosing in mice.

Scheme 1. *In vivo* biodistribution of ¹⁴C-Peptide in solution and ¹⁴C-Peptide PEGylated NP in mice organs at different time intervals post intranasal and intravenous administration



RESULTS

In this project, we formulated and optimised peptide NP which showed hydrodynamic diameter (PS) of ~98.8- 117nm, PDI index ~0.18-0.23 and zeta potential of -21.4 -32.6mV with entrapment efficiency of ~12 %. One-month stability studies confirmed no change in these parameters. A sustained release pattern of peptide from peptide NP was observed with ~12, ~26 and ~35% release after 4, 24 and 48 h. The fluorescently labelled PEGylated peptide NP showed higher diffusion through artificial nasal simulated mucus models as compared to non-PEGylated peptide NP. We successfully synthesized ¹⁴C-peptide with a yield of 74% and 53.35% by weight and radioactivity respectively. The ¹⁴C-peptide PEGylated NP showed significantly higher uptake in brain at 10, 30 and 60 min as compared to free ¹⁴C-peptide post intranasal and intravenous administration in mice suggesting potential benefits of the developed NP formulation, presumably due to enhanced penetration through olfactory and trigeminal nerve pathways in the nasal cavity. Comparative biodistribution data indicated that peptide could be delivered directly into the brain from the nasal cavity, and that intranasal administration of small NP resulted in enhanced brain peptide bioavailability and less distribution into non-targeted organs including liver. Current studies are being undertaken to assess the therapeutic efficacy of the formulation in a relevant disease mouse model.

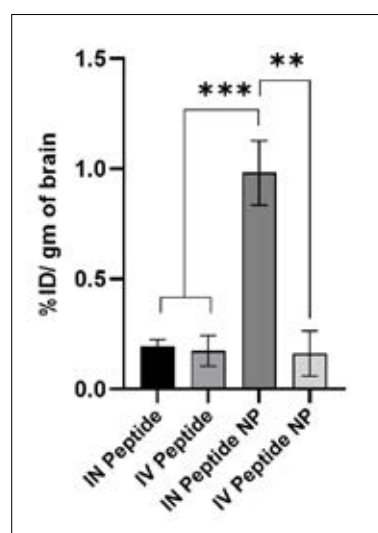


Figure 1. Uptake of ¹⁴C-Peptide in solution and ¹⁴C-Peptide PEGylated NP in brain at 10 min post intranasal and intravenous administration. Results are expressed as %ID per gram of brain. Data are expressed as mean ± SEM, n = 3. Statistical significance is indicated in comparison between each mode at the same time-point. *p < 0.05, **0.01 < p < 0.05; ***p < 0.01 (Student's t-test).

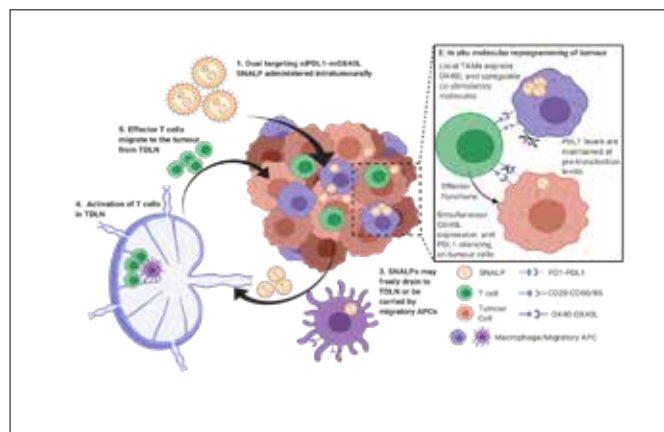
CONCLUSIONS

The current results demonstrated successful formulation of peptide NP with high entrapment efficiency and small PS to be utilized as a potential nanoplatform for NTB delivery of peptide to treat neurological disorders.

MRNA/SIRNA COMBINATORY DELIVERY TO UNBLOCK IMMUNE CHECKPOINT INHIBITION IN SOLID TUMOURS USING STABLE NUCLEIC ACIDS NANOPARTICLES

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Scheme 1. Molecular programming of checkpoint interactions.

INTRODUCTION

Immune checkpoint blockade involves targeting immune regulatory molecules with antibodies. Preclinically, complex multi-antibody regimes of both inhibitory and stimulatory targets are a promising candidate for the next generation of immunotherapy. However, in this setting, the antibody platform may be limited due to excessive toxicity caused by off target effects as a result of systemic administration. RNA can be used as an alternate to antibodies as it can both downregulate immunosuppressive (siRNA) or induce expression of immunostimulatory (mRNA) checkpoints.

METHODS

OX40L mRNA (mOX40L) was synthesized from a mouse TNFSF4 ORF mammalian expression OX40L plasmid. Luciferase mRNA (mLuc) was synthesized from Luciferase-pcDNA3 plasmid. SNALPs were prepared by nanoprecipitation method. SNALPs encapsulating different types of RNA were characterised in terms of size (z-average), polydispersity and surface charge (z-potential) utilizing dynamic light scattering (DLS). Annular Dark Field Scanning Transmission Electron Microscopy (ADF-STEM) was used for imaging. The encapsulation efficiency (EE%) was quantified with the Quant-iT™ Ribogreen™ assay. Cell transection was carried out in B16F10 and J774 macrophages. SNALP biodistribution, *in vivo* transfection, immunogenicity, tumour growth delay, survival and challenge were carried out in B16F10 tumour bearing mice after local administration.

RESULTS

In this study we demonstrate that the combination of both siRNA and mRNA in a single formulation can simultaneously knock down and induce expression of immune checkpoint targets thereby reprogramming the tumour microenvironment from immunosuppressive to immunostimulatory phenotype. To achieve this, RNA constructs were synthesized and formulated into stable nucleic acid lipid nanoparticles (SNALPs); the SNALPs produced were 140-150 nm in size with >80% loading efficiency. SNALPs could transfect macrophages and B16F10 cells *in vitro* resulting in 75% knockdown of inhibitory checkpoint (PDL1) expression and simultaneous express high levels of stimulatory checkpoint (OX40L) with minimal toxicity. Intratumoural treatment with the proposed formulation resulted in statistically reduced tumour growth, a greater density of CD4+ and CD8+ infiltrates in the tumour and immune activation within tumour draining lymph nodes.

CONCLUSIONS

These data suggest that a single RNA based formulation can successfully reprogram multiple immune checkpoint interactions on a cellular level. Such a candidate may be able to replace future immune checkpoint therapeutic regimes composed of both stimulatory and inhibitory receptor targeting antibodies.

ACKNOWLEDGEMENT

Funding is provided from the Maplethorpe Foundation.

NOVEL STERICALLY STABILIZED NANOLIPOSOMES BASED ON CISPLATIN-CARBOXYLATE CONJUGATE FOR COLON CANCER THERAPY

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Cisplatin (cis-diamminedichloro platinum[II], CDDP) is a widely used therapeutic agent in the treatment of various solid tumors, however, its use is associated with severe toxicities including neurotoxicity, nephrotoxicity, and hematological toxicity. Thus, numerous nano-based drug delivery vehicles have emerged for improving CDDP selective targeting and tumor accumulation through EPR effect (enhanced permeability and retention) and to reduce its systemic toxicity. To this aim, various liposomal formulations have been developed which showed insufficient release and inefficient therapeutic response.

Table 1. Liposomes characterization

Formulations	PDI ± SD	Z-average ± SD (nm)	Zeta Potential ± SD (mv)	EE%	Loading method
F1	0.148 ± 0.01	152.20 ± 3.46	-14.20 ± 0.81	27.32	1
F2	0.171 ± 0.01	156.90 ± 4.21	-18.22 ± 0.08	19.60	1
F3	0.149 ± 0.021	134.38 ± 6.41	-15.39 ± 1.21	11.72	1
F4	0.169 ± 0.03	150.50 ± 7.21	-20.90 ± 0.086	12.94	2
F5	0.283 ± 0.024	197.20 ± 4.65	-18.20 ± 0.073	14.20	2
F6	0.220 ± 0.019	187.20 ± 5.12	-15.80 ± 0.096	18.84	2

Introducing weakly basic functional groups has shown to improve the loading of small molecules into performed liposomes. In the current report, several conjugates of CDDP-carboxylate using aspartic acid (AA), glutamic acid (GA) and calcium acetate (CaA) were examined to develop PEGylated CDDP liposomes. For this, various lipids including HSPC, DPPG, mPEG₂₀₀₀-DSPE and cholesterol (55/5/5/35) were used to prepare liposomes. For CDDP active loading, drug powder was incubated with the pre-formed liposomes bearing AA, GA and CaA (200 mM, pH 5.5) at 65 °C for 2 hours and then for 48 h at 37 °C (method 1 :F1 to F3). Liposomes were also prepared by hydrating lipid films with conjugates of CDDP-carboxylate of AA, GA or CaA solutions (200 mM, pH 5.5) at 65 °C (method 2 : F4 to F6). FT-IR spectra of CDDP-conjugates was run on Spectrometer using KBr pellets. The physicochemical characterization of liposomes using DLS showed nanoliposomes size ranged from 130 to 200 nm with poly dispersity index (PDI) of 0.1 to 0.3. All liposomes demonstrated negative zeta potential within the range of -14 to -20 mV (Table 1).

The cytotoxicity of liposomes on C26 cells indicated that F1 to F4 were significantly toxic and the highest IC₅₀s were attributed to CDDP complexes.

Figure 1. *In vitro* cytotoxicity of cisplatin-loaded liposomes against C26 cells. The IC₅₀ was measured by MTT assay following 48h treatment. The data represented as the means ± SD (n = 3). P Value com-

pared using one-way ANOVA. All formulations are compared to free Cisplatin.

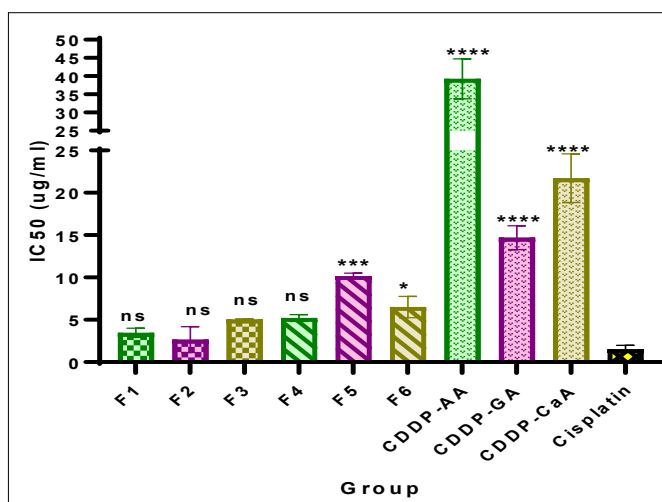
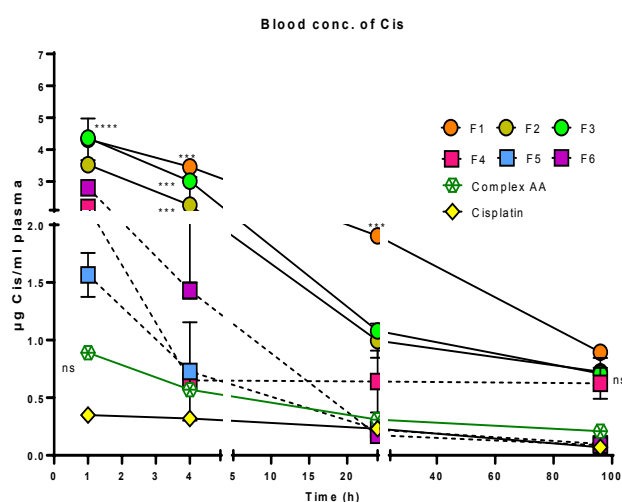


Figure 2. Concentration–time profile of cisplatin in plasma following administration of free cisplatin, pt-complexes and cisplatin liposomal formulations containing (3 mg/kg). Data are means \pm SD (n = 4).



The plasma concentration of total platinum as a matter of time is represented in figure 2. Free and cisplatin complex showed the lowest plasma levels within the first hour following injection and promptly cleared within the first 4 h. Higher levels of platinum in plasma were detected following liposomal administration and the highest cisplatin concentration at 1 h was attributed to F1, F3 and F2. At 4 h, CDDP levels were still higher in the aforementioned formulations compared to CDDP, while significantly reduced in F4 and F5. We therefore investigated the efficacy of the liposomal formulations against C26 colon carcinoma in mice animal model. When tumor volume reached 100 mm³, mice were injected via lateral tail vein once weekly for 3 weeks. Figure 3 illustrates that cisplatin-loaded liposomes successfully reduced the rate of tumor growth compared to control mice as well as free cisplatin groups. Mice in F1, F2 and F3 groups showed a considerable delayed in tumor growth compared to free cisplatin as well as other liposomal formulations. From the survival data on figure 4, it is clear that the survival time was significantly prolonged with liposomal formulations as compared to free cisplatin. The best survival was observed with F1 formulation, which was in consistent with tumor inhibition results.

Figure 3 In vivo anti-tumor efficacy of cisplatin-loaded formulations, free cisplatin, CDDP-complexes and PBS in female BALB/c mice after i.v. administration of three doses of 3 mg/kg cisplatin after tumor inoculation. Data represented as mean \pm SD.

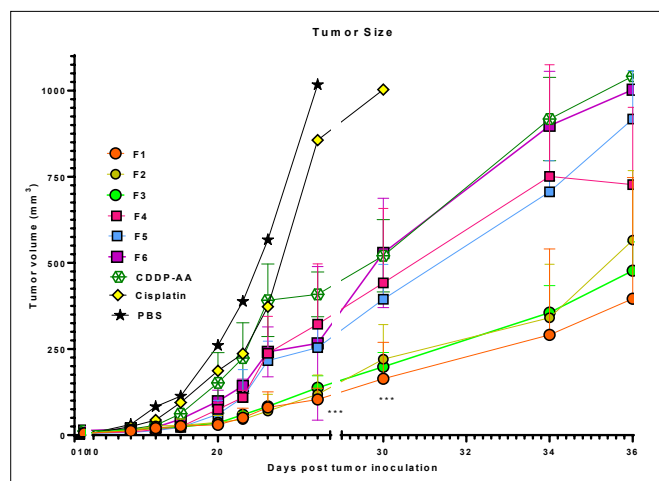
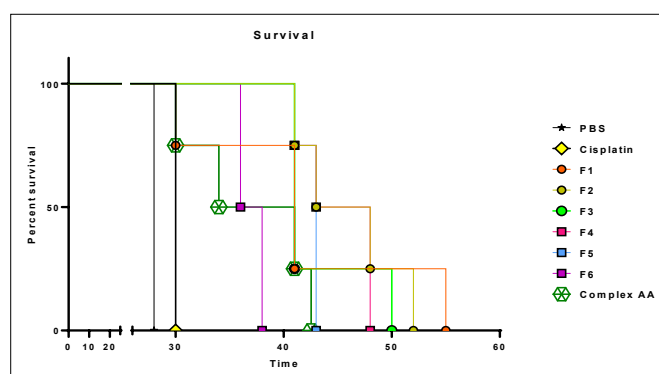


Figure 4 In vivo survival of cisplatin-loaded formulations, free cisplatin, pt-complexes and PBS in female BALB/c mice after i.v. administration of three doses of 3 mg/kg cisplatin after tumor inoculation. Data represented as mean \pm SD.



In conclusion, anti-tumor efficacy studies in C26 tumor bearing mice demonstrated the potential of cisplatin-loaded liposomes prepared with the active loading method (F1 to F3) in inhibiting tumor growth compared to free unencapsulated cisplatin. Previous studies indicated that carboxylic acid functional groups in poly (ethylene glycol)-poly (glutamic acid) block copolymers could form a coordination bond with platinum (II) atom in CDDP. In this complex, the chloride ions in CDDP are substituted with carboxylates, forming a less toxic derivative, which could readily release CDDP upon accumulation within the tumor area. The same release mechanism might contribute to the liposome prepared using active loading. CDDP-complexes were not as effective as liposomal formulations and rapidly release cisplatin in blood which showed the crucial role of liposomes in maintaining cisplatin concentration within blood circulation and targeting tumor cells in the tumor microenvironment.

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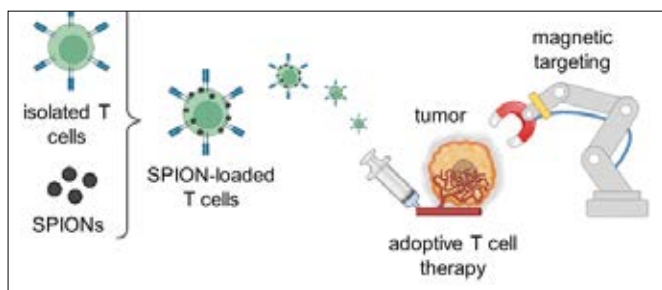
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CANCER-IMMUNOTHERAPY USING IRON OXIDE NANOPARTICLES

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The immune system can effectively induce antitumor immune responses in cancer patients. Usually, T cell infiltration into a tumor is associated with a good clinical prognosis. Immunotherapies such as adoptive T cell transfers shall increase the number of intratumoral T effector cells, but the immunosuppressive tumor microenvironment may impair T cell infiltration and their activation. The targeted accumulation of T cells by external magnetic forces might increase the amount of T cells in the tumor and improve the patient's prognosis. We showed that the loading of T cells with citrate-coated superparamagnetic iron oxide nanoparticles (SPIONs) enables us to control the cells by magnetic forces. The incubation of SPIONs with freshly isolated CD3⁺ T cells led to an intracellular iron amount of 1.4 pg per cell, which was sufficient for magnetic steering *in vitro* under static as well as dynamic conditions in a peristaltic flow system. Transmission electron microscopy showed that nanoparticles were both located intracellularly in vesicles as well as attached to the plasma membranes. Importantly, nanoparticles did not spill from loaded to non-loaded cells. Multiparameter stainings in flow cytometry revealed that SPION loading did not impair T cell viability, activation, proliferation, and cytokine release (IFN- γ , TNF- α) after polyclonal stimulation (CD3/CD28/CD2 and IL-2). The magnetic accumulation of SPION-loaded cells in the tumor region might improve immune cell infiltration, turning initially non immune-infiltrated "cold" tumors into immune-infiltrated "hot" tumors (Figure 1). Since SPION-loaded cells can be monitored in magnetic resonance imaging (MRI), an imaging control of successful T cell targeting might be a vision for future application in adoptive T cell therapy *in vivo*.

Figure 1: Adoptive T cell therapy with magnetic targeting of SPION-loaded T cells into the tumor region.



DEVELOPMENT, PROCUREMENT AND RESPONSIBLE MANAGEMENT OF NEW ANTIMICROBIALS – THE EUROPEAN PULL INCENTIVE

CHRISTINE ÅRDAL

Antibiotic resistance is one of the world's most pressing health threats. While resistance is on the rise, antibiotic innovation has almost come to standstill due to an unattractive business case. WHO identified 43 antibiotics in the clinical pipeline in 2020, but only two of these are active against the most pressing priorities. The market failure of antibiotics has been well documented, and experts recommend the adoption of "pull" incentives, i.e., economic models that provide increased revenues for high value antibiotics. Four countries are currently trialing different types of pull incentives, and the EU through Horizon Europe has selected a consortium to perform the preparatory work leading to the establishment of a European pull incentive. This session will focus on a potential design for the European pull incentive, including eligibility, stewardship, and access requirements.

3D-(BIO-) PRINTING FOR ADVANCED IN VITRO SYSTEMS TO INVESTIGATE NANO-ANTIBIOTICS AGAINST BACTERIAL INFECTIONS

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INTRODUCTION

In the last decade, 3D-printing gained more and more popularity in biopharmaceutical research for various applications. Here, we provide two novel approaches to apply the technology for the design of *in vitro* models. Firstly, we designed a 3D *in vitro* organ culture of human hair follicles. Conventional hair follicle models, where single follicles are "free floating" in a medium are not suitable for studying follicular penetration of nanoparticles. To overcome that, we provide a 3D model with perpendicularly oriented hair follicles to be applied for penetration studies of nanocarriers (1,2), in particular for treating severe follicular diseases, such as Acne Inversa or Folliculitis Decalvans. Secondly, we established a method to 3D bioprint bacterial biofilms. Biofilm-forming bacteria are a major threat for patients suffering from chronic infections. The field of nanotechnology field has shown promising results to design new anti-infective approaches. Current biofilm *in vitro* models for testing of anti-infectives lack in reproducibility and design possibilities (3,4). Thus, the objective of this work was to develop a method to transfer bacterial biofilms in any required shape and dimension via the technology of 3D bioprinting.

METHODS

Human anagen hair follicles were isolated from skin biopsies originating from cosmetic surgery*. Then, they were implanted perpendicularly into a 3D printed collagen matrix scaffold, emulating the dermis (Fig. 1 A,B). Cultivability was assessed via hair growth in the model and compared to the conventional culturing method. To make a proof of concept, fluorospheres were applied on the model to observe follicular transport. Additionally, the system was tried to be infected by drop infection with *Staph. aureus*, to have a treatable infectious model. A self-established characterized gelatin-alginate bioink was inoculated with *E.coli* MG1655. Biofilms were formed in a syringe for 3 days and were bioprinted afterwards (Fig. 2). Imaging, antibiotic susceptibility assays and metabolic profile analysis were performed to confirm biofilm properties.

RESULTS

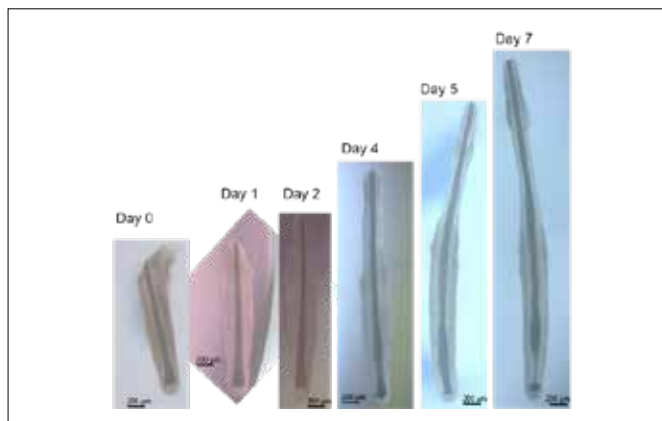
Inserted hair follicles showed similar length increase on the respective days compared to conventional cultivation. Preliminary experiments with fluorospheres showed follicular transport after 4 h. Drop infection showed follicular colonization of *Staph. aureus*. We showed, that biofilms could be printed in the hydrogel in various shapes and dimensions. Biofilm properties were confirmed via imaging, stronger antibiotic resistance than bioprinted planktonic bacteria and by analysis of their metabolic profiles.

CONCLUSION

3D (Bio-)printing could facilitate future design of *in vitro* models. Here, we showed a novel 3D hair follicle model in a 3D printed scaffold system, which could serve potentially as a platform to test topical transport systems against hair follicle disease related strains. Furthermore, we plan to optimize and elaborate the model (e.g. adding an epidermis, inclusion of cells). Additionally, we showed a novel approach to bioprint ready bacterial biofilms to investigate anti-infectives against biofilm infections. On the long-term, we aim to bioprint these biofilms on human epithelial cells to mimic a chronic infection *in vitro*. *Ethics approval and patient consent in place

Figure 1: A) Growing isolated human hair follicle B) Scheme of a 3D hair follicle *in vitro* organ culture

A



B

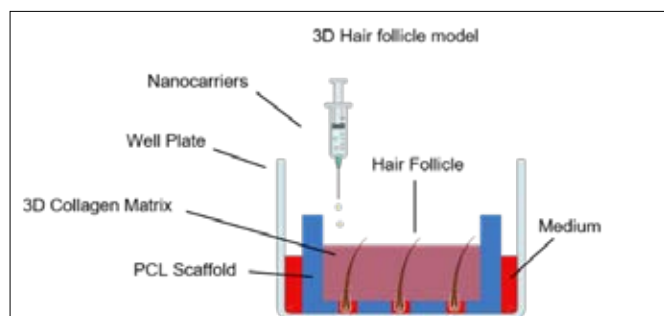


Figure 2: Biofilm inside a gelatin-alginate hydrogel printed in various shapes on an agar plate and cultured over night. Scale bar 5 mm



Acknowledgement: We would like to acknowledge the excellent technical support of Pascal Paul and Annette Boese.

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DESIGN AND OPTIMISATION OF A DENDRIMER-CONJUGATED DUAL BCL-2/BCL-XL INHIBITOR, AZD0466, WITH IMPROVED THERAPEUTIC INDEX

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Dual Bcl-2/Bcl-xL inhibitors are expected to deliver therapeutic benefit in many hematological and solid tumors, but their clinical application has been limited by tolerability issues, including thrombocytopenia. AZD4320, a potent dual Bcl-2/Bcl-xL inhibitor, showed good efficacy but encountered dose limiting cardiovascular toxicity in preclinical species, and had challenging physicochemical properties which prevented its clinical development. Nanocarriers can provide prolonged circulation time, controlled release, tumor accumulation and retention. Consequently, they have been explored to improve the therapeutic index of small molecules in oncology. This talk describes the design and development of AZD0466, a novel drug-dendrimer conjugate, where AZD4320 is chemically conjugated to Starpharma's DEP® dendrimer platform, a 5-generation PEGylated poly-lysine dendrimer via a hydrolytically labile linker. Release of AZD4320 is through hydrolytic cleavage of the linker, which is characterized by a "release half-life", defined as the time to release 50% of the active moiety. This release half-life can be modified through linker design.

Initially, three drug-dendrimer conjugates with a range of AZD4320 release half-lives (from 1.7 h to 217 h) were synthesised and efficacy was investigated in C.B-17 SCID mice bearing RS4;11 tumors while cardiovascular parameters and tolerance were assessed in a telemetered rat model. A mathematical model was developed and used to optimize the desired release rate of the active moiety, AZD4320, from the dendrimer conjugate for maximal therapeutic index in terms of preclinical anti-tumor efficacy and cardiovascular profile. Based on this modeling, AZD0466, with a release half-life of 25.5 h, was synthesised and selected for further *in vivo* efficacy and tolerability assessment.

Efficacy studies in the RS4;11 xenograft model showed similar efficacy to AZD4320, while cardiovascular studies in rat and dog demonstrated that AZD0466 was tolerated at doses of active-moiety that are more than ten-fold higher. In addition, it can be easily formulated for intravenous administration due to significantly enhanced solubility.

The AZD4320-dendrimer conjugate, AZD0466, identified in this study has resulted in an improved therapeutic index and enabled progression of this promising Bcl-2/Bcl-xL inhibitor into clinical development.

CARBON NANOMATERIALS TO MANIPULATE NEURONAL NETWORKS AND SYNAPSES AT THE NANOSCALE

LAURA BALLERINI, SISSA, Trieste

Carbon Nanomaterials applied to interface neuronal functions might propel the development of new biomedical devices, enabling nervous system modulation. In recent years we have gained an improved understanding of micro and nano-engineered materials developed to investigate emergent biological adaptive and integrated behaviours. This new class of materials can improve exploring fundamental biological phenomena as well as contribute to biomedical and clinical applications. I will present our results concerning multi-walled carbon nanotubes (MWCNTs) and graphene interfacing neurons, I will describe the effects of such materials on neuron signalling when cell maturation, axon growth and synapse formation are driven by integrating genuine biosystems and the nanomaterials *in vitro* and *in vivo*.

LITTERATURE AND FAKE SCIENCE – WHAT TO WATCH OUT FOR?

LAJOS BALOGH

This talk will address the increasing disorder in scholarly publications. To detect potential problems requires understanding today's publishing landscape. Publications used to be for sharing information and knowledge, and authors, editors, and publishers all guarded this essential feature. Since then, publications have also become an existential issue for every scientist. Moving up on the professional ladder is a must for every scientist, but the present system encourages quantity at the expense of quality. Unfortunately, for some, the "publish or perish" attitude means "whatever it takes." Commercialization of scholarly publishing and the promotion and graduation rules set by funders and institutions led to the dilution of scientific literature. It also contributed to the skepticism of the general public towards "science." The ease of online publishing, the rise of "open science" and preprints (especially fueled during the pandemic) gave rise to spreading false information, fake publications, and outright disinformation by papermills and predatory publishers. The speaker has worked for more than 20 years in the publishing field and will speak about his criteria for selecting useful information.

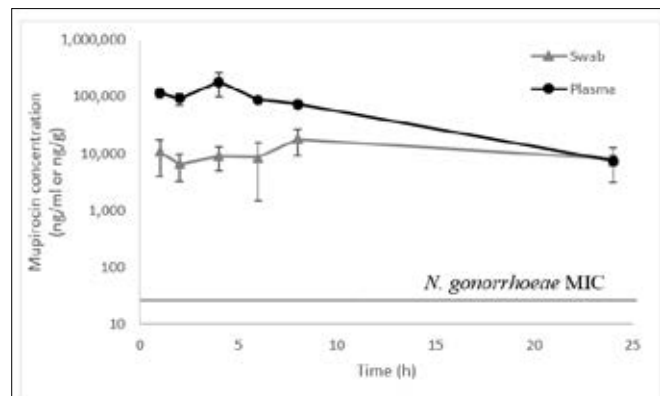
INJECTABLE MUPIROICIN LOADED PEGYLATED NANO LIPOSOMES (NANO-MUPIROICIN) IS POTENTIALLY EFFICACIOUS AGAINST INFECTIONS INVOLVING MULTIDRUG-RESISTANT BACTERIA

YECHZKEL BARENHOLZ¹, Ahuva Cern¹, Yaelle Bavli¹, Atara Hod¹, Daniel Zilbersheid¹, Shazad Mushtaq², Ayelet Michael-Gayego³, Dinorah Barasch⁴, Yael Feinstein Rotkopf⁵, Allon E. Moses³, David M. Livermore⁶

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Antibiotic resistance is a global health threat. There are a few antibiotics under development, and even fewer with new modes of action and no cross-resistance to established antibiotics. Accordingly, reformulation of old antibiotics to overcome resistance is attractive. Nano-mupirocin, a PEGylated nano-liposomal formulation of mupirocin, was developed and fully characterized¹. Mupirocin is a unique antibiotic having a unique mode of action². However due to its rapid metabolism and high binding to plasma proteins its clinical use is limited to topical administration³. Reformulating mupirocin to Nano-mupirocin, allowed the parenteral use of mupirocin in deep infections, as previously demonstrated by us in several animal models⁴⁻⁶. Nano-mupirocin showed no cross-resistance with other antibiotics and retained full activity against vancomycin-, daptomycin-, linezolid- and methicillin- resistant *Staphylococcus aureus* against vancomycin-resistant *Enterococcus faecium*, and cephalosporin-resistant *Neisseria gonorrhoeae*^{6,7}. Following Nano-mupirocin injection to rats, plasma levels greatly exceeded relevant MICs for >24h, and a biodistribution study in mice showed that mupirocin concentrations in vaginal secretions also greatly exceeded the MIC₉₀ for *N. gonorrhoeae* (0.03 µg/mL)

for > 24 h, as described in the Figure below. In addition, the use of fluorescent Nano-liposomes revealed that intact liposomal mupirocin reached the mice vagina. In summary, Nano-mupirocin has excellent potential for treatment of several infection types involving multiresistant bacteria. It has the concomitant benefits from utilizing an established antibiotic and PEGylated nano-liposomes of the same size and lipid composition as of Doxil[®], an anticancer drug product now used for the treatment of over 700,000 patients globally. Accordingly, Nano-mupirocin should be seen as a formulation with considerable potential and low development risks.



Mupirocin concentration (sum of free and liposomal) in vaginal secretions (ng/g) and plasma (ng/mL) following IP administration of Nano-mupirocin 50 mg/kg to mice (mean ± SE).

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ROLE OF CELL RECEPTORS AND PROTEOGLYCANS IN NANOPARTICLE UPTAKE BY CELLS

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The capacity of nano-sized materials to distribute within organisms and enter cells opened up tremendous opportunities for their application as drug delivery systems. However, many aspects of how these materials are processed by cells are still unclear. In order to gain new insights into this question, for the first time in this field in our laboratory we have applied the genome-wide forward genetic screening developed by Carette et al. 2009^[1] but the diploid genome of mammalian cells has precluded large-scale gene disruption. We used insertional mutagenesis to develop a screening method to generate null alleles in a human cell line haploid for all chromosomes except chromosome 8. Using this approach, we identified host factors essential for infection with influenza and genes encoding important elements of the biosynthetic pathway of diphthamide, which are required for the cytotoxic effects of diphtheria toxin and exotoxin A. We also identified genes needed for the action of cytolethal distending toxin, including a cell-surface protein that interacts with the toxin. This approach has both conceptual and practical parallels with genetic approaches in haploid yeast.¹ container-title": "Science (New York, N.Y. to identify potential novel molecules involved in the uptake of nano-sized objects by cells (Montizaan et al., 2022 under revision).

The method uses a retroviral gene trap vector and human haploid HAP1 cells to create a genome-wide library of mutagenized cells. The library of mutagenized cells is exposed to nanoparticles, then cells with reduced uptake are sorted using fluorescent activated cell sorting (FACS). This step was repeated for several rounds (Fig.1). Next generation sequencing was used to identify the enriched random insertions and consequently the proteins responsible for the lower uptake.

The screening was performed using silica nanoparticles with a human serum corona which we previously showed enter cells via interactions with the LDL receptor mediated by the corona adsorbed on their surface (Francia et al., ACS Nano 2019)^[2]. Interestingly, we also showed that these nanoparticles enter cells via a mechanism that is not clathrin-mediated, as opposed to what it is usually observed for the LDL receptor and its endogenous ligands.

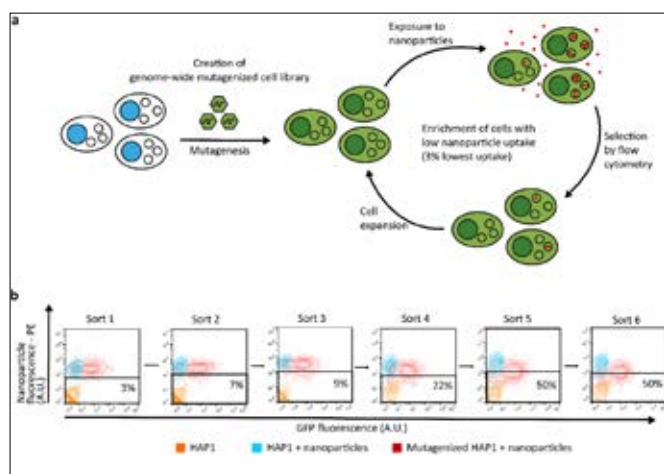


Figure 1. HAP1 cells randomly mutagenized by retroviral transduction (with GFP), exposed to nanoparticles overnight and selected

using flow cytometry. This was repeated multiple times in order to enrich the phenotype. From Montizaan et al, 2022, under revision.

More than 80 different genes were significantly enriched and pathway analysis showed that several of the identified targets were involved in intracellular trafficking, and cholesterol and glycosaminoglycan metabolism. A panel of the identified targets was further validated using three different cell lines and nanoparticles with diverse characteristics in order to clarify their role. In particular, the results showed that next to the known LDLR, other receptors are involved in the uptake of these nanoparticles. Additionally, we found that cell surface proteoglycan also mediate nanoparticle uptake via specific interactions that depend on the cell type, nanoparticle type as well as the corona adsorbed on the nanoparticles. Interestingly, a similar role for cell surface proteoglycans has been recently discovered also for the uptake of certain viruses and exosomes, suggesting that engineered and natural nano-sized materials share important aspects in the way they are processed by cells. At a broader level, the fact that multiple receptors, including cell surface proteoglycans, mediate nanoparticle uptake within the same cells clearly show the importance of disentangling all interactions at the cell membrane in order to be able to design truly targeted nanoparticles.

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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 be 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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MICORTISSUE PLATFORM TO STUDY CRUCIAL EVENTS IN TISSUE GROWTH

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Tissue growth is essential for multicellular life and delicately organized across length-scales by spatio-temporally controlled cell proliferation and differentiation until homeostasis is reached. As the cells are embedded in extracellular matrix (ECM), which they synthesize and remodel, tissue growth and remodeling processes can only be understood if the intimate crosstalk between cells and their environment is revealed. Little is known how ECM gradients are formed, or remodelled, and how these gradients steer cell phenotype transitions during tissue growth. Myofibroblastic phenotypes play central roles in tissue growth, but are also associated with many inflammatory or fibrotic diseases. How myofibroblasts orchestrate tissue growth processes, and how the ECM vice versa facilitates their disappearance or drives persistent myofibroblast activation and disease progression is still elusive. As 2D cell culture approaches do not resemble the biophysical characteristics of tissues, we developed a 3D μ Tissue platform that allows to investigate tissue growth and maturation processes at high spatiotemporal resolution. We asked how tissue growth and maturation is regulated by the tensional state of the ECM fibers which they produce, cell phenotypetransitions and certain transiently expressed ECM components. Using de novo grown μ Tissues, we identified crucial sequential events that steer tissue growth and maturation, the latter being associated with a disappearance of myofibroblasts. Understanding how ECM gradients regulate tissue growth and the cell phenotype transition is crucial to developing novel treatment strategies to optimize wound healing, and counter fibrosis and cancer progression.

EMA'S APPROACHES FOR NANOMEDICINES AND NANOSIMILARS

KEVIN BLAKE, Scientific Officer Clinical Pharmacology; Scientific Evidence Generation Department; European Medicines Agency

About: Within the European Union (EU) non-biological complex drug products may usually be anticipated as being authorised as hybrid applications. These can be submitted within a centralised procedure i.e. assessed on an EU-wide basis and resulting in a single Marketing Authorisation (MA) or a Mutual recognition/Decen-

tralised procedure (MRP/DCP) resulting in a mutually recognised product with an MA in specified Member States. The implications of this will be outlined as will associated EMA efforts to harmonise approaches within the European Union including product-specific bioequivalence guidelines and other guidance documents. In addition EMA perspectives on global efforts at convergence on understanding of non-biological complex drug products will be shared including those on cluster meetings for complex generics and the parallel scientific advice initiative with FDA.

ENABLING VACCINE SUPPLY THROUGH INTERNATIONAL COOPERATION: LESSONS LEARNED

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In a globalized world, the surge of a new and highly transmissible pathogen quickly results in rapid spread of infection and difficulties in controlling pathogen transmission. In the context of a pandemic, vaccine development needs to face multiple challenges in order to deliver a safe and cost-effective product. The current SARS-CoV-2 derived pandemic not only placed an immense strain on health-care systems worldwide but is also the cause of an unprecedented socio-economic crisis due both to strict isolation policies and sanitary measures adopted that have led to an economic recession. The US Food and Drug Administration (FDA) issued the Emergency Use Authorization for Pfizer-BioNTech COVID-19 vaccine only one year after the discovery of the SARS-CoV-2 virus, which is the fastest development of a vaccine ever recorded.

However, obstacles had and still have to be overcome especially with regard to manufacturing, distribution, transport and accessibility to such vaccines. Low and middle income countries (LMICs), specifically, have been left behind in the race to vaccine accessibility due to high prices of such products, extreme conditions of storage and the hoarding of vaccine doses by developed countries. Furthermore, the lack of infrastructure prevents LMICs to manufacture their own vaccines, enforcing a dependency on manufacturing in high-income countries. In order to overcome these issues, the World Health Organization (WHO), in collaboration with the Coalition for Epidemic Preparedness and the Gavi alliance initiated the COVAX program with the goal to make 1.8 billion COVID-19 vaccine to be distributed in LMICs by the end of 2021.

Here we describe two projects between Switzerland, Indonesia and Thailand on the development of pandemic flu and SARS-CoV-2 DNA vaccines. The projects involved a technology transfer between Switzerland and Indonesia that led to the establishment of a pandemic flu vaccine formulation platform in Surabaya (Indonesia), which involved the transfer of equipment and expertise to enable research and development of adjuvanted vaccine formulations and delivery systems^[1]. The interaction with Chulalongkorn University hospital (Bangkok, Thailand) has led to the pre-clinical testing of a generic liposomal formulation of a DNA vaccine^[2].

Through this presentation, we aim to share the "lessons learned" from these projects to both support and inspire future scientific collaborations of a similar nature.

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PERSPECTIVES OF THE INTERNATIONAL ASSOCIATION OF PHARMACEUTICAL TECHNOLOGY (APV)

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APV e.V. is a scientific non-for-profit association with its headquarter located in Mainz, Germany. According to its constitution, the most important goals are to deepen scientific understanding about the development, manufacture, distribution and use of medicinal products and to impart this knowledge. APV is an independent, international and interdisciplinary scientific organisation with presently about 1,750 individual members working in pharmaceutical companies or universities. Hence, APV promises to bring together the scientific and practical aspects to enable manufacture of the best medicines: "Making Science Work".

Nanomedicines are addressed at different bodies of APV: by direct cooperation with other associations such as the CLINAM, by planning and organising scientific event on nanomedicine-related topics, e.g. by our Focus Groups on Drug Delivery (chair: Dr. Georg Böck, Boehringer Ingelheim) or Pharmaceutical Biotechnology (chair: Dr. Hans-Georg Eckert, Valicare/Syntegon). The focus group members are appointed by the APV Executive Council consisting on distinguished academic and industrial members from different companies and universities.



The present Executive Council of APV (from left to right) Prof. Dr. Jörg Breitkreutz (Heinrich Heine University Düsseldorf), Prof. Dr. Heribert Häusler (formerly Boehringer Ingelheim, University of Mainz), Prof. Dr. Sandra Klein (University of Greifswald), Dr. Kathrin Bartscher (CSO Nextpharma), Dr. Hans Lindner (CEO Nuvisan), Dr. Martin Lück (Vice-president Grünenthal), Dr. Karoline Bechtold-Peters (Novartis), Prof. Dr. Johannes Bartholomäus (pharmakreativ, University of Braunschweig).

APV Task-forces are another opportunity to collaborate on the Nanomedicines topic. Here, any APV member may contribute independent from the career level, may propose a topic and invite interested colleagues to meet just for an exchange of ideas or for organizing an event supported by the APV Office in Mainz. Some time ago, a task-force on Nanoformulations has been formed, but need some refreshment in order to re-start its activities. A recently very active task-force is working on the nanotoxicology of titanium dioxide particles in medicines and potential replacement opportunities.

A strong focus on nanomedicines is also given at the World Meeting on Pharmaceutics, Biopharmaceutics and Pharmaceutical Technology (PBP World Meeting) with usually around 1,200 participants and the European Conference on Pharmaceutics (ECP) with usually around 600 participants which are jointly organized by APV together with its partner associations APGI (France) and ADRITELF (Italy) and significant support of many more scientific associations throughout Europe. The last PBP World Meeting took recently place in March 2022 at the Ahoy conference center in Rotterdam, the Netherlands. Invited talks on nanoparticulate formulations included insights into vaccination strategies with lipoplexes of mRNA vaccines, dendrimers and lipid nanoparticles, biomimetic approaches for nanoparticulates, nanoformulations for improved solubility, stability or bioavailability in the gastrointestinal tract and site-specific delivery to different mucosas. The next PBP World Meeting is scheduled for the year 2024 in Vienna, Austria. The ECP will take place in 2023 in Marseille, France.

APV will further contribute to the future of the pharmaceutical sciences and industrial pharmacy by our culture of an open and cooperative exchange of knowledge and ideas among energetic and enthusiastic colleagues from different areas. Our slogan „Making Science Work“ is an individual and collective experience, personally satisfying to all those involved.

HIGH-THROUGHPUT IN-VIVO SCREENING TO ENABLE EXTRA-HEPATIC DELIVERY OF LNPs

LUIS BRITO

Systemic delivery of first generation LNPs accumulate primarily in the RES with a very large percentage of the dose ending up in the liver. The field has been looking at developing delivery systems to accumulate in non RES tissues or rare cell types such as hematopoietic stem cells. At Beam we are developing a highly sensitive high throughput barcoding method to assess biodistribution of several LNPs in-vivo with the expectation of identifying rare delivery events in large numbers of nanoparticle libraries. In this talk I will walk through our methodology and highlight some examples.

TOWARDS NANOMEDICINE IN PREGNANCY: ESTABLISHING A PHYSIOLOGICALLY RELEVANT IN VITRO PLATFORM TO ASSESS PLACENTAL TRANSFER AND SYSTEMIC EFFECTS AT THE MATERNAL-FETAL INTERFACE

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The development of safe and effective therapies in pregnancy is a great challenge since the health of two sensitive individuals is at stake. Tremendous safety and ethical concerns led to the current situation that pregnant women are largely excluded from clinical trials and have hindered the development of new treatments in pregnancy. A further issue is that even if medications are available, they mostly lack safety and efficacy data for their use in pregnancy. Still, drug intake by pregnant women is widespread despite growing evidence that prenatal exposure to supposedly safe drugs (e.g. paracetamol) might alter fetal development and increase the risks of some neurodevelopmental, reproductive and urogenital disorders ^[1].

Nanoparticle (NP)-based drug carriers hold great promise for novel targeted therapies to specifically treat the mother, the placenta or the fetus with reduced side effects as compared to conventional therapies ^[2]. To pave the way towards novel obstetric nanotherapeutics, reliable and predictive preclinical data on NP transfer and safety at the maternal-fetal interface are indispensable in particular since animal testing does not necessarily correlate with clinical outcomes. Therefore, we aimed to 1) identify relevant mechanisms of developmental effects and toxicity of NPs and 2) to use this knowledge to establish a microfluidic platform for embryotoxicity testing *in vitro*.

To better understand potential developmental effect pathways of NPs we performed an extensive literature review, which indicated that developmental toxicity is not only induced from NPs crossing the placental barrier but can be mediated by indirect mechanisms if NPs accumulate in maternal and placental tissues and affect their function and release of mediators ^[3]. We thus examined indirect placenta-mediated signalling pathways of NPs (TiO₂, SiO₂ and diesel exhaust particles (DEPs)) in exposed human placental tissue explants including a secretomics profiling study, which revealed interference of particles with the secretion of various vascular, endocrine and inflammatory factors in dependence of gestational stage (first trimester versus term). Moreover, preliminary results suggested that the conditioned explant medium depleted of NPs could affect angiogenic and neurodevelopmental processes relevant to fetal wellbeing.

These findings prompted us to develop a novel microphysiological placenta-embryo-chip that incorporates both, a placental trophoblast barrier (BeWo monolayer) and an embryonic 3D cell culture model (murine embryoid bodies (EB)) to capture the direct as well as indirect effects of NPs including transiently stable and highly reactive intermediates (e.g. reactive oxygen species) ^[4] (Figure 1). Assessing biological effects in a dynamic model was critical to achieve realistic dosing and exposure conditions and a first proof-of-concept study with plastic microparticles (500 nm polystyrol particles) revealed differential toxicity of microplastics on EBs (ATP content) in the presence or absence of a placental barrier. Specifically, viability of EBs was only reduced after indirect exposure in the presence of a placental barrier and since no particle transfer across the placental barrier was observed, it is possible that embryotoxicity was mediated by the release of placental factors.

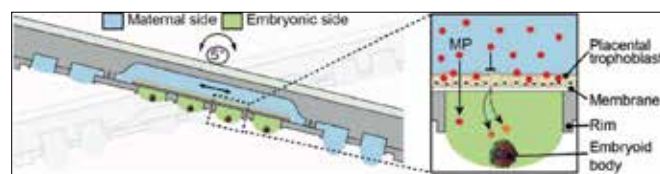


Figure 1: We developed a novel microphysiological model to recapitulate the maternal-placental-embryonic axis. Placental trophoblasts were cultured on top of the membrane on the maternal side, whereas EBs were formed and cultured in hanging drops underneath the porous membrane. Gravity-driven flow through the platform was induced by continuously tilting the chip back and forth by $\pm 5^\circ$. The immediate interaction of the placental trophoblast barrier and the EB allowed for studying direct and indirect toxicity effects triggered by maternal microplastics (MP) exposure.

In parallel we were also pushing the development of novel microporous membrane supports for cell barrier cultivation since commercial track-edged polymer membranes or porous PDMS membranes often constitute a major barrier to the free transfer of NPs. Here, we have fabricated free-standing nanofibrous electrospun chitosan membranes on cell culture inserts and confirmed their superior permeability for macromolecules and NPs. We are currently assessing the feasibility to incorporate these membranes into our microphysiological chip and to use more physiologically relevant, human-based placenta and embryo models to further push the predictive power of the platform.

In summary, our holistic approach to drive mechanistic insights on NP transport and developmental effects along with the development of microphysiological co-culture models of the maternal-placental-embryonic axis will set important groundwork to establish valuable pre-clinical safety and efficacy data for nanomedicines in pregnancy.

Acknowledgments:

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RADIOLIGAND THERAPY IN PROSTATE CANCER – RECENT CLINICAL TRIAL RESULTS AND DEVELOPMENTS

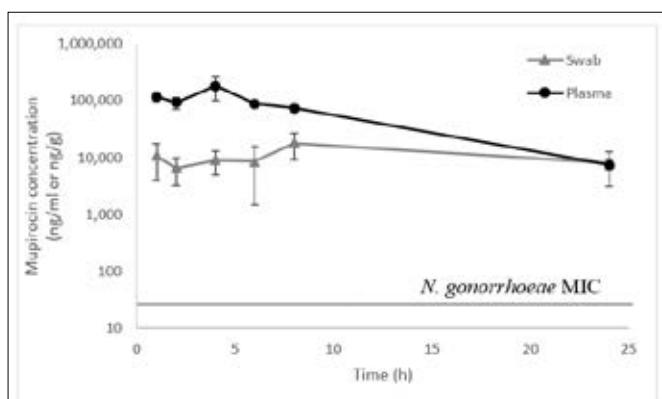
ANDREW CAVEY

Radioligand therapy represents a potential new treatment modality in prostate cancer. In this talk, we will explain the history of radioligand therapy development, share recent results from the phase III VISION study exploring ¹⁷⁷Lu-PSMA-617 in prostate cancer, and discuss ongoing developments in the field.

INJECTABLE MUPIROCIN LOADED PEGYLATED NANO LIPOSOMES (NANO-MUPIROCIN) IS POTENTIALLY EFFICACIOUS AGAINST INFECTIONS INVOLVING MULTIDRUG-RESISTANT BACTERIA

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Mupirocin concentration (sum of free and liposomal) in vaginal secretions (ng/g) and plasma (ng/mL) following IP administration of Nano-mupirocin 50 mg/kg to mice (mean \pm SE).

Antibiotic resistance is a global health threat. There are a few antibiotics under development, and even fewer with new modes of action and no cross-resistance to established antibiotics. Accordingly, reformulation of old antibiotics to overcome resistance is attractive. Nano-mupirocin, a PEGylated nano-liposomal formulation of mupirocin, was developed and fully characterized¹. Mupirocin is a unique antibiotic having a unique mode of action². However due to its rapid metabolism and high binding to plasma proteins its clinical use is limited to topical administration³. Reformulating mupirocin to Nano-mupirocin, allowed the parenteral use of mupirocin in deep infections, as previously demonstrated by us in several animal models⁴⁻⁶. Nano-mupirocin showed no cross-resistance

with other antibiotics and retained full activity against vancomycin-, daptomycin-, linezolid- and methicillin- resistant *Staphylococcus aureus* against vancomycin-resistant *Enterococcus faecium*, and cephalosporin-resistant *Neisseria gonorrhoeae*^{6,7}. Following Nano-mupirocin injection to rats, plasma levels greatly exceeded relevant MICs for >24h, and a biodistribution study in mice showed that mupirocin concentrations in vaginal secretions also greatly exceeded the MIC₉₀ for *N. gonorrhoeae* (0.03 μ g/mL) for > 24 h, as described in the Figure below. In addition, the use of fluorescent Nano-liposomes revealed that intact liposomal mupirocin reached the mice vagina. In summary, Nano-mupirocin has excellent potential for treatment of several infection types involving multiresistant bacteria. It has the concomitant benefits from utilizing an established antibiotic and PEGylated nano-liposomes of the same size and lipid composition as of Doxil[®], an anticancer drug product now used for the treatment of over 700,000 patients globally. Accordingly, Nano-mupirocin should be seen as a formulation with considerable potential and low development risks.

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TRAINED IMMUNITY IN CANCER IMMUNOTHERAPY

TRIANAFYLLOS CHAVAKIS, Institute for Clinical Chemistry and Laboratory Medicine, University Hospital and Faculty of Medicine, Technische Universität Dresden, Dresden, Germany

Trained immunity represents a form of innate immune memory. Certain trained immunity agonists, such as fungal beta-glucan, enhance the responses of myeloid cells to subsequent stimuli. We have previously shown that beta-glucan-induced trained immunity involves immunometabolic and epigenetic rewiring of myelopoiesis progenitors in the bone marrow (Mitroulis et al., Cell 2018). Furthermore, trained granulopoiesis leads to production of neutrophils that inhibit tumor growth (Kalafati et al., Cell 2020) and could therefore be exploited in cancer immunotherapy. The presentation will discuss the role of trained immunity in cancer immunotherapy.

NOVEL HUMAN SINGLE DOMAIN ANTIBODY DRUG CONJUGATES FOR ONCOLOGY

MARK CHIU

We have developed a platform using conformation-selective human single domain antibodies that have high specificity to tumor cells as compared to normal cells to minimize systemic toxicity; designed for endocytosis and internalization for intracellular delivery to enhance potency; and engineered tunable pharmacokinetic profiles for better efficacy. We present the rationale for our particular designs for the solid tumor microenvironment.

A BROAD-SPECTRUM ANTIVIRAL PEPTIDE FOR COMBATING EMERGING VIRAL PATHOGENS

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Viral infections are a leading cause of global morbidity and mortality that urgently need effective therapeutic strategies. While there have been important advances in antiviral drug development over the past few decades, there remain major challenges associated with the large number of emerging and re-emerging viruses as well as with the rise of drug-resistant virus strains. Developing broad-spectrum antiviral strategies that work against multiple viruses is a high priority to counter emerging viral threats. One promising strategy involves utilizing antiviral agents that target the lipid membrane surrounding a wide range of enveloped viruses such as Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2), Zika (ZIKV), and Dengue (DENV). Unlike other antiviral targets, the lipid envelope is derived from host cell membranes and there is a high barrier to the emergence of drug-resistant virus strains. In this talk, I will present ongoing work to develop a membrane-active peptide that exhibits broad-spectrum antiviral activity against medically important viruses by selectively destabilizing high-curvature viral membranes. By utilizing biophysical assays, we have characterized the mechanism of action of drug candidates down to the single-virus particle level with real-time measurement readouts. Based on these characterization efforts, we have identified a lead peptide drug candidate that exhibits potent, in vitro antiviral activity against ZIKV and DENV (all four serotypes) at nanomolar concentrations whereas it is nontoxic to mammalian cells at 1000-fold higher concentrations. The therapeutic efficacy of the peptide was also evaluated in a lethal ZIKV mouse model and treatment started three days after infection. Therapeutic administration of the peptide not only significantly reduced mortality, clinical symptoms, viremia, and inflammation, but also prevented neurodegeneration and brain damage. Furthermore, in a humanized mouse model of DENV infection, peptide treatment reduced viremia levels in vivo to nearly undetectable levels. Other arboviruses as well as filoviruses and SARS-CoV-2 have also proven to be susceptible to this targeting strategy. Collectively, our findings support that selective targeting of viral membranes holds great potential for combating emerging viral threats, including SARS-CoV-2 and beyond.

THE ROAD FOR HANDLING PANDEMIC PATIENTS IS STREWN WITH BIOETHICAL ISSUES.

AARON CIECHANOVER

The recent COVID-19 pandemic has taught us that science alone, as advanced as it might be, is not sufficient to handle all the problems that arise with a large mass of patients. Among the many problems we encountered are heavy bioethical issues. Among them are, for example:

Priorities in selecting patients for artificial respiration when equipment and expert teams are short; 2. Neglected areas like other

diseases or urgent issues like climate change; 3. Anti-vaxxers; 4. Misinformation and disinformation; 5. Racism; and 6. Inequality in disbursing vaccines;

Solution of these problems is essential; for our success in defeating future Pandemics, and requires, among other factors – trust in science, leadership, and national and international solidarity.

ADDRESSING THE COLD REALITY OF mRNA VACCINE STABILITY

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Keywords: COVID-19, lipid nanoparticle (LNP), lyophilization, mRNA, shelf life, storage stability, structure, vaccine

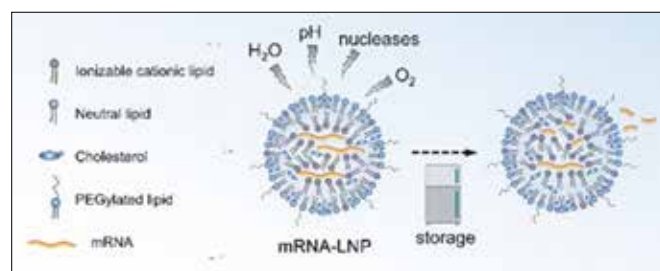
A drawback of the current mRNA-lipid nanoparticle (LNP) COVID-19 vaccines is that they have to be stored at (ultra)low temperatures (2). Understanding the root cause of the instability of these vaccines may help to rationally improve mRNA-LNP product stability and thereby ease the temperature conditions for storage. In this presentation we discuss proposed structures of mRNA-LNPs, factors that impact mRNA-LNP stability and strategies to optimize mRNA-LNP product stability. Analysis of mRNA-LNP structures reveals that mRNA, the ionizable cationic lipid and water are present in the LNP core. The neutral helper lipids are mainly positioned in the outer, encapsulating, wall. mRNA hydrolysis is an important driver for mRNA-LNP instability. It is currently a matter of debate whether water in the LNP core can freely interact with the mRNA and to what extent the degradation prone sites of mRNA are protected through a coat of ionizable cationic lipids. To improve the stability of mRNA-LNP vaccines, optimization of the mRNA nucleotide composition should be prioritized. Secondly, a better understanding of the milieu the mRNA is exposed to in the core of LNPs may help to rationalize adjustments to the LNP structure to preserve mRNA integrity. Moreover, drying techniques, such as lyophilization, are promising options still to be explored.

As vaccines turn out to be the major weapon against the COVID-19 viral attack, the urge to develop more stable formulations is still growing and alternative, not-mRNA based products, may come to the forefront in situations where the (ultra)cold chain cannot be guaranteed.

This is an adapted abstract from references

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LIPID NANOPARTICLES, GENE THERAPY AND THE PFIZER/BIONTECH COVID-19 VACCINE

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Gene therapies employing genetic drugs such as small interfering RNA (siRNA) for gene silencing and mRNA for gene expression have the potential to cure most diseases. However, sophisticated delivery systems are required to enable clinical use of nucleic acid polymers as they are readily broken down in biological fluids, do not accumulate at sites of disease and cannot penetrate target cells even if they arrive at target tissues. Lipid nanoparticle (LNP) technology is increasingly enabling the clinical potential of genetic drugs by packaging the nucleic acid polymer in well-defined nanoparticles that protect the nucleic acid payload *in vivo* and facilitate intracellular delivery following uptake into target cells by endocytosis. This approach has received clinical validation with the approval of Onpattro by the FDA in 2018. Onpattro consists of an LNP containing siRNA to silence transthyretin in hepatocytes, thereby arresting and reversing the disease transthyretin induced amyloidosis (hATTR), a disease that was previously untreatable and was fatal within five years of diagnosis. In this talk I will describe the design features that were followed to develop Onpattro and how related technology is being employed to enable mRNA-based drugs. A notable example is the development of the Pfizer/BioNTech mRNA vaccine, which is playing a leading role in alleviating the Covid-19 pandemic.

A FLEXIBLE POLYMERIC MICROMESH FOR THE INTRACRANIAL DELIVERY OF SMALL MOLECULES, ANTIBODIES AND NANOMEDICINES AGAINST GLIOMAS

PAOLO DECUZZI, Ph.D. Senior Researcher and Professor, Director, Laboratory of Nanotechnology for Precision Medicine, Italian Institute of Technology

Despite tremendous advancements in early cancer diagnosis, targeted therapies, robot-assisted surgery, image-guided radiation therapies and the advent of cancer immunotherapies, glioblastoma and pediatric low-grade gliomas continue to be the most aggressive and less curable forms of any cancer. The current standard of care has not changed over the past 20 years, still relying on maximal resection of the malignant tissue followed by adjuvant radiotherapy and chemotherapy. This complex, expensive treatment plan provides only a modest improvement in life expectancy (a few months) and various degrees of therapy-induced complications, including the deterioration of physical, emotional, and social functions. Key challenges in the treatment and management of brain tumors are uniquely related to the specific biological complexity and anatomical barrier. In this talk, a novel compartmentalized, flexible micro-implant – microMESH – for the sustained and localized delivery of a variety of therapeutic molecules, and their combinations, will be described. First the fabrication process of microMESH will be detailed, followed by its physico-chemical and mechanical characterization. Then, the therapeutic efficacy of microMESH will be demonstrated on primary cell cultures and patient derived organoids as well as in orthotopic murine models of the disease. Data on small molecules, nanomedicines and antibodies will be presented.

A PORCINE MODEL OF RARE ALLERGIC REACTIONS TO LNP-mRNA-BASED COVID-19 VACCINES

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INTRODUCTION

A minor percentage of people immunized with mRNA-containing liposomal (LNP-mRNA) vaccine develop allergy-like symptoms after vaccination. This can lead to a severe reaction. The exact mechanism is unknown. The time course and spectrum of symptoms are similar to those of the pseudoallergy to i.v.-administered nanomedicines, in which the activation of the complement system (C) plays an important role, and is therefore called C-activation-related pseudoallergy (CARPA). Pigs provide a natural model of hypersensitivity to study HSRs. The aim of the present experiments was to investigate the development of CARPA-like reactions after administration of Comirnaty (CMT), an LNP-mRNA type vaccine.

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 O₂ flow. In spontaneously ventilating animals, the pulmonary arterial pressure (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 and ECG were continuously monitored using an AD Instruments (ADI) PowerLab System. Mean PAP, SAP, HR and ECG 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 K₃-EDTA blood tubes, of which samples for TXB2 analysis were containing indomethacin. Aliquots of 100 µl blood were drawn into tubes with K₃-EDTA for haematological analysis, performed by an Abacus (Diatron) analyser. 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: Doses of 1x, 2x and 5x of the human dose of CMT vaccine were given as an i.v. bolus injection. Zymosan (0.1 mg/kg), a direct complement activator was utilized as positive control inducing CARPA.

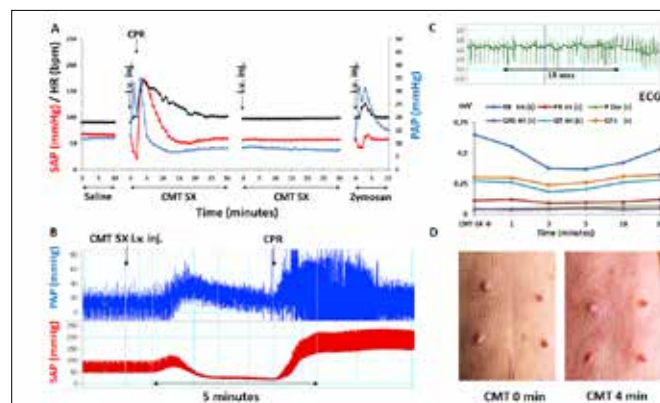


Figure 1. Anaphylaxis with tachyphylaxis in a pig injected with 5x human dose. CMT-Comirnaty vaccine, CPR-cardiopulmonary resuscitation. A. Mean PAP (blue), SAP (red), and HR (black) during the experiment. B. Real-time pulse pressure recording during the initial 10 min. C. Top: A 25 s ECG recording during the reaction showing arrhythmia; Bottom: Changes in ECG parameters after the first injection of 5x CMT up to 30 min. D. Photographs of baseline (CMT 0) and skin flushing caused by CMT at 4 min (CMT 4 min) after i.v. injection.

During *in vitro* incubation of CMT with porcine serum, the anaphylatoxin C3a and C-terminal complex sC5b-9 were significantly elevated (data not shown).

CONCLUSIONS

This study investigated the immune reactive properties of Comirnaty (CMT), an LNP-mRNA type vaccine. CMT administration induced HSRs showing all characteristic properties of CARPA. Thus, this phenomenon may be a contributing factor to the HSR to CMT and potentially other vaccines.

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FINANCIAL SUPPORT

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IMMUNOLOGICAL PROPERTIES OF THERAPEUTIC NUCLEIC ACIDS AND LIPID-BASED NANOPARTICLES

MARINA A. DOBROVLSKAIA, Nanotechnology Characterization Lab., Frederick National Laboratory for Cancer Research, Frederick, MD, USA; marina@mail.nih.gov

Nanotechnology carriers improve the delivery of therapeutic nucleic acids (TNAs) by improving their stability and targeting the cells and tissues of interest. Lipid-based nanocarriers such as liposomes and lipoplexes are commonly used to deliver TNAs. This presentation will focus on the immunological properties of nucleic acid-based nanoparticles (NANPs) and lipid-based nanocarriers. Complement activation, cytokine responses, and recognition by Toll-Like Receptors will be discussed. Complementary and overlapping properties will be identified in the context of both NANPs and their lipid-based nanocarriers.

Acknowledgments: Supported by NCI contracts HHSN261200800001E and 75N91019D00024.

THE PHOSPHOLIPID RESEARCH CENTER – CURRENT RESEARCH IN PHOSPHOLIPIDS AND THEIR USE IN DRUG DELIVERY

SIMON DRESCHER

This talk shortly introduces the Phospholipid Research Center Heidelberg (Germany) and summarizes the research on phospholipids and their use for drug delivery. The focus is on projects that have been approved by the Phospholipid Research Center since 2018 and are currently still ongoing or have been recently completed. The different projects cover all facets of phospholipid research: from basic to applied research, including the use of phospholipids in various forms of application such as liposomes, mixed micelles,

emulsions, and extrudates, up to industrial application-oriented research. The projects presented also include all route of administration, namely topical, oral, and parenteral.

ENGINEERING PERSONALIZED TISSUE IMPLANTS: FROM 3D PRINTING TO BIONIC ORGANS

TAL DVIR

In this talk I will describe cutting-edge bio and nanotechnologies for engineering functional tissues and organs, focusing on the design of new biomaterials mimicking the natural microenvironment, or releasing biofactors to promote stem cell recruitment and tissue protection. In addition, I will discuss the development of patient-specific materials and 3D-printing of personalized vascularized tissues and organs. Finally, I will show a new direction in tissue engineering, where, micro and nanoelectronics are integrated within engineered tissues to form cyborg tissues and bionic organs.

THE IMMUNOTHERAPY REVOLUTION: LESSONS FROM MELANOMA FROM ADVANCED TO (NEO) ADJUVANT STRATEGIES

ALEXANDER EGGERMONT, University Medical Center Utrecht, Netherlands; Comprehensive Cancer Center Munich, Germany

In the last 10 years an immunotherapy revolution has unfolded that has no precedent in cancer medicine. The discoveries of two key-regulator molecules, anti-CTLA4 and anti-PD(L)1, which enhance T-Cell priming and protect T-Cell Effector function have changed cancer medicine. Anti-PD(L)1 is the central key molecule in > 3000 clinical trials: in combination with anti-CTLA4 (>400 trials), chemotherapy (>400 trials), radiation therapy (>200 trials), targeted agents (>400 trials), vaccines (>300 trials), and various other agents (> 300 trials). It all started in melanoma where basic principles of moa and rationale of combinations have been explored. Moreover a direct translation has taken place from advanced disease to application in the adjuvant setting. Anti-PD1 has more than 100 FDA approvals across > 25 different tumor types.

Currently a **true revolution in neoadjuvant immunotherapy strategies** is unfolding in multiple tumor types. The results of anti-PD1 and the combination of anti-CTLA4+anti-PD1 in stage resected III/IV melanoma patients are unprecedented in terms of response rates (> 70% pathologicCRs and nearCRs; reduction of relapses; and reduction of surgical interventions : in other words more cures, shorter treatments and less surgery !). Lessons from melanoma hold true for multiple tumors and new treatment paradigms: Extraordinary activity in MSI-Colorectal cancers (>90% CRs and subsequent reduction of mutilating surgical interventions); similar results in advanced cSCC inHead and Neck region and reduction of mutilating surgery) neoadjuvant combotherapies for esophageal, GEJ and Gastric cancers; up to 50% CR rates in invasive bladder cancers; and so on. Neoadjuvant Immunotherapy will dominate the clinical trial scene for at least 5 years to come and will profoundly change the way we manage cancer patients with solid tumors. Lessons from melanoma hold true for multiple tumors and new treatment paradigms. The development of Immuno-Combos involves the innate immune system, in particular by blocking its immunosuppressive “wound-healing tumor promoting aspects”. The quickest way to learn is in the neo-adjuvant setting and in “window of opportunity studies”. In summary: Lessons from melanoma can be drawn for virtual all new strategies (**ref**).

Neoadjuvant immunotherapy is reshaping cancer management across multiple tumour types: The future is now! Maio M, Blank C, Necchi A, Di Giacomo AM, Ibrahim R, Lahn M, Fox BA, Bell RB, Tortora G, Eggermont AMM. *Eur J Cancer* 2021;**152:155-164**.

A NANOFUIDIC DEVICE FOR RELIABLE AND QUANTIFIABLE DIAGNOSTICS FOR COVID-19

YASIN EKINCI, Paul Scherrer Institute

The outbreak of SARS-CoV-2 underlined the importance of point-of-care diagnostics, as well as reliable and cost-effective serological antibody tests to monitor the viral spread and contain pandemics and endemics. We developed a three-dimensional (3D) nanofluidic device for rapid and multiplexed detection of viral antibodies. The device is designed to size-dependently immobilize particles from a multi-particle mixture at predefined positions in nanochannels through capillary forces only, resulting in distinct trapping lines. We show that individual lines can be used as an on-chip fluorescence-linked immunosorbent assay for multiplexed detection of serological immunoglobulin antibodies against viral proteins with high sensitivity and specificity. Further versatility is exhibited by on-bead color multiplexing for simultaneous detection of IgG and IgM antibodies in convalescent human serum. The particle sorting is further leveraged to enable concurrent detection of anti-spike (SARS-CoV-2) and anti-hemagglutinin (Influenza A) antibodies. In general, the device offers a platform of performing immunoassays for rapid, simple, multiplexed detection of proteins of small amounts. The device's applications can be further extended to detect a plethora of diseases simultaneously in a reliable, quantitative, and straightforward manner.

FROM BENCH TO BEDSIDE: D-PLEX₁₀₀ LIMITS ANTI-MICROBIAL RESISTANCE OCCURRENCE IN RANDOMIZED BLINDED PHASE 2 TRIAL IN COLORECTAL SURGERY PATIENTS.

NOAM EMANUEL^{1*}, Shlomo Nedvetski¹, Olga Belotserkovsky¹, Yafit Stark¹, Goldi A. Kozloski¹, Malka Reichert¹, Anthony J. Senagore²

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Despite significant advances in infection control guidelines and practices, surgical site infections (SSIs) remain a substantial cause of morbidity, prolonged hospitalization, and mortality. Among patients undergoing elective colorectal surgeries, SSI rates are particularly high due to the exposure of intraluminal bacteria. Combination of oral and systemic antibiotic prophylaxis have been shown to reduce the incidence of post-operative soft tissue incisional infections, however given the potential complications associated with systemic antibiotic exposure there is a significant unmet need for effective, local prophylactic options.

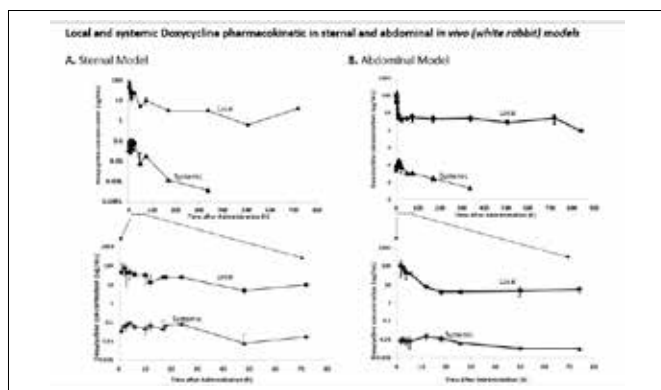


Figure 1. Local vs systemic Doxycycline released from D-PLEX100 in sternal and abdominal surgical defect models.

D-PLEX100 is a novel, drug-eluting polymer-lipid matrix, designed as a single application, directly at the surgical wound in a form of paste prior to surgical closure. D-PLEX100 uniquely provides a high

local concentration of doxycycline (DOX) in a constant, zero order rate, for four weeks. The prolonged release profile was confirmed *in vivo* in custom designed SSI rabbit models, where local wound microdialysis pharmacokinetic (PK) parameters of DOX released from D-PLEX100 (AUC and C_{max}) were analyzed and validated by HPLC-MS/MS. The *in vivo* results indicated robust and prolonged local exposure profile that is similar to the *in vitro* profile.

Lower graphs represent a detailed insert of the first 3-4 days. Animal sternal and abdominal SSI models were challenged with the common SSI associated pathogens (gram-negative, gram-positive, and multi-drug resistant strains including DOX-resistant methicillin-resistance Staphylococcus aureus (MRSA) and DOX-resistant Klebsiella pneumoniae). Treatment with D-PLEX100 resulted in statistically significant reduction of the challenged bacteria compared with controls Table 1.

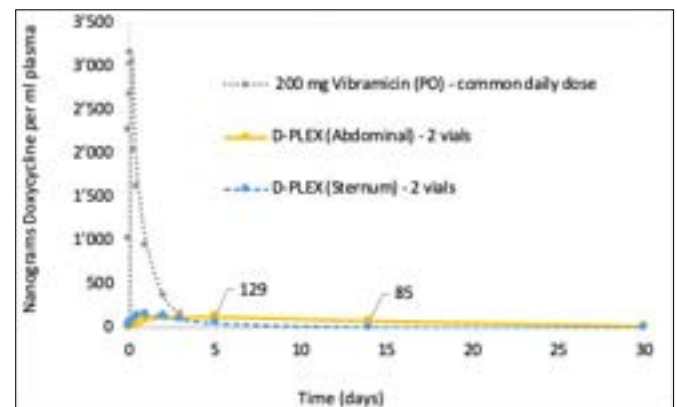
Bacteria	Sternal model				Abdominal model				P-value	
	MIC to Doxycycline (µg/ml)	Bacteria/Unit	Control	D-PLEX	Bacteria/Unit	Control	D-PLEX	log10 Reduction		
Enterobacter aerogenes (ATCC #13048)*	0.98	1.2 × 10 ⁸	1.39 × 10 ⁷	39	3.47				<0.0001	
Escherichia coli (ATCC #15922)*	0.98	1.5 × 10 ⁸	3 × 10 ⁷	8	8.84	1.2 × 10 ⁷	5.99 × 10 ⁷	1.76	5.52	<0.0001
Klebsiella pneumoniae (ATCC #700603)*	31.25	1.5 × 10 ⁸	1.7 × 10 ⁷	4.85 × 10 ⁷	2.14				<0.0001	
Methicillin-resistant Staphylococcus aureus (MRSA ATCC #12228)	15.63	1.2 × 10 ⁸	0.74 × 10 ⁷	7.74 × 10 ⁷	3.05	1.2 × 10 ⁷	3.55 × 10 ⁷	8.84 × 10 ⁷	3.6	<0.0001
Staphylococcus epidermidis (ATCC #12228)	0.49	1.5 × 10 ⁸	4.3 × 10 ⁷	7.52 × 10 ⁷	1.74				<0.0001	
Enterococcus faecalis (ATCC 29211)	3.90	1.5 × 10 ⁸	1.51 × 10 ⁷	1.29 × 10 ⁷	3.08				<0.0001	
Vancomycin resistant Enterococcus faecium (ATCC #700221)	0.49					1.2 × 10 ⁷	2.53 × 10 ⁷	8	4.4	<0.0001

Table 1. Bacterial recovery summary in rabbit SSI models

Animal safety histology studies indicated considerable reduction in local necrosis in the D-PLEX₁₀₀ treated vs control animals. The plasma PK values in both animal models indicated 2-3 orders of magnitude reduction in systemic vs local exposure.

Minimal systemic exposure from D-PLEX₁₀₀ was also confirmed in patients treated with D-PLEX₁₀₀ (Average dose of 2 vials, 126 mg DOX hyclate) where DOX exposure (mean C_{max}) was 183ng/mL, significantly lower than the reference standard of 200mg DOX oral dose 3160 ng/mL (Figure 2).

Figure 2. Comparative mean plasma doxycycline concentration levels: D-PLEX₁₀₀ vs. oral doxycycline.



A phase 2 clinical trial in patients undergoing elective colorectal surgery evaluated SSI prevention by D-PLEX100. Patients were randomized 1:1 to D-PLEX₁₀₀ plus standard of care (SOC) or SOC alone (ClinicalTrials.gov identifier NCT03633123). The SOC included prophylactic IV antibiotics 30-60 minutes prior to surgery, and D-PLEX100 was applied based on the length of surgical incision at the time of surgical closure. The study results indicated a 64% reduction in SSI rate in the D-PLEX100 group (N=7/88 [7.9%]) vs SOC alone (N=20/91 [21.9%]); p<0.05. There were no mortalities in the D-PLEX100 arm, and in the SOC, 3 (3.0%) within 30 days (p=0.1213) and total 5 (5%) within 60 days (p=0.029). D-PLEX₁₀₀ was well tolerated. No serious adverse events were deemed related to the study drug.

D-PLEX₁₀₀ effect on SSI reduction in terms of the causative organisms that are isolated from the infected wound sites were evaluated between the treatment arms. The majority of SSI pathogens isolated in both study arms were drug resistant (70%), including DOX resistant strains.

To determine if prolonged D-PLEX₁₀₀ exposure may select for or increase the frequency of DOX resistance or multi-drug resistant organisms (MDROs), patients were assessed by rectal swab tests before and after D-PLEX₁₀₀ treatment. There was no significant difference in rectal swab colonization with MDROs between groups.

Together, these data demonstrate that D-PLEX₁₀₀ provides an effective novel approach to SSI prophylaxis which is effective across wound classes, extends activity to bacteria that is functionally resistant to traditional systemic administration strategies, and avoids microbiome pressure for the potential development of additional resistant bacterial strains. As such, D-PLEX₁₀₀ may be a promising addition to established colorectal SSI bundles for reducing SSIs without the risks associated with both oral and systemic antibiotic exposure. *PolyPid Ltd.* is currently conducting a phase 3 global multicenter clinical trial in colorectal surgery to further evaluate the safety and efficacy of D-PLEX₁₀₀ in SSI prevention.

T-CHARGE™, NEXT-GENERATION CAR-T PLATFORM WITH FIRST-IN-HUMAN DATA

BORIS ENGELS, Novartis Institutes for BioMedical Research, Cambridge, MA, USA

Extended T-cell culture periods *in vitro* deplete the CAR-T final product of naive and stem cell memory T-cell (Tscm) subpopulations that are associated with improved antitumor efficacy. YTB323 is an autologous CD19-directed CAR-T cell therapy with dramatically simplified manufacturing, which eliminates complexities such as long culture periods. This improved T-Charge™ process preserves T-cell stemness, an important characteristic closely tied to therapeutic potential, which leads to enhanced expansion ability and greater antitumor activity of CAR-T cells.

A Phase I, multicenter, dose-escalation study (NCT03960840) is evaluating safety and preliminary efficacy of YTB323 in patients with B-cell malignancies. In diffuse large B cell lymphoma (DLBCL) YTB323 recruitment is ongoing at dose level (DL) 3. At DL2, YTB323 showed promising efficacy and a favorable safety profile. Current data support continued development of YTB323.

The novel manufacturing platform T-Charge™ used for YTB323 is simplified, shortened, and expansionless. It thereby preserves T-cell stemness, associated with improved *in vivo* CAR-T expansion and antitumor efficacy. Compared to approved CAR-T therapies, YTB323 has the potential to achieve higher clinical efficacy at its respective lower doses. T-Charge™ is aiming to substantially revolutionize CAR-T manufacturing, with concomitant higher likelihood of long-term, deep responses.

WHY DID FATHER UNIVERSE AND MOTHER NATURE BEGET LIPIDS?

ALFRED FAHR

All biological entities have to demarcate themselves from the environment; this is mainly done by biomembranes. In the overwhelming majority of cases, biomembranes consist of phospholipids. At the latest since Alec Bangham's experiments (1965) we also know that phospholipids can spontaneously form vesicles (liposomes) the size of bacteria. Did phospholipids exist from the beginning of chemical evolution or were they tailored during evolution?

Various theories have been developed and, in a way, they have undergone their own evolution.

The famous Miller-Urey experiment (1953) and subsequent experiments (e.g. Ferus et al. 2017) showed that amino acids, peptides and nucleobases can indeed be formed in the primordial atmosphere under lightning, but amphiphilic products that could be considered precursors to phospholipids were hard to detect. However, fatty acids and other vesicle-forming substances could be found in meteorites (e.g. Deamer 1985), which were probably formed during the dangerous, often torrid journey of the meteorites to Earth. Under violent simulated hydrothermal conditions (in terms of pressure and temperature), membrane-forming amphiphilic substances can also be obtained (e.g. McCollom 1999).

What seems clear, however, is that phospholipids were not present in prebiotic soups or meteorites. This was then a result of the further evolution of the first membrane-forming elements.

During this evolution, phospholipids acquired astonishing abilities that go far beyond compartment-forming properties. For example, liposomes can release another enclosed liposome in response to a purely physical stimulus (Moroz 1997) or when phospholipids aggregate to form liposomes, proteins are concentrated in great density in the liposome (de Souza et al. 2011).

In medicine today, liposomes are used in many forms as successful drug carriers. The disadvantage of the fragility of liposomes, often claimed by died-in-the-wool chemists, is mostly irrelevant and often surpasses the functionality of polymer or other particles. If the amazing properties of phospholipids, which have only been briefly hinted at here, can be exploited in future drug carriers, phospholipid-containing vesicle systems have an even greater potential.

REGULATION OF COMPLEX NON-BIOLOGICAL MEDICINES AND THEIR GENERICS IN AUSTRALIA BY THE THERAPEUTIC GOODS ADMINISTRATION (TGA)

ANNE FIELD

The Therapeutic Goods Administration (TGA) is part of the Health Products Regulation Group (HPRG) in the Australian Government Department of Health and is the national regulator responsible for regulating the quality, safety and efficacy of therapeutic products imported into, exported from, and supplied or manufactured in Australia. Regulation of medicines, medical devices, blood and blood products as well as biologicals is underpinned by the *Therapeutic Goods Act 1989 ('the Act')*. The Department of Health aims to provide an affordable, accessible, efficient and high-quality health system through regulations that protect the health and safety of the community while minimising unnecessary compliance burdens. Access to generic medicines can provide Australians with greater choice and convenience, may safeguard medicine supply in times of shortage, and reduce costs. However, regulators at the TGA have faced challenges applying the Australian regulatory framework to the evaluation of complex generic products, and a flexible and pragmatic approach has been taken. As a general rule, EU scientific guidelines are adopted by the TGA, but reference may be made to product specific guidance or draft guidance developed by other major international regulatory agencies when appropriate. In 2020, the Act was amended to allow applicants to request pre-submission advice on aspects of quality, safety or efficacy of a medicine prior to lodging a registration application. At present, this advice is limited to requests for biowaiver justification, including justifications to use an overseas reference product in a bioequivalence study. However, this scheme may be expanded in future to include other aspects of quality, safety and efficacy.

While many Australian standards are based on international standards, further alignment with the processes of other regulatory

agencies is possible. International alignment offers potential benefits through reduced regulatory burden, including by improving opportunities for work sharing with comparable overseas regulators. The TGA is a member of the Access Consortium, a coalition of regulatory authorities that work together to promote greater regulatory collaboration and alignment of regulatory requirements (the other members of the Consortium are Health Canada, Health Sciences Authority of Singapore, Swissmedic and the UK's Medicines and Healthcare products Regulatory Agency). An example of a recent international work sharing experience resulting in approval of a complex generic product will be discussed.

In conclusion, the trend towards globalisation of therapeutic product industries and the rapid emergence of new technologies have created an increased need for regulatory bodies to communicate with each other routinely. This maximises the use of up-to-date technical expertise, and ensures a consistent, contemporary approach to assessing the benefits and risks associated with the use of therapeutic products.

FIGHTING VECTOR-BORNE DISEASES IN IBEROAMERICAN REGIONS WITH NANOFORMULATIONS

FABIO ROCHA FORMIGA, Research Scientist and Professor, Oswaldo Cruz Foundation (FIOCRUZ) and University of Pernambuco (UPE), Recife, Brazil.

According to World Health Organization (WHO), vector-borne diseases account for more than 17% of all infectious diseases, causing more than 700 000 deaths annually. They can be caused by either parasites, bacteria or viruses, and include highly prevalent diseases such as malaria, leishmaniasis, yellow fever, dengue, and more recently, chikungunya and Zika virus infections. Currently, the approaches used to prevent and treat these conditions are limited. From therapeutic standpoint, conventional drugs are toxic and presents lack of specificity, reduced patient compliance and emerging resistance. By facing this scenario, nanotechnology has provided significant insights towards novel approaches able to prevent and treat vector-borne diseases in a safe and effective manner. This conference talk covers the development and biological assessment of nanotechnology-based drug delivery systems for fighting vector-borne diseases in Iberoamerican regions.

CELL-DERIVED VESICLES AS NOVEL CARRIERS FOR ANTIBIOTICS

GREGOR FUHRMANN^{1,2}

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Extracellular vesicles (EVs) are cell-derived lipid membrane particles decorated with surface and membrane proteins. EVs are nature's way to deliver information as they transfer protein and nucleic acid based cargoes selectively to their target cell. Moreover, EVs feature a naturally derived composition; can potentially bypass complement activation and coagulation factors leading to reduced immunogenicity and increased stability in biological fluids. In addition, they often transit to their specific target cell rendering them promising candidates for drug delivery applications in cancer, inflammation or infection research.

In my presentation, I will discuss challenges associated with loading EVs with antimicrobial drugs. Moreover, I will introduce a group

of bacterial vesicles derived from non-pathogenic soil bacteria that produce inherently antimicrobial EVs. We have studied these vesicles in different infection models, including intracellular infections and bacterial biofilm; and we assessed their biocompatibility *in vitro*. Our results indicate that EVs are promising carriers that are able to overcome physiological barriers and deliver drugs with high efficiency and at the cellular level.

DRUG CO-ENCAPSULATION IN LIPID NANOPARTICLES FOR A MULTIMODALITY APPROACH TO CANCER THERAPY

ALBERTO GABIZON, Affiliation: Shaare Zedek MC and Hebrew University, Jerusalem, Israel.

Introduction: Alendronate (ALD) is a potent amino-bisphosphonate that has demonstrated direct tumoricidal activity and immune modulatory effects. Reformulation of alendronate by encapsulation in liposomes has been found to increase the anticancer efficacy of cytotoxic chemotherapies and adoptive T cell immunotherapies in murine cancer models. Co-encapsulation of ALD and doxorubicin (DOX), a potent chemotherapeutic agent with non-overlapping mechanisms of activity and toxicity, could lead to a chemo-immunotherapeutic, multi modality platform, with major pharmacologic advantages.

Methods: Methods to manufacture an upscalable liposomal formulation co-encapsulating ALD and DOX (PLAD) at 10-fold laboratory batch size (0.1-0.5 L) were developed. ALD was passively loaded in the liposome water phase. DOX was remote loaded with a transmembranal ammonium alendronate gradient.

Results: Optimal encapsulation efficiency of DOX (>95%) and stability of the complex ALD-DOX was achieved by adjusting the ALD:DOX molar ratio during the remote loading process. CryoTEM images of PLAD show spherical vesicles of average diameter ~90 nm with intraliposomal electron-dense rods which are shorter and thicker than those commonly seen in Doxil (Caelyx™) liposomes (Figure). PLAD was stable with no detectable leakage in a plasma stability assay. *In vitro* cytotoxicity tests in several human and murine cancer cell lines indicated greater potency for PLAD compared to Doxil. High and comparable plasma levels of DOX were obtained after injection of PLAD and Doxil in mice. The *in vivo* antitumor efficacy of PLAD was investigated in a multidrug resistant mouse tumor model (M109R) and in a mouse sarcoma model (WEHI-164). PLAD was superior to Doxil and free DOX in the growth inhibition of tumor implants at study endpoint.

Conclusions/Impact: These results open the way for further development of PLAD towards clinical applications of a unique product that blends chemotherapeutic and immune-boosting properties.

REDEFINING CANCER WITH INTEGRATIVE TUMOR IMMUNOLOGY

JÉRÔME GALON, Director of Research, French National Institute of the Health and Medical Research (INSERM), Chief of laboratory of Integrative Cancer Immunology, Cordeliers Research Center, Paris, France.

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. Analyses revealed a large inter- and intra-metastatic tumor cell and immune heterogeneity. We further demonstrated the significant role of Immunoscore and immunoediting in affecting metastatic clonal dissemination. We hence proposed a "parallel immune selection model" of tumor evolution incorporating the effects of the immune system in shaping and driving metastatic spread. We proposed a continuum of cancer immunosurveillance from pre-cancer to metastasis, and novel concepts underpinning tumor evolution at the pre-cancerous stages will be advocated.

SELF-ASSEMBLY IN INBORN ERROR OF METABOLISM DISORDERS: NEW THERAPEUTIC PATH FOR RARE DISEASE

EHUD GAZIT, Ph.D. FRSC FNASc OSSI, Founding Director, BLAVATNIK CENTER for Drug Discovery Incumbent, Chair for Biotechnology of Neurodegenerative Diseases, Tel Aviv (I)

Various genetic error of metabolism disorders, such as phenylketonuria, tyrosinemia, maple syrup disease, and homocystinuria, are characterized by the accumulation of various metabolites in blood, tissues, and organs. It was recently established that this accumulation could lead to the formation of amyloid-like structures by the process of self-assembly. Such assemblies could be observed as deposits in the brain of affected individuals. This discovery paves the way toward new therapeutic interventions by targeting the self-association events. The applicability of the method was confirmed using animal models. The concept of metabolite assembly is now suggested to be relevant also to age-related disorders including Alzheimer's disease and Parkinson's disease. This could clarify certain unexplained epidemiological associations between abnormal levels of metabolites and the tendency to develop pathological conditions. This plenary presentation will describe inborn error of metabolism disorders which result in the abnormal accumulation of metabolites. We will discuss the role of metabolite nano-assemblies in the pathology of the disease, describe new therapeutic avenues and the relationship of the nanostructures to age-related maladies.

COVID-19: THE FIRST PARADIGM OF PERSONALIZED ANTI-IL-1 THERAPY

EVANGELOS GIAMARELLOS-BOURBOULIS, 4th Department of Internal Medicine, National and Kapodistrian University of Athens, Medical School, Greece
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The new era of theragnostics requires the use of a biomarker which is informative of the activation of a specific pathway in the human host and which guides treatment decisions in order to target this pathway. The SAVE-MORE trial has paved a new way in this personalized treatment for infectious diseases and it is the first theragnostics strategy which has been endorsed by one large regulatory authority i.e. the European Medicines Agency. This strategy is aiming to early recognize the activation of the interleukin-1 pathway in adults hospitalized for COVID-19 pneumonia using the biomarker suPAR (soluble urokinase plasminogen activator receptor). Plasma concentrations 6 ng/ml or more guide the immediate start of treatment with anakinra; anakinra is the recombinant non-glycosylated form of the antagonist of the IL-1 receptor. This strategy increases by 64% the odds for full recovery from the infection and decreases by 54% the odds for remaining at severe illness or having died after 28 days.

CONFRONTING AMR BEYOND THE COVID-19 PANDEMIC

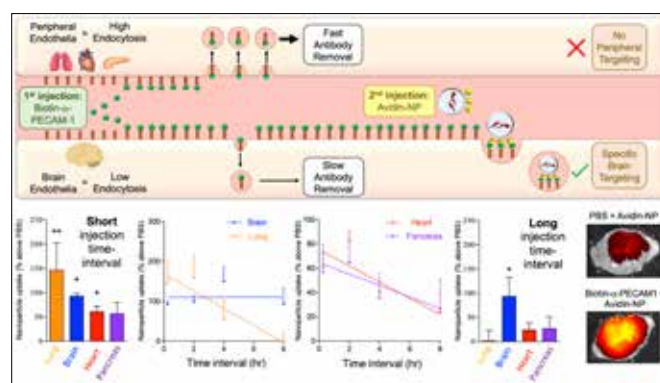
VALERIA GIGANTE

The presentation will focus on the WHO role in shaping R&D to fight AMR and ensure access to new antimicrobial agents guiding research efforts and investments by defining and updating priorities of global public health importance. Within the WHO tools available to researchers, pharmaceutical companies and policy makers on antibacterials, the upcoming WHO annual pipeline review will be presented along with research priorities established and maintained in the WHO Priority Pathogen Lists (WHO BPPL). In addition, the application of nanotechnologies as antimicrobial agents will be discussed as they might be an effective tool against pathogens listed as a priority.

EXPLOITING THE BLOOD-BRAIN BARRIER IMPERMEABILITY TO GENERATE ARTIFICIAL BRAIN TARGETS FOR NANOPARTICLE DELIVERY

DANIEL GONZALEZ-CARTER^{1,4*}, Xueying Liu¹, Theofilus Tockary¹, Anjaneyulu Dirisala¹, Kazuko Toh¹, Yasutaka Anraku^{1,2}, Kazunori Kataoka^{1,3}

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Nanoparticle (NP) brain-delivery strategies targeting proteins over-expressed at the brain micro-vasculature (e.g. TfR1, Glut1) have substantial specificity limitations due to significant protein expression in peripheral organs¹. We have developed a new strategy² to target NPs to the brain by instead selectively labelling the brain microvasculature. We exploit the lower endocytic rate of brain endothelial cells (BEC)³ to promote retention of free ligands (i.e. labels) selectively on the surface of BEC. NPs capable of recognizing the endothelial label are subsequently targeted to the brain without peripheral targeting (scheme 1). We demonstrate the *in vivo* feasibility of this strategy by injecting biotinylated α -PECAM1 antibodies (to label endothelial cell surfaces) followed by injection of avidin-functionalized nanoparticles (Avidin-NP) at increasing time-intervals. While short time-intervals result in avidin-NP targeting to the lung, brain, heart and pancreas, long time-intervals result in avidin-NP targeting only to the brain.

The present work therefore provides the basis for a new targeting strategy which exploits the physiology of BEC to generate the required NP targeting specificity.

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FUTURE LEADERS AGAINST AMR

ANNA GOVETT, Project Director, will be presenting the 'Future Leaders Against AMR' programme and discussing the importance of youth engagement with AMR given the unique position of young people as influential community leaders both at present and beyond. There is a real need for an interdisciplinary and collaborative approach to AMR which actively invests into young people as future leaders in the field.

'Future Leaders Against AMR' is the first global programme focused on supporting the professional development of the next generation of change-makers in AMR. The 10-week programme, running from January to March 2022, involves 40 young people from a range of backgrounds, with priority having been given to those from LMIC and backgrounds outside of the biological sciences. The programme includes 32 lectures and panels from experts, 14 small projects with mentorship from professionals, soft-skills training sessions, group discussions, and guided independent readings. Due to high demand (320 applications for 40 places), one lecture a week was opened to all who applied and other interested parties, acquiring an active audience of over 300.

The aim of the programme is to give the participants a solid understanding of the complexities of AMR and the necessary skills required to enact meaningful change in the field throughout their own professional careers. The programme values cross-border and cross-disciplinary collaboration, aiming to instill these values in the next generation of AMR change-makers.

More information: <https://futureleadersagainstamr.org>



BIORESPONSIVE DRUG DELIVERY

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Spurred by recent advances in materials chemistry, molecular pharmaceutics and nanobiotechnology, stimuli-responsive "smart" systems offer opportunities for precisely delivering drugs in dose-, spatial- and temporal-controlled manners. In this talk, I will discuss our ongoing efforts in developing physiological signal-triggered bioinspired drug delivery systems. I will first present the glucose-responsive synthetic systems for biomimetic delivery of insulin for diabetes treatment. Development of smart insulin patches will be emphasized. I will further discuss the local and targeted delivery of immunomodulatory therapeutics for enhanced cancer therapy. Our latest studies utilizing platelets, cell conjugates and sprayed gels for delivery of immune checkpoint inhibitors will be specifically introduced.

FIGHTING CHRONIC BACTERIAL INFECTIONS WITH NATURE'S FINEST NANOMACHINES

ALEXANDER HARMS

Bacteriophages are fierce viral predators with no regard for pathogenicity or antibiotic resistance of their bacterial hosts. Despite early recognition of their therapeutic potential and the current escalation of bacterial multidrug resistance, phages have so far failed to become a regular treatment option in clinical practice. One reason is the occasional discrepancy between poor performance of selected phages *in vivo* despite high potency *in vitro*. Similar resilience of supposedly drug-sensitive bacterial infections to antibiotic treatment has been linked to persistence of dormant cells inside patients. Given the abundance of non-growing bacteria also in the environment, we wondered whether some phages can infect and kill these antibiotic-tolerant cells. As shown previously, most phages failed to replicate on dormant hosts and instead entered a state of hibernation or pseudolysogeny. However, we isolated a new *Pseudomonas aeruginosa* phage named Paride with the exciting ability to directly kill dormant, antibiotic-tolerant hosts by lytic replication, causing sterilization of deep-dormant cultures in synergy with the β -lactam meropenem. Intriguingly, efficient replication of Paride on dormant hosts depends on the same bacterial stress responses that also drive antibiotic tolerance. We therefore suggest that Paride hijacks weak spots in the dormant physiology of antibiotic-tolerant bacteria that could be exploited as Achilles' heels for the development of new treatments targeting resilient bacterial infections.

DITHRANOL AS NOVEL CO-ADJUVANT FOR NON-INVASIVE DERMAL VACCINATION

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Vaccinations are one of the most successful medical interventions in case of the prevention of formerly serious infectious diseases¹. The current Covid-19 pandemic outbreak is a daily reminder of the need for easy-to-use vaccines to establish protective and long-lasting immunity. In this context, transcutaneous immunization methods (TCI) have come into focus, as they integrate many desirable properties of an ideal vaccination strategy. As needle-free immunization method, it enables the painless application of vaccines onto

intact skin. Besides the advantage of self-medication, TCI strategies avoid accidents caused of needle sticks for medical personnel and patients. WHO prioritizes this issue because of the high socioeconomic and medical consequences of needle stick injuries^{2,3}. Moreover, the application onto the intact skin targets skin-resident APCs that promotes the formation of systemic cytotoxic T lymphocyte (CTL) responses that are highly desirable in the therapy of viral infections or cancer⁴.

In our previous works, we established a TCI method with the TLR7 agonist imiquimod (IMQ) as an adjuvant, inducing a prophylactic or therapeutic effective cytotoxic T-cell response⁵⁻⁷. Since the applicability of imiquimod-based TCI is limited by the commercial formulation Aldara, we have recently developed two new imiquimod-containing formulations. One is a gel-based formulation called IMI-Gel⁸, which elicits a comparable T cell response in mouse models, and the other is a solid nano-emulsion called IMI-Sol⁹. Compared to the commercially available imiquimod formulation Aldara – that is a. o. used for the treatment of psoriasis-, IMI-Sol elicits an enhanced T cell response and is also capable of eliciting not only MHC class I-mediated T cell responses, but also MHC class II-mediated T helper responses. However, transcutaneous immunization with imiquimod does not result in a long-lasting cellular memory response. Most recently, we developed a novel transcutaneous immunization procedure that elicits a targeted, systemic immune response (“triple vaccine”) through the combined application of two immuno-stimulatory agents (imiquimod (IMQ) and dithranol) with a defined target structure (synthetic peptide). We termed this new vaccination approach dithranol /IMQ-based transcutaneous vaccination (DIVA). When applied prior to imiquimod, dithranol strongly enhances specific T-cell responses and fosters the induction of long-lasting preventive and therapeutic immune responses by generating a large number of high-quality memory immune cells directed against a target peptide (fig.1 B and C). In this context, CD4 help promotes optimal CD8 T cell activation (fig 1 D).

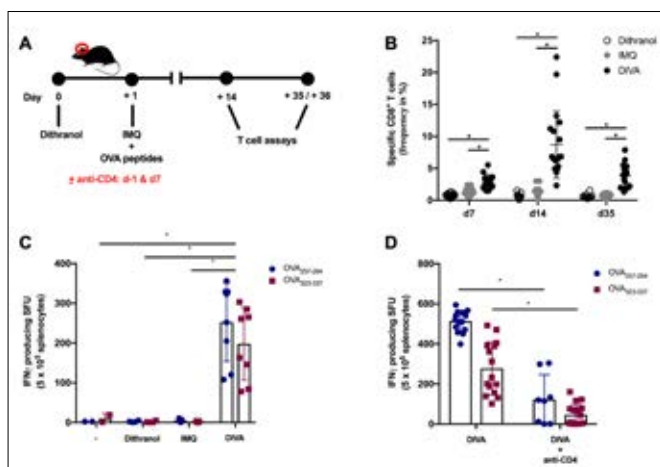
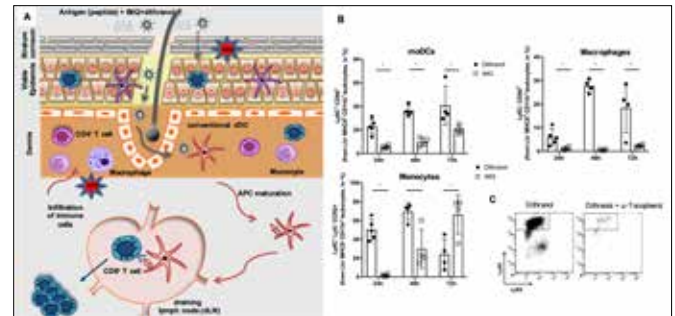


Figure 1: DIVA induces potent memory T cell responses

(A) Schematic overview of the DIVA protocol. C57B6/J wildtype mice were immunized on both ears with dithranol (day 0) and IMQ together with OVA₂₅₇₋₂₆₄ & OVA₃₂₃₋₃₃₇ (day 1) on two consecutive days, treated with the single agents or left untreated. (B) The frequency of OVA₂₅₇₋₂₆₄ specific CD8⁺ T cells in the blood was assessed on day 7, 14 and 35 (n=9-19). (C) Splenocytes were *ex vivo* restimulated with either OVA₂₅₇₋₂₆₄ or OVA₃₂₃₋₃₃₇ peptide and IFN- γ producing cells were analyzed by ELISpot assays on day 36. Bars represent mean and SD of data collected from at least two independent experiments. (D) IFN- γ producing cells after restimulation with OVA₂₅₇₋₂₆₄ or OVA₃₂₃₋₃₃₇ were quantified in splenocytes on day 35 after DIVA with or without CD4 T cell depletion (500 μ g GK1.5 i. p. as indicated). * p<0.05 by one-way ANOVA with Bonferroni's post-hoc test. Having a closer look at the underlying mechanisms (fig.2A), dithranol induces a massive skin inflammation characterized by an early influx of inflammatory monocytes and macrophages into the side of vaccination that is crucial for the immunization efficiency.

Figure 2: Dithranol mediated transcutaneous immunization (DIVA)



(A) Schematic overview about DIVA TCI, adapted from¹⁰. Dithranol induces a local skin inflammation and promotes the recruitment of monocytes and macrophages into the skin. This mechanism is depended of oxidative stress (ROS). Antigen-presenting cells (APC) migrate into the draining lymph nodes, where T cells are primed and antigen-specific cytotoxic T cells are generated. (B) Mice were treated once with dithranol or IMQ on both ears. After 24 hours the ears were digested with collagenase type IV in a GentleMACS dissociator. The percentages of monocytes (MHCII- CD11b+ Ly6C+ Ly6G- CCR2+), monocyte-derived DCs (MHCII+ CD11b+ Ly6C+ CD64+) and macrophages (MHCII+ CD11b+ Ly6C- CD64+) in the living leukocytes in the skin were determined by flow cytometry. (C) Mice were treated with dithranol or IMQ for 24 hours. When indicated the radical scavenger α -Tocopherol was administered (30 mg, i.p.). Treated murine ears were then digested with collagenase type IV in a GentleMACS dissociator. The total number of monocytes (MHCII- CD11b+ Ly6C+ Ly6G- CCR2+) was determined by flow cytometry. Bars represent mean and SD of data collected from at least two independent experiments. *Significant difference with p<0.05 by t-Test.

DIVA creates in the skin a local oxidant dependent (fig. 2C) inflammatory milieu to attract CCR2⁺ monocytes (2B) and favors a pro-inflammatory M1-like macrophage differentiation that most likely induces the further differentiation of the attracted monocytes into inflammatory conventional DC2 (inflammatory-cDC2)¹¹.

In summary, we established a new transcutaneous immunization platform DIVA that might be a groundbreaking method for new needle free immunization methods. The combined application of imiquimod and dithranol, both already well established in the clinic, induces a potent and long-lasting antigen-specific T-cell response, that are of great importance in the fight against persistent and acute viral infections and tumor diseases.

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INORGANIC ANTIBIOTICS – NANOZYMES COMBAT BACTERIA HIDING WITHIN HUMAN MACROPHAGES

INGE HERRMANN, ETH Zurich and Empa

Antimicrobial infections are a global health concerns and bacterial resistances are set to aggravate the issue in the coming years. Various bacterial strains evade antibiotic treatment by hiding inside cells. Conventional antimicrobial agents are unable to penetrate or be retained in the infected mammalian cells. Recent approaches to overcome these limitations have focused on load-carrier systems, requiring a triggered discharge leading to complex release kinetics. The union of potent antimicrobial activity with high mammalian cell compatibility is a prerequisite for intracellular activity, which is not well-met by otherwise well-established inorganic systems, such as silver-based nanoparticles. In this presentation, I will introduce a load and carrier are combined into one functional inorganic nanoparticle system, which unites antimicrobial activity with mammalian cell compatibility. These multicomponent nanohybrids based on cerium oxide are produced in one step, yet unite complex materials. The nanoparticles form suprastructures of similar size and surface charge as bacteria, therefore facilitating the uptake into the same subcellular compartments, where they unleash their antibacterial effect. Such intrinsically antibacterial nanohybrids significantly reduce bacterial survival inside macrophages without harming the latter. Furthermore, blocking of nanoparticle endocytosis and subcellular electron microscopy elucidate the mechanism of action. This first demonstration of antibacterial activity of ceria-based nanoparticles inside of mammalian cells offers a route to straightforward and robust intracellular antibacterial agents that do not depend on payload delivery or biological constituents.

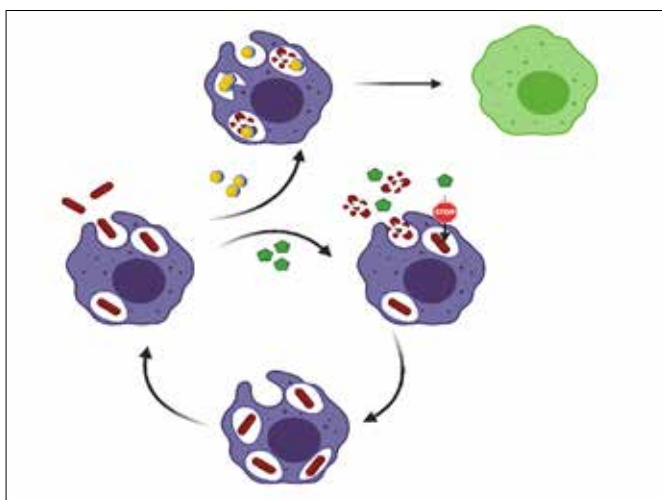


Figure 1. Intracellular infections are hard to treat with conventional antibiotics due to their poor membrane permeability. Nanohybrids with intrinsic antimicrobial activity tend end up in the same compartment as bacteria hiding inside the cells because of their similar size and surface charge. Treating intracellular infections with endocytosed nanohybrids has the prospect to bring significant benefit. Figure adapted from Matter *et al.* *Nanoscale*, 2021

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MOLECULAR K-EDGE NANOPROBES FOR MULTIPLEXED PHOTON COUNTING IMAGING OF SUBDURAL HEMATOMA IN HUMAN BRAIN TISSUES

NIVETHA GUNASEELAN

Epidemiological studies indicate that chronic subdural hematoma (SDHT) has an overall incidence of 1.7–20.6 per 100,000 persons per year and is more common among veterans and elderly persons than among members of the general population and is an important reversible cause of dementia and disability in the elderly.¹⁻³ Incidence of chronic SDHT is increasing, and for up to 20% of patients, neurological outcomes are deceptively poor, resulting in significant disability such as seizures, breathing problems, loss of consciousness and even coma. Since bleeding occurs slowly in older patients, symptoms may not appear for weeks or months. Among elderly patients who undergo treatment with a drainage intervention, the 1-year mortality rate is 32%. The outcome is even worse if chronic SDHT converts to acute SDHT. Apart from older adults, chronic SDHT is also common in athletes, people who take blood thinners, hemophiliacs, alcoholics and babies.¹⁻³ Neurosurgeons must be prepared to face the inevitable increased workload that will accompany the increased incidence of chronic SDHT. A sufficiently high level of clinical suspicion and prompt radiographic evaluation may therefore require for timely treatment to avoid poor outcomes. Noninvasive neuroimaging techniques have become an indispensable part of the diagnostic work up for chronic SDHT patients because an early diagnosis can significantly alter the clinical course.⁴⁻⁶ In this regard, CT is the primary imaging modality of choice to diagnose hematomas. However, while conventional CT can detect hemorrhage and edema, it is unable to pick up the subtle pathology changes associated with the shear-strain effects since CT provides very poor soft tissue contrast. Hematoma may not be depicted in CT scans because the attenuation may be similar to the adjacent inner table of the skull and usually goes unnoticed, sometimes for 2 to 4 weeks post injury. If undiagnosed, the buildup of blood can cause pressure on the brain which can lead to breathing problems, paralysis and death if not treated on time. Although more recently magnetic resonance imaging (MRI) has been shown to be more sensitive in diagnosing cerebral pathology in SDHT patients, this is an expensive, limiting imaging modality and generally requires much larger acquisition time than CT.⁷⁻⁸ Given that conventional CT is plagued by serious limitations such as strong influence from calcifications, beam-hardening effects, metal artifacts from bone and poor soft tissue contrast, **there is a clear unmet clinical need for the accurate diagnosis of subdural hematoma at the chronic stage.**

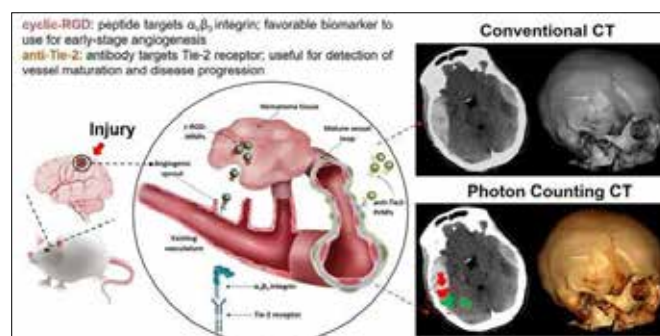


Fig 1. Overall concept of the development of multicolor imaging probes for multiplexed spectral information targeting two biomarkers of interest, i.e., $\alpha V\beta 3$ integrin and Tie-2 receptor with tailor made hafnium and praseodymium nanoparticles respectively.

While there has been some progress in identifying biomarkers of chronic SDHT, there is a paucity in the development of molecularly targeted agents to determine injury level identified by imaging angiogenic vessel growth in the hematoma tissue. Multiplexing *in vivo* allows integration of imaging results from different targeted probes offering opportunities to detect complimentary biological activities and simultaneous events. Each probe alone provides important biological and molecular information and when combined, the visualization can help to better understand the full complexity of biological processes. However, *in vivo* multiplexing process is challenging and till now only restricted to fluorescence and Raman-based techniques.⁹ The new advancement in this field is the emergence of photon counting CT (PCCT) that allows material decomposition, artifact correction and offers other advantages over conventional CT.¹⁰⁻¹¹ PCCT brings a universal, quantitative set of “colors” to CT scans which apply to all applications and span clinical and preclinical research. A prime benefit of PCCT is its ability to separate more than two materials based on the difference of K-edge energy of elements.¹²⁻¹⁴ Photon counting K-edge imaging in combination with targeted contrast agents that carry K-edge material (e.g., gold or hafnium) is expected to be a revolutionary improvement for quantitative pathologic characterization.¹⁵⁻¹⁸ We and others have previously developed and studied targeted nanoparticles in a variety of animal models for both cardiovascular and oncologic disease.¹⁵⁻¹⁸ **Thus, a strategy that offers the multiplex targeted *in vivo* PCCT imaging of different biomarkers with potential to contribute across diseases and target tissues, is the focus of this study.**

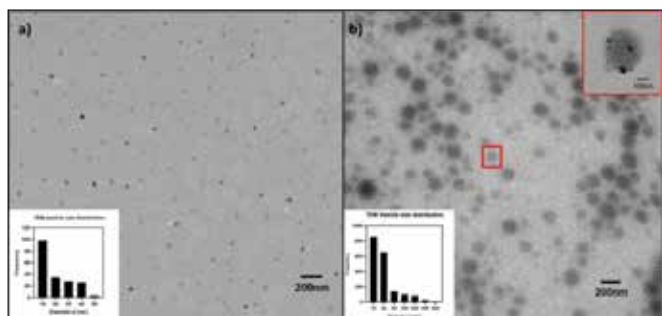


Fig 2. TEM images of prepared nanoparticles **a)** Hafnium oxide nanoparticles; inset shows particle size distribution (nm) **b)** Praseodymium nanoparticles showing vesicle formation; top right inset shows individual praseodymium particles encapsulated in a lipid vesicle; bottom left inset shows size distribution (nm)

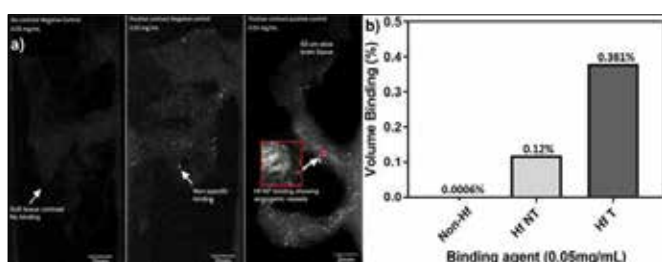


Fig 3a) Micro-CT images of 60µm sections of human SDHT tissue samples treated with no contrast non-Hf particles, non-targeted Hf particles and c-RGD conjugated Hf particles targeting angiogenic vessels **b)** quantitative analysis of micro-CT data showing % difference in Hf binding between tissue samples

Since a variety of high atomic number materials can be considered in PCCT imaging, materials were selected according to their K-edge energies to improve their discrimination *in vivo*. The selection of two materials with larger K-edge energy separation helped to design probes with wider energy bins to optimize the SNR in the K-edge ratio parametric maps. This enabled precise identification of multiple contrast agents *in vivo* when they simultaneously target different biomarkers. While the choice of iodine may seem obvious as it is widely used in radiology, e.g., blood pool agent, simulations have shown that other metals would yield highly increased base SNR when imaged with a binned photon-counting system. Hafnium (Hf; Z=72) possessing well-positioned K-edge energy (65.3

keV) provides a superior overall performance at clinical CT tube potential (80-140 kVp) in comparison to other commonly explored CT contrast agents comprising iodine, bismuth and other elements.¹⁹ Utilizing our previous expertise in molecular probe development for photon counting CT (Pan¹⁵⁻¹⁸), we have further chosen praseodymium (Pr) with a K-edge energy of 42.9 keV as another common metallic element for generating “soft” type nanocolloids i.e. praseodymium oxide (PrOC) nanocolloid, for use as a novel *in vivo* contrast agent.

Innovation, Technical details and Preliminary data:

We have developed multicolor photon counting imaging probes (Hf, K-edge: 65.3keV and Pr, K-edge: 42.9keV) for *in vivo* multiplexing of two molecularly targeted biomarkers, i.e., angiogenesis marker ($\alpha_v\beta_3$) and mature membrane marker (Tie2), in chronic subdural hematoma (SDHT) tissue. The choice of Hf and Pr are deliberate so that the overlap between the two energy bins can be minimized and materials separation can be efficiently achieved. **We hypothesize that the outcome will generate quantitative and spatially specific images that may transform the early subdural hematoma diagnosis without increasing the complexity of clinical workflows (Fig. 1).** This strategy would allow the physicians to directly visualize the damage and potentially be used to accurately predict the extent of angiogenesis in the subdural hematoma tissue. Furthermore, due to the quantitative nature of the technique, it has the potential for use in longitudinal studies on patients to assess the effectiveness of therapeutic interventions. To test this hypothesis, we will investigate the utility of photon counting CT technique in combination with two molecularly targeted contrast probes in a chronic subdural hematoma (SDHT) rat model and corroborate PCCT findings with micro-CT data of human SDHT samples and tissue histopathology. Hf and Pr nanoparticles were synthesized and conjugated with cyclic-RGD targeting the angiogenesis marker ($\alpha_v\beta_3$ integrin) and Tie2 antibody targeting the mature membrane marker (Tie2 receptor) respectively. Detailed characterization of these NPs was performed by multiple analytical techniques such as DLS, Zeta and TEM. **Fig 2** shows TEM images and anhydrous particle size distribution of HfO₂ and PrO₂ nanoparticles with an average size of 10 nm which makes them the ideal nanoprobe that combine the visibility of contrast agents with the ability to cross the blood brain barrier (BBB) and selectively target the damaged tissue while still maintaining their radio-opacity.²⁰ DLS measurements showed the hydrodynamic diameters of HfO₂ and PrO₂ NPs as 38.47 ± 6.1 nm and 31.85 ± 4.7 nm respectively in aqueous solution. Zeta potential measurements of NPs demonstrated a positive ζ -value of 37.6 mV due to the polymer coating added to improve nanoparticle hydrophilicity. We then obtained quantitative preliminary data using the cyclic-RGD-HfNPs to monitor angiogenesis specific binding in subdural hematoma human tissue samples. **Fig 3a** shows micro-CT images of 60 µm thick sections of human subdural hematoma tissue samples treated with no contrast non-targeted particles (non-Hf), positive contrast non-targeted hafnium particles (Hf-NT) and positive contrast c-RGD conjugated hafnium particles (Hf-T) targeting angiogenic vessels. It has been observed that the angiogenic vessels are being specifically illuminated by the targeted particles under CT. **Fig 3b** shows a quantitative comparison of metal binding observed in these three samples. Almost three-fold increase in HfNP binding was observed as compared to the non-targeted particles due to increased level of angiogenesis in the tissue sample. The CT data was further corroborated with tissue histopathology studies. **Fig4(a-i)** show immunohistochemistry images obtained for three representative SDHT patient samples with varying angiogenic levels and stained for VEGFR2 (angiogenesis), Tie2 (mature membrane) and PECAM (microvasculature) markers. The samples show low, medium, and high level of angiogenesis which is also corroborated from the Hf-T mediated micro-CT images reflecting low, medium, and high angiogenesis (**Fig 4 j,k,l**). The respective Pr-T mediated CT images were also acquired those highlight the mature membrane profile of these samples (data not shown). Thus, after optimizing treatment strategies under *in vitro* and *ex vivo* conditions, treatments have to be established in animal models to establish the efficacy of the adopted strategy. Through this fellow-

ship, the translational relevance of these SDHT agents (Hf, Pr) will be evaluated in a pilot animal study using a subdural hematoma rat model. Here, *in vivo* brain accumulation of c-RGD-HfNPs and anti-Tie2-PrNPs administrated intracranially (n=8/group, saline, non-targeted HfNPs, cRGD-HfNPs, anti-Tie2-PrNPs) will be comprehensively evaluated and corroborated with immunohistochemistry. Adult male Sprague-Dawley rats (n=80, 250-350g) will be used to induce chronic subdural hematoma. Chronic SDHT will be induced as described in this publication²¹. The rats will be scanned using micro-CT and PCCT to view angiogenic vessels in the induced hematoma tissue and quantify nanoparticle binding at varying time points chosen to see the effectiveness of the agents in detecting level of angiogenesis at the chronic and acute stages.

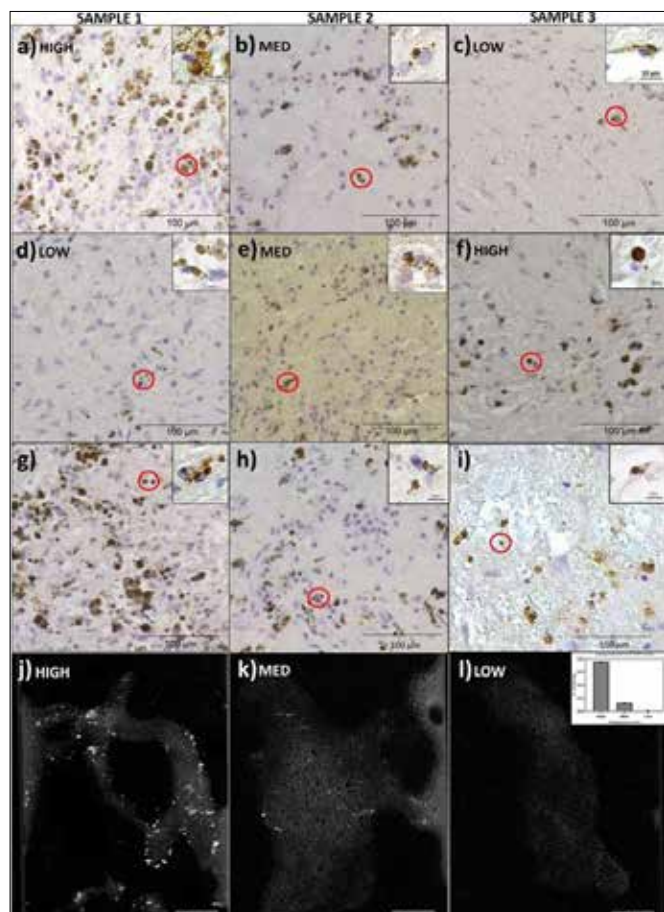


Fig 4 Immunohistochemistry images showing **a,b,c** VEGFR2 staining **d,e,f** Tie2 staining **g,h,i** PECAM staining **j,k,l** Micro-CT images of 60µm sections of human SDHT tissue samples treated with c-RGD conjugated Hf particles targeting angiogenic vessels; inset shows quantitative analysis of micro-CT data showing % difference in cRGD-Hf binding between tissue samples (0.05 mg/mL).

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RNA FORMULATION & DRUG DELIVERY DURING COVID: ONGOING DEVELOPMENTS BEYOND LNPS

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The fast and successful development of the mRNA based COVID-19 vaccines has impressively demonstrated the potential of mRNA therapeutics. So far, LNP formulations for vaccination purposes are in the focus of public attention. In order to bring also other mRNA based therapeutic products into clinical practice, further, tailored, delivery systems are required, where structural and functional characteristics, molecular composition, manufacturing and several other aspects have to be adjusted. In the presentation, strategies for controlled assembly of lipid and polymer based RNA delivery systems will be discussed, with examples from existing and potential future products.

NEXT-GEN LIPOSOMAL DOXORUBICIN (TALIDOX): FROM BIOPHYSICS TO CLINICS

STEFAN HALBHERR, CEO/Country Manager Switzerland, Inno-Medica

Biodistribution and tissue accumulation of nanoparticles is complex, integrating multiple parameters with high variability at once. Nanoparticle size, as well as lipid composition, drug loading and release, but also surface design form a multidimensional design space that enables previously unseen pharmaceutical effects which can be exploited to favorably affect drug tolerance, efficacy, or both. Talidox (Targeted Liposomal Doxorubicin) is makes use of three major design tweaks that positively interact with each other. The resulting design was found to favorably affect biodistribution, pharmacokinetics, drug tolerance, and also efficacy in some cases.

First of all, the liposome size was scaled down to the curvature limit for cholesterol-rich phospholipid membranes of approx. 30 nanometers. A small nanoparticle size brings along multiple desirable features such as a greater capacity to accumulate in solid tumors, but also a faster transcytotic and endocytotic process. This, comes with faster drug release due to the enhanced surface availability. In a second step, drug loading per liposome was reduced in order to increase the drug release capacity after triggers such as endocytosis. Another desirable effect that came along with the reduction of drug-to-lipid ratios was a reduction of the drug load in clearance organs such as the liver and spleen. As a third element, the liposome nanosurface was densely decorated with PEG2000 and an outward-only orientation of the PEG molecule was ensured, as inward facing PEG2000 was regarded as non-functional and thus should be avoided. The high surface density of PEG2000 again further reduced the drug load in clearance organs and greatly prolonged serum half-life to previously unseen degrees. It is subject of ongoing research how prolonged pharmacokinetics connect with tumor tissue uptake over time.

In a phase I safety study with a total of 21 patients with solid tumors it was found that the median half-life of Talidox was longer than the half-life of currently marketed Doxil/Caelyx. Taking into account the largely reduced particle diameter this was seen as a potentially positive drug characteristic, as small nanoparticles would usually clear faster from blood. Longer drug residence time in blood was considered a potentially promising sign, as the availability of the nanodrug in blood forms a prerequisite to enable interactions of the nanomedicament with tumor vasculature and adjacent tumor tissue. At the same time, unwanted adverse drug effects could be reduced due to the increased timeframe that the body is given to metabolize the drug load. Considering the late stage of disease and many prior lines of treatment the treated population had, the clinical activity profile of Talidox was noteworthy. Further studies are

being conducted or in planning to better understand the potential benefits that this nanocarrier design might bring. The phase I trial is currently being continued with a comparative PK-part evaluating the two liposomal formulations Talidox and Doxil/Caelyx head-to-head (<https://clinicaltrials.gov/ct2/show/NCT03387917?term=talidox&draw=2&rank=1>). At the same time, a large phase II study is being set up to generate additional efficacy, safety and quality-of-life data in a more homogeneous patient population with early-stage metastatic breast cancer, holding Talidox 2:1 against a calibrator arm representing the standard-of-care.

In sum, Talidox is pivoting a new approach to make use of nanomedicine in oncology through a set of well-known design parameters, ushering a next generation of advanced nanodrug delivery systems.

THE LANDSCAPE OF PRECISION MEDICINE – FROM TARGET TO DOSE TO COMPLEX PATIENT SCENARIOS

PATRICK HUNZIKER

“Maintaining health, preventing, diagnosing and curing disease requires precision at every dimension of life from molecular bottom up to societal scale: Precisely interacting with molecular targets, targeting specific cell populations, taking specific disease phenotypes up to pathophysiology at the body scale, and human-to-human interaction, the environment and societal scale is proving to be the key to mastering the Covid-19 pandemic but has implications to most diseases.

Knowledge-based medicine based on the converging science approach is thus both, precise and comprehensive and promises to replace current, one-size-fits all paradigms. “

TRANSLATIONAL MODELS TO EVALUATE PHARMACOKINETIC PROPERTIES OF NANOMEDICINES

JÖRG HUWYLER, Professor of Pharmaceutical Technology, University of Basel, Switzerland

The transition from nanoparticle design to *in vitro* assessment and finally *in vivo* experiments in higher vertebrates remains a challenge. Phenomena such as protein binding, cellular uptake and intracellular processing are of prime importance since they may have a strong impact on biodistribution and immunogenicity of nanoparticles. Therefore, particle characteristics should be studied in an environment that simulates the situation encountered in biological systems. We will discuss the use of zebrafish (*Danio rerio*) larvae as a vertebrate screening model to assess the systemic circulation and extravasation of nanoparticulate drug delivery systems *in vivo*. To validate this novel approach, monodisperse preparations of fluorescent labelled liposomes with similar size and zeta potential were injected into transgenic zebrafish lines expressing green fluorescent protein in their vasculature. Phosphatidylcholine based lipids differed by fatty acid chain length and saturation. Circulation behavior and vascular distribution pattern were evaluated qualitatively and semi-quantitatively using image analysis. The circulation patterns in the zebrafish model did correlate with published and experimental pharmacokinetic data from mice and rats. Our findings indicate that the presented translational approach can be used to predict the *in vivo* performance of particulate drug delivery systems.

GENERIC DRUG DEVELOPMENT AND BRIDGING GLOBAL REGULATIONS

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Generic drugs provide significant public health benefits by providing high-quality, more affordable alternatives to brand name drugs. Approximately 90 percent of prescriptions dispensed in the United States are generic drugs that often are the product of an intricate global supply chain. The Office of Generic Drugs (OGD) is involved in efforts intended to advance the international harmonization of scientific, technical and regulatory standards for generic drug development. OGD continues global efforts with a focus on prioritizing future topics for generic harmonization that will include more complex generic drugs. The envisioned outcome of these global efforts is reduced time and cost of product development consequently improving patient access to more affordable medicines.

Over the last few decades, international dialogue has increased, and three types of initiatives have gained traction: bilateral, regional, and global. The European Union and the United States have a long history of cooperation and harmonization. Pharmaceutical regulatory harmonization could reduce clinical trial duplication, drug development, and regulatory expenditures, as well as expedite the approval of medications and pharmaceutical innovation. ICH has also played a key role in the development of worldwide regulatory harmonized guidances that will allow a more convergent path to accessibility of medications. The path to harmonization is paved with rigorous dialogue, gap analysis and negotiations. Reasonable, effective, and predictable regulatory structures are becoming increasingly evident as vital to the successful development of generic medications including more complex products such as nanomedicines. OGD global affairs identifies opportunities and challenges as those national regulations are being developed and implemented positioning regulators proactively on the path of convergence.

NANOMEDICINES – ENSURING PATIENT SAFETY THROUGH REGULATORY CLARITY

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Keywords: Advocacy programme, centralised regulatory procedure, hybrid application, nanomedicines, nanosimilars, follow-on products

Given that nanomedicines and follow-on nanosimilars have complex manufacturing processes and heteromolecular structures, the question is being raised in ever increasing frequency as to whether the current European regulation of medicines for human use is robust enough to authorise these medicinal products and their follow-ons. Until this can be achieved, there is a potential for patient safety to be compromised¹. The current situation is that nanomedicines have the potential for being assessed under four different types of procedures: the national procedure, the decentralised procedure, the mutual recognition procedure, and the centralised procedure. In this context, it is important to note that a survey published in 2018 reported “strong regional differences in the regulation of nanomedicines and confirmed the need for a harmonisation of information requirements on nano-specific properties” (Bremer-Hoffmann et al., 2018)².

Given their complex nature and the fact that each nanomedicine will have unique features, there is currently a lack of guidelines or

protocols so that these medicines can be appropriately processed, which will provide a marketing authorisation (MA) that meets the demanding standards of today and thus ensure patient safety (Nanomedicines and Nanosimilars, 2021)³.

The EU Nanomedicines Regulatory Coalition (Nanomedicines Regulatory Coalition, 2021)⁴ currently comprising seven pan-European organisations is therefore advocating for all nanomedicines to be assessed by the EMA Centralised regulatory procedure (Patient Safety and Nanomedicines, 2020)⁵. This is equally true of the off-patent follow-on copy products, or nanosimilars, as they are also called. Within this context, a centralised regulatory process that addresses this, is needed at the EU level, and in the absence of a tailored regulatory pathway similar to that of the biosimilars, the European Alliance for Access to Safe Medicines (EAASM) strongly believes that all future nanosimilars should go through the Hybrid Application process (10.3) and not the Generics Application process (10.1). This pathway, if consistently applied and aligned to the draft guidance (European Medicines Agency, 2015) which the EMA has produced for specific types of nanomedicines, would ensure that follow-on copies are therapeutically similar to their originator and therefore improve patient safety.

This paper endeavours to lay out the critical elements of an advocacy programme that will enable a centralised procedure for nanomedicines and nanosimilars to be achieved whilst also fostering actions that will:

- Harmonise the information requirements of regulators in order to correctly characterise nanomedicines
- produce a scientific consensus on definitions for nanomedicines across Europe
- improve education and awareness of the complexity and sophistication of nanomedicines among policymakers, prescribers, payers and patients. This is especially relevant when it centres on issues of interchangeability

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COMPLEX GENERICS CONTAINING NANOMATERIALS: DEVELOPMENTS IN 2021 AND 2022

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, some recent progress made in regulatory research, guidance development, and approval of complex products containing nanomaterials by U.S. FDA will be discussed.

NANOMEDICINE EX MACHINA: DESIGN STRATEGIES BASED ON CLINICAL RELEVANCE

JORDAN ANDREAS

In recent years, nanomedicines have conquered the global healthcare market. Still, they represent an expensive niche technology with a limited application range. Among other examples, they have been used to reduce the toxicity of anticancer agents, overcome physiological barriers, or stabilize drug molecules that are sensitive to degradation. Current design strategies are widely based on our experiences with the first generation of innovator drug products. With a growing knowledge of their clinical performance, computer-based design strategies can be implemented into the drug development pipeline. This includes not only the selection of suitable models and algorithms but also the design of *in vitro* assays to predict their behavior *in vivo*. In this regard, clinical performance is the ultimate benchmark that needs to be implemented into the design process at an early stage. The talk will highlight the biopredictive methodologies we have to date and explain common limitations and pitfalls.

BIOINSPIRED HYALURONIC ACID-COATED GOLD NANOPARTICLES TO TARGET LUNG CANCER CELLS

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Cellular targeting by nanoparticles has shown wide promise, but there are still many challenges to overcome for a successful clinical translation. Herein we test the efficacy of gold nanoparticles (Au NPs) coated with a functional bioinspired coating, i.e., hyaluronic acid (HA), for targeted drug delivery to cancer cells. HA, the ligand for the cluster of differentiation 44 (CD44) receptors that are overexpressed in cancer cells, is a biocompatible, biodegradable, non-immunogenic, and anti-oxidative natural biopolymer. Prior to the HA conjugation, the Au NPs were loaded with a widely used and effective FDA-approved anti-cancer drug, doxorubicin hydrochloride (DOX). After rigorous centrifugation and dialysis, the unbound DOX was quantified and found to be below the detection limit, verifying the DOX loading amount was appropriate and the conjugation was successful, using fluorescence high-performance liquid chromatography (limit of detection: 145 ng/column) and a fluorometer (limit of detection: 50 ng mL⁻¹). The presence of HA on the Au NP surface was verified with nuclear magnetic resonance. The uptake and the fate of the DOX-loaded NPs inside the cells were evaluated using A549 lung epithelial cancer cells and non-cancerous Met-5A mesoepithelial cells. We demonstrated that significantly more HA-coated NPs were internalized by A549 cells than by the Met-5A cells. Furthermore, lysosome tracking analysis revealed that the fate of uncoated versus HA-coated Au NPs inside the cell differs. An interesting finding was that the HA coating prevented DOX from being released from the particles and translocating into the nucleus; therefore, the role of HA on the fate of DOX inside the cell needs further investigation. As a result, for targeted drug delivery applications, while the emphasis is on the selective internalization of a drug molecule by cancer cells, the fate of the cargo post-internalization needs to be assessed thoroughly.

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THE CMC PERSPECTIVE OF MRNA BASED MEDICINES-HOW STABLE ARE mRNAs IN AND OUTSIDE THEIR DELIVERY VEHICLE?"

MICHAEL KELLER

"The overwhelming success of mRNA vaccines as a major contributor to fight the COVID-19 pandemic has triggered widespread interest in the scientific community to further develop this technology, also for non-vaccine applications. Here we are reporting preliminary data investigating the properties of commercial GFP encoding mRNA from the CMC perspective. The optimization of an mRNA/LNP formulation process and subsequent investigation into the stabilities of the resulting nanoparticles is reported. Analytical challenges and first steps towards solidifying our understanding of mRNA in and outside LNPs are reported."

MICROVASCULAR CLOTS AS A NEW TARGET FOR NANO-SCALE DRUG-DELIVERY INTO INJURED BRAIN

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Background: The blood-brain-barrier (BBB) limits the targeting of drugs to the CNS. Lipid nanoparticles (NPs) may represent an option for drug delivery to the brain, however the underlying mechanisms of NP-BBB interactions are not clearly understood. We previously demonstrated that loading the cationic dyes with their bulky hydrophobic counterions causes excellent brightness enabling tracking individual NPs *in vivo* (Khalin I., et al, ACSNano, 2020) as well as *ex vivo* (Khalin I., et al. Nanomedicine: NBM, 2022) in mouse brain. Moreover, we showed that acutely after brain injury, NPs extravasate into the brain parenchyma (Khalin I., et al, ACSNano, 2020), suggesting the crossing the BBB. In current study, we further investigated how NPs cross the BBB, using different animals' models and imaging modalities.

Aim: To directly visualize at subcellular resolution whether and how individual super-bright fluorescent NPs cross the BBB under various pathological conditions *in vivo*.

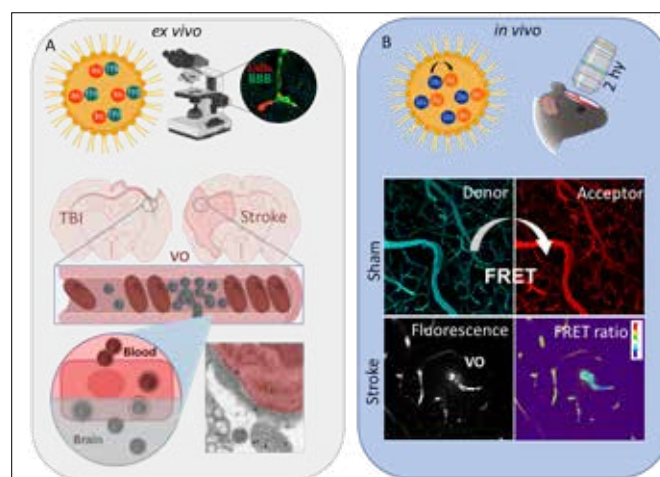
Methods: Lipid nanodroplets demonstrated high stability and brightness in blood and absence of dye leakage (Kilin et al, Biomaterials 2014). We prepared Super-bright fluorescent lipid nanodroplets (SBFLnDs; diameter: 30- and 80-nm) loading with a counterion-coupled rhodamine dye or with two dyes, F888 and cyanine-3.5, undergoing Förster Resonance Energy Transfer (FRET). SBFLnDs were injected systemically into mice following brain trauma or ischemic stroke and visualized by *in vivo* by 2-photon, confocal microscopy or correlative light-electron microscopy (CLEM). Brain trauma was performed using mouse controlled-cortical impact model, ischemic stroke – filament middle cerebral artery occlusion, targeted mini stroke – combination of magnetic iron oxide NPs and mini magnet.

Results: Shortly after brain injury microvascular occlusions (VO) formed within the cerebral microcirculation (Figure 1A). SBFLnDs accumulated within VOs in still viable brain tissue, crossed the BBB, and entered the brain parenchyma reaching healthy neurons (Khalin et al., in revision). 30-nm NPs passed the opened BBB more readily than 80-nm NPs. Using lectin-labelled vessels as a landmark, VOs with extravasated SBFLnDs were correlated to its ultrastructure by

automated tape collecting ultramicrotomy (ATUM). This allowed precise re-localization and ultrastructural volume analysis of the VO enabling observation of exact way of BBB crossing. CLEM revealed that 30-nm NPs crossed the BBB at microvascular clots via endothelial transcytosis. Using FRET NPs (Figure 1B) and targeted mini-stroke model, we were able to demonstrate *in vivo* that NPs decomposed inside the VO and the brain parenchyma implicating specific accumulation and releasing the cargo in brain diseases accompanied with microthrombosis.

Conclusion: The current study demonstrates that NPs accumulate and enter the injured brain at sites of VOs. Thus, VOs unlock the BBB for NPs functioning as a size-selective gate to the brain. Therefore, taking advantage of this process may pave the way for novel drug-delivery approach for brain disorders.

Figure 1. A. *Ex vivo* fluorescent microscopy and correlative light-electron microscopy revealed that 30-nm lipid nanodroplets were accumulated in vascular occlusions following the stroke or traumatic brain injury and crossed the blood-brain barrier via endothelial vesicular transcytosis. B. Using intravital 2 photon microscope, Förster Resonance Energy Transfer demonstrated that accumulated in vascular occlusion lipid nanodroplets were decomposed inside vascular occlusion; and extravasated - inside brain parenchyma.



Abbreviations: Rh – rhodamine; TFB – tetraphenylborate; LnD – fluorescent lipid nanodroplets; BBB – blood-brain barrier; TBI – traumatic brain injury; VO – vascular occlusion; Do – donor fluorophore; Ac – acceptor fluorophore; FRET - Förster Resonance Energy Transfer.

DEVELOPMENT AND VALIDATION OF 3.3' – DIINDOLYLMETHANE NANOFORMULATION FOR THE TREATMENT OF INFLAMMATORY DISEASES

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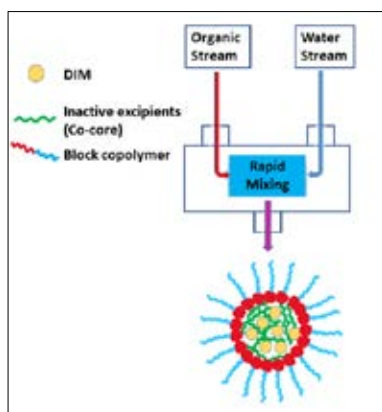


Fig. 1. Schematic of DIM_NP Formulation via FNP.

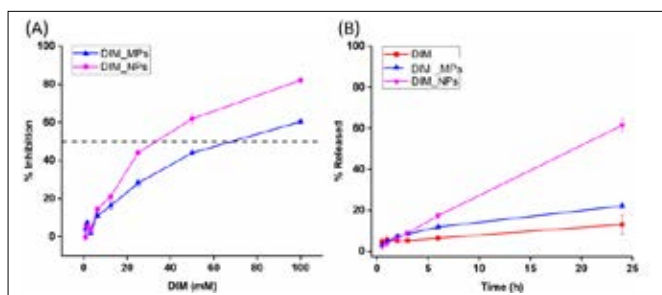


Fig. 2. Comparing (A) ROS scavenging effect and (B) Release kinetics of NPs and MPs. Unpublished data.

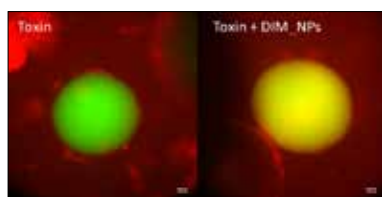


Fig. 3. Retention of NPs in inflamed human enteroids 6h post-toxin injection. Lumen of enteroids is injected with either FITC-Dextran with (right) or without DIM_NPs (left).

Overlay of fluorescence from FITC-Dextran (green) and DIM_NPs (red) resulted in yellow colors in the right panel. Unpublished data.

3.3' -diindolylmethane (DIM) is a phytochemical that has demonstrated anti-inflammatory and anti-tumor effects in variety of pre-clinical models. Despite its therapeutic potentials, delivery of DIM in clinical setting is challenging due to the crystalline nature of the chemical. A microparticle formulation of DIM (DIM_MPs) has enhanced bioavailability relative to crystalline DIM in pre-clinical and clinical studies. However, the bioavailability is still low and is below values that are required to ensure efficacy. Therefore, there is unmet need to develop DIM formulation to better investigate its biological activity *in vivo*. Nanoformulation of oral drug exhibits improved pharmacodynamics compared to micronized drug formulations due to the improved bioavailability. Herein, we have developed a muco-diffusive nanoformulation of DIM (DIM_NPs) using scalable encapsulation process called FlashNanoPrecipitation (FNP) (Fig. 1). DIM_NPs had good resuspendability and photostability (data not shown). DIM_NPs exhibited reactive oxygen species (ROS) scavenging activity two times stronger than DIM_MPs (Fig. 2A). DIM_NPs also released three times higher amount of DIM in

fed state simulated intestinal fluid than BR_DIM during human GI transit time (Fig. 2B). To validate the superior anti-inflammatory effect of DIM_NPs, we will employ human enteroids *ex vivo* and a murine colitis model *in vivo*. Our preliminary study has evidenced the retention of DIM_NPs in inflamed enteroids (Fig. 3) and restoration of a junction protein E-cadherin (ECAD) (Fig. 4). Future studies will involve the investigation of changes in expression for proteins comprising the tight junctions in the epithelial barrier as well as key pro-inflammatory cytokines (IL-1 β and IL-18). The functionality of barrier properties will also be investigated by measuring the leakage of FITC-Dextran. DIM_NPs have a potential to become a modular platform for the treatment of variety of inflammatory diseases as well as cancers.

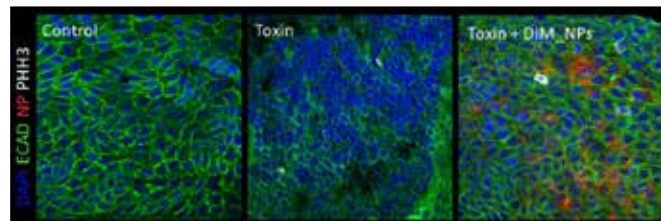


Fig. 4. Restoration of E-cadherin 6h after DIM_NP treatment.

NANOMATERIAL REPROGRAMMING OF IMMUNE CELLS TO UNLOCK THE EFFICACY OF IMMUNOTHERAPY

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Immunotherapy has substantiated its promise and achieved striking clinical efficacy in at least a subset of patients in several cancer types. However, in many patients and in those with certain cancer types such as glioblastoma multiforme, immunotherapies have lacked effectivity as a consequence of highly immunosuppressive "cold" tumour microenvironments and the potential for severe systemic immunotoxicities when trying to overcome this. Small, thin graphene oxide (GO) sheets possess notable pharmacokinetic properties including passive accumulation in lymphoid tissues such as the spleen following systemic administration^[1], and deep translocation throughout the whole tumour area following local administration^[2]. Here, we will demonstrate how we exploited this pharmacokinetic profile along with the versatile and efficient biomolecule loading capacity of this nanomaterial for the development of several strategies to unlock the efficacy of immunotherapeutics in both aggressive melanoma and glioblastoma.

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FROM FULLERENES, TO CARBON NANOTUBES TO GRAPHENE: BIOMEDICAL APPLICATIONS OF CARBON NANOTECHNOLOGY

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A brief historical perspective will be offered on how the three key carbon nanostructures, fullerenes, carbon nanotubes and graphene (and various other structural alternatives) have evolved as the field of nanotechnology has been maturing. Among the myriad application areas seeking to utilise their properties, use of carbon nanomaterials in medicine has been explored with varying degrees of success and adoption. Despite the fact that pharmaceutical and biotechnology adoption of these nanostructures has not (yet) materialised, there are many extremely useful lessons that have been learnt that apply to all novel nanomaterials as they develop into clinically useful biomaterials. Particular emphasis will be placed on the combination of properties offered by graphene-based nanomaterials and the recent efforts to clinically induct them into first-in-human trials.

REALISTIC IN VITRO MODELS AS WAY TO REAL INDIVIDUALIZED MEDICINE

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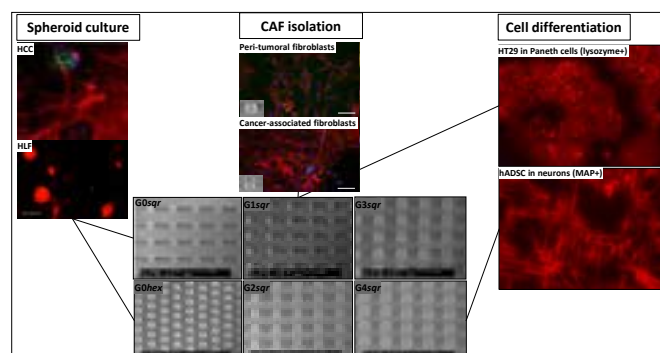
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For personalized medicine to be predictive for the treatment of a patient, the *in vitro* models need to be as close as possible to the real situation in the patient's body. So far, personalized medicine means to identify distinctive patient's genetic or biomarker set-up to predict response to certain treatments such as antibody therapy. However, the vision is that each patient might in future be treated with drugs or drug combinations to which the tumour responses best. While the gold standard in cell culture is still two-dimensional, it is well-recognized that *in vitro* tumour spheroids or organoids are more predictive for the real drug response and xenografts of patient-derived tissue (PDX) in immune-deficient mice reflect *in vivo* the real conditions in the human body. However, there are limitations which makes these models unsuitable for use in every patient. On one hand the spheroid culture as well as the PDX model is time-consuming leading to a gap between diagnosis and treatment. On the other hand, it is too expensive and unacceptable to use animals for screening different therapeutic regimes in mice for each patient. A possible solution would be a fast-growing spheroidal *in vitro* model that is predictive for the patient's response to therapy. We present a novel inorganic topographic platform to grow complex *in vitro* models in a short time. The surface allows the cells to grow as natural as possible. Cells which grow in tissue in sheets (intestinal endothelial cells) while cells forming cell clusters will grow in 150-200 micron-sized spheroids or small organoids on the same surface. No surface pre-treatment or growth factors are necessary. The spheroids grown from hepatocellular carcinoma (HCC) showed morphological similarities with an HCC *in vivo* such as small clusters of tumour cells enwrapped by a layer of cancer-associated fibroblasts (CAF).

The topographic features can be modified to present increasing complexity (see figure 1). The difference in complexity results in different cell culture applications. The simplest topographic struc-

ture is optimal for spheroidal growth, while the more complex topography can be used to isolate in a one-step process CAF without contaminating tumour cells⁵. The surfaces can induce cell differentiation in susceptible cells such as adipose-derived stem cells or HT29 de-differentiated intestinal tumour cells without differentiation factors or chemical agents.

Figure 1: Overview of the inorganic topographic surface and its applications in complex cell culture



The shorter growth time as well as the ease to use the platform might allow in future to culture *in vitro* primary tumour cells and form spheroid which then can predict the response of the patients tumour to treatment.

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BIOMIMETIC AND BIOFUNCTIONAL POLYPEPTIDE-BASED SELF-ASSEMBLIES

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We present here an overview of the self-assembly of biomimetic amphiphilic block copolymers developed in our laboratory, focusing on polymersomes, and their contribution in nanomedicine. We pay particular attention to block copolymer vesicles based on polysaccharides, polypeptides and proteins, that can advantageously be loaded with drugs and present a surface with multivalent presentation of bioactive saccharide moieties. Recent developments at the interface of bioengineering and polymer science, based on elastin-like polypeptides, relevant for regenerative medicine, glycoproteins and lipoprotein mimetics, will also be proposed.

NOVEL NEUROIMMUNOMODULATORY THERAPY FOR DEMENTIA MODEL MOUSE INTERPRETED BY SPATIAL TRANSCRIPTOMICS

DONG SOO LEE,
Seoul National University, Seoul Korea

The roles of brain cells their genes to the pathogenesis of Alzheimer's disease can now be examined using spatial transcriptomics with brain section, sequencing RNAs and allocating them to the 4,900 spots (100 μ x 100 μ) per section. Quantitative pseudocount of 2-3,000 variable genes expressed in each type of brain cells were imaged on the brain section of 40 control and dementia model mice. Region or state-specific RNAs of the expressed genes delineated the brain areas, both anatomical and functional. Modular expression disclosed the regional/ontogenical annotation and/or homeostatic/reactive status of glial cells as well as neurons. Seurat/Cell-DART programs and web-GUI platform were adopted and developed in house to explore and investigate the action on the brain cells of novel therapeutics in control, disease-control and in mouse models.

Behavior improved with novel trials of small molecule drug and cell therapy. 1. Off-label drug (Fxx) effect was found to be on the dorsal striatum for certain inhibitory/excitatory neuron subtypes, microglia and astrocytes in 5xFAD mice (mutated human APP/PS genes in mouse). Oligodendrocytes responded to the oral administration of Fxx, which were considered to act on lymph nodes but ambiguous on brain/spinal cord. Neuroimmune interaction on the brain, borderline leukocytes, and central/peripheral immune organs. 2. NK cells were also found to influence amygdala neurons, aging-related microglia, and astrocytes (esp. C4b). NK cells did not affect tissue (brain)-resident NK cells or T cells of the recipient mice. Systemic administration of NK cells in 5xFAD mice had been speculated to act upon bone marrow/spleen leukocytes with further brain action unknown, however, we disclosed how the specific brain cells in certain areas of the brain responded to the novel trial of NK cell therapy in dementia mouse model.

This suggests that we need to find the target action of novel immunomodulatory therapeutics to explain behavior improvement in mouse model considering their possible mechanistic action on neuroimmune interface/interaction based on the observed changes of transcriptomes of brain cells using the spatial transcriptomics platform described in this study.

NANOCARRIERS FOR COMBATING AND PREVENTING INFECTIOUS DISEASES

CLAUS-MICHAEL LEHR, Professor at Saarland University, co-founder, and Head of the department Drug Delivery at the Helmholtz Institute for Pharmaceutical Research Saarland (HIPS), co-founder of Across Barriers GmbH, CEO, PharmBioTec GmbH, not-for-profit contract research subsidiary of Saarland University; Saarbrücken (D)

Not ignoring the current pandemic, the threat of antimicrobial resistance might be even worse and the problem is still increasing. Ironically enough, the number of new antibiotics and even the number of companies engaging in those is decreasing. Besides the need for new targets and actives there is also a need to deliver those across biological barriers. Besides the body's outer epithelia, like e.g., gut, skin and lung, there are also the bacterial cell envelope itself, as well as non-cellular barriers, such as mucus and bacterial biofilms. To study the cellular interactions of drugs and nanoparticles after deposition in the deep lung, our group has pioneered human alveolar epithelial cell models, including primary cells and as cell lines. Complex human in-vitro models allow to mimic diseases like inflammation and bacterial infections. Novel self-assembling nanocarriers, capable to co-deliver Tobramycin and modern quorum sensing inhibitors, allow to significantly reduce the dose of the antibiotic for completely eradicating the bugs and thus also the risk of inducing antimicrobial resistance (14,15).

ACCELERATED DEVELOPMENT AND CLINICAL READINESS OF GENOMIC MEDICINES

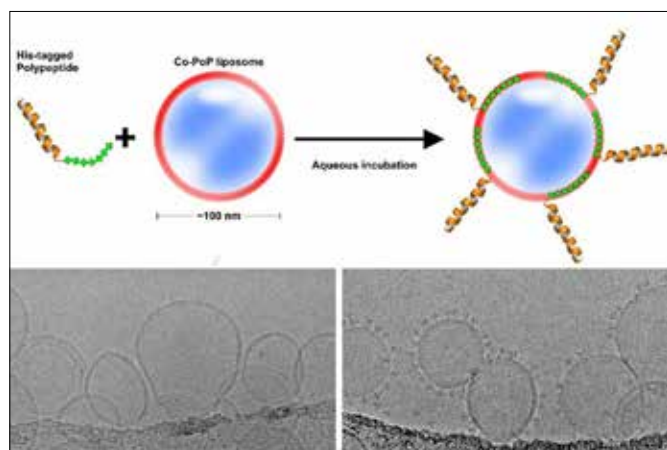
JEFFS LLOYD

RNA can be designed to silence, express, and edit specific genes providing a flexible and powerful approach to treating diseases. RNA vaccines for COVID-19, while an extraordinary accomplishment, have revealed that rapid development and scale up – and access to necessary technologies – are needed to keep up with emerging variants and to help usher in new RNA medicines in other areas of unmet medical need. For this reason, we have developed a “genetic medicine toolbox” for the end-to-end development of RNA-lipid nanoparticles (RNA-LNP). The toolbox comprises an RNA drug substance platform, a lipid nanoparticle drug delivery platform, and a microfluidic manufacturing platform, which are made accessible to scientists of different backgrounds by leveraging in-house expertise in RNA-LNP formulation, manufacturing, process and analytical development to engineer both our know-how and ease-of-use into these tools. Additionally, our experts, who are developing a self-amplifying RNA vaccine on behalf of the Canadian Government, are available through our Biopharmaceutical Services to support and supplement each step in your development process. Here, we provide examples from our R&D to demonstrate the versatility of the toolbox for the rapid development of vaccines, gene therapies and cell therapies from idea to approved drug product.

LIPOSOME-DISPLAY OF ANTIGENS: A VERSATILE APPROACH FOR VACCINE DEVELOPMENT

JONATHAN LOVELL

Display of protein-based antigens in nanoparticle format has attracted interest for improving vaccine immunogenicity. We have developed cobalt-porphyrin-phospholipid (CoPoP) as a lipid-like excipient that enables the rapid and biostable anchoring of his-tagged peptides and proteins with simple admixture of proteins and liposomes. This approach has recently moved into clinical testing in the EuCorVac-19 COVID-19 vaccine. Recent findings on this approach will be discussed.



ULTRA-HIGH DRUG NANOFORMULATIONS: HOW ARE THEY POSSIBLE AND WHY DOES IT MATTER?

ROBERT LUXENHOFER¹

¹ Soft Matter Chemistry, Department of Chemistry, and Helsinki Institute of Sustainability Science, Faculty of Science, Helsinki University, 00014 Helsinki, Finland

A large proportion of active pharmaceutical ingredients (API) suffer from poor water-solubility and concomitant poor bioavailability. Polymeric micelles and nanoparticles are well known to increase the apparent water solubility of insoluble compounds, however, the amount of API that can be stably dispersed is often rather limited, limiting the clinical benefit.

We have previously reported on a polymer platform which allows to formulate unusually large amounts of the extremely water insoluble paclitaxel^[1-3] and many other APIs and API combinations^[4] with up to 50 wt.% incorporated in the polymer micelles. In some cases, higher drug loading was beneficial for therapeutic efficacy^[3]. We have identified pronounced effects of small structural variations in the polymers on the formulation capacity for a variety of different drugs^[5-8]. In addition, we have found that the hydrophilic shell of the polymer micelles is strongly involved in the drug interactions^[9], which, depending on the polymer structure, can severely compromise drug loading, stability and dissolution rates^[10].

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A LESSON FROM NATURE TOWARDS LIFE IN EXTREME CONDITIONS – FROM NANOSCALE TO COMPLEX SYSTEMS

MIRA MARCUS-KALISH

The last challenging years that combined two major threats – the COVID pandemic and accelerating climate change – forced us to learn some new lessons from Nature regarding our survival, both physically and mentally.

The Dead Sea salt lake – the lowest point on Earth, with extreme salinity can teach us some lessons from Nature, in a broad range of areas from the nanoscale to complex systems. These lessons arise from the rare and unparalleled combination of air, sun, water and soil and its impact on our everyday mental and physical life. The air - higher in partial oxygen pressure, rich in aerosols, non-polluted and non-allergenic; the safer sun exposure, the water - salty and mineral rich and the special structure of the mud. How and what can we learn from this “not so dead” lake and region, from its living biome, bacteria, algae, fungi, archaea and others, to adjust and survive in extreme conditions? Such unique conditions give rise to materials that can be used to develop Nano carriers, delivering insoluble molecule cargo through lipid environment, while crossing phase barriers - applied to drugs, cosmetic and other agents, etc. In general, the goal is to provide healing, physical and mental treatment benefits to the community, especially to the vulnerable groups, the elderly and the young affected by the pandemic, through work on respiratory disease, arthritis, paraplegia, skin conditions, and more.

A NEED FOR BACTERIOPHAGE THERAPY FOR AMR INFECTIONS AND THE COMPLEX CHALLENGES ASSOCIATED WITH THIS ENDEAVOR

CARL R. MERRIL, M.D., NIH Emeritus Scientist and CAPT USPHS (ret), Chief Scientific Officer, Adaptive Phage Therapeutics

For most of human history, survival from bacterial infections was determined by an individual's natural defense systems and general health. The first bacterial pathogen (*Bacillus anthracis*) was identified in 1876, and this was rapidly followed by identification of other bacterial pathogens. These discoveries led to efforts to treat these bacterial infections with: synthetic chemicals in 1910, with bacteriophages (phages), first reported in 1917, and with organic molecules synthesized by fungi (antibiotics), in the 1940s. While antibiotics initially proved highly effective, their widespread use has resulted in selection for resistance factors, many of which arose millions of years ago. The current situation has been exacerbated by a slowdown in the discovery and development of new antibiotics, such that bacterial diseases are again a major cause of medi-

cal morbidity and mortality. Given this situation, it may be prudent to again consider the development of “therapeutic phages.” We now recognize that there are almost an infinite number of phages >10³¹, on the planet and they are the greatest predators of bacteria on the Earth. While these factors increase the probability that phages offer a respite from the current increase in bacterial associated morbidity and mortality, their use will require a significant effort to succeed, as bacteria have had billions of years to develop defense mechanisms against the phages.

A promising approach to counter this widespread distribution of anti-phage bacterial defense systems involves the use of phage banks (collections of phage strains) that collectively provide poly-microbial broad-spectrum coverage, including strains with capabilities to overcome diverse bacterial defense systems. In addition, it is recognized that phage banks will need to have a capacity to grow to address an ever-increasing diversity of bacterial defense mechanisms as the use of phage becomes more widespread. To assure that phage strains included in these banks do not contain potentially deleterious genes such as antibody resistance or toxin genes, the genomes of phage strains collected for inclusion in the banks, need to be annotated, analyzed, and subsequently approved by regulatory agencies. Once phage strains are selected for the collection, they will need to be amplified, purified, and formulated such that they can be stored and shipped without loss of clinical efficacy. In addition, the clinical use of phage banks will require development and deployment of rapid phage-bacterial host range assays, to assure that phage strains are appropriate for each individual patient’s therapeutic needs. In addition, despite the discovery of the anti-bacterial potential of phages in 1917, phages are only now being subjected to controlled, prospective, double blind, clinical studies, with reviews by regulatory agencies. Development, manufacturing and testing of phage strain collections require significant expertise and resources to be successful. Given the ubiquitous and evolutionary basis of bacterial defense systems, phage banks and associated companion diagnostics may be the only viable option for us to effectively address the antimicrobial resistance crisis.

CAN ARTIFICIAL INTELLIGENCE (AI) ASSISTED COMPUTER LEARNING AID IN THE RAPID IDENTIFICATION OF BACTERIOPHAGE STRAINS FOR THE TREATMENT OF BACTERIAL INFECTIONS IN INDIVIDUAL PATIENTS?

DR. CARL R. MERRIL, M.D., NIH Emeritus Scientist and CAPT USPHS (ret), Chief Scientific Officer, Adaptive Phage Therapeutics

Identification of phage strains that may be efficacious for the treatment of bacterial infections currently requires isolation and culturing of pathogenic bacteria from infected individuals, followed by testing for phage susceptibility. This matching assay currently takes hours to perform and analyze. There is a possibility that AI assisted computer learning may be able to accelerate identification of clinically efficacious phage strains. This possibility may be enabled by the existence of clinically relevant phage banks that contain genomically sequenced phages, and genomically sequenced bacterial pathogens that are now being determined in some hospitals by their use of whole-genome bacterial sequencing, to discriminate between highly related lineages of pathogenic bacteria. AI and computer learning may be able to use such information in the future to assist in the rapid identification of phage strains for the treatment bacterial infections in individual patients. Several publications have reported the use of AI to match phage genomic information with bacterial genomes. Unfortunately, these reports have been restricted to in-silico studies. They have not employed experimental phage-bacterial host range testing or clinical efficacy data for phage tested against genomically sequenced bacterial pathogen isolated from patients. Development of AI assisted computer selection of phage therapeutic phage strains for individual bacterial infections will require the development and use of “learn-

ing pairs” that contain sequenced: specific phage and bacterial host strains, where the “learning pairs” contain host bacterial strains that are susceptible to phage killing or inhibition (based on in-vitro host range testing and clinical efficacy data). In addition, AI learning will also require “learning pairs” where the host bacterial strains are not susceptible to phage killing or inhibition. Development of AI and computer learning to assist in the rapid identification of bacteriophage strains for the treatment of bacterial infections may be made even more difficult, for bacterial strains that use complex mechanisms, such as genetic switches, that allow some bacterial strains to shed their outer capsule, thereby eliminating specific phage attachment sites. In clinical practice, such defenses may be dealt with by monitoring for phage resistance following initial phage treatments and then by the selection of “new efficacious” phage strain(s) to counter such defense systems. Complex bacterial defense may require additional time and resources for development of AI and machine learning systems that have a capacity to deal with such multifaceted bacterial defense systems.

THE ROLE OF STERIC STABILISATION IN VACCINE FORMULATION AND PERFORMANCE

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Steric stabilisation is a surface engineering approach conferring stability to colloidal particles by non-ionic macromolecules such as block co-polymers of ethylene oxide/propylene oxide (Pluronic), poly(ethylene glycol)-lipid conjugates, polysorbates (Tweens), and hyperbranched polyglycerols. Colloidal stabilisation arises primarily from elastic (volume restriction) and osmotic contributions. Elastic effect is the result of loss of conformational entropy when two surfaces approach each other, caused by a reduction in the available volume for each polymer. Entropy loss (and increase in enthalpy) increases free energy of mixing causing particle separation. However, for very short polymer chains, the Van der Waals attraction force is rarely important in determining stabilisation by surface-bound polymers. The osmotic contribution is due to increase in polymer concentration on compressing two surfaces. This induces an influx of water into the region forcing particles apart. Steric stabilisation with non-ionic macromolecules remains an effective strategy in conferring colloidal stability to non-viral and viral vaccines during production, storage and in biological milieu, where stabilisation can be optimised depending on materials surface chemistry and macromolecular type. Furthermore, the strength of the steric barrier and the conformation of surface projected polymers can not only control the rate of drainage of intramuscularly and subcutaneously injected particles intended for vaccination into the initial lymphatic vessels, but also modulate the extent of particles interaction with immune cells at interstitium and in the local lymph nodes. Particle curvature also controls architectural display of surface adsorbed polymers, but the overall conformation of surface projected polymers (or the adlayer thickness) could be optimised. Polymer conformation and density also play a modulatory role in complement activation, where the complement activation product C3d exerts adjuvant activity by activating B cells and inducing long-term memory. Considering the modulatory role of dendrimers in innate immunity, surface fabrication of particles with dendrimers might offer alternative means for controlling stability (through steric as well as ionic stabilisation, depending on dendrimer functionality) as well as generating defined patterns and ordered lattices for precision

interaction with the pattern-recognition receptors and causing pathway-specific activation of the complement system. Thus, dendrimeric and dendron-mediated engineering initiatives could form the basis of a new generation of safer designer subunit and genetic vaccines for infectious diseases and beyond. Finally, sterically stabilised nonlamellar liquid crystalline aqueous nanodispersions (e.g., hexosomes, cubosomes) are also promising platforms for vaccine development. Here, the packing mode of the hydrophobic moiety of the stabilising macromolecule (e.g., PEG-lipid) in the oily phase (or bilayer) of the nanodispersion can modulate mobility and stretching of surface projected polymer chains. In summary, the relationship between surface properties of particles (i.e., projected polymer density and adlayer thickness) and their lymphatic kinetics provides rational means for simultaneous vaccine stabilisation, adjuvanticity and optimisation in lymphoid targeting.

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COVID VACCINES AND ALLERGIC REACTIONS: FACTS AND MYTHS

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Allergic reactions to vaccines are very rare. Vaccination against SARS-CoV-2 has shown a swift response in controlling COVID-19 pandemic globally, and anaphylaxis has been reported rarely following COVID-19 vaccination. According to the FDA, COVID-19 vaccines should be contraindicated in individuals with a severe allergic reaction to the first dose of vaccine, or with known history of hypersensitivity to any component of the vaccine. History of previous allergies should also be scrutinised. Nevertheless, the molecular basis of these rare allergic reactions to COVID-19 vaccines currently remains unknown, but equivocal suggestions have held anti-PEG antibodies as the culprit. Pfizer-BioNTech and Moderna COVID-19 vaccine contain low amounts of PEGylated lipids, whereas adenoviral-based COVID-19 vaccines (e.g., Oxford/AstraZeneca and Johnson & Johnson) are stabilised with Tweens, which share some structural similarities to PEG. Previously, allergic responses in some patients undergoing treatment with PEGylated medicines such as pegnivacogin have been noted where the involvement of anti-PEG antibodies was considered. Unlike COVID-19 vaccines, large amounts of PEG (64 mg PEG for an 80 kg patient) were administered in a single bolus dose of pegnivacogin. However, not all patients with high levels of the so-called anti-PEG antibodies experienced allergic reactions to pegnivacogin. This either suggests differences in susceptibility to antibody-triggered reactions among individuals (e.g., signalling threshold and/or desensitisation through FcγRs on binding of pegnivacogin-immunoglobulin complexes) or inter-individual differences in properties of circulating anti-PEG antibodies. Nevertheless, it should also be stressed that human sera contain low levels of anti-PEG IgG and IgM antibodies, but the titre of these antibodies has been well over-estimated in

many studies using inappropriate methodologies, lack of validation and characterisation of the immobilised substrates, and the use of exceedingly high concentration of secondary anti-human antibodies, giving the impression of high prevalence of anti-PEG antibodies in the general population. Also, not much information is available on cross-reactivity of other antibodies (e.g., anti-phospholipid and anti-microbial lipid antibodies) with PEG substrates. Thus, without further evidence, the role of anti-PEG IgG- and IgM-mediated allergic reactions in COVID-19 vaccination should be viewed cautiously (*Molecular Therapy* 2021, 29, 898–900). The idea of an IgE-mediated allergy to PEG has also been proposed, but the available evidence with COVID-19 mRNA vaccination do not support this hypothesis either. However, it is still possible that in some individuals other IgEs that cross-react with a heterogeneous group of allergenic determinants could recognise different epitopes on lipid nanoparticles in Pfizer and Moderna COVID-19 vaccines and trigger anaphylactoid reactions. If PEG is proven responsible for triggering allergic responses, then alternative stabiliser such as dendrimers or even dendrimer-based vaccines may be the way forward. Indeed, dendrimers through their Angstrom-scale spacing arrangement of end-terminal motifs, and hence controlled pattern presentation, might regulate and dampen untoward immune responses (*Nature Communications* 2021, 12, 4858). Finally, another plausible hypothesis with Pfizer-BioNTech and Moderna COVID-19 vaccine is the tissue damage/inflammation mediated by their lipid nanoparticles, which might trigger interleukin-33 release and induce anaphylaxis through the orphan receptor ST2 in some individuals. The benefits of COVID-19 vaccination clearly outweigh the risks, but better understanding of anaphylaxis mechanisms will surely improve and accelerate development of safe vaccines in the future.

SHORT INTRODUCTION BY THE CHAIR TO THE TOPIC 6: ACHIEVING GLOBAL VACCINE EQUITY (CLINAM 2022)

STEFAN MÜHLEBACH

Vaccines are a mainstay to control or even eradicate diseases by viral infections.

The COVID pandemic has shown that 1st thanks the existing and growing knowledge of biotechnology and nanotechnology and 2nd to an adopted parallel authorization process within almost one year highly efficacious, safe, and high-quality mRNA vaccines could be approved. They can protect the vulnerable and other groups of the population from severe disease. The production of these vaccines could be increased massively in short time to respond the high demand. Nevertheless, due to the very large request, the difficult manufacturing with a high number of different starting materials, shipment and handling requirements for the vaccines, and the necessary financial resources, a global vaccine equity could not be achieved despite international initiatives in favor also to the lower income countries. Therefore, and in contrast to the Western countries, where a high vaccination rate and a sufficient supply of vaccines could be achieved, this is not yet the case e.g., for Africa or other areas with limited health care and infrastructure resources. The learnings from the actual pandemic with the challenges for an appropriate international collaboration to provide such vaccines on an equity base require actions.

IMAGING AND IMAGE ANALYSIS FOR STEM CELL-MEDIATED DISTRACTION OSTEOGENESIS

BERT MÜLLER

Distraction osteogenesis aims at dedicated bone formation to improve jaw's function. This treatment, however, is complex and time-consuming. In order to increase the distraction rate, we propose the application of human mesenchymal stromal cells (hMSC) before distraction. A custom-made distraction device bilaterally placed in the mandibular angle of four nude rats was employed. After a latency period of five days, the distraction rate of two rats was set at 1.0 mm on the first day and subsequently 0.5 mm per day. For the other two rats, the distraction speed was 1.5 mm per day. In each of these two groups, one animal obtained one million hMSC per side. Once a distraction gap of 6.0 mm was reached and the consolidation phase terminated, the four rats were sacrificed and the bone formation studied via conventional and synchrotron radiation-based micro computed tomography (μ CT) as well as histology. Semi-automatic registration of the μ CT provided a bivariate histogram for direct comparison between the two preparations, i.e. before and after decalcification. The identification of the location of the H&E-stained slide within the μ CT data was achieved via non-rigid 2D-3D registration. In the related tri-variant histograms we have identified four clusters related to the anatomical features from bone and soft tissues. Synchrotron radiation-based μ CT, non-destructively performed after tissue embedding and before histological sectioning, suitably complements the protocols employed in histology.

ASSESSMENT OF ALLERGIC AND ANAPHYLACTIC REACTIONS TO mRNA COVID-19 VACCINES

KARI CHRISTINE NADEAU

During the COVID-19 pandemic, nanoparticle-based mRNA vaccines were developed, and several billion doses have been administered. A few individuals developed allergic reactions to these vaccines, although the mechanisms remain undefined. To understand COVID-19 vaccine-mediated allergic reactions, we enrolled 13 controls and 19 participants with allergic reactions. Using standard hemolysis assays, we demonstrated that sera from allergic participants induced stronger complement activation compared to non-allergic subjects following *ex vivo* vaccine exposure. In a subgroup of allergic individuals, vaccine-mediated complement activation correlated with anti-PEG IgG levels while depletion of IgG suppressed complement activation. To investigate the effects of vaccine and vaccine excipients on basophil function we employed a validated indirect basophil activation test, which stratified the allergic populations into high and low responders, with high responders exhibiting the shortest time to allergic reaction development. Complement receptor blockade in this system suppressed basophil response, providing strong evidence for complement involvement in vaccine-mediated basophil activation.

NOVEL MICRORNA-RELEASING LIPOPLEXES ENCAPSULATED IN INJECTABLE HYDROGEL FOR CARDIAC REGENERATIVE MEDICINE

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INTRODUCTION

Cardiovascular diseases, including myocardial infarction (MI), represent one of the leading causes of death worldwide. MI is caused by a reduction or blockage of blood flow to the left ventricle, causing the massive death of cardiomyocytes and the formation of dysfunctional fibrotic scar tissue, which consequently may lead to heart failure^[1]. To date, heart transplantation represents the only available therapeutic option for end-stage heart failure, although associated with several limitations. Hence, extensive research is being conducted to develop novel strategies for post-infarct cardiac regeneration, including the design of tissue engineering scaffolds, cell therapies and novel "reprogramming" approaches. In this regard, the local release of specific microRNA (miRNAs) has arisen considerable interest as a new strategy for myocardial regeneration, promoting cardiomyocyte proliferation or the trans-differentiation of cardiac fibroblasts (CFs) into induced cardiomyocytes (iCMs)^[2,3]. Previous studies have shown that human cardiac fibroblasts can be directly reprogrammed into induced cardiomyocytes (iCMs) by transient transfection with four microRNA mimics (miR-1, 133, 208, and 499, termed "miRcombo")^[3]. However, the delivery of naked miRNAs is limited by their low retention at the target site due to their fast degradation and the inability to enter the cells due to their negative charge. The encapsulation of miRNAs into nanoparticles may address such drawbacks for an efficient miRNA release into the target cells. In this regard, lipoplexes (LPs) are interesting for direct cell reprogramming as their composition can be tailored to achieve efficient cell transfection, preserving biocompatibility. Injectable hydrogels could additionally be exploited as a promising option for controlled *in situ* delivery of miRNA-loaded LPs. Alginate-based injectable hydrogels are currently being investigated in clinical trials to favour cardiac tissue remodelling after MI^[4]. However, alginate presents some limitations such as low degradability *in vivo* because of the absence of alginate-degrading enzymes in mammals and reduced cell adhesion because of the lack of cell-interacting regions in its structure. To address these drawbacks, oxidized alginate has been used as an alternative drug delivery system, leading to the formation of alginate dialdehyde hydrogels (ADA hydrogels)^[5].

In this work, LPs based on a mixture of cationic and helper lipid were initially formulated for more efficient encapsulation and release of miRNAs to CFs compared to a commercial agent^[6]. Then, miRNA-loaded LPs were encapsulated into an ADA-based hydrogel, properly designed to obtain a controlled release of miRNA-loaded LPs. Potentially, this approach could be exploited for *in situ* miRNA-mediated transdifferentiation of CFs into iCMs (Figure 1).

METHODS

Initially, LPs based on a cationic and a helper lipid containing negmiR or miRcombo were prepared via spontaneous electrostatic interaction at different amino to phosphate groups (N/P) ratios. Their hydrodynamic size, zeta potential and PDI were analysed by Dynamic Light Scattering (DLS). miRNA encapsulation ability and *in vitro* release kinetics were measured using Qubit™ 4 Fluorometer. Adult human CFs (AHCFs) were treated with miRNA-loaded LPs and cell viability, miRNA cell uptake and transfection efficiency were analysed. A commercial lipidic transfection agent was tested for

comparison. Finally, miRcombo-loaded lipoplexes were first produced and physically characterized, and then their ability to promote direct reprogramming of AHCs into iCMs was investigated by analysing gene expression of cardiac and fibroblasts markers post-transfection using droplet digital PCR (ddPCR) analysis.

Alginate dialdehyde (ADA) was prepared by controlled oxidation of sodium alginate with sodium metaperiodate in an equal volume of ethanol–water mixture [5]. ADA was chemically characterized through ATR-FTIR analysis, MAS NMR spectroscopy and via the iodine–starch test to determine the degree of oxidation [5]. ADA hydrogels were obtained by ionic crosslinking with calcium ions using a double syringe mixing apparatus and rheologically characterized. Finally, Cy5-siRNA-loaded LPs were physically encapsulated into ADA hydrogels [6]. Release studies from ADA hydrogels encapsulating Cy5-siRNA were performed in Milli-Q water at 37°C using Qubit™ 4 Fluorometer and DLS analysis.

RESULTS AND DISCUSSION

MiRNA-loaded LPs with different N/P ratios were characterised by DLS analysis, showing a progressive decrease of the hydrodynamic diameter from 876 nm to 372 nm and a gradual increase of Z-potential from -26 mV to +40 mV with increasing the N/P ratio (0.35-3.0) (Figure 2A). LPs showed a significantly higher miRNA encapsulation efficiency (~99%), compared to a control commercially available transfecting agent. Based on their superior physical stability (analysed by DLS), miRNA-loaded LPs with 3.0 N/P ratio were selected for *in vitro* cell tests, showing high biocompatibility and the ability to efficiently deliver a fluorescently labelled model miRNA into cells (Figure 2B). Moreover, transient transfection of AHCs with miRcombo-loaded LPs showed their transdifferentiation into cardiomyocyte-like cells. Indeed, after 15 days post-transfection, cells showed significantly enhanced expression of TNNT2 encoding for cardiac Troponin T, a cardiomyocyte marker, compared to a commercial transfection agent (Figure 2C).

ADA was prepared by periodate oxidation of sodium alginate. ADA preparation process was optimised, varying alginate molecular weight, achieving an overall yield of 68% w/w and an oxidation degree of 23%. FTIR and 13C MAS NMR analyses of ADA confirmed the successful formation of free reactive aldehyde groups, through the presence of a more intense absorption band at 1740–1725 cm⁻¹ and a new peak at 99 ppm compared to sodium alginate spectra, respectively. To develop ADA injectable hydrogels, ADA solution concentration and crosslinking degree were optimised to obtain viscoelastic properties close to alginate-based devices currently investigated in clinical trials for cardiac remodelling (Figure 2D) [4]. Model Cy5-siRNA-loaded LPs were encapsulated into ADA-based hydrogels and, then, Cy5-siRNA release was analysed, showing 100% cumulative release after 24h (Figure 2E), similarly to what found for non-encapsulated Cy5-siRNA-loaded LPs (data not shown). DLS analysis of collected release media suggested that intact Cy5-siRNA-loaded LPs were not released from ADA hydrogels. This result suggested the need for tailoring the surface charge of miRNA-loaded LPs through a coating strategy, to minimize the electrostatic interactions between ADA and the positively charged miRNA-loaded LPs, which is currently in progress [7].

CONCLUSION

In this work, we studied a novel delivery system for miRNAs, consisting of miRNA-loaded LPs encapsulated in ADA-based injectable hydrogels for prospective cardiac regenerative medicine applications. Surface modification of microRNA-loaded lipoplexes is in progress to obtain successful release of intact miRNA-loaded LPs from ADA hydrogels. Future work will involve *in vitro* and *in vivo* biological characterization of the developed materials. The final aim is their application for inducing *in situ* direct reprogramming of AHCs into iCMs.

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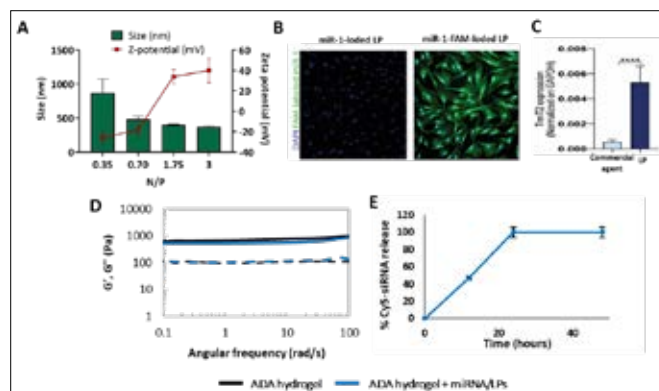


Figure 1. Schematic representation of the preparation of microRNA-releasing LPs encapsulated into an injectable hydrogel for *in situ* direct reprogramming of fibroblasts into induced cardiomyocytes for cardiac regenerative medicine.



Figure 2. (A) Hydrodynamic diameter and zeta potential of miRNA-loaded lipoplexes prepared at different N/P ratios, measured by Dynamic Light Scattering. (B) Representative fluorescence microscopy images showing FAM-labelled miR-1 uptake by AHCs, mediated by miRNA-loaded lipoplexes after 12 h transfection. Non-transfected cells were used as control. Nuclei were counterstained with DAPI (blue). (C) Expression of TNNT2 cardiomyocyte marker at 15 days for cells treated with miRcombo-loaded LPs, evaluated by ddPCR. (D) Frequency sweep analysis of optimised ADA hydrogel composition and ADA hydrogels encapsulating miRNA-loaded LPS. (E) Cy5-siRNA release from Cy5-siRNA-loaded LPs encapsulated into ADA hydrogels, performed at 37°C in Milli-Q water.

THE MULTIPLE CHALLENGES IN THE IMPLEMENTATION OF SCHISTOSOMIASIS AND SOIL-TRANSMITTED HELMINTHS' CONTROL INTERVENTIONS IN THE ITURI PROVINCE, DRC

MAURICE NIGO MUTRO

The Democratic Republic of Congo (DRC), a big country in the center of Africa, is among the poorest countries in the world. It is divided into 26 provinces. DRC is one of the world's highest burdens of neglected tropical diseases. The exact burden of NTDs remains unknown. Some estimates show that DRC ranks the third position in Africa concerning the burden of schistosomiasis. For instance, information on the burden of *S. mansoni* infection is scarce. This hinders the implementation of adequate control measures. Ituri province in the Northeast is one of the poorest provinces. It is exposed to decades of civil war and unrest. Recently, were conducted cross-sectional studies in a total of 59 communities, including both rural and urban communities in Ituri province. Up to five stool and one urine samples were collected from more than 3,500 participants. *S. mansoni* eggs were detected using the Kato–Katz technique. A point-of-care circulating cathodic *S. mansoni* antigen (POC–CCA) urine test was also used to detect light infections. Individual questionnaires were used to collect demographic, socioeconomic, environmental, behavioural, and knowledge data. Some participants underwent clinical and an abdominal ultrasonography examination to diagnose hepatosplenic morbidity. The examined population sample was randomly selected. Infection prevalence and intensity of both *S. mansoni* were assessed in the surveyed communities. Through these large scale and community-based studies, we assessed the geographical distribution of *Schistosoma mansoni* infection across the Ituri province. We also determined the prevailing risk factors, the morbidity burden, and the associated hepatosplenic morbidity of schistosomiasis in the province. Community visits were used to identify and treat patients with severe schistosomiasis. We found alarmingly high overall *S. mansoni* infection rates, beyond 60%; in villages along water bodies even in Bunia city, the provincial capital city. In fact, prevalence ranged from 4.0 to 95.0% across the visited health districts. Infection prevalence increased from north to south and from west to east. Exposure to the waters of the lake and the villages' altitude above sea level were associated with the distribution. Infection prevalence and intensity peaked in the age groups between 10 and 29 years. Preschool children were highly infected (62.3%). Key risk factors were poor housing structure, close proximity to water bodies, long-term residence in a community, lack of latrine in the household, and swimming and washing in local water bodies. Enlargement of liver and spleen were common; more than 30% in these areas. Intestinal morbidity reported in the two preceding weeks was very frequent, and included abdominal pain (52.7%), diarrhoea (23.4%) and blood in the stool (21.5%). Hepatosplenic morbidity consisted of abnormal liver parenchyma patterns (42.8%), hepatomegaly (26.5%) and splenomegaly (25.3%). Liver pathology was positively and significantly associated with *S. mansoni* infection. Hepatomegaly and splenomegaly were positively but not significantly associated with *S. mansoni* infection at the individual level. At the village level, *S. mansoni* prevalence was positively associated with the prevalence of hepatomegaly and splenomegaly. High-intensity *S. mansoni* infections were associated with diarrhoea, blood in the stool, hepatomegaly, splenomegaly, and liver parenchyma pathologic patterns. Four study participants were diagnosed with ascites and five reported hematemesis. Patients with severe chronic schistosomiasis were present in all these communities. Dispensaries reported frequent death due to blood vomiting. The national schistosomiasis control program had overlooked the severity of the schistosomiasis burden in this most neglected province. Our results show that *S. mansoni* is highly endemic and a major health concern in Ituri province, DRC. Infection prevalence and intensity, and the prevailing socioeconomic, environmental, and behavioural risk factors in Ituri reflect intense exposure and alarming transmission rates. A robust plan of action is urgently needed in the province. Due to security and

unrest reasons, we could not follow up all the patients. However, for the second round, we can follow up our patients in Bunia city.

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FABI INHIBITORS: PATHOGEN-SPECIFIC ANTIBIOTICS FOR THE TREATMENT OF ANTIMICROBIAL RESISTANT INFECTIONS

RICCARDO NISATO

Debiopharm invests in innovation and the development of new drugs to tackle and cure cancer and bacterial infections. Specifically, in the antibacterial domain, Debiopharm is investigating enoyl acyl carrier protein reductase (FabI) inhibitors, to address the need for new antibiotics. FabI inhibitors disrupt the bacterial fatty acid biosynthetic pathway preventing bacterial growth and have the potential to be the first pathogen-specific drugs to treat multi-resistant bacteria included in the critical and high priority list of the WHO, such as *Staphylococcus aureus*, including MRSA and VRSA, *Acinetobacter baumannii*, including carbapenem-resistant strains and multi-resistant *Neisseria gonorrhoeae*. The available data on the antimicrobial activity of FabI inhibitors in our pipeline highlight the potential of the bacterial fatty acid biosynthetic enzymes as a source of novel antibacterial targets for the 21st century.

Broad-spectrum antibiotic therapy decimates the gut microbiome, resulting in a variety of negative health consequences. One key challenge for current and future development of antibacterials is to design drugs that minimize disturbances to the microbiome. Afabycin (Debio 1450), the most advanced FabI inhibitor in our pipeline, is being developed to treat staphylococcal infections, including those caused by resistant strains of *S. aureus* and coagulase negative staphylococci. It demonstrates a staphylococcus-specific spectrum of activity, and treatment does not cause significant changes in the composition of gut microbiota in mice or humans. Therefore, one potential key benefit of afabycin treatment is the reduction of antibiotic-associated complications such as antibiotic associated diarrhea and *C. difficile* infections.

The efficacy and safety of afabycin was demonstrated in a double-blind Phase 2 trial when compared with vancomycin/linezolid in the treatment of acute bacterial skin and skin structure infections (ABSSSIs) caused by staphylococci. These promising results prompted Debiopharm to further investigate afabycin in hard-to-treat staphylococcal infections such as bone and joint infections (BJI). A randomized phase 2 trial in BJI is currently ongoing.

A TITLE FOR MY TALK IS 'PRECISION POLYMER NANOPARTICLES' AND MY ABSTRACT IS BELOW

RACHEL O'REILLY

Crystallization-driven self-assembly (CDSA) is a powerful tool in the solution polymer self-assembly toolbox and has been utilized to create an impressive range of hierarchical block copolymer structures. Unlike in conventional solution self-assembly, where the range of morphologies obtained are determined by varying the relative block composition of each block, in polymers assembled via CDSA, the formation of micelles with low interfacial curvature is favored. However, despite advances in CDSA there are relatively few examples where the aggregate morphology can be readily controlled to form nanostructures whose size can be controlled in 2 dimensions. Our group has the CDSA of poly(ester) based block copolymers. In this work we present the CDSA of a range of polylactone block copolymers which form a range of self-assembled nanostructures including 2D nanostructures. Using these we have further explored the design rules for the synthesis of such 2D nanomaterials and demonstrated their epitaxial growth, which highlights their potential as biocompatible nanomaterials.

ESTIMATING THE APPROPRIATE SIZE OF GLOBAL PULL INCENTIVES FOR ANTIMICROBIAL MEDICINES

KEVIN OUTTERSON, Professor of Law & N. Neal Pike Scholar in Health and Disability Law - Boston University Executive Director, CARB-X

Antibacterial medicines should be foundational for modern medicine, a key part of the infrastructure of contemporary practice. Recently, however, antibacterials have struggled commercially. Even with "push" incentives (grants paid before regulatory approval), antibacterials have failed on the market because revenues are tied to volume sold. There are policy initiatives under way in the United States and United Kingdom that explore paying for exceptional antibacterials with "pull" incentives (paid after regulatory approval) by delinking the payments from volume via other payment formats such as market entry rewards and subscriptions. This speech discusses these initiatives but also proposes an expected net present value model for calculating the global incentives required to create a functional antibacterial market, exploring options such as antibacterial subscriptions, market entry rewards, push incentives, higher prices, and drug development through charitable efforts. The model estimates that current push incentives should be continued, but governments must also enact pull incentives that will add several billion dollars to the global revenue stream of a highly innovative antibacterial, reduced by any grants received supporting clinical development of that product. The amounts in the proposed Pioneering Antibiotic Subscriptions to End Upsurging Resistance (PASTEUR) Act of 2021 and a UK pilot program are well within the bounds of an effective antibacterial pull incentive.

REGULATORY MATTERS: AN INTERNATIONAL OVERVIEW

MARISA PAPALUCA AMATI

The Covid 19 pandemic hit as a shock society as well as in the regulatory ways to carry out scientific evaluation of vaccines and antiviral treatments.

Since at least a decade cutting edge scientific and clinical investigations have been ongoing in various pathological condition to develop new applications for genomics/RNA transcripts, gene transfer, nanotechnology. and the necessary supportive digital eco-system. In spite of risk-averse regulatory and societal environment already

at the onset of the pandemic innovative platforms rapidly were identified as the tools to be deployed in emergency to save lives and prevent the occurrence of a devastating viral disease.

International regulatory collaboration with stakeholders in the fight against the pandemic acted as catalyst for un-precedented changes in innovative products development and deployment.

PROGRESS IN REGULATORY SCIENCE RESEARCH AND COLLABORATIVE INTERNATIONAL STANDARDS DEVELOPMENT IN NANOTECHNOLOGY

ANIL PATRI, Director, Nanocore and Chair, Nanotechnology Task Force, National Center for Toxicological Research, U.S. Food and Drug Administration, USA.

The U.S. FDA has made significant progress in learning about nanomaterial and nanotechnology through regulatory science research, collaborations, and engagement with stakeholders. Standards are invaluable resource for industry and FDA staff. The use of standards can increase predictability, streamline premarket review, and facilitate market entry of safe and effective products. This presentation is aimed at summarizing recent research and progress in standards development in support of public health.

Disclaimer: The views expressed in this presentation do not necessarily represent those of the U.S. Food and Drug Administration

RNA THERAPEUTICS GOING BEYOND THE LIVER: FROM GENE SILENCING TO GENE EDITING

DAN PEER, Director, Laboratory of Precision NanoMedicine Tel Aviv University

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 we 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 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. I will also share some data on mRNA vaccines for COVID19 and Finally, I will share new data showing very high efficiency genome editing in glioma and metastatic ovarian cancer. This modular delivery platform can serve as a milestone in turning precision medicine feasible.

TALINEUREN: WORLD'S SMALLEST GLYCOLIPID-BASED NANO DRUG AGAINST NEURODEGENERATIVE DISEASES: A JOURNEY TO CLINICS AND BEYOND

CAMILLE PEITSCH, Research & Development Manager, Inno-Medica Schweiz AG

Talineuren (TARgeted LIposomal NEUro REgeneration) 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, and immuno-modulation. Treatment with GM1 has been documented to be well tolerated and effective in several indications such as spinal cord injuries and stroke and in Parkinson's Disease with potentially disease-modifying effects. However, therapeutic breakthrough has been hampered by the limited availability of the molecule in the central nervous system and the feasibility of chronic daily treatments.

Talineuren has been designed to address the issue of 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.

To date Talineuren has been investigated in a number of *in vitro* and *in vivo* Parkinson's Disease and Amyotrophic Lateral Sclerosis (ALS) models. As of December 2021, a safety trial with 12 Parkinson patients has been initiated (<https://clinicaltrials.gov/ct2/show/NCT04976127>). 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 free GM1 dosed at 30 mg/kg while therapeutic efficacy was still superior in the Talineuren group compared to free GM1.

We were intrigued to note that in the C9orf72 mouse model of amyotrophic lateral sclerosis (ALS) Talineuren presents disease modifying potential by significantly rescuing the motor performance assessed on a rotarod protocol compared to the respective genetic background healthy control. Several tissue analyses were conducted to corroborate the observed rotarod results. PolyGA aggregates diminution could be analysed in the soma of the motoneurons and the activation of the unfolded protein response could be significantly reduced. ER stress could also be significantly reduced. Ultimately, muscle fibre analysis showed that Talineuren prevents muscular death and thus also shows a potential disease modifying effect for ALS. With these results, an orphan drug designation status was assigned to Talineuren's GM1 in ALS by the US-FDA, the EMA and Swissmedic. Clinical translation of Talineuren was started with a safety study in Parkinson patients and start of the clinical trial with Talineuren in ALS is planned for Mai 2022.

Unlike the majority of therapeutic approaches in Parkinson and ALS, Talineuren does not address a specific mechanism of disease but instead aims for an endogenous pathway with multipronged stimulation of neuronal survival through its pleiotropic properties. Talineuren could become a dogma changing medicine and make disease-modifying therapy in conditions such as Parkinson and ALS possible and open the doors to treatments for other severe neurodegenerative conditions such as Alzheimer.

SYNTHESIS, FORMULATION AND CHARACTERIZATION OF IMMUNOTHERAPEUTIC CGAMP-DENDRIMER COMPLEXES FOR CD206 TARGETED DELIVERY TO M2 MACROPHAGES

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Generation of anti-tumor responses can be achieved via the stimulation of the immune system, a therapeutic approach called cancer immunotherapy. Pharmacological activation of endogenous anti-tumor pathways is a way to implement cancer immunotherapy. The stimulator of interferon genes pathway (STING) generates intrinsic antitumor immunity upon detection of cytosolic tumor-derived nucleic acids. Activation of the effector STING leads to the productions of type I interferons in antigen-presenting cells (APC) of the tumor microenvironment (TME), specifically antigen presenting cells (APC). In addition, few studies have shown correlational increase of tumor specific CD8 + T cells after dendritic cell activation and macrophage shift from pro-tumor M2 to anti-tumor inflammatory M1 macrophages. Pharmacological activation of this pathway using natural STING agonists, such as the eukaryotic 2'3'cGAMP, is limited by the molecule's high hydrophilicity and poor stability. These characteristics difficult their delivery to the TME, showing the need for a transfection carrier.

To circumvent these barriers, we designed poly(amidoamine) (PAMAM) nanoparticles encapsulating a STING agonist for local delivery to the tumor micro-environment. Surface engineering of PAMAM dendrimers with glucuronic acid was investigated to decrease the polymer's toxicity and improve its transfection efficacy via mannose-mediated CD206 endocytosis, specific for M2 macrophages. We discuss the physicochemical characterization of PAMAM, coated or not with the glucuronic acid targeting ligand and two different molar charge ratio PAMAM-Glu /cGAMP complexes. The toxicity and efficacy of the formulations was further investigated through *in vitro* and *in vivo* experiments.

GRAPHENE MATERIALS FOR VISUAL PROSTHESES AIMING AT RESTORING VISION

SERGE PICAUD, Sorbonne Université, INSERM, CNRS, Institut de la Vision, 75012 Paris, France;

Visual restoration is certainly the greatest challenge for brain-machine interfaces because images require at least 600 pixels and they need to be applied at a refreshing rate ranging from 13Hz to 30Hz. At the retinal level, efficient prostheses are already in clinical trials. However, highly conductive electrodes could further increase the resolution of such visual prostheses. We have therefore investigated how semiconductive graphene materials could contribute to the development of novel high-resolution devices. Our investigation includes efficiency of graphene electrodes to activate the retinal tissue along analysis of the material biocompatibility. The results support the use of graphene electrodes for long-term use in brain machine interfaces for visual restoration or other neurological applications.

ENGINEERING "TAIL-FLIPPING" LIPOSOMES TO TARGET AND RE-PROGRAM TUMOR-ASSOCIATED MACROPHAGES IN PRECLINICAL ANIMAL MODELS

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Tumor-associated macrophages (TAMs), representing M2-like phenotype, are the key cells in the tumor microenvironment that drive the tumor progression, invasion and metastasis. Efficient technologies to target and alter M2-like TAMs are of high interest to realize novel effective immunotherapeutic strategies against cancer.

The aim of this study is to design liposomes to target TAMs specifically and use them to modulate TAMs to inhibit their tumor-promoting functions. Earlier, we have identified several scavenger receptors including Scarb1 upregulated on the surface of M2 macrophages compared to M1 type. To achieve targeting to these receptors, we designed novel engineered nanoliposomes biomimicking the macrophage-mediated recognition and uptake of cell membrane phospholipids undergoing peroxidation via specific scavenger receptors. We show that incorporation of a phospholipid possessing a terminal carboxylate group at the sn-2 position, into the bilayer of nanoliposomes allowed specific uptake by M2 macrophages. Molecular dynamics simulation of the lipid bilayer revealed that the polar tail flips towards the aqueous phase within nanoseconds. In triple negative 4T1 orthotopic breast tumor model, the engineered nanoliposomes, distributed to the M2 macrophages, as shown with immunofluorescent co-localization staining and flow cytometry. Furthermore, we used this distribution pattern to deliver specific compounds to alter M2-TAMs *in vivo*. After confirmation of tumor accumulation specifically in TAMs, we encapsulated a Stat6 inhibitor AS1517499 in the aqueous layer of liposomes or muramyl-tripeptide phosphoethanolamine (MTP-PE) into the lipid bilayer of liposomes. *In vitro*, we have found that both AS-containing or MTP-PE containing liposomes inhibited TAMs activation or converted into M1-like macrophages. *In vivo*, we examined the effect of AS-targeted or MTP-PE targeted liposomes in 4T1 breast tumor model and found that AS-targeted liposomes inhibited the pre-metastatic niche formation in lungs and livers compared to free drug or AS-containing non-targeted liposomes. Furthermore, MTP-PE targeted liposomes reduced the tumor growth significantly while MTP-PE containing non-targeted liposomes did not show reductions in tumor growth. The effects on the metastasis and metastatic niche with MTP-PE liposomes are under analysis. In conclusion, we have successfully developed liposomes targeted to M2 macrophages (TAMs) which have specific binding and uptake in M2 macrophages *in vivo*. These engineered "tail-flipping" nanoliposome appears to be versatile nanocarriers to specifically target and alter pro-tumoral activity of M2-TAMs and warrant further development as novel cancer immuno-therapeutics.

PHAGE THERAPY: HOPE OR HYPE?

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The rapid emergence and worldwide spread of multidrug resistant bacteria is a major public health challenge. Several alternative/complementary strategies to antibiotics have been proposed over the last decade as potential answers to antibiotic resistance, but only a few of them have reached the patient bedside. Bacteriophages, bacterial viruses known as phages, are currently considered to be one of the most promising alternatives in this context and have increasingly been given to patients as compassionate treatments, although randomized controlled trials (RCT) conducted according to existing regulatory requirements have failed to demonstrate any efficacy and/or superiority of phages over either placebo or stan-

dard of care. During this presentation I will give an overview on the phage therapy, with a special focus on the issues that should be solved to avoid the next RCT to fail again.

OPTIMISATION OF STABLE NUCLEIC ACID LIPID NANOPARTICLES FORMULATIONS FOR IN VITRO AND IN VIVO DELIVERY OF PLASMID DNA AND SIRNA TO CANCER USING DESIGN OF EXPERIMENTS

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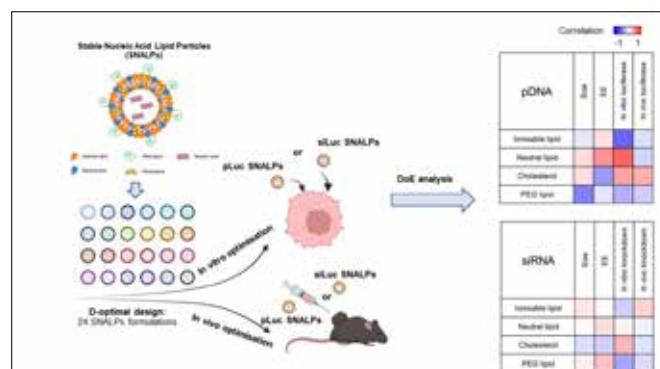
INTRODUCTION

Cellular delivery of genetic molecules has been widely used in diverse applications during the past two decades. However, naked RNA and DNA molecules are negatively charged and have limited cellular uptake, low biological stability, and unfavourable pharmacokinetics. Therefore, the safety and efficiency of nucleic acid (NA) delivery to specific tissues or cell types are the two most critical hampers for gene-delivery applications. Recently, a new class of NA lipid particles called stable nucleic acid lipid particles (SNALPs) have been explored to efficiently and safely deliver NA both *in vitro* and *in vivo*. Small interfering RNA (siRNA) and messenger RNA (mRNA) showed promising ability in a wide range of applications and have been thoroughly investigated and optimised using design of experiments (DoE). Rare studies however can be found on lipid nanoparticles optimisation by DoE for pDNA delivery. Moreover, majority of the research on siRNA lipid particles optimisation investigated the effect of manufacturing methods on the physicochemical properties not the transfection efficiency *in vitro* or *in vivo* studies.

METHODS

SNALPs formulations encapsulated pDNA or siRNA were prepared to contain four lipid components (ionisable cationic lipids, neutral lipids, cholesterol and PEG-lipid) by ethanol injection method. Size, PDI and zeta potential of SNALPs were measured by dynamic light scattering (DLS) and electrophoretic mobility, respectively. Encapsulation efficiencies (EE%) of NA in SNALPs formulations were measured using Ribogreen assay. B16F10 and B16F10-LUC cells were transfected with SNALPs encapsulating luciferase reported genes pLuc and siLuc, respectively. For *in vivo* studies, SNALPs encapsulating pLuc and siLuc were intratumourally injected to C57BL/6 mice implanted with B16F10 or B16F10-LUC cells, respectively. The transfection efficiencies of SNALPs *in vitro* and *in vivo* were detected by the microplate reader and IVIS imaging system, respectively. The effect of lipid compositions on the size, EE%, *in vitro* and *in vivo* transfection efficiency were analysed using a design of experiments approach.

Graphical abstract



RESULTS

The lipid compositions affected SNALPs particles size, *in vitro* and *in vivo* transfection efficiency for both pDNA and siRNA SNALPs formulations. pDNA but not siRNA encapsulation was affected by the lipid compositions. The lipid compositions of SNALPs of optimal performance was identified and was not identical for pDNA/siRNA delivery nor *in vitro/in vivo* transfection.

CONCLUSIONS

The design of experiment approach allowed us to select a middle ground SNALP formulation which can achieve high pDNA transfection and gene silencing *in vitro* or *in vivo*. This will aid our future studies to simultaneously deliver therapeutic siRNA and pDNA for cancer therapy.

Acknowledgement

This work acknowledges funding from King's-China Scholarship Council (CSC).

INHIBITING INTRACELLULAR TARGETS WITH NANOMEDICINE

DIANA FERNANDES DE SOUSA RAFAEL

Nowadays, the use of antibodies in cancer treatment relies mainly on targeting molecules at the surface of cellular membranes. It is not common the use of antibody therapy for intracellular targets since the antibodies by itself cannot efficiently enter the cells. This drawback can be overcome by using adequate nanocarriers, which due to its small size could reach the tumor through the Enhanced Permeation and Retention (EPR) effect. Moreover, nanocarriers will protect the antibody integrity and efficiently internalize it into the desired cells. Until now any carrier demonstrated to be totally efficient for the intracellular delivery of antibodies *in vivo*. In the present work we propose the use of polymeric micelles (PM) for the intracellular delivery of anti-KRAS Ab and anti-SMC2 Ab to reduce the tumorigenicity of different types of cancer cells. Based on preliminary studies performed by the group in the last months, we believe that the developed work has the potential to contribute with important advances in the field of intracellular delivery of antibodies and bring a new solution for the so-called undruggable targets in cancer therapy. The present strategy already demonstrated high efficacy, in terms of tumorigenicity inhibition, both *in vitro* and *in vivo*, both in bulk tumor cells and cancer stem cells populations. Moreover, the biodegradability and biocompatibility and the easy and low-cost production of the proposed micelles, make it an ideal carrier platform for the intracellular delivery of antibodies in combination with another drugs or biomolecules, accordingly to the clinical application.

BNT211: A PHASE I TRIAL EVALUATING SAFETY AND EFFICACY OF CLDN6 CAR-T CELLS AND CARVAC-MEDIATED IN VIVO EXPANSION IN PATIENTS WITH CLDN6-POSITIVE ADVANCED SOLID TUMORS

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Background: BNT211 comprises a chimeric antigen receptor (CAR)-T cell product candidate targeting the tumor specific antigen claudin 6 (CLDN6) and a CAR T cell-Amplifying RNA Vaccine (CARVac). CARVac mediates expansion of adoptively transferred CAR-T cells, resulting in improved persistence and functionality.

Methods: BNT211-01 (NCT04503278, sponsor BioNTech), a first-in-human, open label, multi-center trial involves a bifurcated 3+3

design with separate CLDN6 CAR-T cell dose escalations (single flat-dose) for monotherapy (Part 1) and combination with CARVac (Part 2). In Part 2, CARVac is applied every 3 weeks from Day 4 post-transplantation including a one-step intra-patient dose escalation. Patients with CLDN6-positive relapsed or refractory solid tumors without further standard treatment options and ECOG 0-1 are eligible for recruitment.

Results: As of 18th Nov 2021, 15 patients had been treated. Part 1, dose levels (DLs) 1 and 2 and Part 2, DL1 had been completed, while Part 2, DL2 was ongoing. Few adverse events related to the drug product and a single DLT mainly linked to the lymphodepletion regimen have been reported. Pronounced cytopenia occurred in testicular cancer patients previously treated with high-dose chemotherapy and autologous stem cell transplantation. For these patients a lymphodepletion-free cohort was recently opened. Manageable cytokine release syndrome (grade 1-2) without any signs of neurotoxicity has been observed in 7 patients. Transient, moderate elevations of IL-6 serum levels occurred in remaining patients. Notably, CARVac resulted in flu-like symptoms resolving within 24 h. Analysis of CAR-T cell frequency in peripheral blood revealed robust engraftment in all patients. Preliminary efficacy data for 10 evaluable patients 6 weeks post-infusion showed 4 partial responses, 1 progressive disease and 5 stable disease. Most responses were seen in testicular cancer patients; remaining patients had stable disease.

Conclusions: CLDN6 CAR-T cells ± CARVac show a favorable safety profile at doses tested and encouraging signs of clinical activity.

Acknowledgements: BNT211-01 is funded by BioNTech Cell & Gene Therapies GmbH.

Trial registration: Clinicaltrials.gov: NCT04503278

Ethics approval: Ethics & Institutional Review Board approvals were obtained from the re-spective participating countries prior to initiation of the trial.

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CONCEPTION TILL CLINICAL TRANSLATION OF CORE-CROSSLINKED (CRIPEC[®]) POLYMERIC MICELLES AND FUTURE DIVERSIFICATION – RESULTS UP TO AND INCLUDING PHASE 2

CRISTIANNE RIJCKEN

Cristal Therapeutics is a highly innovative biotech company that applies three distinct and interconnected technologies together with biological insight to improve the therapeutic profile of our own and partners' products in development. Based on over 10 years of experience, Cristal's CliCr[®], CriPec[®] and CriVac[®] technologies provide superior conjugation, enhance target specificity and engender highly selective immune responses, thereby increasing efficacy and

reducing toxicity. The company aims to be the partner of choice for overcoming challenges and enabling the full potential of e.g. chemotherapeutic agents, immuno-oncology treatments and vaccines, amongst a broader range of therapeutics, tuned to modality and indication.

In this talk, the conception of the CriPec technology (based on core-crosslinked polymeric micelles) up until phase 2 clinical evaluation will be presented. More explicitly, this will comprise of generic clinical pharmacokinetic, safety and efficacy assessment of the clinical product candidate (CriPec docetaxel) next to unique tumour accumulation studies. In one clinical trial, a head to head comparison of the tumour uptake of a nanomedicinal version of docetaxel versus conventional docetaxel was performed, whilst in another clinical study the biological fate of the CriPec docetaxel was monitored by non-invasive PET imaging. All studies demonstrated the significantly extended systemic circulation of CriPec docetaxel, the on average 4-fold higher tumour uptake, the superior safety profile as well as signs of efficacy in a heavily pretreated group of ovarian cancer patients. Inherently to their rational design and extensive experience, our technologies can overcome various challenges and generate therapeutic value for a broad range of biopharmaceutical products.

WHY HARMONIZATION IS NEEDED: GLATIRAMER ACETATE, A CASE FROM REALITY

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Keywords: NBCD; complex drug; glatiramer; glatiramoid; regulatory science

1. INTRODUCTION

For policy makers and competent Authorities, the challenge of copies/follow-on complex drugs revolves around the nature and extent of data required for authorization. On the one hand, redundant and non-relevant data results in unnecessary costs; on the other hand, not enough relevant data results in risking a poor assessment of the risk/benefit ratio (Figure 1).

In general, complex drugs may be either biological, if the active ingredients are derived from a biological source, or non-biological, if obtained by chemical synthesis. In both cases, their quality depends considerably on the manufacturing process. In particular, for Non-Biological Complex Drugs (NBCDs), complexity may arise either from the active substance, as in the case of glatiramer acetate (GA), or from other sources, such as the formulation, as in the case of liposomes [1].

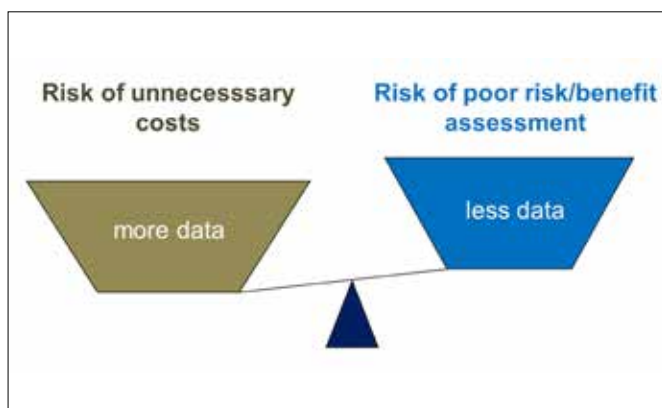


Figure 1. The effect of the extent of data on costs and the quality of risk/benefit assessment

GA is approved, in the US and the EU, as a disease-modifying treatment for patients with relapsing forms of Multiple Sclerosis. It is a heterogeneous mixture of not fully characterized synthetic poly-

peptides, containing L-alanine, L-lysine, L-glutamic acid, L-tyrosine in the constant molar ratio 0.43:0.34:0.14:0.09, with an average molecular weight from 5 to 9 kDa and distribution range from 2.5 to 20 kDa [2]. The amino acid sequences are not completely random, being the result of both the physicochemical properties of the starting materials and the fundamental reaction scheme. However, they are not completely conserved from batch to batch, even when the process is tightly controlled. Indeed, along with conserved characteristics - such as amino acid molar ratio - other characteristics - such as the specific amino acid sequences - will show batch-to-batch variability [1].

To address this complexity, for the marketing of GA copies, US and EU regulatory agencies have chosen a generic approach integrated with additional data. However, the implementation is different in the two jurisdictions (Figure 2).

2. RESULTS

The originator GA was first authorized in Israel and then in the United States in 1996. In the EU, the 20 mg/ml was initially approved in the UK, in 2000, and then in other Member States by a Mutual Recognition Procedure starting in 2004. Copies are now marketed in many countries. In the USA, they have been approved following an Abbreviated New Drug Application (ANDA) and are considered generics. In the EU, they have been approved following a hybrid application and are considered as generics in some member states [1,3].

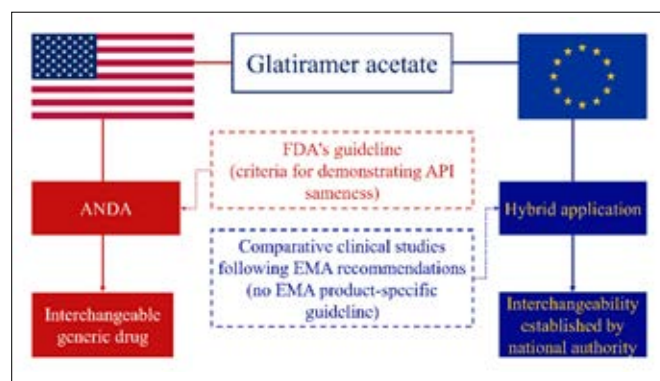


Figure 2. Regulatory pathways for Glatiramer Acetate in the US and EU. ANDA = Abbreviated New Drug Application, API = Active Pharmaceutical Ingredient

In the United States, the FDA required the demonstration of both pharmaceutical equivalence and bioequivalence. Demonstration of pharmaceutical equivalence, though, relies on the fact the two products contain the same active pharmaceutical ingredients (APIs). Currently, there is no single physicochemical or biological assay that can be used to demonstrate API sameness between the originator and a copy. However, FDA's position has been that API sameness can be demonstrated using a battery of orthogonal methods and an approach based on four criteria, published in product-specific guideline, which may be used to demonstrate API sameness even when the manufacturer of a copy does not entirely know the manufacturing steps used by the manufacturer of the originator [1].

In the EU, the first copy of GA 20 mg/ml was approved in 2016 with a decentralized procedure, following a hybrid application. Unlike the US case, no product-specific guideline exists in the EU for the production of GA copies, and the nature and extent of the studies required is determined on a case-by-case basis. The national regulatory agencies required a comparative characterization study with the originator [1,3]. The Applicant, in agreement with the EMA, also provided non-clinical and clinical data in support of similarity. As for the non-clinical aspects, it provided data from an EAE mouse model, two 28-days studies and one 90-days comparative toxicity study performed in rats. As for the clinical aspects, following EMA's recommendation, the applicant performed a comparative clinical trial to assess the efficacy, safety, and tolerability of both

prolonged treatment with the copy (GTR) and switching from the originator 20mg OD to GTR 20 mg OD. The 9-month randomized clinical trial on 794 patients, named Glatiramer Acetate Clinical Trial to assess Equivalence with Copaxone® (GATE) [4], was followed by 15 months open label follow-up. To support the hybrid application for GTR 40 mg/ml, which could not be based only an extrapolation of the results from the GATE study, Synthon used bridging scheme which involved GATE clinical study (comparing Copaxone® 20 mg/ml to GTR 20 mg/ml), the GALA clinical study (comparing Copaxone® 40 mg/ml to placebo) and four other published clinical studies (partly used in the application for Copaxone® 40 mg/ml).

3. CONCLUSIONS

For the approval of GA copies, regulatory agencies in the US and the EU are currently oriented toward a generic approach supplemented by additional data. However, this path has been implemented differently in the two jurisdictions (Figure 2).

In the US, this has immediate consequences on interchangeability, as the decision is taken by the FDA during approval. In the case of GA, the additional data required is listed in a product specific guideline and copies have been approved by the FDA as generics based on an ANDA and assigned an "A" code in the Orange Book. In the EU, a product approved based on a simplified dossier is automatically considered interchangeable. If, on the other hand, it follows a hybrid application, it is not interchangeable per se and, as in the case of GA copies, EMA leaves the decision about interchangeability and substitution to the individual member states. For GA copies, national regulatory agencies followed a hybrid approach requiring an additional comparative study, except for one case where an informed consent application could be used [3].

In conclusion, differences in US and EU policies for follow-on NBCDs still exist. While current regulatory frameworks are adequate to guarantee Quality, Safety and Efficacy, an international harmonization would represent a step forward in lowering costs and foster economic sustainability. Indeed, a process of alignment of US and EU policies is already in place for some products, such as GA. Moreover, neither the FDA nor the European agencies have introduced an ad hoc regulatory class for NBCDs. As a prerequisite of future harmonization, it is crucial that, in the EU, NBCDs are centrally regulated and assessed and they could be one of the new classes entering the centralised procedures.

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WHAT MACROPHAGES CAN DO WITH INTERNALIZED NANOPARTICLES

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Understanding the cellular responses upon direct exposure of (human) cells to engineered nanoparticles (NPs) is a prerequisite for their safe-by-design and successful use in biomedical applications, where targeting efficacy and low side effects are important. In particular, in the field of nanoscience, microscopic methods and analytical tools play an important role in gaining insights into the interaction of NPs with structures at the single cell level, including quantification of intracellular NPs. The detection, localization and quantification of NPs within cells is important to study how physico-chemical parameters of the particles might influence the interaction with a particular cell type and the induction of cell response. A plethora of studies focuses on NP uptake mechanisms (i.e. endocytosis) [1, 2]. Much less data is available on the intracellular dynamics of NPs and the potential transformation of the NPs in the endosomal-lysosomal system. In particular, the NPs fate in macrophages, the key cells in the human body responsible for NP clearance, is still poorly understood. The aim of this presentation is to provide an overview of methods, e.g. fluorescence and electron microscopy, flow cytometry, isolation of lysosomes, that can be used to study the fate of NPs in macrophages, with a focus on lysosomes and potential pitfalls associated to studying NP-lysosome interactions.

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IMMUNO-MODULATION & MRNA-BASED PROTEIN REPLACEMENT THERAPY PROGRAMS FOR TREATMENT OF PULMONARY DISEASE

CARSTEN RUDOLPH

Ethris SNIM® RNA is an enabling platform for "Transcript Therapies" in a broad variety of medical indications, from hereditary or acquired metabolic diseases to regenerative medicine. SNIM® RNA circumvent TLR activation and thus enables repeated administration of mRNA. Because of its precursor function, SNIM® RNA yields sustained protein production within the body and overcomes short duration effects of recombinant proteins. Ethris has developed proprietary delivery systems for pulmonary, systemic and local SNIM® RNA administration and will present preclinical results from its activities. Efficient delivery systems and non-immunogenicity are the keys for making mRNA therapeutics reality beyond vaccine applications.

IDENTIFICATION OF CORONA PROTEINS MEDIATING NANOPARTICLE UPTAKE AND CHARACTERIZATION OF THE MECHANISM OF ENDOCYTOSIS

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Nano-sized materials are used in nanomedicine to deliver drugs more efficiently to their site of action. In order to improve their efficacy, a better understanding of how cells internalize and process nano-sized materials is required.

Within this context, our research is focused on characterizing the molecular details of the early interactions and recognition of nano-sized materials at the cell membrane, and the subsequent mechanisms of uptake and intracellular trafficking. To this aim, we combine classic transport studies with inhibitors¹ and RNA interference to genetic screening and proteomic-based methods. Our results show that the corona molecules adsorbing on the nanoparticle surface once applied in serum can interact with specific cell receptors and in this way they also affect the mechanism cells use for their internalization.² Thus, the same nanoparticles when coated with a different corona interact with different receptors and are internalized by cells using different mechanisms.

Based on such observations, we used a panel of liposomes and nanoparticles of different size and charge to form different coronas and correlate corona composition and uptake by cells. In this way, corona proteins promoting or reducing nanoparticle uptake can be identified (Figure 1).^{3,4}

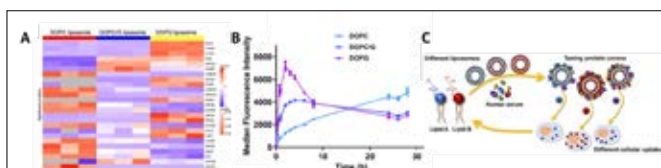


Figure 1. Correlating corona composition and uptake by cells. Adapted from Yang et al.³ Liposome of different charge are used to form different coronas in serum (A) and their uptake by cells is quantified (B). Thus, by correlating corona composition and cell uptake efficiency, corona proteins promoting or reducing nanoparticle uptake can be identified (C). Results reproduced from Yang et al.³

Additionally, we show that the free proteins in solution also affect nanoparticle interactions with cells, as they can compete with the nanoparticles for cell receptors.⁵ Finally, we found that even when interacting with specific receptors, nanoparticles may be internalized by cells via different mechanisms than what it is usually observed for their endogenous ligands. For instance, nanoparticles interacting with the LDL receptor (LDLR) via their corona are internalized by cells via a mechanism that is not clathrin-mediated, as usually it is observed for this receptor.²

These findings highlight the importance of understanding how nanoparticles are modified by biological environments and how cells interact with and process nano-sized materials, in order to be able to design nanomedicine with improved efficacy.

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ENTRY OF NANOPARTICLES INTO CELLS: SMALL VARIATIONS IN STRUCTURE DICTATE THE CELLULAR RESPONSE

KIRSTEN SANDVIG

Nanoparticles can be used to deliver drugs or other substances both *in vivo* and *in vitro*^(1,2). To enter cells the particles exploit the endocytic machinery, and they have been demonstrated to induce changes in cellular uptake and intracellular transport^(3,4). 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. 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 many different types of endocytic mechanisms⁽⁵⁾, 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⁽⁵⁾, and studies of nanoparticle uptake in nonpolarized cells may not give the same results as if uptake in polarized cells is investigated. 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^(5,6), but is involved in several endocytic processes including macropinocytosis^(1,5). Also, robust methods to determine whether a particle is internalized or only at the cell surface or in channels connected to the surroundings are important to provide the investigator with correct data about uptake efficiency and the mechanisms involved^(5,7). Even apparently small differences in the chemical composition of particles may contribute to different types of cell death and have differential effects on autophagy^(8,9) as well as on the proteome of cancer cells incubated with the corresponding drug-containing particles⁽¹⁰⁾. Understanding the complex situation *in vivo*, after intravenous injection of nanoparticles with or without drug, is a challenge. Although the toxic effect of drug-loaded particles on cells in culture does not necessarily differ from each other or from free drug, the structural variations are likely to play a role for the effects observed on biodistribution in mice⁽¹¹⁾, and importantly, drug-containing nanoparticles were found to be more efficient in inhibiting the growth of a PDX model of human breast cancer⁽¹²⁾.

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MANAGING BRAIN MALIGNANCIES IN 3 DIMENSIONS

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Despite the remarkable efficiency of immune checkpoint modulators against metastatic melanoma, there is a low percentage of responders and clinical trials report severe immune-mediated side effects and disease relapse. Recent evidences show that non-tumor cells within the tumor microenvironment (TME), including tumor vasculature and immune stromal cells, dictate the overall therapeutic efficacy. We synthesized off-the-shelf and cost-effective nano-sized polymeric platform that combines a cancer vaccine with the targeted inhibition of molecular and/or cellular immune suppressive players. These precision nano-sized medicines aim to re-educate and harness patient T-cell response against tumors, leading to an immunological memory able to control tumor relapse without any follow-up treatment. The design of these advanced immunotherapies is guided by the identification of lead immune suppressor factors and tumor specific antigens using novel 3D bio-printed tumor-immune spheroids developed in our lab. Our first nano-immunotherapy candidates sensitized melanoma mouse models to immune-checkpoint modulators, dramatically increasing disease-free survival rates.

SYSTEMIC MRNA-LNP VACCINES FOR ANTITUMOR IMMUNITY BY ENGAGING SPLENIC IMMUNE CELLS

RAYMOND SCHIFFELERS, S. Bevers, S. Kooijmans, E. Van de Velde, M. Evers, S. Seghers, J. Gitz-Francois, N. Van Kronenburg, M. Fens, E. Mastrobattista, L. Hassler, H. Sork, T. Lehto, K. Ahmed, S. El-Andaloussi, K. Breckpot, M. Maes, D. Van Hoorick, T. Bastogne, S. De Koker.

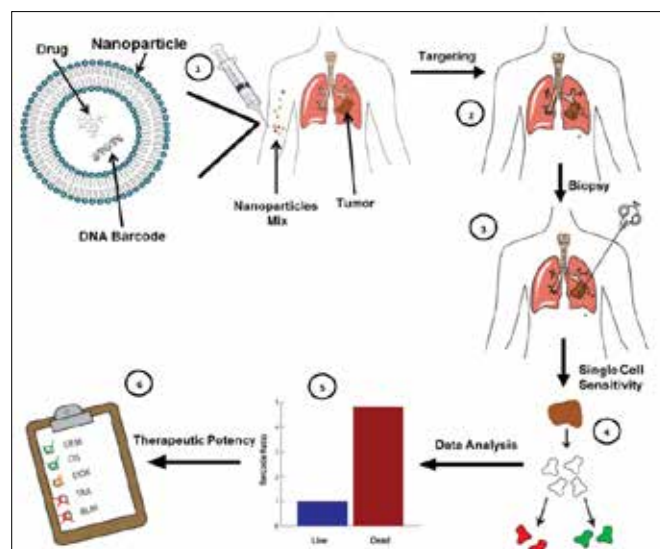
Lipid nanoparticles (LNPs) are currently at the forefront for the delivery of nucleic acid therapeutics, as exemplified by patisiran and the COVID-19 vaccines. We made a library of lipid nanoparticles loaded with mRNA and measured physicochemical characteristics. In vivo tissue distribution was determined after intravenous administration in mice using fluorescent RNA payloads and correlated to functional tissue distribution determined by reporter protein expression. Finally, therapeutic immunogenic mRNA payloads were used to examine the magnitude of an antigen-specific T cell response. From these data a model was built to correlate LNP composition to efficacy. Based on the model a suboptimal and optimal composition was established to validate the model. These compositions were tested for antitumor activity, which demonstrated strong antitumor activity of the optimal LNP composition and validated the model.

SYNTHETIC LIPID NANOPARTICLES OR EXTRACELLULAR VESICLES FOR RNA DELIVERY

Extracellular vesicles can transfer biological cargo between cells, including RNA. It may be an attractive alternative to synthetic delivery system for RNA. I will present a couple of examples of successful transfer by natural vesicles and synthetic lipid nanoparticles in the framework of a project on regeneration of the heart after myocardial infarction. One of the difficulties in comparing the efficiency of both systems was the lack of an assay for quantitative read-out of functional delivery. We recently developed the CROSS-FIRE system based on CRISPR/Cas9 mediated editing that allows to quantitatively compare these systems based on color change of fluorescent reporter proteins.

BARCODED NANOPARTICLES FOR PRECISION CANCER MEDICINE: EFFECTS OF TUMOR TYPE AND PATIENT SEX ON ANTICANCER EFFICACY

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 liposomes can be used for degrading the pancreatic stroma to allow subsequent drug penetration into pancreatic adenocarcinoma, and how nanoparticle' biodistribution and anti-cancer efficacy is impacted by patient' sex and more specifically, the menstrual cycle.

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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SELF-ASSEMBLED PEGYLATED AMPHIPHILIC POLYPEPTIDES FOR GENE TRANSFECTION

STEPHANIE SCHUBERT, Paul Klemm, Mira Behnke, Jana I. Solomon, Colin Bonduelle, Sébastien Lecommandoux, Anja Träger

Polymers play an important role in the development of new gene delivery vehicles for efficient and safe transfection to heal various diseases. In particular, naturally inspired polymers such as synthetic polypeptides are very promising materials due to their biocompatibility and potential biodegradability. In this study, amphiphilic poly(ethylene glycole) (PEG) polypeptide block copolymers with different distributions of L-Lys (60, 43 and 23 %) and Bzl-L-Glu (40, 57 and 77 %) were synthesized by ring opening polymerization of the corresponding amino acid NCAs (Figure 1).

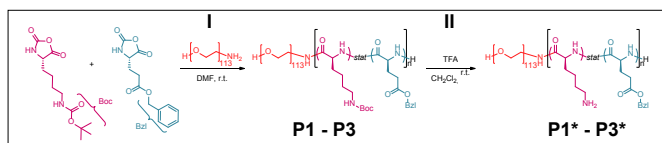
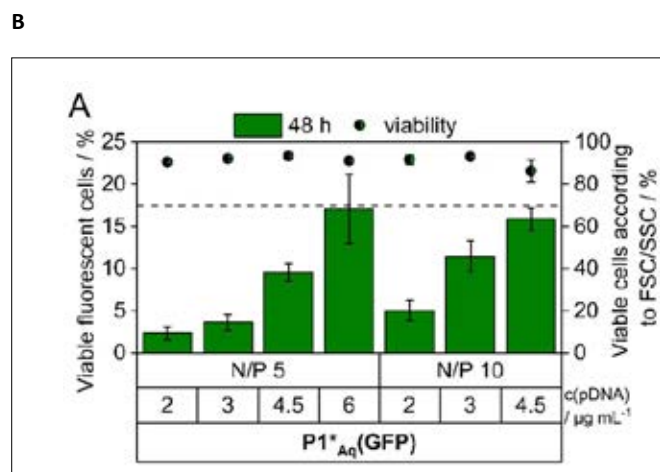
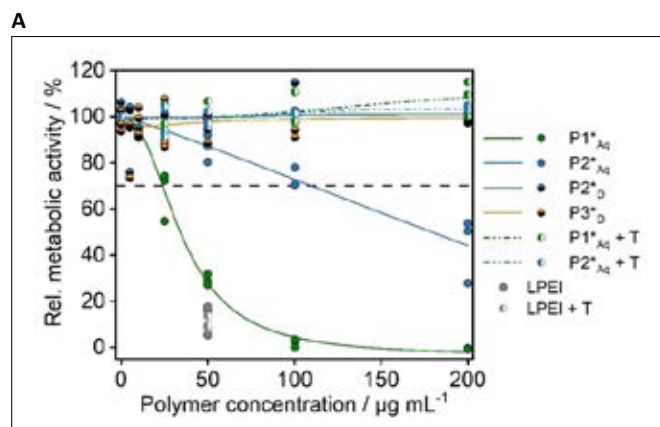


Figure 1: I Reaction formula of HO-PEG-NH₂ initiated ring opening polymerization of Boc-L-Lys and Bzl-L-Glu in DMF at r.t. followed by II deprotection of the Boc-group at r.t.

The conversion of the two monomers in different compositions was investigated in kinetic studies and all copolymer candidates were characterized with respect to their chemical and structural properties by NMR spectroscopy, SEC, SEC MALS, IR and CD spectroscopy. By the latter, it was observed that the helicity of the deprotected polymers increases by up to 71 % with increasing content of hydrophobic Bzl-L-Glu, while higher content of L-Lys favors random coil formations. Different techniques were evaluated to complex and encapsulate pDNA by the synthesized polypeptides. P1* and P2* were suitable for the preparation of pDNA loaded complexes

using an aqueous pH-controlled formulation. pDNA-polymer complexes with different N/P ratios (5, 10, and 20) were prepared. Regardless of the N/P ratio, the aqueous complexes showed sizes around 100 nm and low polydispersity index (PDI) values around 0.2. Nanoparticles of P2* and P3* were prepared by nanoprecipitation using different water-miscible solvents or by emulsion using a bio-based solvent (Cyrene). Particle sizes above 200 nm were obtained by centrifugation, while after dialysis the sizes of the NPs were between 60 and 170 nm. DLS measurements resulted in PDI values above 0.24 up to 0.78. The zeta potential of the NPs ranged from 13 to 30 mV. In our gel electrophoresis studies, all selected polyplexes and most of the nanoparticle samples were suitable for efficient complexation or encapsulation of pDNA. In addition, it was possible to show the release of the genetic material using heparin as competing polyanion. Dissolved P1* showed the highest cytotoxicity of the samples tested, but was less toxic compared to the control sample LPEI (Figure 2A). Formulated nanoparticles of P2* and P3* showed lower cytotoxicity in comparison to the polypeptides dissolved in acetate buffer, potentially caused by a lower number of amine groups accessible for membrane interactions when formulated within a nanoparticle. The biodegradability of the polypeptides was demonstrated by the absence of cytotoxicity after incubation of solubilized P1* and P2* with trypsin.

Figure 2: A) Evaluation of cytotoxicity and biodegradability of polypeptides. Cytotoxicity was investigated by measuring the metabolic activity (PrestoBlue™ assay) after 24 h incubation in L-929 cells as standard cell line for cytotoxicity evaluations (ISO 10993-5). L-929 cells were incubated with P1* and P2* polymer dissolved in acetate buffer (P1*_{Aq}, P2*_{Aq}) and P2*D and P3*D NPs without pDNA. Biodegradability of P1*_{Aq} and P2*_{Aq} was evaluated by incubation with trypsin for 1 h at 37 °C and subsequent incubation with L-929 cells followed by a PrestoBlue™ assay using metabolic activity as read-out (P1*_{Aq} + T, P2*_{Aq} + T). LPEI as non-degradable polymer without and with trypsin treatment was used as control. B) Transfection efficiency of P1*_{Aq}(GFP) in HEK293T cells is displayed as percentage of viable fluorescent cells of all viable single cells measured via flow cytometry. The dashed line indicates 70 % cell viability.



The transfection efficiency of a collection of m-EGFP-N1 pDNA-loaded NPs / complexes was evaluated in HEK293T cells (Figure 2B). The formulated NPs did not show transfection. However, the polyplexes of P2*_{Aq} (GFP) showed a low transfection efficiency of 5 % at N/P 20 and a pDNA concentration of 4.5 µg mL⁻¹. In contrast, P1*_{Aq} (GFP) showed the highest transfection efficiency of 17 % at the low N/P 5, making especially P1* a promising biodegradable carrier for gene transfection. Within the polypeptides in this study, the percentage of α-helical structures and hydrophobic groups decreases from P3* > P2* > P1* while the percentage of L-Lys groups and therefore cationic charge density increases in the same manner (P3* < P2* < P1*). The highest transfection efficiencies are observed for P1*, revealing the highest charge density, but at the same time the lowest amount of hydrophobicity and α-helical structures. The polypeptide P1* is therefore a promising candidate as gene delivery vector showing high transfection efficiencies and at the same time biodegradability.

This study clearly demonstrates that a proper characterization of the initial polymers but also of the resulting formulations reveals important information on structure-property relationships that are essential for the development of new gene carriers with high efficiencies and good compatibilities. The formulation protocol and consequently the characteristics of the nanoparticles have a strong impact on the performance of the polypeptide-based nanoparticle suspensions. In addition, the maintenance of a balance between cationic groups that are necessary for binding genetic material and membrane active properties such as hydrophobicity and α-helical structures is crucial for successful gene delivery by polypeptides. Within our further studies, we are investigating the cellular uptake of polypeptide-based nanoparticles by electron- and fluorescence microscopy to gain more insights on the nanoparticle-cell interactions for further improving the nanoparticle performance.

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BEYOND POLYESTER NANOPARTICLES FOR TARGETED ANTI-INFLAMMATORY STRATEGIES

ULRICH SCHUBERT

State-of-the art polymer nanoparticles for the delivery of hydrophobic actives are often composed of the biodegradable polyester PLGA and the “stealth polymer” PEG. In spite of their high potential, encapsulation of more hydrophilic active pharmaceutical ingredients (API) can be challenging. Aside from adjusting the polyester type or the formulation conditions,^[1] in particular degradable hydrophobic polymers promoting specific interactions with the API might help to overcome that. Polyesteramides, synthesized by polyaddition of bisoxazolines and dicarboxylic acids,^[2] enabled hydrogen bonding through the amide moieties with the anti-inflammatory API indomethacin, as shown by experimental as well as in silico data.^[3] The organocatalyzed ring-opening polymerization (ROP) of morpholine-2,5-diones yielded polyesteramides composed of α-amino acid and glycolic acid repeating units.^[4] In addition, the use of a hydrophilic polyoxazoline (POx) macronitiator for the ROP enabled access to amphiphilic block copolymers functionalized with a PEG alternative.^[5] Able to encapsulate novel anti-inflammatory API such as BRP-187 in aqueous nanoparticle formulations, these polymer materials are promising as the entire synthesis procedure is based on living polymerizations. As such it facilitates the attachment of targeting ligands at the POx a-end group.

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PERSPECTIVES OF NANO-CARRIER DRUG DELIVERY SYSTEMS TO OVERCOME CANCER DRUG RESISTANCE IN THE CLINICS

SIMO SCHWARTZ

Advanced cancer is still considered an incurable disease because of its metastatic spread to distal organs and progressive gain of chemoresistance. Even though considerable treatment progress and more effective therapies have been achieved over the past years, recurrence in the long-term and undesired side effects are still the main drawbacks of current clinical protocols. Moreover, a majority of chemotherapeutic drugs are highly hydrophobic and need to be diluted in organic solvents, which cause high toxicity, in order to reach effective therapeutic dose. These limitations of conventional cancer therapies prompted the use of nanomedicine, the medical application of nanotechnology, to provide more effective and safer cancer treatment. Potential of nanomedicines to overcome resistance, ameliorate solubility, improve pharmacological profile, and reduce adverse effects of chemotherapeutic drugs is thus highly regarded. Their use in the clinical setting has increased over the last decade. Among the various existing nanosystems, nanoparticles have the ability to transform conventional medicine by reducing the adverse effects and providing a controlled release of therapeutic agents. Also, their small size facilitates the intracellular uptake. Prostate cancer (PCa), one of the leading causes of cancer-related deaths, currently lacks effective treatment for advanced-stage disease. Paclitaxel (PTX) is a highly active chemotherapeutic drug and the first-line treatment for PCa; however, conventional PTX formulation causes severe hypersensitivity reactions and limits PTX use at high concentrations. In the pursuit of high molecular weight, biodegradable, and pH-responsive polymeric carriers, one conjugates PTX to a polyacetal-based nanocarrier to yield a tert-Ser-PTX polyacetal conjugate. tert-Ser-PTX conjugate provides sustained release of PTX over 2 weeks in a pH-responsive manner while also obtaining a degree of epimerization of PTX to 7-epi-PTX. Serum proteins stabilize tert-Ser-PTX, with enhanced stability in human serum versus PBS (pH 7.4). In vitro efficacy assessments in PCa cells demonstrate IC₅₀ values above those for the free form of PTX due to the differential cell trafficking modes; however, *in vivo* tolerability assays demonstrate that tert-Ser-PTX significantly reduces the systemic toxicities associated with free PTX treatment. tert-Ser-PTX also effectively inhibits primary tumor growth and hematologic, lymphatic, and coelomic dissemination, as confirmed by *in vivo* and ex vivo bioluminescence imaging and histopathological evaluations in mice carrying orthotopic LNCaP tumors. Overall, the results suggest the application of tert-Ser-PTX as a robust antitumor/antimetastatic treatment for PCa (*Adv Healthc Mater.* 2021 Oct 27;e2101544; *Cancer Drug Resist* 2021;4:44-68).

EVOLUTION OF NANOMEDICINE IN THE GLOBAL PIPELINE: LEARNINGS AND MARKET INSIGHTS

KURT SEDO, Vice President Operations PharmaCircle LLC

This presentation will discuss the current nanomedicine landscape in terms of global technologies, pharmaceutical pipeline, recent products approvals and market insights.

NANODIAMOND MAGNETOMETRY: A NOVEL TOOL FOR HIGH-PRECISION DETECTION OF FREE RADICAL PRODUCTION IN SINGLE CELLS

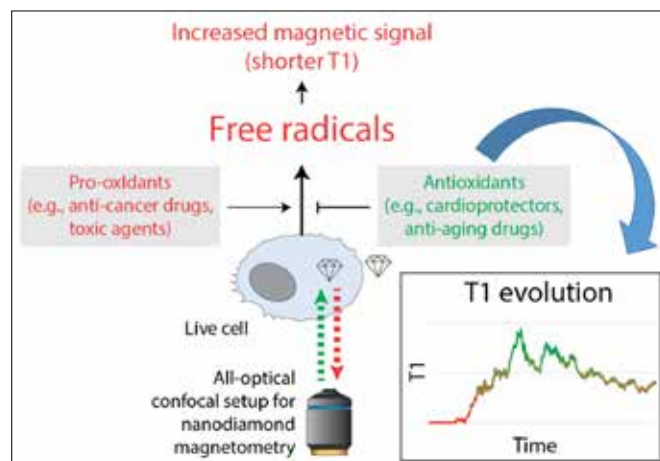
ALINA SIGAEVA¹, Felipe Perona-Martinez¹, Yue Zhang¹, Nikos Mougios¹, Simon D. Pouwels², Irene H. Heijink², Romana Schirhag¹

As we learn more about the complex biological processes underlying pathological changes in our bodies, the importance of personalized, high-precision medical treatment becomes more and more obvious. There is a wide range of techniques for highly targeted interventions, which account for the patient's unique biology and the complexity of cellular networks, implicated in a specific condition. We recognize the diversity of cell types and states that can be found and affected in any pathological condition, from a viral infection to a malignant tumour. These cell states are usually characterized by the expression of specific molecules or by the metabolites they produce. However, nanotechnology can offer us a glimpse into the single-cell physiology.

Fluorescent nanodiamonds (FNDs) have recently been attracting the attention of researchers for their unique properties. These nanosized diamond particles show excellent biocompatibility. They are easily internalized by the cells and can be retained in the cytoplasm for weeks^[1]. FNDs do not show significant cytotoxicity, both *in vitro* and in a wide range of *in vivo* models, including humans^[2]. Moreover, these nanoparticles do not interfere with such delicate biological processes as cell differentiation and embryonic development^[3]. The exceptionally stable fluorescence of FNDs, which does not show bleaching or blinking, makes them an attractive tool to track and visualize the cells over long time periods. This property has already been used to track transplanted cells^[4], to detect quiescent cancer stem cells^[5], or to follow the differentiation of progenitor cells into pneumocytes^[6]. However, there is more to the FND fluorescence than just its stability. The fluorescent properties of FNDs stem from nitrogen-vacancy (NV) centers – the defects of diamond crystal structure, where one of the carbon atoms is replaced by a nitrogen atom, and a neighbouring position remains vacant. The fluorescence of these centers strongly and reversibly depends on their physical environment. NV centers have already been used to visualize the changes in temperature, pH, and, recently, free radical production in biological samples. Free radicals are atoms and molecules with an unpaired electron. Free radicals occurring in live systems are generally considered to be deleterious, as they can interact with various cell components and cause their oxidation. At the same time, free radicals are found in any healthy tissue and organ, playing important roles that are not yet fully understood. Moreover, it is sometimes necessary to ramp up free radical production – for example, to eliminate a cancer cell during chemotherapy. Due to the importance of free radicals, the scientific community has developed a variety of methods for their detection in biological samples. However, these approaches generally either lack specificity and sensitivity or cannot be performed in small samples and require extensive training^[6].

Diamond magnetometry is a technique developed in our group, which allows for all-optical detection of free radicals in live cells. FNDs are introduced into the sample – next to or inside the cells. Next, a specific sequence of green laser pulses, focused on a single FND, brings the NV centers in the particle into a specific quantum state. The fluorescence of NV centers, measured right after this optical “pumping”, is relatively bright. However, if the laser is switched off, the NV centers will gradually return to a darker, less fluorescent state. This relaxation occurs faster in the presence of external magnetic fields, such as those generated by the unpaired electrons of free radicals. The rate of relaxation can be described by the time constant T1: shorter T1 corresponds to faster relaxation and higher radical load. NV centers are extremely sensitive, in principle allowing for the detection of single spins^[7]. This sensitivity rapidly declines with distance between the source of magnetic field and the NV center, leading to the spatial resolution on the order of

10 nanometers. Importantly, there is no chemical interaction between the diamond nanosensor and the free radicals, so the measurements do not disturb the possible physiological functions of the radicals. This, together with the high biocompatibility, makes FNDs a promising tool for high-precision detection of free radical production on the single-cell level.



We have used this technology in several biological models. In the first case, FNDs were internalized by J774 murine macrophages. This has allowed us to study the production of biologically relevant radicals by the immune cells under normal conditions or in the presence of inhibitors.

In the second case, we used HT-29 colon adenocarcinoma cells. These cells can be brought into a re-differentiated state, resembling normal enterocytes. We were able to track the changes in the free radical production during the re-differentiation and obtain characteristic values of free radical load for the cells with varying degrees of differentiation. Cell differentiation state has been closely linked to the malignancy of the tumour and general prognosis for the cancer treatment. Moreover, there is a dire need for the new techniques for early diagnostics of colon cancer, as the success rate of the treatment strongly depends on the stage of the disease. Finally, as most of the approaches for cancer therapy involve the production of free radicals, it would prove useful to measure the free radical load before and after the treatment. Our method allows to dissect the clinical samples into functional subpopulations of cells and test the responsiveness of those to the proposed medical intervention.

In the final study, we worked with primary lung epithelial cells, obtained from healthy donors and from the subjects with chronic obstructive pulmonary disease (COPD). We were able to compare the free radical load between the two conditions, as well as between the donors with the same health status. We assessed the individual response of the cells to an external stressor (various concentrations of cigarette smoke extract) and compared the potential of the cells to adapt to this stressor.

These case studies demonstrate the versatility of our method and the potential range of applications in (pre-)clinical setting. Nanodiamond magnetometry allows one to find the cells with an altered free radical load in the sample, assess their reaction to an external intervention, and, importantly, preserves the cells for other assays. As the cells are not perturbed by the measurements, the same sample can, in principle, be tested with other single-cell techniques, including sequencing or proteomic analysis. Nanodiamond magnetometry can thus become a useful addition to the toolbox of high-precision personalized medicine.

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DEVELOPMENT OF NANOPARTICLES FOR CLINICAL USE: EVALUATION OF BIODISTRIBUTION STUDIES AND 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^(1,2,3,4). More interdisciplinary collaboration to improve the quality is required.

With a long experience from pharmaceutical R&D, I will discuss some improvements that should be made in biological studies with NPs. Biodistribution, metabolism and excretion (ADME) studies are extremely important not only to generate such data, 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⁾. Concerning biodistribution of NPs (and during recent years also extracellular vesicles; EVs) it is a challenge to measure the biodistribution of many NPs and EVs without using labels that may change the particle surface properties such that labelled particles will have a biodistribution different from the unlabelled particles. It is also a challenge to know if the signals obtained come from the labelled intact particles and not from a label no longer associated with the particles. Some opportunities, challenges and pitfalls with ADME studies will be discussed^(3,5).

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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SPLEEN TARGETING OF LIPOSOMAL ANTIGENS IN CANCER IMMUNOTHERAPY

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Cancer is the second leading cause of death globally. In recent years the potency of the immune system to attack cancer has become increasingly clear by the success of checkpoint inhibitor therapy. However, still only a minority of the patients respond to this treatment and this is correlated with a lower (pre-existing) immune response against tumor antigens. Cancer vaccines can be used to enhance anti-tumor immune responses and can be applied in combination with existing immunotherapies. However, the efficacy of cancer vaccines is not optimal. Cancer vaccines that efficiently can elicit cytotoxic CD8+ T cells could be used in cancer patients to stimulate anti-cancer immune responses and can be combined with other immunotherapies such as checkpoint inhibitors to enhance the clinical efficacy.

In our previous studies CD169-expressing antigen presenting cells were identified as important mediators for the activation of T and B cell responses. CD169+ macrophages are present in lymphoid organs (spleen and lymph nodes) and filter the blood and lymph fluid for particulate antigens such as viruses and exosomes. Antigens captured by CD169+ macrophages are transferred to dendritic cells (DCs) that are specialized in CD8+ and CD4+ T cell activation. Since CD8+ T cells are cytotoxic and can recognize and kill tumor cells, cancer vaccines should efficiently stimulate these immune cells. This talk will focus on our work to utilize liposomes for antigen targeting to splenic CD169+ macrophages to achieve this goal.

FROM DATA TO ACTION – CATALYZING THE TRANSITION FROM EVIDENCE TO GLOBAL AMR POLICY DECISIONS

RALF SUDBRAK

Antimicrobial resistance (AMR) is one of the top 10 global public health threats facing humanity and an ever-present and growing socio-economic burden. Recent work by the Global Research on Antimicrobial Resistance Project estimates that 1.27 million deaths in 2019 were the direct result of drug resistant bacterial infections – a significant and increasing burden that is projected to grow globally, but with a disproportionate impact on low and middle income (LMIC) countries.

Given the urgency of the situation, there is a need for coordinated efforts in AMR across the value chain from research and development (R&D) efforts to the incentives being implemented globally that aim to improve the functioning of markets responsible for the development and distribution of priority health technologies for addressing AMR.

Recognising the critical nature of AMR has led to increased discussion of the AMR challenge at the national and international level, including within fora such as the UN General Assembly, the World Health Assembly, the G7, the G20, and by multi-lateral organisations such as the WHO, OIE and the FAO, resulting in high political interest and commitment. There is collective recognition that investments in R&D are crucial for developing solutions in terms of effective interventions and products resulting in the prevention, containment or reduction of AMR, including the use of nanomedicine tools and approaches. However, in order to promote the development of health technologies and strategies addressing AMR, resources need to be employed even more effectively than at present.

In July 2017, the G20 called for a new international R&D Collaboration Hub to maximise the impact of existing and new basic and clinical antimicrobial research initiatives, which led to the establishment of the Global AMR R&D Hub (www.globalamrhub.org). The Hub is catalysing the international momentum to support global priority-setting and evidence-based decision-making on allocation of resources for AMR R&D through the identification of gaps, overlaps and cross-sectoral collaboration.

ALLERGIC REACTIONS TO COVID-19 VACCINES: EVIDENCE FOR A CONTRIBUTING ROLE OF ANTI-PEG ANTIBODIES AND COMPLEMENT ACTIVATION IN MAN AND IN A PORCINE MODEL

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A tiny fraction of people immunized with lipid nanoparticle (LNP)-enclosed mRNA (LNP-mRNA) vaccines develop anaphylaxis or other allergic symptoms following their first or subsequent vaccinations. These reactions resemble complement (C) activation-related pseudoallergy (CARPA) to i.v. administered liposomes, for which pigs provide a naturally oversensitive model. Using this model, we have shown that i.v. injection of the human vaccination dose (HVD) of BNT162b2 (Comirnaty, CMT) or its 2-fold (2x) or 5-fold (5x) amounts can trigger transient pulmonary hypertension along with thromboxane A2 release into the blood and other hemodynamic and blood cell changes, including hypertension, granulocytosis, lymphopenia, and thrombocytopenia, providing a typical fingerprint of CARPA. Since CMT contains polyethylene-glycol (PEG), one possible cause of CARPA is classical pathway C activation due to the presence of anti-PEG antibodies in blood that bind to vaccine-PEG. We have obtained multiple lines of evidence that this process can indeed occur. Notably; (i) we have identified people who developed allergy to an mRNA vaccine and had exceptionally high anti-PEG antibodies in their blood, (ii) found that Comirnaty is a strong activator of C, which effect is greatly amplified in the presence of anti-PEG Abs, and (iii) pigs immunized with PEGylated liposomes (Doxebo) to rise anti-PEG Abs in their blood developed, without exception, anaphylactic shock to a portion of CMT injection. Furthermore, we show that anti-PEG antibodies can be induced, or their preexisting level may rise in a small portion of people immunized with PEG-containing vaccines, thus raising the risk not only for further vaccine reactions but also for reactions to all PEG-containing drugs, contrast agents and food or sanitation/cosmetic products. Taken together, these data suggest that CARPA may be an important contributing mechanism to these reactions, and that anti-PEG antibody assays may be useful to predict at least one major risk for allergic reactions to PEG- (or polysorbate)-containing vaccines. The lecture will address alternative explanations for these allergic

reactions, including the “local immune cell excitement” and “rapid phagocytosis response” myths.

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ANTIBACTERIAL DRUG R&D IN THE RESISTANCE ERA

URSULA THEURETZBACHER

Antibacterial Drug R&D focuses on the most relevant resistant bacteria globally. The WHO list of critical priority pathogens for R&D provides guidance for needed R&D strategies from a global health point of view. Numerous challenges pave the way to more robust pipelines. In addition to funding and economic problems scientific hurdles, especially for Gram-negative bacteria, require deep knowledge and experience in the drug discovery process. Novel antibiotics of a new chemical class/mode of action/binding site are urgently needed and researched in academic groups and small companies. Several initiatives aim at supporting and incentivising antibacterial drug R&D. Future success remains to be seen.

VIRTUAL 3D SURGERY PLANNING AND 3D PRINTING OF PATIENT-SPECIFIC "SMART IMPLANTS" AT THE POINT-OF-CARE

FLORIAN THIERINGER

For reconstructive surgery in the oral and cranio-maxillofacial region, e.g., after trauma or tumors, the intact human anatomy is the best blueprint for 3D surgical planning, minimal-invasive surgical procedures, and the production of patient-specific (PSI) high-performance implants. PSI comprises PEEK, titanium, or 3D-printed resorbable bone graft substitutes up to bioprinting processes. Smart implants are personalized bio-implants characterized by customized shapes, special functions in combination with flexible and durable biomaterials. Ideally, smart implants can be produced quickly and cost-effectively at the point-of-care (at the hospital). In this presentation, indications, characteristics and the specific features of the treatment process with Smart Implants will be discussed.

REFLECTIONS ON THE EMERGENCE OF DENDRIMERS IN NANOMEDICINE

DONALD A. TOMALIA

Nearly four decades ago, the first examples of a new class of polymeric architecture after traditional: (I) linear, (II) cross-linked and (III) branched polymer types were reported. They are recognized as a distinct fourth new architectural category (IV), referred to as dendrimers/dendritic polymers. As would be expected, this novel architectural polymer class has revealed many unprecedented features and unexpected properties including quantized critical nanoscale design parameters (CNDPs) such as: nanometric sizes, surface functionality, persistent shapes, rigidity/flexibility and elemental compositions, as well as non-traditional intrinsic luminescence (NTIL), drug solubility enhancement, unimolecular encapsulation and macromolecular monodispersity rivaling proteins/DNA. Many of these unique properties have been exploited in nanomedicine and now constitute the basis for many familiar nanotherapeutic concepts such as active, receptor mediated drug targeting, unimolecular drug encapsulation, non-viral gene delivery, NMR contrast agents, solubility enhancing excipients, anti-viral/microbial agents, anti-inflammatory agents, non-complement activating, stealthy

nanoparticles and cardio-diagnostics, to mention a few. The role that these unique dendritic properties play in current nanomedical products will be overviewed including: Starpharma's DEPTM targeted delivery therapy for cancer, anti-microbial agents (i.e., VivaGel™), anti-viral agents (i.e., Viraleze™), Siemen's, cardio-diagnostics (i.e., Stratus™), Tibia's dendrimer-based mRNA delivery for flu/COVID-19 vaccines and NanoSynthons, API solubility enhancing excipients (i.e., SupraPlex™). The lecture will conclude with the recent use of simple surface functionalized PAMAM dendrimers as fluorescing agents for neurological and whole body imaging based on non-traditional intrinsic luminescence (NTIL) imaging principles

INHALATION OF ANTIBIOTICS IN CYSTIC FIBROSIS; DEVELOPMENTS, FACTS AND FICTION.

TOUW DAAN, University Medical Center Groningen

Cystic fibrosis (CF) is a genetic disorder in which abnormal mucus in the lungs is associated with susceptibility to persistent bacterial infections. Haemophilus influenzae and Staphylococcus aureus are usually the first pathogens encountered in childhood. As the lung disease progresses, colonization by Pseudomonas aeruginosa (P.aeruginosa) will follow. After a period of intermittent colonization the colonization with P. aeruginosa will become chronic accompanied by inflammation of the lungs. Inhaled antibiotics, i.e. colistin, tobramycin, aztreonam lysine and levofloxacin, have improved respiratory diseases in CF, however, lung infections are still the leading cause of death.

After the development of chronic P. aeruginosa infection, inhaled antibiotics are used as maintenance or exacerbation treatment for CF patients. Their use offers advantages over systemic therapy since a relatively high concentration of the drug is delivered directly to the lungs, thus enhancing the pharmacokinetic/pharmacodynamic parameters and decreasing toxicity.

Inhaled antibiotics as maintenance therapy: Current guidelines recommend inhaled antibiotics for individuals with CF and persistent P. aeruginosa infection who are aged six years or older. The aim is to reduce bacterial load in the lungs so as to reduce inflammation and deterioration of lung function.

Inhaled antibiotics as therapy for exacerbations: Pulmonary exacerbations are when symptoms of infection become more severe. Antibiotics are an essential part of treatment for exacerbations and inhaled antibiotics may be used alone or in conjunction with oral antibiotics for milder exacerbations or with intravenous antibiotics for more severe infections. Inhaled antibiotics do not cause the same adverse effects as intravenous antibiotics and may prove an alternative in people with poor access to their veins.

Because different antibiotic inhalation therapies exist with different modes of action, alternating treatment with inhaled antibiotics may represent an interesting strategy for avoiding bacterial resistance and improving patient outcomes.

A NOVEL MULTIDRUG NANOPARTICLE FORMULATION ASSESSMENT IN A CARDIAC ISCHEMIA REPERFUSION MODEL

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Introduction

Acute myocardial infarction (MI), caused by a sudden occlusion of a coronary artery, is responsible for the death and disabilities of millions of people worldwide yearly¹. Although reperfusion of the occluded artery is essential to maintain cardiomyocyte viability and restore cardiac function, it induces another form of injury named ischemia/reperfusion (I/R) injury². Pharmacologic strategies involve the administration of active molecules such as adenosine³ or antioxidants in high doses⁴. However, limited protective effects are observed, as these molecules are either rapidly metabolised when delivered to the blood flow, or are whole-body biodistributed. In this context, nanoparticles loaded with a therapeutic drug have emerged as valuable tools, since they can protect the drug from rapid degradation and enhance specific organ accumulation. Previously, our team has demonstrated an interesting protective effect of squalene multidrug nanomedicines, containing adenosine and vitamin E in a rodent model of uncontrolled inflammation⁵. In this current project, we aim to evaluate if a cardioprotective effect is obtained when administered in a rodent model of cardiac ischemia/reperfusion (MIR).

Experimental Methods

Nanoparticle preparation and characterisation

Multidrug NPs were obtained after the bio-conjugation of adenosine with squalene, a natural lipid, followed by the nanoprecipitation in the presence of vitamin E (an antioxidant) in ratios of 50/50, 60/40 and 75/25 squalene adenosine bioconjugate (SqAd) and vitamin E (Vit E) respectively. These preparations were evaluated for their stability during a period of 15 days via dynamic light scattering (DLS) measurements of size and zeta potential.

In vitro assessment

Cytotoxicity

To assess the cytotoxicity of the different compositions of Sq Ade Vit E NPs, MTT assays were conducted on two cardiac cell lines. MCEC and H9c2 cells were seeded at 6,000 and 10,000 cells per well respectively in 96-wells plates and left to adhere for 24 h. Cells were then treated with different kinds of SqAd Vit E NPs in several concentrations and cytotoxicity was evaluated after 24 h incubation.

Antioxidant effect

A DCFDA assay kit was used according to the manufacturer's instructions to assess the antioxidant capacities of the different compositions of Sq Ade Vit E NPs. MCEC cells were seeded in 96-wells plates and incubated with different concentrations of the NPs for 2 h. We then kinetically recorded the cells' production of reactive oxygen species (ROS) within cells after triggering their production with H₂O₂.

In vivo assessment

Based on our *in vitro* results, we decided to proceed to evaluate SqAd Vit E 50/50 NPs' cardioprotective effect *in vivo*. By occluding the myocardial left anterior artery, rats were subjected to ischemia for 30 min, followed by ischemia for 24 h or 7 days. SqAd Vit E 50/50 NPs were injected intravenously 5 – 10 min after reperfusion.

Plasma evaluation

Blood plasma samples taken before surgery and after reperfusion

of 24 h were analysed. Oxidative damage through free radicals was determined by measuring lipid peroxidation with an MDA kit in plasma collected before and after surgery. In plasma, TNF alpha and IL-6 levels were quantified to assess acute phase pro-inflammatory response and IL-10, as an anti-inflammatory cytokine produced in later stages of inflammation. Further, plasma was analysed for markers for heart failure, troponin I and pro NT-proBNP.

Echocardiography

We evaluated cardiac function by using transthoracic echocardiography before surgery and after 24 h or 7 days of reperfusion. Two-dimensional brightness mode (B-mode) and M-mode echocardiograms were taken in parasternal long-axis and short-axis views. We measured the intraventricular septum thickness, left ventricle internal diameter and the left ventricular posterior wall thickness in diastole and systole to calculate left ventricular ejection fraction (LVEF), in order to assess left ventricular cardiac function. Cardiac remodelling was evaluated based on LVEF values and comparisons of pre- and postoperative diastolic and systolic wall thickness and diameters.

Determination of infarct size and area at risk

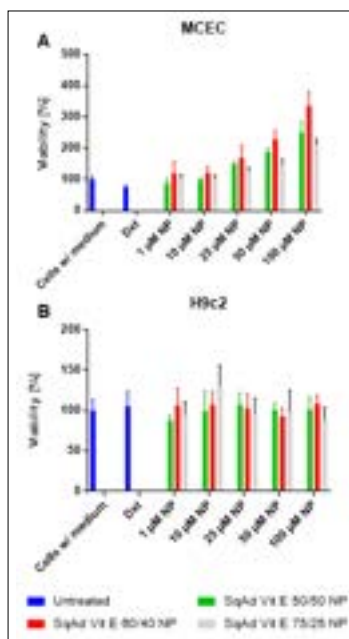
After 7 days of reperfusion, the animals were euthanised. The heart was stained with a 2% Evan's Blue solution, excised and sliced. Images were taken before incubation in tetrazolium chloride (TTC) and processed with Image J software to evaluate infarct size and area at risk (AAR).

Results and Discussion

In vitro assessment

Nanoparticle preparation and characterisation

After storage at room temperature between 15 – 20 °C for up to 15 days, DLS measurements confirmed all NP compositions' stability.



Cytotoxicity

In MTT assays, all multidrug formulations tested on MCEC and H9c2 cells do not show any cytotoxic effects after incubation for 24 hours (Fig. 2). Further, we noted a different response between these cell lines, as an enhanced viability is shown in MCEC when incubated with increasing concentrations of NPs. This can be explained by the MCEC cells' higher capacity for endocytosis⁶.

Fig. 2. Cytotoxicity assessment of SqAd Vit E NP after 24 h incubation with the different compositions. (A) In MCEC cells. (B) In H9c2 cells.

Antioxidant effect

DCFDA assays demonstrated that NPs containing SqAd and Vit E in a 50/50 ratio exhibited an enhanced antioxidant effect on MCEC exposed to H₂O₂, compared to other compositions (Fig. 3).

In vivo assessment

Based on our *in vitro* results, we decided to proceed with the administration of Sq Ad Vit E 50/50 NPs in an *in vivo* model of MIR in rats. After 24 h of reperfusion, we performed plasma analysis. However, our results were inconclusive, as detected absorptions were too low to calculate the corresponding values. Interestingly, our preliminary results in echocardiography and determination of infarct size and area at risk (Fig. 4) after 24 h and 7 days reperfusion demonstrate a cardioprotective effect in animals receiving the nanomedicines compared to untreated animals.

Fig. 3. Evaluation of the antioxidant capacity of the SqAd Vit E 50/50 NP preparation.

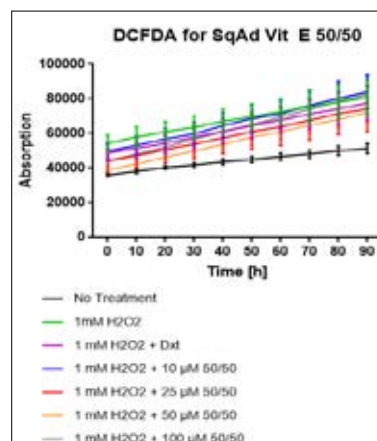
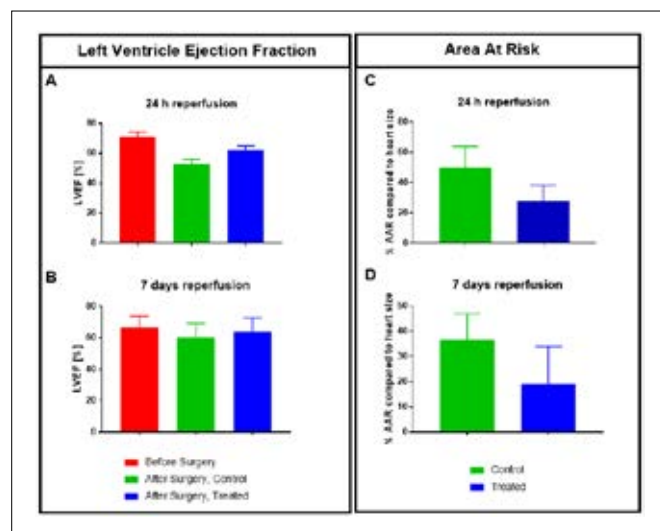


Fig. 4. Left ventricular ejection fraction (LVEF) calculated by using echocardiography and area at risk calculations (AAR) on animals before and after surgery. (A) LVEF after 24 h reperfusion. (B) LVEF after 7 days. (C) AAR after 24 h reperfusion. (D) AAR after 7 days reperfusion.



In conclusion, our preliminary results obtained *in vivo* showed an interesting cardioprotective effect. Deeper analysis is ongoing, in order to understand the molecular mechanisms of this cardioprotection.

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DENDRIMERS: FROM INNATE IMMUNITY TO CARDIOLOGY, CARDIOVASCULAR MEDICINE AND BEYOND – THE ASSA PHENOMENON

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Atherosclerotic cardiovascular diseases, such as coronary artery disease (CAD), carotid artery disease, and peripheral artery disease, are by far the leading cause of human death and morbidity worldwide. Atherosclerosis is the underlying pathology that builds up atherosclerotic plaques in the arterial wall. This leads either to progressive narrowing of the arterial lumen limiting the blood flow to organs and tissues (stable atherosclerotic plaques), or in the vast majority of the most lethal cases leads to rupture of the inflammatory atherosclerotic plaques and to subsequent acute thrombotic blockage of the blood flow to vital organs and tissues (vulnerable atherosclerotic plaques).

The EU FP7 NMP funded large-scale CosmoPHOS-nano Project had as major objective the research and development of a disruptive nanotechnology-enabled system (the CosmoPHOS System) for the early diagnosis, therapy, and therapy monitoring of the vulnerable atherosclerotic plaques applicable to CAD and wider atherosclerotic cardiovascular diseases. Particularly, the CosmoPHOS System was designed to stabilize and passivate the vulnerable atherosclerotic plaques thus preventing their rupture and acute thrombosis. From a clinical perspective, it is imperative that the nanomedicines that would be used should not elevate locally the inflammatory burden of the vulnerable atherosclerotic plaques following intra-plaque accumulation. Many nanomedicines, however, trigger local complement activation at diseased sites raising serious concerns, since uncontrolled activation of the human complement system, a key component of the human innate immunity, promotes inflammatory cascades and disease progression. The CosmoPHOS System is dendrimer-enabled. Dendrimers are synthetic nanostructures that grow like trees displaying surface motifs typically spaced less than 1 nm from each other¹⁻⁴. This Angstrom-Scale Spacing Arrangement (ASSA) of the end-terminal motifs endows dendrimers to escape sensing by the human complement pattern-recognition molecules^{1,2}. Among many outcomes of the CosmoPHOS-nano Project was the design, engineering and selection of a library of complement-safe dendrimeric nanomedicines^{1,2}, which was successfully applied for diagnosis, therapy, and therapy monitoring of the atherosclerotic plaques in validated rabbit models of atherosclerosis.

Finally, from a broader perspective, complement escape through the ASSA phenomenon opens new avenues for improved design and development of safer stealth nanomedicines and biomedical devices not only for diagnostic, therapeutic, and theranostic applications in cardiology and cardiovascular medicine, but also in wider pathological conditions where local complement activation is problematic and promotes disease progression (e.g., cancer, autoimmune/inflammatory diseases, diabetes mellitus, neurodegenerative diseases, and infectious diseases)^{1,2}.

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EMULSOMES IMPROVE ANTILEISHMANIAL ACTIVITY OF BISNAPHTHALIMIDOPROPYL (BNIP) DERIVATIVES AGAINST LEISHMANIA INFANTUM PARASITES

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Leishmaniasis is a widespread disease, affecting 12 million people around the world with about 1–2 million estimated new cases occurring every year. Chemotherapy is the most efficient strategy in fight against leishmaniasis; however, treatment remains insufficient as the current anti-leishmanial agents have several limitations including low efficacy, toxicity, adverse side effects, drug-resistance, length of treatment and cost lines^[1]. Delivery of the anti-leishmanial drug to the target cell selectively is highly regarded as the prominent strategy. Combined with the discovery of new anti-leishmanial drug candidates with improved efficacy, drug delivery systems are expected to serve the WHO's aim of eliminating Leishmaniasis on many regions around the world. However, the need for better anti-leishmanial agents and alternative, low-expense drug delivery systems is still present.

Among bisnaphthalimidopropyl (BNIP) derivatives, BNIPDaoct and BNIPDanon recently came forward with antileishmanial activities beyond the standard, commercialized antileishmanial therapies^[2,3]. However, high-level toxicity on macrophages plus poor aqueous solubility and poor bioavailability of the compounds limit their application in therapies. Emulsome is considered as a prominent drug delivery strategy to overcome the drug solubility and toxicity limitations of bisnaphthalimidopropyl (BNIP) derivatives for their potential use in antileishmanial therapy.

Emulsome is a lipid-based drug delivery system comprising of a solid fat core surrounded by phospholipid multi-layers^[4]. Its intrinsic features include biocompatibility, prolonged release profile, good safety and high encapsulation capacity for lipophilic drug compounds^[4-6]. Accordingly, emulsome formulations were prepared with the presence of BNIP compounds. The average diameters of BNIPDaoct- and BNIPDanon-loaded emulsomes were found as 363.1 and 337.4 nm, respectively; while empty emulsomes differed with a smaller average particle diameter, i.e., 239.1 nm^[7]. All formulations exhibited a negative zeta potential value. The formulations achieved the encapsulation of BNIPDaoct and BNIPDanon at approximately 0.31 mg/ml and 0.24 mg/ml, respectively. The delivery of BNIP within the emulsomes improved the antileishmanial activity of the compounds. BNIPDaoct-loaded emulsome with 50% inhibitory concentration (IC50) value of $0.59 \pm 0.08 \mu\text{M}$ was in particular effective against *Leishmania infantum* promastigotes compared to free BNIPDaoct ($0.84 \pm 0.09 \mu\text{M}$), free BNIPDanon ($1.85 \pm 0.01 \mu\text{M}$), and BNIPDanon-loaded emulsome ($1.73 \pm 0.02 \mu\text{M}$) [7]. Indicated by at least ≥ 2 -fold higher 50% cytotoxic concentration (CC50) values, the incorporation of BNIP into emulsomes significantly reduced the toxicity of BNIPs against macrophages, corresponding to up to 16-fold improvement in selectivity index (CC50/IC50) for *L. infantum* promastigotes. The infection rates of macrophages were determined using dual-fluorescent flow cytometry as 68.6%^[7,8]. Both BNIP formulations at concentration of $1.87 \mu\text{M}$ reduced the parasitic load nearly to 40%, whereas BNIPDaoct-loaded emulsomes could further decrease the parasitic load below 20% at $7.5 \mu\text{M}$ and above.

In conclusion, the incorporation of BNIPDaoct and BNIPDanon into emulsomes resulted in water-soluble dispersed emulsome formu-

lations that do not only successfully facilitate the delivery of BNIP compounds into the parasites and the Leishmania-infected macrophages *in vitro* but also enhance antileishmanial efficacy as proven by the decline in IC50 values. The selectivity of the formulation for *L. infantum* parasites further contributes to the challenging safety profile of the compounds. The promising *in vitro* antileishmanial efficacy of BNIP-loaded emulsomes highlights the potential of the system for the future *in vivo* studies.

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ARTIFICIAL ANTIGEN PRESENTING CELLS WITH TOPOLOGICAL CONTROL

JAN VAN HEST

Artificial antigen presenting cells (AAPCs) have received much interest as an effective way to modulate the immune system. One of the most common modes of application is as mimics of dendritic cells that engage with T cells via the display of activating signals. Although a range of different AAPC platforms have been developed, a systematic study at how size, shape and signal density affect the T cell response is still lacking in the field, which is of key importance in order to develop a platform with reliable performance. In this contribution we present our AAPC platform based on nanometer-sized polymer vesicles, or polymersomes. We have developed an effective way to regulate both the size and shape (spherical and tubular) of these nanoparticles. Furthermore, via an advanced conjugation strategy we have been able to control the density of two main signaling moieties on the particle surface. Using an *in vitro* cell activation assay we have demonstrated that larger particles simultaneously displaying both signals are the most effective in generating a T cell response. This opens up the way to further applications of this platform in an *in vivo* setting.

LIPOSOMES AND LNP'S: THE BEST NANOTECHNOLOGY?

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Based on the number of products and ongoing clinical trials with liposomes and LNPs (Lipid Nanoparticles), liposomes and LNPs can be considered as the most successful drug-carrier systems in nanotechnology so far. With the development of products such as Doxil[®], AmBisome[®], and Comirnaty[®], the hurdles that had to be overcome enabling reproducible large-scale cGMP production of these liposome and LNP products were addressed. These products show clinically proven efficacy and low toxicity. Besides manufacturing factors, for instance, efficient incorporation of the drug in the lipid particles, control of the particle size and particle size distribution of the liposomes/LNPs, and handling of solvents during production, the large-scale availability of pharmaceutical grade phospholipids and designer lipids was certainly another unlocking key success factor enabling large-scale production of liposome and LNP products. For parenteral products based on lipid-carriers, natural phospholipids, derived from soybean and egg yolk, and synthetic phospholipids are being used. When required also cationic lipids including pH-sensitive amino-lipids may be applied. Alternative non-lipid carriers are not that advanced in pharmaceutical industry, because many of the hurdles described above have not been overcome yet. This seminar compares the benefits and state of the art of lipid-based drug carriers with alternative nano-carriers and draws the conclusion that academic and industrial research in nanotechnology carriers should focus on lipid carriers.

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NEW NANOPARTICLE FORMULATION FOR CYCLOSPORIN A: IN VITRO ASSESSMENT

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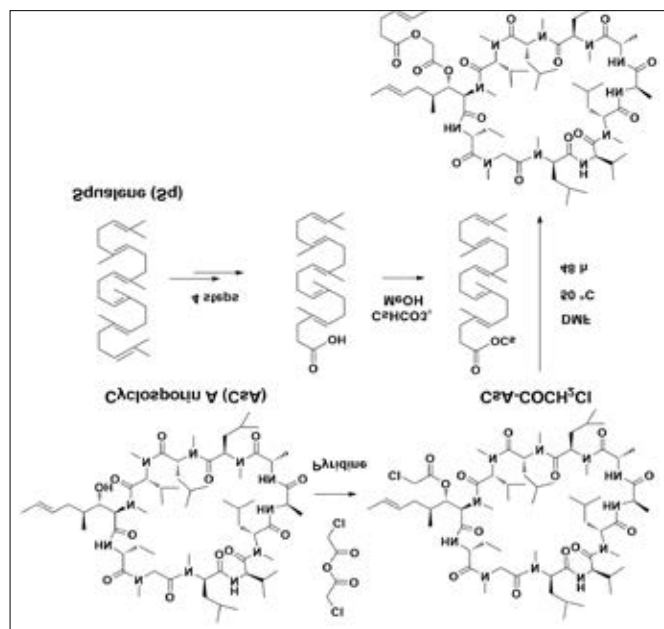
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INTRODUCTION

Cyclosporin A (CsA) is a hydrophobic neutral cyclic peptide with well-known immunosuppressive properties. Additionally, CsA has also shown to inhibit the opening of the mitochondrial permeability transition pore (mPTP) by binding to cyclophilin D, a major protein which regulates mPTP¹. In a proof-of-concept phase 2 clinical trial,

it was shown that the administration of CsA just before cardiac reperfusion reduced the myocardial infarct size². Still, these results were mitigated in larger clinical trials^{3,4}, probably because of the whole body biodistribution and low accumulation of CsA into ischemic heart. Our laboratory has developed an innovative method based on the chemical encapsulation of various drugs. This method is preferred to the physical encapsulation since improves drug loading and avoid drug burst release⁵. In this work, we have applied the “squalenylation strategy” to obtain a squalene-CsA (Sq-CsA) bioconjugate, which was further nanoprecipitated in aqueous solution to obtain NPs.

Figure 1 : Synthetic scheme of the Sq-CsA bioconjugate



EXPERIMENTAL METHODS

Bioconjugate synthesis

The Sq-CsA bioconjugate was obtained by conjugation of the 1,1,2-trisnorsqualenic acid onto the alcohol group of the MeBmT amino acid (Figure 1 : Synthetic scheme of the Sq-CsA bioconjugate1). Since, this alcohol group is hindered in the CsA cycle, it was first functionalized with chloroacetic anhydride in pyridine to obtain a CsA chloroacetic ester. Then, the CsA was linked to the 1,1,2-trisnorsqualenic acid cesium salt synthesized from squalene using the Van Tamelen method⁷.

Nanoparticle preparation and characterization

Sq-CsA nanoparticles (NPs) were prepared using the nanoprecipitation technique. Briefly, the Sq-CsA bioconjugate was dissolved in absolute ethanol and added drop by drop to a 5% (w/v) dextrose solution under vigorous stirring to give an aqueous suspension of Sq-CsA NPs. The morphology, size and zeta potential of the NPs were obtained using cryogenic transmission electron microscopy (cryoTEM) and Zetasizer Nano ZS, respectively. The, CsA release from the NPs was assessed in foetal bovine serum (FBS), using a reversed-phase HPLC corona CAD system.

In vitro assessment

Cytotoxicity of Sq-CsA NPs

The cytotoxicity of Sq-CsA NPs was evaluated on two cardiac cell lines (Mouse Cardiac Endothelial Cells (MCEC) and rat cardiomyoblasts (H9c2)) by using MTT assays. Different concentration ranging from 0,6 to 60 µg/mL were tested for up to 24 hours.

Cellular uptake

To evaluate the cellular uptake, both cell lines were seeded in 8 well plates. Twenty-four hours later, the cells were incubated with fluorescently labelled Sq-CsA NPs.

After incubation the cells were processed, and images were obtained using an inverted Confocal Laser Scanning Microscope Leica

TCS SP8.

Cytoprotective effect

The cytoprotective effect was performed on the two cell lines after incubation with Sq-CsA NPs for 24 hours, followed by 6 hours incubation in hypoxic conditions (1% O₂, without serum and glucose). Some plates were submitted to reoxygenation for 30 minutes or 1 hour respectively. Cell viability was assessed by MTT and LDH assays.

RESULTS AND DISCUSSION

The Sq-CsA bioconjugate was obtained with a yield of 35% and gave NPs with a size of approximately 150 nm that were stable for at least 1 month at room temperature. Moreover, those NPs have a spherical shape and can encapsulate 73%⁶ of CsA using a diglycolic linker that was relatively stable in FBS because no free CsA was detected even after 48 h of incubation.

In vitro studies showed that Sq-CsA NPs were internalized by MCEC and H9c2 cells and reached a peak at 24 hours (Figure 2 : Sq-CsA NPs uptake assessment in MCEC and H9c2 cell lines.2).

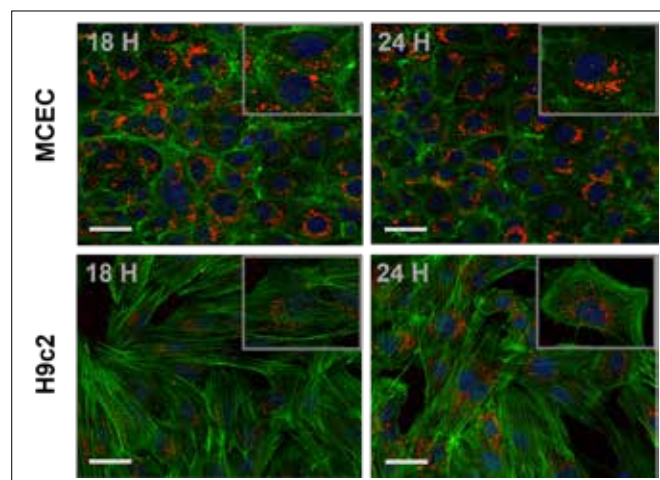


Figure 2 : Sq-CsA NPs uptake assessment in MCEC and H9c2 cell lines.

The NPs accumulated faster in MCEC (2h) than in H9c2 cells (7h) which could be explained by the fact that MCEC cells have a higher capacity for endocytosis.

Sq-CsA NPs also exhibited a protective effect on H9c2 cells under hypoxia and after hypoxia followed by reoxygenation (Figure 3 : Cardioprotective effect assessment of Sq-CsA NPs using MTT assays on MCEC and H9c2 cells.3). We noted that the protection was stronger for cells undergoing hypoxia/reoxygenation than hypoxia alone.

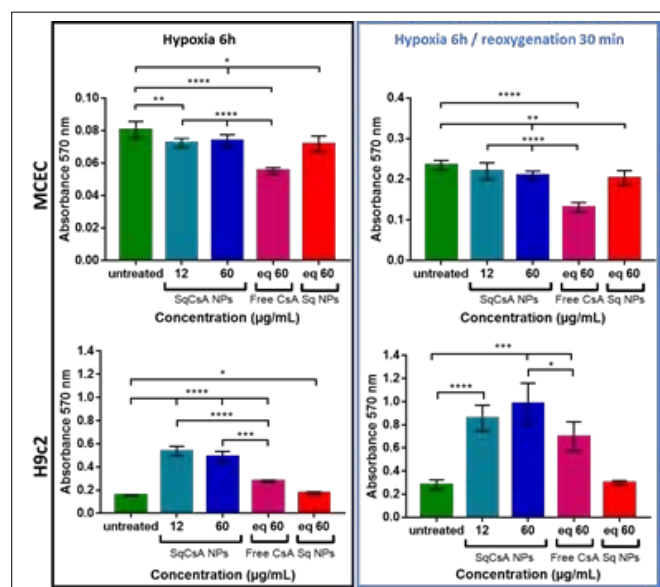


Figure 3 : Cardioprotective effect assessment of Sq-CsA NPs using MTT assays on MCEC and H9c2 cells.

CONCLUSION

In conclusion, we obtained a new nanoformulation of CsA, based on the covalent linkage between Sq and CsA. This bioconjugate allows the obtaining NPs of controlled size in an aqueous medium. Our first results have demonstrated a strong *in vitro* protective effect on different cell lines tested. Understanding the outcome of NPs in the cells and the mechanism of CsA release from the NPs is essential for a possible future medical application.

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DESIGNING PERSONALIZED POLYMER-BASED COMBINATION NANOMEDICINES FOR ADVANCED STAGE BREAST CANCER PATIENTS.

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Breast cancer, the most prevalent tumor in women worldwide¹, still lacks effective treatment approaches that increase survival rates and reduce side effects. The implementation of polypeptide-based polymer-drug conjugation strategies represents a promising approach².

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^{3,4}.

Conjugation of combined anticancer drugs with biodegradable polypeptides offers many advantages when compared with small molecules therapies. It has been already demonstrated that polypeptide-based combination therapy ensures the arrival of two or more drugs at a synergistic ratio to the same cell at the same time, yielding to an improved therapeutic index of already clinically established agents. Importantly, the use of bioresponsive linking chemistry, in particular, towards tumor-specific microenvironmental stimuli, allows the control on drug release kinetics profile and consequently, enhance drug(s) therapeutic output⁵.

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APPRECIATING AND EXPLOITING THE MECHANICAL DESIGN OF PROTEINS

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Even though life is happening far out of equilibrium, very few cell signaling diagrams indicate which signaling pathways or protein functions are activated or destroyed by the stretching of proteins out of equilibrium. Mechanical forces drive essential life processes, from the first steps in fertilization all the way to shaping growing cellular assemblies into organisms. External and cell-generated forces also orchestrate tissue homeostasis in healthy organs, or if misbalanced, result in a range of degenerative diseases. Yet, our knowledge of proteins in biology, pharmaceutical sciences and medicine is still mostly based on knowledge of their equilibrium structure-function relationships. This notion is increasingly challenged as proteins can have amazing mechanical properties as well, as many extracellular and intracellular proteins serve as mechanochemical switches when stretched by cell generated forces. Reciprocal mechanical signaling between cells and their environment is thus key to the ability of cells to sense their 3D microenvironments and thus to the spatio-temporal coordination of tissue growth and regenerative processes. If miss-balanced, physical factors can even override chemical stimuli and this can tip cell niches towards pathological transformations. While enormous progress has been made in the last decades how biochemical factors regulate cell signaling pathways and the transcriptional responses of cells, combining the biochemical and mechanobiological viewpoints is essential to advance the field of regenerative medicine as will be illustrated here with recent discoveries.

NANOMEDICINE EX MACHINA: DESIGN STRATEGIES BASED ON CLINICAL RELEVANCE

MATHIAS WACKER

In recent years, nanomedicines have conquered the global healthcare market. Still, they represent an expensive niche technology with a limited application range. Among other examples, they have been used to reduce the toxicity of anticancer agents, overcome physiological barriers, or stabilize drug molecules that are sensitive to degradation. Current design strategies are widely based on our experiences with the first generation of innovator drug products. With a growing knowledge of their clinical performance, computer-based design strategies can be implemented into the drug development pipeline, reducing cost and making these advanced carriers more predictable. These methodologies not only include suitable models and algorithms but also the design of *in vitro* assays to predict their behavior *in vivo*. In this context, clinical performance is the ultimate benchmark that needs to be implemented into the design process at an early stage. The talk will highlight the biopredictive methodologies we have to date and explain common limitations and pitfalls.

GUIDING MRNA-LNP DRUG PRODUCTS FROM EARLY R&D PROGRAMS TO THE MARKET – HOW POLYMUON CONTRIBUTES TO PRODUCTION OF MRNA VACCINES

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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, like Covid-19 related 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.

During the early days of the Covid-19 pandemic, industry partners reached out to Polymun to set up production processes for mRNA-LNPs together with the respective analytical test methods. Within weeks, a robust and scalable process has been developed and first animal trial materials have been provided to initiate toxicology studies. Just a few weeks later, GMP batches were produced, which entered first in man studies around mid-April.

Data will be presented, which describe hurdles and solutions throughout these processes.

BIOMEDICAL NANOPARTICLE BIOTRANSFORMATION: ANALYSIS OF THE EARLY DYNAMIC EVENTS IN SITU

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Nanoparticle (NP) colloidal stability plays a crucial role in biomedical application not only for human and environmental safety but also of NP efficiency and functionality. Minor changes in the physicochemical properties of NP and their buffer systems can have tremendous influence on their colloidal stability, a prerequisite for medical applications. Despite many investigations on NP agglomeration and the effective parameters related to their stability, there is a lack of information about the early stage of the dynamic behavior

during the media changing from a stable colloidal solution to a biological environment like body fluids. Therefore, the precise label-free and non-disruptive monitoring of the early-stage of interactions, namely agglomeration process, is an essential requirement for a better understanding of the underlying mechanism.

In this presentation, the methodology and the major outcome of gold nanoparticles biotransformation will be explained and discussed.

In brief, the novel microfluidic-based mixing device is developed connected to a quartz capillary allowing subsequent SAXS analysis. With this setting, suspension of NPs around 20 nm and smaller can be mixed with any buffer system or body fluid mimicking solutions. SAXS measurement along the capillary enables to follow the early dynamic events in situ, label-free and in a time-resolved manner, as shown with silica^[1] and gold particles of different size and surface functionalization^[2].

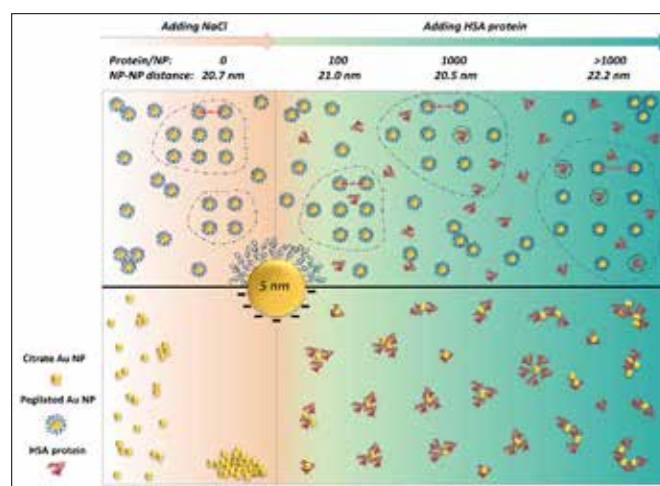


Figure 1: Schematic overview of the main findings of 5nm gold particles either citrate or PEG modified after mixing with physiological NaCl or human serum albumin (HSA) buffer with increasing HSA content. PEG gold remain a stable dispersion with 3D self-assembled cubic-ordered structures with interparticle distance of 20.7 ± 0.01 nm. This distance is increasing by the presence of HSA. Citrate stabilized gold particles do start to form agglomerates when transferred to NaCl or HSA buffer systems^[2].

Our investigation with the model particles like silica or gold of different size and surface functionalization can be seen as proof of concept for further analysis of more complex particle systems like particles with a core shell architecture^[3] or more complex NP biotransformation like dissolution and re-precipitation of NP released ions^[4].

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TAILORING LIPID NANOPARTICLE SYSTEMS FOR DELIVERING NUCLEIC ACIDS TO A VARIETY OF TISSUES

DOMINIK WITZIGMANN,

CEO & Co-Founder, NanoVation Therapeutics

We are at the most exciting point in human history with respect to the launch and development of gene therapies. In our lifetimes, we will see major diseases including rare genetic, infectious, autoimmune, malignant and age-related disorders become treatable or even cured. The major issue we currently face is access to state-of-the-art platform technologies to safely and effectively target the diseased tissue. To enable the delivery of nucleic acids to a variety of (extra)hepatic tissues, we are developing a lipid nanoparticle (LNP) toolbox. Our flagship technology, the long-circulating LNP (IcLNP™) platform, enables functional nucleic acid delivery to extrahepatic tissues such as bone marrow, tumors, or skin. Underlying this and other technologies are high-yield synthesis pathways for specialty lipids and increased therapeutic indices compared to commercial standards. We at NanoVation are aggregating and creating new technologies to get 5 or 10 or even 100 gene therapies out of one LNP platform to treat a pinwheel of diseases.

GRAPHENE TRANSISTORS RECORDING AND MONITORING EPILEPSY

ROB WYKES

DC shifts and infraslow activity (ISA) are associated with pathological brain states including cortical spreading depression (CSD) and seizures. Recording ultraslow signals (frequencies <0.1 Hz) with microelectrodes is severely hampered by current electrode materials and technology primarily due to limitations resulting from high electrode impedance and voltage drift. Few studies have attempted to investigate CSD or the involvement of ISA in seizure initiation and propagation in awake brain. A major limiting factor has been the absence of experimental tools that allow concurrent recordings of both ISA and high frequency activity associated with seizures. Graphene transistor arrays (gSGFETs) have full-band recording capabilities resulting from the direct field-effect coupling of the active transistor in contrast to standard passive electrodes, as well as from the electrochemical inertness of graphene (Masvidal-Codina, E et al Nature Materials, 2019). We report the ability of epicortical and intracortical gSGFETs arrays for studying two types of pathological brain activities, CSD and seizures, in awake head-fixed mice. Chemoconvulsants (Picrotoxin 10mM and 4-AP 50mM) were used to induce epileptiform activity and seizures in awake mice and electrographic recordings made using either epidural or penetrating depth gSGFETs. Combined recordings using both epidural and penetrating gSGFETs allow us to determine how local activity across and through cortical columns become entrained during seizures. DC shifts were frequently observed either just before, at onset, or during seizures. Around 20% of seizures were followed by a post-ictal CSD. Additionally gSGFETs recorded cortical high frequency oscillations (HFO) ~200-500Hz. These novel devices therefore permit recording concurrently ictal baseline shifts and HFOs, markers identified as useful in localising seizure onset zones (SOZ). We are now implanting these devices in mouse models of focal cortical dysplasia to determine their usefulness in identifying SOZ; and in mouse models of migraine to detect spontaneous CSDs. They may also aid in preclinical investigations examining the link between post-ictal

NEXT GENERATION ANTIBIOTICS

ADA YONATH

Ribosomes are the universal cellular multicomponent particles that translate the genetic code to proteins. Owing to their high significance they are targeted by many antibiotics. Structures of complexes of bacterial and eubacterial ribosomes with the commonly used antibiotics that paralyze them, illuminated common pathways in their inhibitory-actions, synergism, differentiation and resistance. Comparisons of structures of ribosomes from multi-resistant pathogens to those of harmless bacteria illuminated unique features that may become sites for the design of novel, next generation, species-specific antibiotics, thus microbiome preserving, and degradable, thus eco-friendly.

RNA THERAPEUTICS IN OPHTHALMOLOGY – TRANSLATION TO CLINICAL TRIALS

CYNTHIA YU WAI MAN

The use of RNA interference technology has proven to inhibit the expression of many target genes involved in the underlying pathogenesis of several diseases affecting various systems. First established in *in vitro* and later in animal studies, small interfering RNA (siRNA) and antisense oligonucleotide (ASO) therapeutics are now entering clinical trials with the potential of clinical translation to patients. Gene-silencing therapies have demonstrated promising responses in ocular disorders, predominantly due to the structure of the eye being a closed and compartmentalised organ. However, although the efficacy of such treatments has been observed in both preclinical studies and clinical trials, there are issues pertaining to the use of these drugs which require more extensive research with regards to the delivery and stability of siRNAs and ASOs. This would improve their use for long-term treatment regimens and alleviate the difficulties experienced by patients with ocular diseases. This presentation provides a detailed insight into the recent developments and clinical trials that have been conducted for several gene-silencing therapies, including ISTH0036, SYL040012, SYL1001, PF-04523655, Sirna-027, QR-110, QR-1123, QR-421a and IONIS-FB-LRX in glaucoma, dry eye disease, age-related macular degeneration, diabetic macular oedema and various inherited retinal diseases. We aim to explore the potential of these nanomedicines whilst evaluating their associated advantages and disadvantages, and to discuss the future translation of RNA therapeutics in ophthalmology.

BEYOND HERD IMMUNITY: UTILIZING PRECISELY ORGANIZED NANOIMMUNOGEN TO CREATE A PERSONALIZED VACCINE FOR IMMUNOCOMPROMISED INDIVIDUALS.

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Current vaccines for SARS-CoV-2 are extremely **effective**, but their inflammatory nature is a complication in individuals with immune disorders. Moreover, herd immunity towards novel pathogens has proven unreliable and difficult to achieve, leaving immunocompromised individuals at risk. This highlights the need for vaccine plat-

forms that can be individualized and adapted for immunocompromised individuals and individuals with autoimmune disorders. ***Our strategy is based on short peptide epitopes that generate immune responses precisely aimed at the critical components of the SARS-CoV-2, limiting the adverse effects.*** The immunogen is the nano assembly in which one DNA molecule acts as a scaffold to short DNA oligonucleotides (probes) that are annealed in a sequence-specific manner. Each DNA probe carries a specific epitope (peptide) covalently conjugated to the end. The peptides are selected from reported neutralizing linear B-cell epitopes from the SARS-CoV-2 S-protein. Along with these DNA-peptide probes, the DNA nano-scaffold contains the T-helper epitope (PADRE peptide), which plays a critical role in T-helper-mediated immune response initiation and activation. Biophysical approaches validated the assembly of the nano-immunogen. Conjugation to gold nanoparticles was utilized to improve the uptake and biological stability of the construct. These validated nano-immunogen formulations were further tested for their ability to induce an antigen-specific immune response in mice. The results demonstrate that this assembly is immunogenic and generates neutralizing antibodies against SARS-CoV-2 wild type and its variants of concern (VOCs). Importantly, the selected peptide epitopes do not carry any of these mutations. This finding suggests that our vaccine should be equally efficient for all current variants, including the Omicron variant. The modular basis of the DNA nano-scaffold immunogen has many attractive features. Various ligands can be incorporated into the immunogen by appropriately modifying the DNA template to tailor the vaccine to the recipient. Finally, new epitopes can be introduced to rapidly modify the vaccine according to the identified mutations in the pathogen.

Key Words: variant of concern (VOC); SARS-CoV-2; peptide, vaccine, PADRE, epitope

ADVANCING REGULATORY SCIENCE BY KNOWLEDGE EXCHANGE ACROSS REGULATORY DOMAINS

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Due to their specific properties and sometimes complex structure, medicinal products utilizing nanomaterials present regulatory challenges with regard to proper quality and safety assessments. The Horizon 2020 project REFINE has identified such challenges and worked on the development of adequate solutions. Other product types utilizing nanomaterials are regulated differently, however, they are facing similar challenges related to the nanomaterials. Knowledge exchange between the various regulatory domains provides the most effective and efficient means to address such challenges. Examples of relevant regulatory domains besides medicinal products are medical devices, chemicals (substances), cosmetics, food and biocides. This was recognized also in the Gov4Nano project. Both projects have worked together to promote this by organizing meetings to bridge the various communities from these domains, including a joint session in January 2022. The high value this kind of knowledge exchange was confirmed by participants. It is the optimal way to advance regulatory science in all relevant domains. From a European policy perspective, this links also to the Chemical Strategy for Sustainability, including amongst others the “one substance-one assessment” ambition.

CLINAM

European Foundation for Clinical Nanomedicine

13/2022
Basel, May 2-4

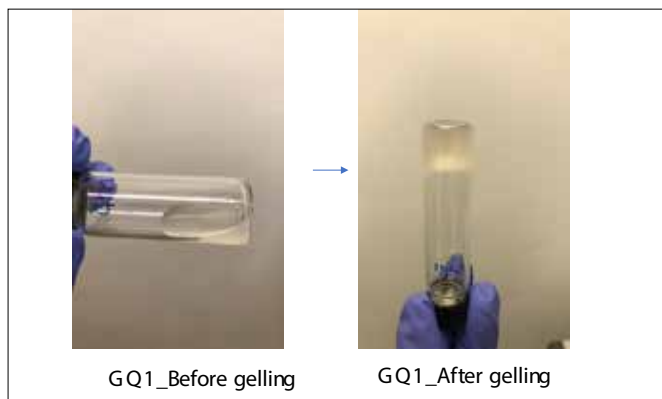
**ABSTRACTS
POSTERS**

CHITOSAN-BASED QUININE THERMOSENSITIVE GELS FOR THE INTRANASAL TREATMENT OF CEREBRAL MALARIA IN RURAL AREAS IN SUB-SAHARAN AFRICA

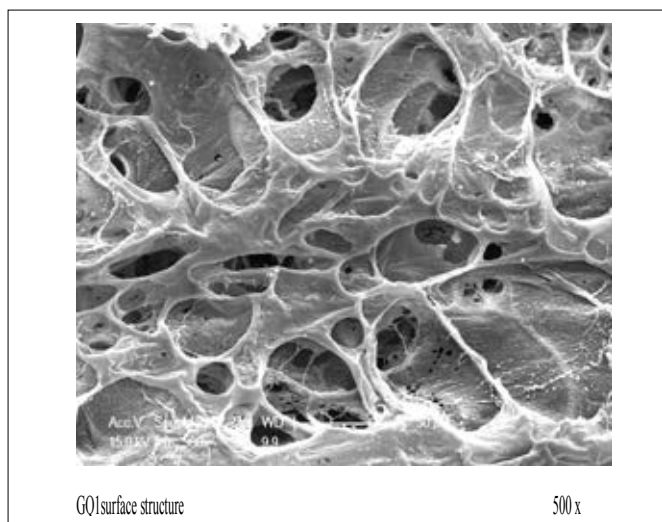
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Gel formation process



SEM Image of quinine TSGs

Background: Intranasal route of drug administration provides a safe and convenient alternative to access the central nervous system for the treatment of diseases such as cerebral malaria. However, nasal clearance of drugs prevents significant permeation of drugs from nasally administered formulations. Chitosan is a natural polymer, a permeation enhancer and a gel forming agent used for the design of drug delivery systems.

Aim: This research was aimed at formulating chitosan-based quinine thermosensitive gels (TSG) having characteristics for successful intranasal administration to serve as an alternative to parenteral quinine for the treatment of cerebral malaria.

Methods: TSGs containing 5% quinine were formulated using 1.5 and 1.8 % of chitosan, and appropriate quantities of cross-linking agent. Rheological behaviour, gelling temperatures and gelling times of formulations at $37 \pm 2^\circ\text{C}$ were determined. Morphological investigations and *in vitro* release and *ex vivo* permeation studies were also conducted.

Result: Quinine TSGs were successfully formulated and demonstrated rapid gelation at $37 \pm 2^\circ\text{C}$ with gelling times being $\leq 4.8 \pm$

0.03 minutes. Scanning electron microscope images of gels showed crosslinking as well as porous gel networks which permitted faster rate of drug release. *In vitro* and *ex vivo* release studies in pig nasal mucosa showed that quinine release was sustained.

Conclusion: Quinine chitosan-based TSGs designed for intranasal administration can serve as an alternative to parenteral route for the treatment of cerebral malaria in remote areas.

SOFT BIOMIMETIC LIPID MEMBRANE-BASED NANOPARTICLE CARRIERS OF NEUROPROTECTIVE COMPOUNDS

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Delivery of natural compounds promoting the neurotrophin receptor signaling in the central nervous system (CNS) present ongoing interest for combination therapy development. Recent research on human SARS-CoV-2 coronavirus has emphasized that COVID-19 can affect the brain depending on the severity of the viral infection. Coronavirus-provoked inflammatory changes, cerebrovascular and ischemic lesions can cause neuronal and axonal damages, which last several months and may lead to post-COVID-19 neuronal dysfunction and neuropsychiatric complications. It can be suggested that nanomedicine-based strategies may help for recovery from the neuronal damages in the post-COVID-19 infection related to early Parkinsonism and other neurological disorders. We investigate the structural advantages of a variety of biomimetic nanoparticulate systems ranging from liquid crystalline lipid nanocarriers with inner self-assembled organization (e.g., cubosomes and spongosomes) to liposomes for encapsulation of multiple neuroprotective compounds. Innovative liquid crystalline lipid nanoparticles were designed and prepared by self-assembly of lyotropic monoglyceride and phospholipids. Examples are presented for the encapsulation of the natural plant-derived antioxidant curcumin, fish oil rich in ω -3 polyunsaturated fatty acids (PUFA), and the neurotrophin brain-derived neurotrophic factor (BDNF). Structural experiments using the synchrotron SAXS method established the dimensions of the inner compartments serving as reservoirs for dual and multidrug-loaded lipid carriers of potential interest for nanomedicine development.

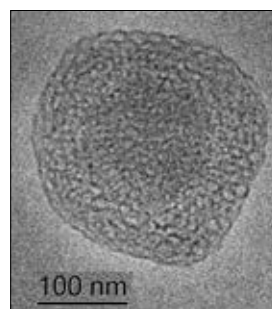


Figure: A cryo-TEM image of a core/shell cubosome particle of a mixed composition peptide/lipid/polymer [1].

ACKNOWLEDGMENT

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of melanoma and this combination therapy may have great potential to improve the immunotherapy in solid tumors.

Keywords: Dendritic Cell vaccine, gp100, Liposome, Celecoxib, Melanoma, Combination Therapy

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LIPOSOMAL CELECOXIB COMBINED WITH DENDRITIC CELL THERAPY IN B16F10 MOUSE MODEL FOR MELANOMA

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Introduction: The immunosuppressive nature of the tumor micro-environment caused by immunosuppressive cells like regulatory T cells (Tregs), inflammation and inhibitory factors limit the effectiveness of anticancer vaccines. Inspired by the role of cyclooxygenase-2 (COX-2) in inflammation in tumor site as well as induction of Tregs, we proposed that normalization of tumor microenvironment by celecoxib as a COX-2 inhibitor might improve the efficacy of dendritic cell (DC) therapy in a melanoma model. In the present study, liposomal celecoxib was combined with ex vivo generated DC vaccines pulsed with gp100 antigen (in liposomal and non-liposomal forms) in both a prophylactic and therapeutic model.

Method: The thin film plus sonication method was used for the preparation of liposomes. Liposomal celecoxib was combined with dendritic cells matured by gp-100 peptide linked to the liposomes. The therapeutic efficacy of this combination was evaluated in B16F10 bearing C57BL/6 mice. Immunological tests such as Enzyme-linked immunospot (ELISpot) assay, flow cytometry and cytotoxicity assay were performed on splenocyte suspensions, and the remaining mice were evaluated for tumor growth and survival analysis. Graph Pad Prism 6 Software was used to analyze the data.

Results: The present study demonstrated that DC vaccines pulsed with gp100 peptide with or without liposomal formulation together with liposomal celecoxib enhanced prophylactic and therapeutic anti-tumor immune responses. The immunological tests showed that the co-administration of dendritic cells + liposomal gp-100 peptide + liposomal celecoxib leads to a significant amount of IFN- γ secretion and increases the number of tumors infiltrated lymphocytes (TILs) and cytotoxic activity. This therapeutic combination demonstrated an effective immune response, effective tumor regression and prolonged survival in C57BL/6 mice bearing B16F10 melanoma (Figure 1).

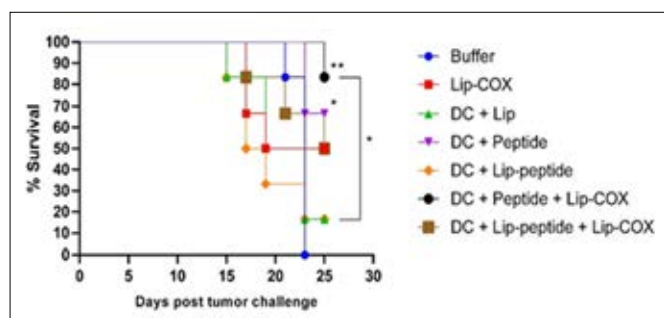


Figure 1 Survival analysis of therapeutic groups were monitored by the multiple comparison log-rank (Mantel–Cox) test. Effects of treatments on survival time were monitored for a period of 30 days among B16F10 tumor model of C57BL/6 mice (n=6).

Conclusions: In conclusion, our findings suggest that DC therapy alongside liposomal celecoxib has a significant effect on immune responses and enhanced therapeutic outcomes in a mouse model

DEVELOPMENT OF INHALABLE RETINOIC ACID-LOADED POLYMERIC NANOPARTICLES AS TARGETED HOST DIRECTED IMMUNOTHERAPY FOR MYCOBACTERIUM TUBERCULOSIS

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Introduction: Tuberculosis (TB) is the top bacterial infectious disease killer and one of the top ten causes of death worldwide. The emergence of strains of multiple drug-resistant tuberculosis (MDR-TB) has pushed our available stock of anti-TB agents to the limit of effectiveness. An adjunctive, host-directed therapy (HDT) designed to act on the host, instead of the bacteria, by boosting the host immune response through activation of intracellular pathways could help address this issue. The integration of multidisciplinary approaches of repurposing currently FDA-approved drugs, with a targeted drug-delivery platform is a very promising option to accelerate new therapeutics reaching the clinic. Previous work conducted by our group showed the efficacy of All Trans Retinoic Acid (ATRA) as a HDT toward TB both *in vitro* and *in vivo* (1,2). The ultimate goal of this project is to develop Poly-Lactic-co-Glycolic Acid (PLGA) nanoparticles (NPs) to target ATRA to the lungs via inhalation and enhance uptake by alveolar macrophages (AM) which are the host cells for *Mtb*.

Methods: ATRA was encapsulated into PLGA nanoparticles using a nanoprecipitation method. Size and zeta potential were measured using a Zetasizer. Efficacy studies were conducted *in vitro* using THP-1 derived macrophages infected with the avirulent *Mtb* strain (H37Ra) and determined by the BACT/ALERT® liquid culture system (3). MTS and propidium iodide (PI) exclusion assays were used to determine the cell viability in THP-1 derived macrophages and A549 alveolar epithelium. Cellular uptake of ATRA-loaded NPs into differentiated THP-1 cells was evaluated using confocal laser scanning microscopy (CLSM). The formulation was integrated with a vibrating mesh nebulizer (Aerogen Solo®, Aerogen, Ireland); aerosol droplet size was characterized using laser diffraction (Spraytec, Malvern Instruments, UK) and apparatus 5/E (Westech, UK). The inhaled dose percent in an adult breathing profile (500 mL

tidal volume, 15 breaths per minute, Inhalation to exhalation ratio: 1:1, and Waveform: sinusoidal) was generated using a breathing stimulator (BRS 2100, Copley Scientific Ltd., UK). A scalable nanomanufacturing approach was optimized using microfluidics mixing (Ignite Nanoassembler[®], Precision nano-systems, Canada).

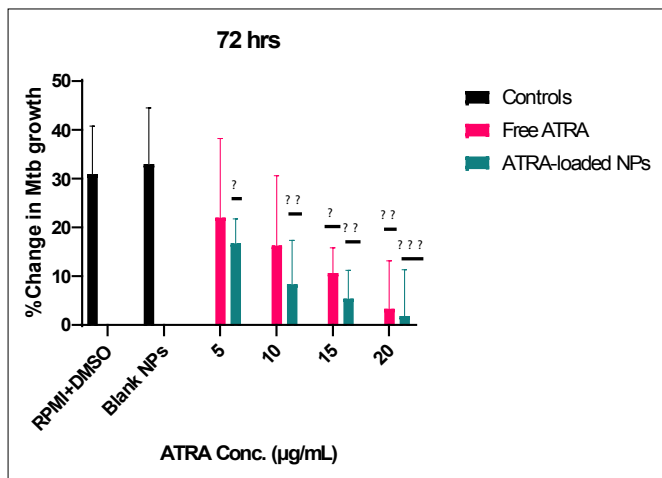


Figure 1 : ATRA treatment arrests growth of *Mtb* (H37Ra) *in vitro*. Baseline infection levels were measured at 3 hrs post-infection of THP-1 derived macrophages infected with H37Ra which was followed by the addition of treatment. Treatment groups included RPMI+DMSO, ATRA solution (20, 15, 10, 5 µg/mL), unloaded-PLGA NPs (Blank NPs), ATRA-loaded PLGA NPs (equivalent to 20, 15, 10, 5 µg/mL of ATRA). Efficacy of treatment was assessed at day 3 post-treatment by monitoring the change in bacterial growth (%), using the BacT/Alert[®] 3D system (BioMerieux), MOI: 1–10/cell (n=3). Statistical analysis was performed using two-way ANOVA with Tukey's post-hoc test comparing RPMI+DMSO and Blank NPs groups as reference. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, and **** $p < 0.0001$

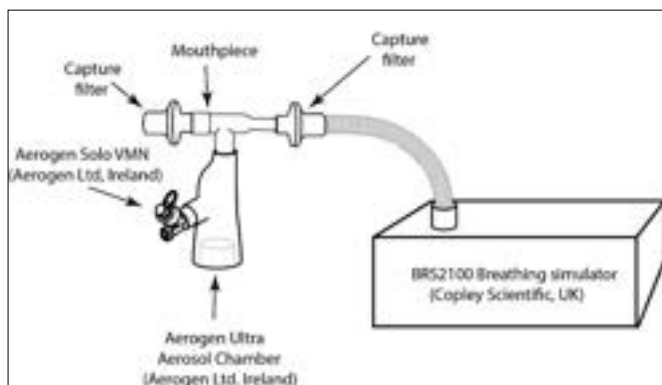


Figure 2: Experimental setup for Inhaled Dose assessment

Results: We successfully developed a host-directed formulation for TB, using ATRA-loaded PLGA nanoparticles with a size of 261.6 and neutral zeta potential. Confocal laser scanning microscopy (CLSM) showed efficient cellular delivery of ATRA-loaded NPs into macrophages and extensive distribution in the cytoplasm. Cell viability studies indicated that the nanoparticles did not lead to significant cytotoxicity in macrophages or alveolar epithelial cells and efficacy studies conducted in an *in vitro* TB infection model have demonstrated a dose dependent reduction in *Mtb* growth (H37Ra). The aerosol had a volumetric median diameter (VMD) of 4.09 µm and mass median aerodynamic diameter (MMAD) of 2.13 µm. 65.1% of the dose was inhaled in an adult breathing simulation experiment.

Conclusion: This type of targeted inhaled HDT offers an innovative approach for TB treatment with the potential to enhance current therapeutic regimens thereby providing bet-

ter prognosis for patients, and reducing the incidence rate of MDR-TB.

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NOSE-TO-BRAIN DELIVERY OF RILUZOLE-LOADED POLYMERIC NANOPARTICLES FOR THE TREATMENT OF AMYOTROPHIC LATERAL SCLEROSIS

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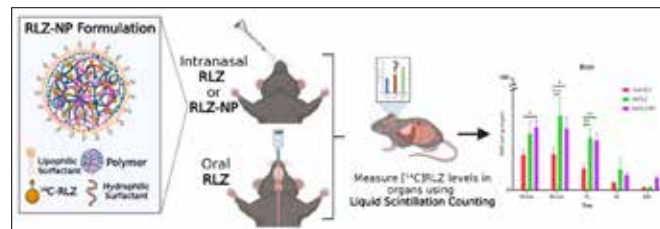


Figure 1 Schematic representation of workflow for testing the biodistribution of formulated riluzole-NP *in vivo*.

Introduction

Amyotrophic lateral sclerosis (ALS) is a deadly heterogeneous neurodegenerative disorder involving progressive loss of function of motor neurons leading to death due to respiratory failure within 3 years of symptoms onset. Riluzole (RLZ), the drug of choice for ALS, has inadequate pharmacokinetics and efficacy despite promising preclinical results. Therefore, we sought to develop a biocompatible nanoparticulate system for RLZ utilising the convenient Nose-to-Brain (N2B) delivery route which provides the opportunity to bypass the BBB and target the brain non-invasively through the olfactory and trigeminal cranial nerves and test the formulation *in vivo*.

Methods

[¹⁴C]RLZ was synthesised in a modified 1-pot chemical synthesis method for *in vivo* biodistribution study. RLZ polymeric nanoparticles (RLZ-NP) were formulated by nanoprecipitation method. Particle size (z-average), polydispersity and surface charge (z-potential) of RLZ-NP were characterised using dynamic light scattering (DLS). The encapsulation efficiency (EE%) was measured using High-Performance Liquid Chromatography (HPLC). [¹⁴C]RLZ radioactivity was measured using Liquid Scintillation Counting (LSC). *In vivo* biodistribution studies were carried out in C57BL/6 mice after intranasal or oral administration at different timepoints. Data were analysed using GraphPad Prism.

Results

RLZ nanoparticles (RLZ-NP) had a particle size <120 nm suitable for N2B delivery and an EE% >10%. RLZ was radiolabelled with [¹⁴C] for *in vivo* pharmacokinetic studies in a >50% synthesis yield using an improved 1-pot method which was confirmed by HPLC, mass spectrometry and ¹H-NMR. RLZ was successfully delivered to the central nervous system *via* N2B with significantly higher amounts in the brain compared with

oral delivery for both free RLZ and RLZ-NP formulations across measured timepoints. RLZ-NP formulation offered an alternative to the 2% DMSO in 20% Tween-80 solubilising agent to RLZ without compromising brain uptake.

Conclusion

In the data shown we demonstrated the formulation of biodegradable nanoparticles encapsulating RLZ with promising physicochemical characteristics appropriate for N2B delivery for the treatment of ALS. N2B delivery of RLZ-NP achieved higher RLZ amounts in the CNS non-invasively, comparable to its free RLZ counterpart but was advantageous in not requiring high amounts of surfactants and harmful organic solvents (i.e., DMSO) per dose, making the delivery system more suitable for clinical use.

ASSESSMENT OF CELL PHENOTYPE FOLLOWING REPEAT EXPOSURE TO NRTIS: FTC AND 3TC

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HIV treatment requires chronic exposure to antiretrovirals, as it is incurable (Ruelas & Greene, 2013). Long-acting formulations of antiretroviral medication can help to improve adherence to medication, resulting in better treatment outcomes (Chandiwana et al., 2021). Currently nucleoside reverse-transcriptase inhibitors (NRTIs): emtricitabine (FTC) and lamivudine (3TC) are being explored in long-acting delivery. A key question in this type of delivery is, with long-acting formulations, does chronic exposure of the drugs to human cells alter their phenotype?

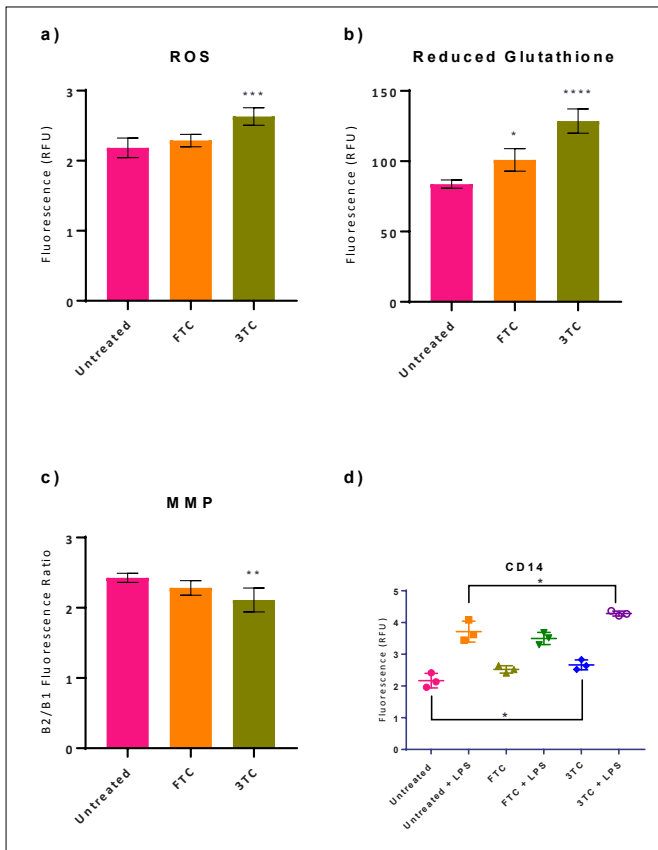


Figure 1: THP-1 cells exposed to FTC and 3TC for 7 weeks and subsequent phenotypic assessment. a) Intracellular ROS, $n=4$, mean \pm SD. b) Intracellular reduced glutathione, $n=4$, mean \pm SD. c) MMP, $n=4$, mean \pm SD. d) CD14 marker expression, as assessed by flow cytometry $n=3$, mean \pm SD. $P < 0.0001 = ****$, $P < = ***$, $P < 0.01 = **$ and $P < 0.05 = *$.

THP-1 (human monocytic cell line), MUTZ-3 (human dendritic cell (DC) line), CEM (human T cell line) and KU812 (human basophil cell line) were cultured in either basal media or media containing either FTC (1.8 $\mu\text{g}/\text{mL}$) or 3TC (2 $\mu\text{g}/\text{mL}$) for 7 weeks, passaged twice-weekly, followed by an assessment of cellular phenotype. Intracellular reactive oxygen species (ROS) was measured using the CellROXTM Green Reagent, intracellular reduced glutathione was measured using the ThiolTrackerTM Violet Dye and mitochondrial membrane potential (MMP) was assessed using the JC-1 reagent (InvitrogenTM). Positive activation controls, specific to each cell type, were included in the evaluation: THP-1 cells were treated with LPS, MUTZ-3 cells were treated with LPS or Resiquimod (R848), KU812 cells were treated with C5a or calcium ionophore/PMA and CEM cells were treated with PHA. Expression of a number of cell-surface markers in each cell line was assessed via flow cytometry.

Intracellular ROS levels were significantly higher (20%) in the 3TC-cultured THP-1 cells than the untreated cells ($P = < 0.001$) (Figure 1a). Intracellular reduced glutathione levels were also significantly higher (20% and 54% respectively) in both the FTC- and 3TC-cultured THP-1 cells compared to those untreated ($P = < 0.05$ and $P = < 0.0001$ respectively) (Figure 1b). 3TC-cultured THP-1 cells also demonstrated a significant reduction (13%) in the MMP ($P = < 0.01$) (Figure 1c). When compared to the untreated THP-1 cells, 3TC-treated cells showed a significantly higher expression of CD14 ($P = < 0.05$) (Figure 1d). When compared to the LPS-treated cells, the 3TC-cultured LPS-treated cells showed significantly more CD14 expression ($P = < 0.05$) (Figure 1d).

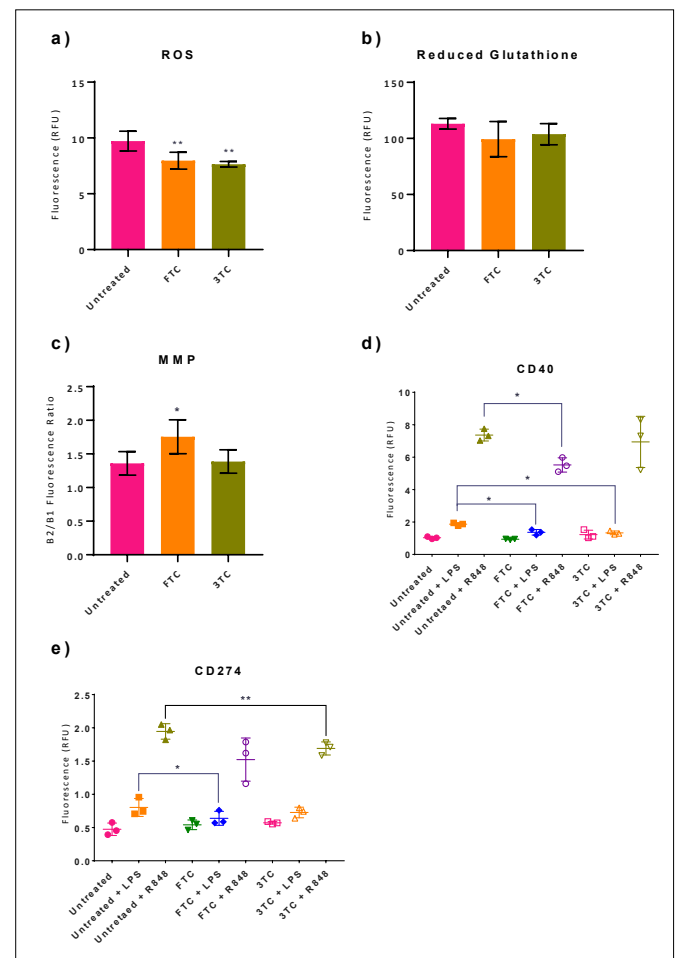


Figure 2: MUTZ-3 cells exposed to FTC and 3TC for 7 weeks and subsequent phenotypic assessment. a) Intracellular ROS, $n=4$, mean \pm SD. b) Intracellular reduced glutathione, $n=4$, mean \pm SD. c) MMP, $n=4$, mean \pm SD. d) CD40 marker expression, $n=3 \pm$ SD. e) CD274 marker expression, $n=3 \pm$ SD. $P < 0.0001 = ****$, $P < = ***$, $P < 0.01 = **$ and $P < 0.05 = *$.

Intracellular ROS levels were significantly lower (18% and 21% respectively) in the FTC- and 3TC-cultured MUTZ-3 cells, than the

untreated cells ($P < 0.01$) (Figure 2a). FTC-cultured THP-1 cells displayed a significant increase (29%) in the MMP ($P < 0.05$) (Figure 2b). When compared to the LPS-treated cells, the FTC- and 3TC-cultured LPS-treated cells showed a significant decrease in CD40 expression ($P < 0.05$) (Figure 2c). R848-treated MUTZ-3 cells in comparison with the FTC-cultured R848-treated cells showed significantly less CD40 expression ($P < 0.05$) (Figure 2d). When compared to the LPS-treated cells, the FTC-cultured LPS-treated cells showed a significant decrease in CD274 expression ($P < 0.05$) (Figure 2e). Untreated, R848 treated cells when the 3TC cultured, R848 treated cells showed a significant decrease in CD274 expression ($P < 0.05$) (Figure 2e).

Higher production of ROS would intuitively be associated with a lower amount of reduced glutathione. In THP-1, however, we have observed the opposite. This may be a consequence of prolonged increased ROS levels, leading to a greater intracellular reserve of reduced glutathione to counteract the deleterious effects of elevated ROS. CD14 expression is known to increase as a result of LPS challenge (Zamani, Zare Shahneh, Aghebati-Maleki & Baradaran, 2013), if following repeated exposure of the cells to 3TC causes a basal increase of CD14 expression, this may potentially impact subsequent exposure of cells to LPS or other pathogens. CD40 is upregulated on activated DCs (Ma & Clark, 2009), a decreased expression of CD40 suggests that both FTC and 3TC may have impacted the MUTZ-3 cells ability to respond to DC-specific positive controls. This could potentially imply that repeated exposure could cause a reduced ability of the bodies DCs to fight off immunological challenges. CD274, also known as programmed death ligand 1 (PD-L1), plays a role in controlling T-cell responses via the receptor PD-1 (Hudson, Cross, Jordan-Mahy & Leyland, 2020), this again poses the question whether repeated exposure may prevent the immune system from carrying out its normal roles. These results have, potential, consequences for long-acting formulations and implants as they show possible effects of repeat exposure to antiretrovirals that may be used in such preparations.

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INTERACTION OF NANOCARRIERS WITH ANTI-PEG ANTIBODIES

MAREIKE DEUKER

Today's gold standard polymer for many biomedical applications is poly(ethylene glycol) (PEG).^[1] PEG is often used to reduce unspecific protein adsorption on nanocarriers (NC) and to prolong their circulation time by the attachment on the NC's surface – the so-called "PEGylation".^[2] It is an important approach to improve the pharmacokinetics and pharmacodynamics of biopharmaceuticals. It can increase their water-solubility and stability, decrease enzymatic degradation, reduce the immunogenicity and extend the blood circulation half-life.^[3] This is due to the reduction of non-specific interactions with their environment (such as protein adsorption) and is known as the "stealth effect".^[4] Nevertheless, there are increasing reports that PEG-binding antibodies can be elicited in humans. Various research groups observed that the administration of repeated

doses of PEGylated NC led to an accelerated blood clearance and weakened efficacy of PEGylated therapeutics.^[5] In contrast to most antidrug antibodies, anti-PEG antibodies were observed in both PEGylated therapeutics-treated patients and healthy (treatment-naïve) individuals. A study by Chen *et al.* investigated pre-existing anti-PEG antibodies in healthy Han Chinese and found that 44.3% participants were positive for anti-PEG antibodies. This high prevalence might be due to exposure to free PEG, which is present in commonly used products including processed food and cosmetics.^[6]

The presence of anti-PEG antibodies has been correlated with reduced efficacy of PEGylated therapeutics in clinical trials. Doxil, a PEGylated liposome, caused immediate hypersensitivity reactions upon first injection in some patients.^[7] Acute severe allergic reactions to pegnivacogin, a PEGylated aptamer, were observed exclusively in those with pre-existing anti-PEG antibodies and were associated with complement activation and tryptase release.^[8] In animal models, a repeated injection of PEGylated liposomes induced the formation of anti-PEG IgM and enhanced clearance of a second dose. Cheng *et al.* raised the assumption that the ethylene oxide repeating unit in PEGylated NC acts as a TI-2 (thymus independent) antigen and could be an immunogenetic epitope of PEG and a binding site for anti-PEG IgM. TI-2 antigens can induce an immunological response by cross-linking the cell surface immunoglobulins of specific B cells, resulting in secretion of IgG and IgM from the B cells.^[9] Binding of IgM can trigger opsonization of complement factors that subsequently promote phagocytosis by Kupffer cells.^[10]

Despite the potential serious consequences of circulating anti-PEG antibodies, their influence on the effect of therapeutics and on related side effects remains an unanswered question. To address this question, a detailed study of pre-existing anti-PEG antibodies in healthy individuals among the German population was performed using an enzyme linked immunosorbent assay (ELISA) (Figure 1). A high prevalence of anti-PEG IgG and IgM was found throughout the samples. Notably, the concentration and prevalence decreased with increasing age. To further evaluate the biological response, the role of anti-PEG antibodies in the protein corona was investigated. The protein corona is the biological coating of the NC that creates its biological identity as recognized by cells.^[11] We investigated the enrichment of anti-PEG antibodies in the protein corona of PEGylated silica nanocapsules (SiNC). An enrichment of anti-PEG antibodies in the protein corona of PEGylated NC compared to non-PEGylated NC could be observed. Additionally, the cellular uptake of PEGylated NC with varying amounts of bound anti-PEG antibodies was monitored. The cell uptake in macrophages increased with the anti-PEG antibody concentration in the protein corona. These results suggest that the existence and concentration of anti-PEG antibodies in the protein corona of different (PEGylated and non-PEGylated) NC should be further evaluated to determine the potential effects *in vivo*.

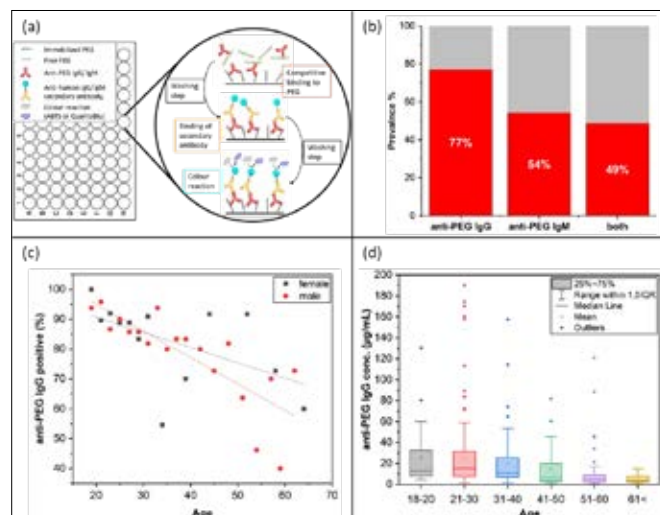


Figure 1: Plasma screening to analyse anti-PEG antibody concentration and prevalence in a sample of the German population ($n = 500$). (a) Schematic setup of the ELISA. (b) Prevalence of anti-PEG IgG antibodies (grey: all samples (100%) red: anti-PEG antibody positive samples). (c) Distribution of anti-PEG IgG positive samples with age (grouping of $10 \leq$ samples per data point). (d) Concentration of anti-PEG IgG antibodies in age groups.

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macological properties of both formulations, their therapeutic efficacy was proven *in vivo* in a mechanically-induced OA mouse model (PTOA) (Fig. 2a and b).

μ PLs, synthesized using 15 mg of PLGA, displayed a squared shape with a length of 20 μ m and a height of 10 μ m (Fig. 1a and b). Mechanically speaking, they showed an apparent Young's modulus of ~ 3 MPa, similar to that of cartilage, and a high damping capability ($\tan \delta = 0.3$) (Fig. 1c and d). Also, both developed formulations demonstrated a good drug loading and a sustained and continuous drug or particles release in biologically relevant volumes, still preserving their pharmacological activity.

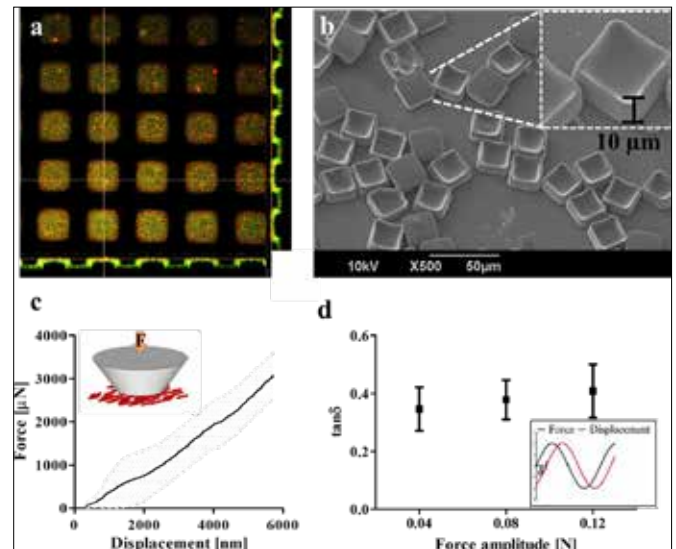


Figure 1: PLGA microPlates (μ PLs) physico-chemical and mechanical characterization. a. Confocal microscopy image of the PVA template containing Cy5-siNPs (red) dispersed within PLGA paste. b. SEM images of the μ PLs released from the PVA template. c. Force-displacement curve for a flat punch indentation experiment on an ensemble of μ PLs (average curve and standard deviation). In the inset, a schematic of the experimental setup is provided. d. Energy dissipation capability of μ PL upon cyclic mechanical loading (frequency 5 Hz) as a function of the force oscillation amplitude. In the inset, a schematic of the testing routine is provided highlighting the phase angle δ -dissipation parameter.

POLYMERIC SQUARED MICROPLATES AS A NEW TOOL FOR THE LOCAL TREATMENT OF POST-TRAUMATIC OSTEOARTHRITIS

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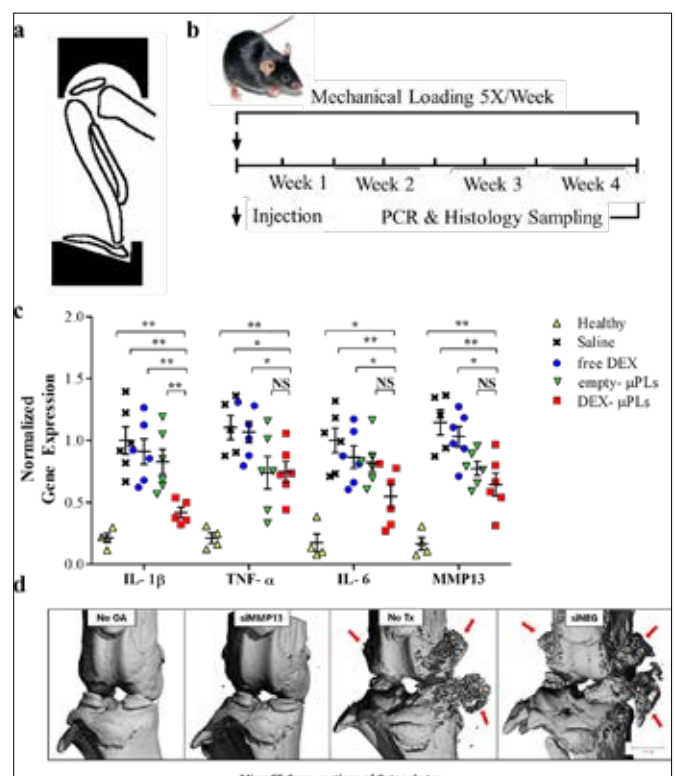
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Osteoarthritis (OA) is a chronic disabling disease that affects people of all age around the world. It is caused by the combination of bio-mechanical factors and genetic predisposition, resulting in substantial pain, function loss and, eventually, permanent disability. So far, therapeutic strategies clinically available help to alleviate symptoms and enhance temporarily joint mobility and function, without reverting OA progression itself.

Within this framework, a top-down strategy was applied for developing micro-sized square -poly(D,L-lactide-co-glycolide) (PLGA) microPlates (μ PLs) for local, slow and continuous releases of small anti-inflammatory molecules or nanoparticles loaded themselves with drugs. Dexamethasone (DEX)² and matrix metalloproteinase 13 (MMP-13) RNA interference nanoparticles (siMMP13-NPs)³ were selected as payloads and two different formulations were developed. After investigating physical-chemical, mechanical and phar-



Both formulations showed good results *in vivo* on PTOA model (Fig. 2). On one side, a single intra-articular (IA) injection of DEX- μ PLs reduced the expression of pro-inflammatory cytokines, such as IL-1 β , TNF- α , IL-6 and MMP-13. Also, they protected articular cartilage and synovial tissues from load-induced histological changes compared to Saline and DEX free groups (Fig. 2c). While on the other, a single IA injection of siMMP13-NPs loaded μ PLs provided 70% of gene silencing efficiency and reduction of related inflammatory markers and damages in the same animal model (Fig. 2d)³.

Figure 2: *In vivo* therapeutic efficacy of μ PLs on PTOA model. a. Schematic of the loading fixture used in the mechanical loading of mouse knee joints to induce PTOA. b. Mechanical loading regimen. c. *in vivo* expression of IL-1 β , TNF- α , IL-6, and MMP-13 after a single IA injection of DEX- μ PLs measured by TaqMan qPCR. d. MicroCT analysis of topographic mineralization and osteophyte outgrowth at 28 days after siMMP13- μ PL treatment.

In conclusion, top-down approach allowed to synthesize shaped-defined μ PLs that can be used as local device for OA treatment, able to act on both biomechanical and pharmacological disease aspects.

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ACHIEVING DENDRITIC CELL SUBSET-SPECIFIC TARGETING *IN VIVO* BY SITE-DIRECTED CONJUGATION OF TARGETING ANTIBODIES TO NANOCARRIERS

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The major challenge of nanocarrier-based anti-cancer vaccination approaches is their targeted delivery of antigens and immunostimulatory agents to cells of interest, such as specific subtypes of dendritic cells (DCs), in order to induce robust antigen-specific anti-tumor responses. An undirected cell and body distribution of nanocarriers can lead to unwanted delivery to other immune cell types like macrophages reducing the vaccine efficacy. An often-used approach to overcome this issue is the surface functionalization of nanocarriers with targeting moieties, such as antibodies, mediating cell type-specific interaction. Numerous studies could successfully prove the targeting efficiency of antibody-conjugated carrier systems *in vitro*, however, most of them failed *in vivo* when targeting DCs that is partly due to cells of the reticuloendothelial system unspecifically clearing nanocarriers from the blood stream via Fc receptor ligation.

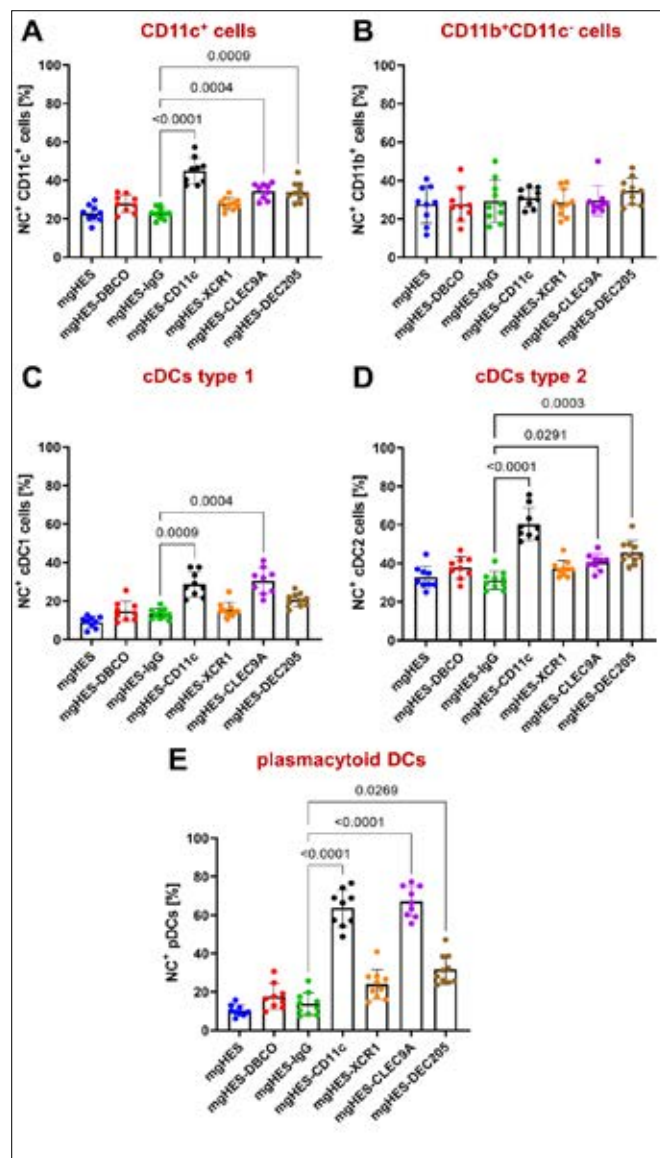


Figure 1. Targeting of dendritic cell subsets *in vivo*. Mice were treated with mgHES nanocarriers functionalized with different DC-targeting antibodies. Nanocarrier uptake by (A) CD11c⁺ cells, (B) CD11b⁺CD11c⁻ macrophages, (C) conventional DCs type 1, (D) conventional DCs type 2, and (E) plasmacytoid DCs within the spleen was determined using flow cytometry. Data represent mean \pm SD. All nanocarrier formulations were compared to the IgG control (mgHES-IgG) and significance was given with $p < 0.05$ using a Kruskal-Wallis test followed by a Dunnett's multiple comparison test. Individual p values are indicated in the graph.

Therefore, this study shows a surface functionalization strategy to site-specifically attach antibodies in an orientated direction onto the nanocarrier surface. Different DC-targeting antibodies, such as anti-CD11c, anti-CLEC9A, anti-DEC205 and anti-XCR1, were conjugated to the nanocarrier surface at their Fc domains. Anti-mouse CD11c antibody-conjugated nanocarriers specifically accumulated in the targeted organ (spleen) over time. Additionally, antibodies against CD11c and CLEC9A proved to specifically direct nanocarriers to the targeted DC subtype, conventional DCs type 1.

In conclusion, site-directed antibody conjugation to nanocarriers is essential in order to avoid unspecific uptake by non-target cells while achieving antibody-specific targeting of DC subsets. This novel conjugation technique paves the way for the development of antibody-functionalized nanocarriers for DC-based vaccination approaches in the field of cancer immunotherapy.

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CO-DELIVERING OF DOCETAXEL AND CURCUMIN USING POLYMERIC NANOCONSTRUCTS FOR THE TREATMENT OF NEUROBLASTOMA

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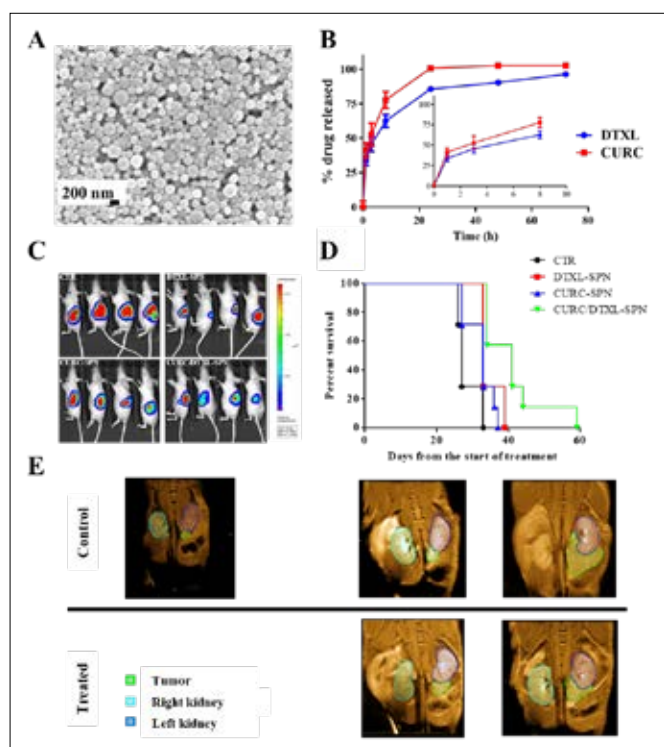


Figure 1. A. Representative TEM image of empty SPN; B. Release profile of CURC and DTXL from CURC/DTXL-SPN in PBS, at pH 7.4 and 37 °C; C. Representative bioluminescence imaging taken 3 weeks post treatment initiation (24h post 9th administration) for the 3 nanoformulation therapeutic groups (CURC-SPN; DTXL-SPN; and CURC/DTXL-SPN) and untreated control (CTR); D. Kaplan-Meier survival curves with corresponding statistical analysis comparing the nanoformulation therapeutic groups with CTR and CURC/DTXL-SPN with SPN monotherapy; E. Longitudinal tumor burden by MR imaging analysis (green contour: tumor; light blue contour: right kidney with tumor; dark blue contour: left kidney with no tumor).

Introduction: Neuroblastoma (NB) is the most common extracranial solid tumor, arising from the sympathetic nervous system. It represents 8-10% of all childhood cancer and 15% of deaths from pediatric tumor. It is characterized by biological and clinical heterogeneity and, despite aggressive therapies, by adverse outcomes. Also, most approved anticancer drugs are being used at maximally tolerated doses, leading to short- and long term toxicity in many patients. [1] These drawbacks highlight the need to search novel

therapeutic strategies with the aim of beating the disease, as well as minimizing chemotherapy-associated toxicity. Nanomedicine and combination therapy could represent a good strategy for pursuing this aim [2]. In this attempt, polymeric biodegradable nanoconstructs (SPNs) for co-delivering Docetaxel (DTXL) and Curcumin (CURC) to NB malignant masses were developed. These nanoconstructs were characterized from physical- chemical point of view and their therapeutic efficacy was tested *in vitro* and *in vivo*.

Methods: Empty, DTXL-SPNs, CURC-SPNs and DTXL/CUR-SPNs were synthesized using an oil-in water emulsion/solvent evaporation technique. [3] Their size and surface zeta potential were estimated by dynamic light scattering (DLS). The SPNs cytotoxicity was assessed by an MTT assay on the human NB cell line SH-SY5Y. Particles *in vivo* efficacy and biodistribution profile were studied in homozygous CD1 nu/nu athymic female mice (4 to 6-weeks old) injected with SH-SY5Y cells in the left adrenal gland.

Results: Empty and drugs loaded particles were characterized by a narrow size distribution (PdI < 0.15) with an average size of about 190 nm (Fig 1A). All formulations presented a negative surface z-potential and were stable for 4 days under physiological conditions. A biphasic release profile was observed for all the 3 formulations, with almost 90% of the total drug mass released within the first 24 hours (Fig 1B). *In vivo* experiments showed that mice treated with CURC/DTXL -SPNs had a significant increase in life span as compared to untreated mice (control) (p=0.0002), mice treated with CURC-SPNs (p=0.0205), DTXL-SPNs (p=0.0391), and free DTXL (p=0.0054) (Fig 1 C and D). Biodistribution results exhibited a 2% ID/g accumulation of the injected dose per tumor mass, regardless of the tumor development stage. This behavior is in agreement with results from a longitudinal Magnetic Resonance Imaging analysis of the malignant masses (Fig 1E).

Conclusion: This work has demonstrated that by combing Nanomedicine with combination therapy it's possible to modulate NB progression with a significant increase in overall survival.

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DEVELOPMENT OF A LIPOSOMAL NANO-FORMULATION FOR THE TREATMENT OF LYOSOMAL STORAGE DISEASES

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Introduction: Lysosomal storage diseases (LSD) have an incidence of 1:5000 births and comprise over 70 inherited disorders caused by various lysosomal dysfunctions. LSD is characterized by the accumulation of metabolic substrates in the lysosomes due to defective or missing lysosomal enzymes, with consequent organ impairment in patients.¹most of which are inherited as autosomal recessive traits. These disorders are individually rare but collectively affect 1 in 5,000 live births. LSDs typically present in infancy and childhood, although adult-onset forms also occur. Most LSDs

have a progressive neurodegenerative clinical course, although symptoms in other organ systems are frequent. LSD-associated genes encode different lysosomal proteins, including lysosomal enzymes and lysosomal membrane proteins. The lysosome is the key cellular hub for macromolecule catabolism, recycling and signalling, and defects that impair any of these functions cause the accumulation of undigested or partially digested macromolecules in lysosomes (that is, 'storage'). Among them, Pompe disease is a rare condition characterized by cardiomegaly and severe hypotonia in children and muscle atrophy in adults, and is caused by the lack of the lysosomal α -glucosidase. Current treatments, such as enzyme replacement therapy, aim at restoring physiological cell function by delivering the missing enzyme. However, patients soon develop an immune response against the recombinant protein, and subsequent treatments are only palliative.² Our aim is to decrease α -glucosidase immunogenicity and improve its therapeutic index by encapsulating it into drug delivery systems, such as liposomes,³ giving patients a chance to benefit from enzyme replacement therapy without incurring in potentially lethal side effects (Figure 1).

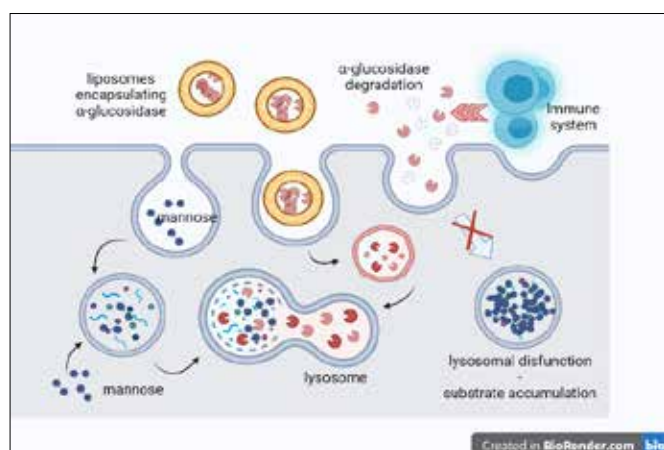


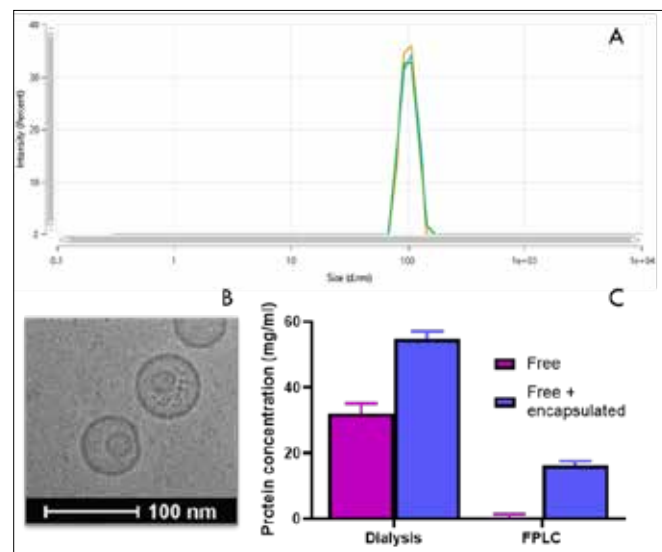
Figure 1: Schematics of the rationale behind this work. Pompe disease patients miss the lysosomal acid α -glucosidase and accumulate substrates in cells. Enzyme replacement therapy with Myozyme, a recombinant α -glucosidase, reverts the phenotype but can cause severe immune reactions after repeated administrations. Encapsulation into drug delivery systems can decrease enzyme immunogenicity and increase its bioavailability in patients.

Methods: To accomplish this, the α -glucosidase currently used in the clinical practice is encapsulated into nanoparticle delivery systems. The formulation used in this study includes clinically approved lipids such as DOPC and DSPE, to reduce the timing of formulation approval. PEG lipids are used to increase the nanoparticle circulation time and decrease its immunogenicity. Lipid film hydration and microfluidics are compared to improve the enzyme encapsulation process, and techniques such as dialysis and FPLC are used to effectively remove the excess of free, unencapsulated protein. Enzyme activity is evaluated during the encapsulation process and *in vitro* on patient-derived cells. Moreover, stability and uptake testing are performed *ex vivo* and *in vitro* in biologically-relevant conditions, such as human serum at physiological concentrations.

Results and conclusions: We optimized a method for the encapsulation of α -glucosidase into liposomes, which allowed us to produce monodispersed formulations of about 100 nm. We were able to effectively remove the free unencapsulated enzyme, that might otherwise trigger an immune reaction in patients (Figure 2). Moreover, we showed that the formulation process does not significantly alter enzyme activity. We are currently testing the stability in relevant biological media of formulations containing a variable lipid ratio and PEG content and we are optimizing an *ex vivo* platform for measuring nanoparticle immunogenicity *ex vivo* in patient-derived samples.

Figure 2: Liposomal Characterization. A) Liposome size is assessed by DLS in PBS, resulting in vesicles of 100 nm in diameter. B) CryoEM

images were taken after FPLC and show the presence of enzymes into the lipid bilayer and their absence outside of the formulation. C) Enzyme concentration measured by BCA assay. Liposomes were dialyzed using a 300 kDa cellulose membrane or run through a Superdex size exclusion chromatography column (FPLC). Protein concentration was measured in the absence (free) or presence (free + encapsulated) of 1% Triton X-100, to release the encapsulated enzyme. Results show that FPLC can remove most of the free enzymes outside the bilayer.



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LIPID NANOPARTICLE FORMULATION OF NICLOSAMIDE (NANO NCM) EFFECTIVELY INHIBITS SARS-COV-2 REPLICATION IN VITRO.

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As exemplified by the COVID-19 pandemic, highly infective respiratory viruses can spread rapidly in the population because of lack of effective approaches to control viral replication and spread. Niclosamide (NCM) is an old anthelmintic drug (World Health Organization essential medicine list) with pleiotropic pharmacological activities. Several recent publications demonstrated that NCM has broad antiviral activities and potently inhibits viral replication, including replication of SARS-CoV-2, SARS-CoV, and dengue viruses. Unfortunately, NCM is almost completely insoluble in water, which limits its clinical use. We developed a cost-effective lipid nanoparticle formulation of NCM (nano NCM) using only FDA-approved excipient and demonstrated potency against SARS-CoV-2 infection in cells (Vero E6 and ACE2-expressing lung epithelium cells).

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Lipid nanoparticle formulation of niclosamide (nano NCM) effectively inhibits SARS-CoV-2 replication *in vitro*. Guankui Wang, Hanmant Gaikwad, Mary K. McCarthy, Mercedes Gonzalez-Juarrero, Yue Li, Michael Armstrong, Nichole Reisdorph, Thomas E. Morrison, and Dmitri Simberg. *Precis Nanomed.* 2021, 4(1), 724–737.

SELECTIVE UPTAKE INTO INFLAMED HUMAN INTESTINAL TISSUE AND IMMUNE CELL TARGETING BY WORMLIKE POLYMER MICELLES

ELENA GARDEY, Fabian H. Sobotta, Johannes C. Brendel, Andreas Stallmach

The limited efficacy and potentially severe side effects associated with the use of systemic anti-inflammatory drugs call for new approaches in the therapy of inflammatory bowel disease (IBD). Selective targeting of inflamed areas in the gastrointestinal tract with local drug release could be an effective treatment that avoids adverse effects. Our studies show that the shape of polymeric nanoparticles (micelles) represents a key to the necessary tissue selectivity in the colon that has received so far little attention. Using human colon biopsies in ex vivo experiments, we demonstrated that wormlike micelles (filomicelles) with a dense poly(ethylene oxide) (PEO) shell composition selectively penetrate inflamed human mucosa without showing significant interactions with healthy tissue. Similarly shaped small spherical micelles (~25 nm) rapidly cross the epithelial barrier but without the necessary selectivity for the inflamed mucosa of patients with IBD. In contrast, large vesicles (~120 nm) are hardly taken up (Fig. 1).

We demonstrated that after crossing the gastrointestinal barrier, the wormlike nanoparticles localize in immune cells of the lamina propria (Fig. 2). Thus, the filomicelles represent an innovative carrier nanoparticle for efficient and selective targeting of inflamed areas and the main proinflammatory cells in the case of IBD. The comparatively large volume of these wormlike nanoparticles compared to spherical nanoparticles also promises a higher transport capacity of anti-inflammatory drugs to these targets, which is currently under investigation. The ability to further modify the large surface area of these nanostructures should further increase selectivity and further enhance accumulation in immune cells of the inflamed mucosa.

Overall, we conclude that the structure of polymeric nanoparticles is a key factor for selective uptake in inflamed areas in the colon. We believe that our study is important for the further successful development of nanoparticle delivery systems for efficient IBD therapy.

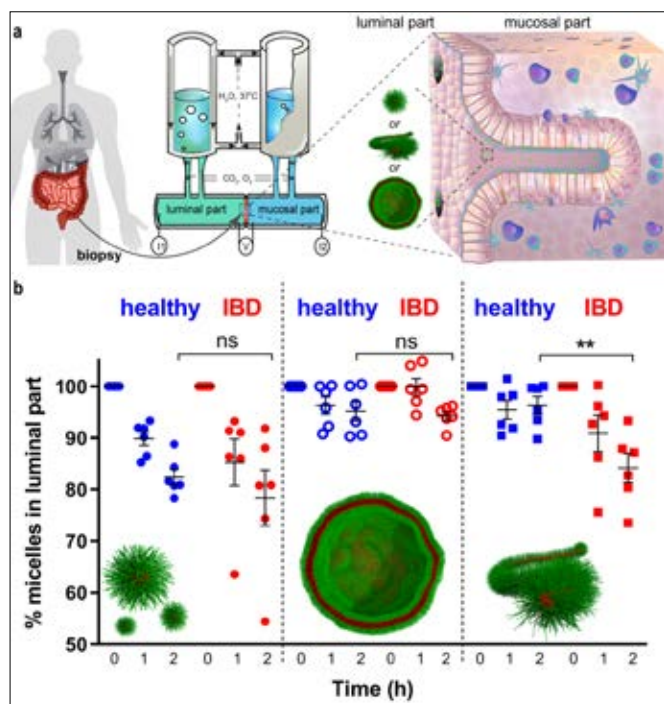


Fig. 1. Ex vivo experiment on nanoparticle interaction with healthy and inflamed human mucosa. a) Schematic representation of the experimental setup using Ussing chambers with mounted biopsies.

Nanoparticles (represented by green dots) were added to the luminal part of the Ussing chambers. The modified Krebs-Ringer bicarbonate buffer in the Ussing chamber is continuously oxygenated and kept at 37°C. Integrity and viability of the biopsy are controlled via inserted electrodes (V, I). b) Time-dependent change of the concentration of spherical micelles (full dots), polymeric vesicles (empty dots), and worms (squares) in the luminal part with healthy (blue) and inflamed (red) tissue. The exposed tissue area is 4.9 mm². The results are presented as percentage of the initial concentration ($M \pm SEM$); ns - not significant, ** $p < 0.001$, $n = 6$ (n - number of different donors/patients in each group). Two-tailed unpaired Student's t-test was used. IBD - inflammatory bowel disease.

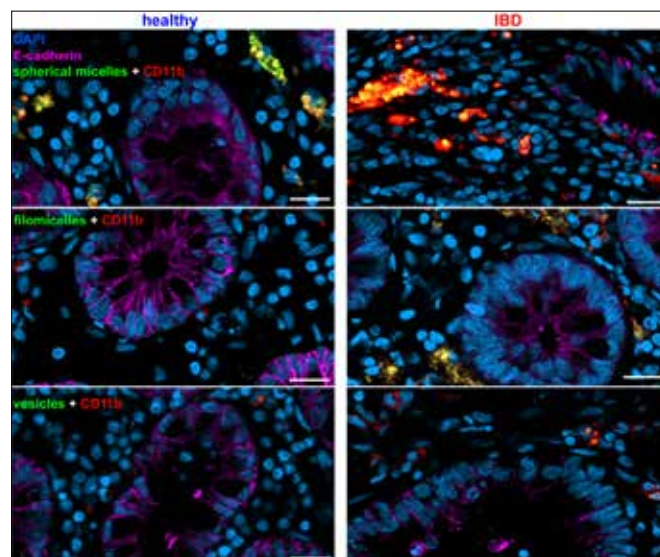


Fig. 2. Localization of nanoparticles in healthy and inflamed human mucosa. Localization of spherical micelles, wormlike micelles and vesicles in human mucosa after 2 h incubation in Ussing chambers. Accumulation of micelles in immune cells of inflamed human mucosa. blue (DAPI) - nuclei; red (CD11b) - immune cells, green: nanoparticles; violet: E-cadherin (epithelium). Scale bar is 20 μ m.

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Elena Gardey, Fabian H. Sobotta, Drilon Haziri, Philip C. Grunert, Maren T. Kuchenbrod, Franka V. Gruschwitz, Stephanie Hoepfener, Michael Schumann, Nikolaus Gaßler, Andreas Stallmach, Johannes C. Brendel, doi: <https://doi.org/10.1101/2021.01.26.428316>

LIPOSOMES – A HIGHLY EFFICIENT DRUG DELIVERY SYSTEM FOR DIFFERENT CLINICAL APPLICATIONS

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Since the first description of vesiculated phospholipid systems in 1965 by Alec Bangham, named “bangosomes”, more than 50 years of research have allowed to transform those vesicles from membrane models into successful drug delivery systems. Many technological advances are based on this evolution and nowadays liposomes are used in diverse areas to deliver antibiotics, anti-cancer, anti-inflammatory and anti-parasitic drugs; macromolecules, such as enzymes and oligonucleotides; in vaccines, imaging and even cosmetics. This success is due to the unique properties of liposomes that can efficiently incorporate different kinds of molecules irrespectively of molecular weight, electric charge or solubility. In addition, they are able to interact with cells, are biodegradable, biocompatible and can be manufactured with different sizes and properties.

Several liposomal formulations have been extensively investigated and designed using drugs already commercialized in order to solve several drawbacks such as instability and low bioavailability. Two examples are rifabutin (1) and paromomycin (2).

Rifabutin has been mostly used for the treatment of mycobacterial infections and its incorporation in liposomes has enhanced the therapeutic effect in different murine models. The observed *in vivo* outcome resulted from a preferential accumulation of the antibiotic in infected macrophages. Recently, *in vitro* studies against *S. aureus* also highlighted the rifabutin therapeutic potential against bacterial infections (3). Indeed, the incorporation of rifabutin in appropriated lipid compositions enabled the interaction and penetration of liposomal formulations into bacteria cell walls. This ultimately promoted the release of the loaded antibiotic and maximizing the anti *S. aureus* effect (3).

Paromomycin is an aminoglycoside with a broad spectrum *in vitro* activity against protozoa and mycobacteria. However, it is poorly absorbed into systemic circulation, after oral administration, and is rapidly eliminated by glomerular excretion after parenteral injection.

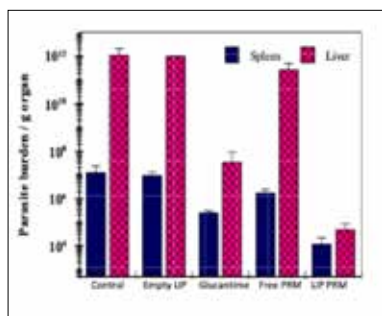


Figure 1. Influence of different formulations on the PB in spleen (blue columns) and in liver (pink columns). BALB/c mice were infected by i.v. injection of 1×10^7 *L. infantum* promastigotes. Groups under study: control group, empty liposomes, Glucantime[®], PRM in free (Free PRM) and liposomal (LIP PRM) forms.

Glucantime was subcutaneously injected at a dose of 45 mg/kg of body weight; PRM in free and liposomal forms was intravenously injected at a dose of 15 mg/kg of body weight. Control group received PBS, and empty liposomes (Empty LIP) were injected with the same lipid dose as mice treated with LIP PRM. All animals received a total number of 5 injections in daily consecutive days. <https://doi.org/10.1016/j.nano.2015.06.008>

In order to tackle this issue the optimization of an adequate lipid composition, enabled paromomycin to be efficiently incorporated in liposomes. *In vivo* studies evidenced huge changes in the biodistribution profile of this aminoglycoside with preferential accumulation in the liver, spleen and lungs.

As a result, the therapeutic effect in a visceral leishmania murine model was greatly enhanced (2) in relation to free paromomycin as demonstrated in Figure 1. The positive results with paromomycin liposomes may constitute an alternative treatment option in the near future. The only liposomal formulation currently available in the market, for visceral leishmaniasis with high therapeutic efficacy, is Ambisome, amphotericin-B liposomes.

The biological evaluation of new synthesized molecules constitutes an appealing strategy, in particular for cancer management. Metal-based complexes or new synthesized hybrid molecules have been evaluated in terms of their antiproliferative properties towards tumor cells and in murine melanoma models (4). A copper-based complex and a new hybrid molecule were efficiently incorporated in long blood circulating liposomes enabling their *in vivo* extravasation through leaky tumor blood vessels. The deficient lymphatic drainage at tumor sites allowed the accumulation of liposomes system via the widely described enhanced permeability and retention effect (5). Biodistribution studies of ¹¹¹In-labelled long circulating liposomes in a syngeneic subcutaneous melanoma model demonstrated that 48 h after i.v. administration 4% of the injected dose (Figure 2) was attained in the tumor thus correlating with the therapeutic effect observed.

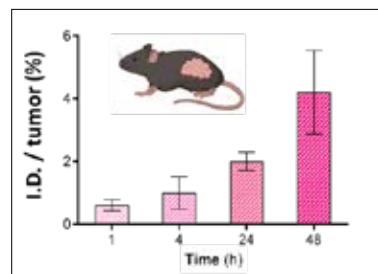


Figure 2. Biodistribution of long circulating liposomes labeled with the radionuclide ¹¹¹In. Data are expressed as percentage of I.D. in total tumor mass 1, 4, 24 and 48 hours after intravenous injection in a syngeneic murine melanoma model.

This work reveals the versatility in producing different kinds of liposomes according to the specific drug and desired target. The design and development of novel liposomal formulations constitutes a stimulating research area. As demonstrated in the present work, lipid-based nanosystems were able to protect the incorporated material, amplifying its activity and reducing its early elimination. These new strategies can raise the interest of the pharmaceutical companies allowing the introduction of novel liposomal products in the market in the near future. Although the developed nanoplat-forms need to undergo a rigorous standardization of the manufacturing process, the high number of marketed liposomal drugs proves the success of transposing liposomal formulations from bench to bedside.

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TITLE: RAMAN SPECTROSCOPY: IN VIVO APPLICATION FOR BONE EVALUATION IN ORAL SURGERY.

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Resume: Our days, there is 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**. It is requested a method adapted for a quik evaluation of the bone and precise in the mean time.

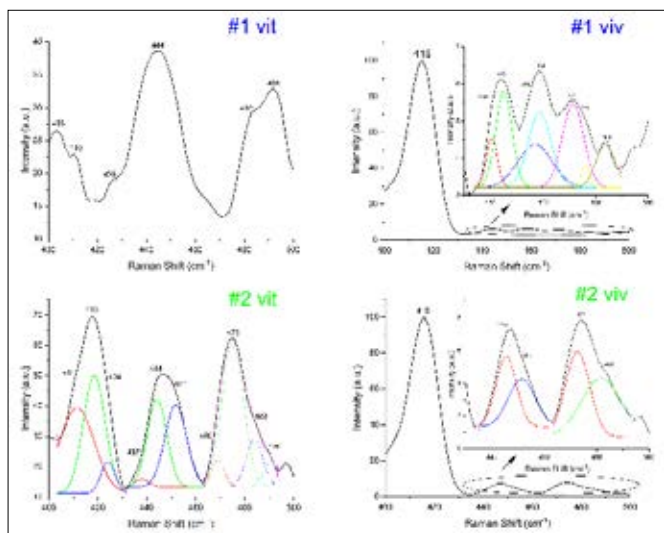
For the bone tissue, at micro level octacalcium phosphate (OCP, $\text{Ca}_8(\text{HPO}_4)_2(\text{PO}_4)_4 \cdot 5\text{H}_2\text{O}$), is considered very important because it is regarded as an *in vivo* precursor of HA. Trying to find traces for phase transition of OCP to HA, the presence of HA nano rods and plate-like HA particles can be utilized as signs of bone good quality evidenced by SEM investigation (Fig. 1 b). The normalized peak intensity values, are related to each compounds concentration.

A group of ten patients was involved to our study. Investigation was performed by RAMAN technique, first *in vivo* and then *in vitro* for the harvested bone samples.

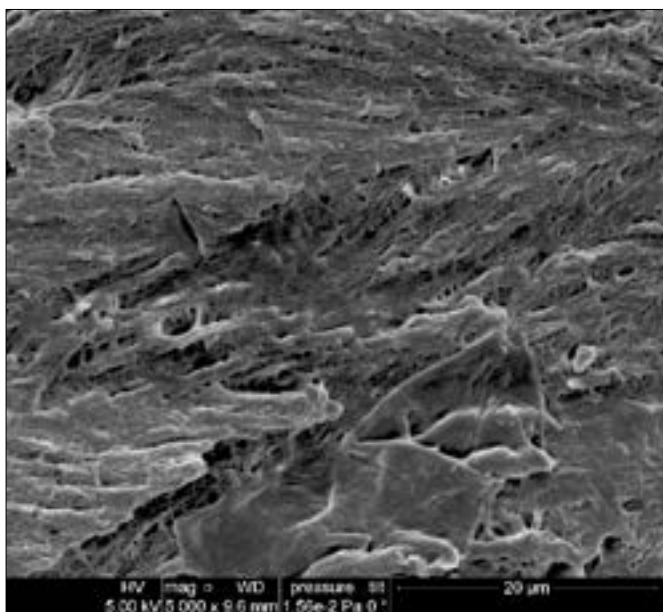
There were evaluated / compared the following peaks, for *in vivo* and then *in vitro* for the harvested bone samples (Fig 1 a):

- 430 – 450 cm^{-1} (ν_2 , PO_4^{3-});
- 955 – 960 cm^{-1} (HPO_4^{2-} , immature bone);
- 960 – 965 cm^{-1} (mineral bone, mature bone);
- 1023 cm^{-1} ($\text{P}_2\text{O}_7^{4-}$; PPI, inorganic pyrophosphate)

Raman method adapted for “*in vivo*” bone quality evaluation, is much less invasive then the well-known CT (computer tomography) or CBCT (con beam computer tomography) already used and more accurate. For this purpose, the Raman probe was modified with a “special cap” in order to assure regular sterilization for *in vivo* use.



(a)



(b)
Fig.1. (a) Raman spectra for patients (#1, #2) *in vitro* and *in vivo*; (b) SEM micrograph patient #1.

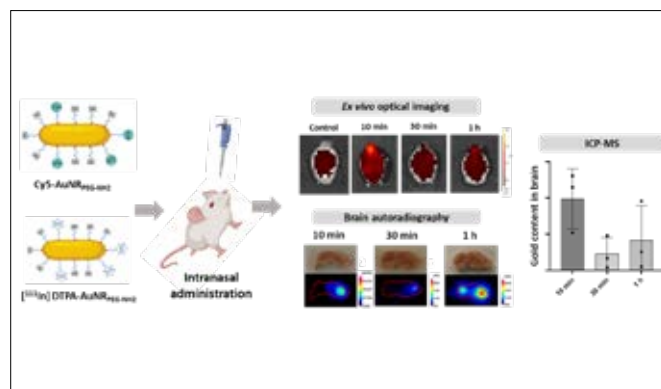
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COMPARATIVE ANALYSES OF GOLD NANOROD UPTAKE IN MICE BRAIN AFTER INTRANASAL ADMINISTRATION

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Scheme 1. comparative analysis of gold nanorod uptake in mice brain after intranasal administration by different modalities.



INTRODUCTION

The delivery of the therapeutic agents to the central neural system (CNS) is often challenged by the presence of the blood-brain barrier (BBB). Intranasal administration (IN) is an alternative route to access the brain non-invasively through the olfactory and trigeminal nerve pathways. IN delivery also offers reduced systemic exposure of the therapeutic agent. Gold nanoparticles are one of the most extensively investigated materials. Gold nanorods (AuNRs) owing to their anisotropic conformation demonstrate attractive optical and biological properties compared to spherical nanoparticles. To the best of our knowledge, the biodistribution of AuNRs after IN administration has never been reported. This study has comprehensively compared brain uptake of AuNRs by different analytical and imaging modalities. Our findings offer insights into the potential application of AuNRs as drug delivery systems of therapeutics to the brain *via* the intranasal route.

METHODS

AuNRs were functionalized with Cyanine5 (Cy5), a fluorescent dye for optical imaging or diethylenetriaminepentaacetic dianhydride (DTPA), a metal chelator to complex ^{111}In *via* polyethylene glycol (PEG) linker. Successful functionalisation was confirmed by FT-IR and $^1\text{H-NMR}$. Brain uptake was measured at 10 min, 30 min or 1 h post intranasal administration of 6 pmoles of AuNRs suspended in 0.5% CMC solution to CD-1 mouse. Brain and major tissue (heart, lung, liver, spleen and kidneys) distribution were semi-quantitatively or quantitatively determined with optical imaging, inductively

coupled plasma mass spectrometry (ICP-MS), gamma scintigraphy or autoradiography.

RESULTS

The functionalized AuNRs (Cy5-AuNR_{PEG-NH₂} or DTPA-AuNR_{PEG-NH₂}) were successfully synthesised and characterised. Both particles demonstrated uniform rod morphology and good colloidal dispersity. *Ex vivo* biodistribution by optical imaging confirmed the highest brain uptake at 10 min post-administration with the highest fluorescence intensity observed in the frontal brain region ($P < 0.05$ vs control). Brain uptake reduced as a function of time with signals reduced to background values after 1h. Gamma scintigraphy provided quantitative information with the majority of the dose detected in the nasal cavity followed by stomach and intestine after 1 h administration. Brain uptake of ~ 0.034 %ID, ~ 0.016 %ID and ~ 0.019 %ID per gram brain tissue was detected at 10 min, 30 min or 1 h, respectively. Brain distribution profile by autoradiography showed the highest signals in the olfactory bulb which extended to other brain regions within 1 h. ICP-MS results showed similar spatiotemporal profiles to the optical and nuclear imaging results. Current studies will evaluate the safety profile of the administered AuNRs in brain tissues.

CONCLUSIONS

Our study has confirmed the first-time brain uptake of AuNRs after intranasal administration and shed the light on their spatiotemporal distribution in brain tissues using a battery of analytical/imaging techniques. This information is crucial for designing drug delivery carriers for nasal application of therapeutics to treat neurological disorders.

ACKNOWLEDGEMENT

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ENHANCING DRUG EFFICACY WITH A HEAT-ACTIVATED DRUG-DELIVERY PLATFORM BASED ON PHOSPHATIDYL-(OLIGO)-GLYCEROL NANOCARRIER

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INTRODUCTION

Liposomes are in the focus of cancer research because of their potential to effectively enhance cancer drug delivery after systemic application and greatly reduce off-site toxicity. Nevertheless, the lack of a release mechanism in traditional liposomes leads to low contents of bioavailable drug within the tumor tissue and therefore limiting therapeutic efficacy^[1]. In order to circumvent this, numerous groups have been investigating lipid compositions to gain stimuli-responsiveness in lipid-based nanocarriers^[1, 2]. Our group focusses on controlled drug release from thermosensitive liposomes (TSL) which rely on phosphatidyl-(oligo)-glycerols (DPPG_n; n = 2,3), synthetic phospholipid excipients. This DPPG_n-TSL drug delivery platform achieves rapid heat-triggered drug release upon mild hyperthermia (41-43 °C; HT) and, after systemic injection, are characterized by prolonged circulation half-life in plasma and ultra-fast drug release in the heated tissue.

RESULTS AND DISCUSSION

Doxorubicin (DOX)-loaded DPPG_n-TSL (DPPG_n-TSL-DOX) were investigated in two animal species: in rats bearing the subcutaneous

rat sarcoma model BN175 and in cats with spontaneous feline sarcoma. Pharmacokinetic studies revealed a good plasma stability in both species. Focusing on biodistribution pattern in the BN175 tumor model, DOX accumulation in heated tumor tissue increased significantly (10-fold vs. non-heated tumors) because of local efficient heat-dependent drug release from DPPG_n-TSL. Combining systemic application and regional HT, DPPG_n-TSL-DOX outperformed non-liposomal DOX, conventional liposomal DOX (Caelyx[®]), and lysolipid-containing TSL-DOX when evaluating tumor growth delay (see figure 1)^[3].

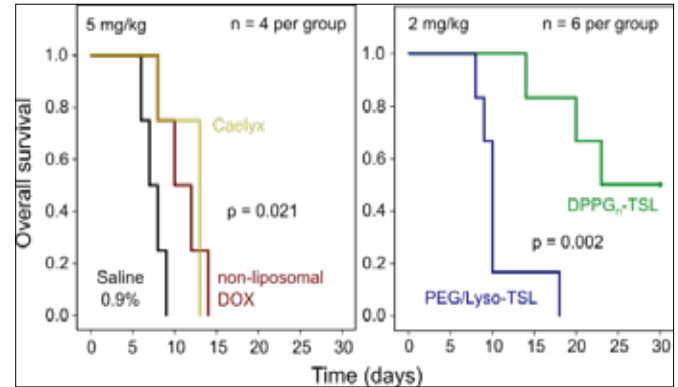


Figure 1: Survival time after systemic application of saline 0,9 % (black), non-liposomal DOX (red), Caelyx[®] (yellow), PEG/Lyso-TSL-DOX (blue) and DPPG_n-TSL-DOX (green) in Brown Norway rats bearing a subcutaneous BN175 tumor.^[3]

In feline patients, therapeutic effectiveness was determined by metabolic and histopathological response, respectively via ¹⁸F-FDG-PET/MRI and tumor resection. Both parameters were significantly better for DPPG_n-TSL-DOX compared to non-liposomal DOX treatment, potentially by overcoming drug resistance based on improved drug distribution within the heated tumor tissue (see figure 2)^[3].

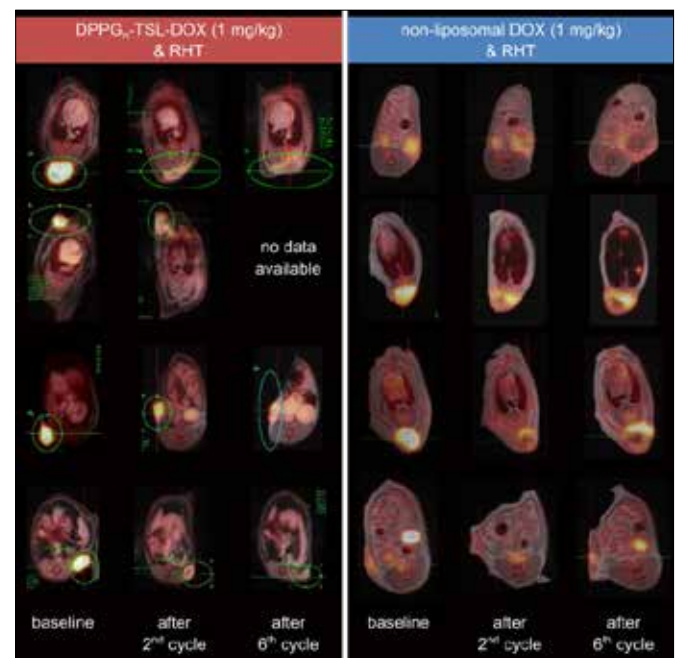


Figure 2: ¹⁸F-FDG-PET/MRI Imaging of feline patients with spontaneous feline sarcoma (baseline) and after the 2nd and 6th cycle of treatment with systemic injection of DPPG_n-TSL-DOX (red) or non-liposomal DOX (blue) combined with regional hyperthermia (RHT).^[3]

CONCLUSION

Our DPPG_n-TSL based drug delivery platform succeeded in enhancing antitumoral efficacy of DOX in pre-clinical studies. Overall, this novel drug delivery strategy has great potential for clinical application in locally advanced tumors.

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MECHANISM BEHIND SELECTIVE INFILTRATION OF LIPID NANOPARTICLES IN THE SPLEEN FOLLOWING ISCHEMIC STROKE

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Sympathetic stimulation of the spleen after ischemic stroke leads to the excessive release of pro-inflammatory cytokines into the circulation and preferential loss of innate immune cells which can further exacerbate tissue damage and predispose patients to infectious complications. Therapeutic approaches currently in use to manipulate the splenic responses are not selective, and their clinical translation potential is still controversial which stresses the need for more selective technologies. Recently, we observed that lipid nanoparticles (LNP) can be selectively targeted to the spleen with more than 90% of the I.D. when administered intravenously into a mouse model of transient middle cerebral artery occlusion (MCAo). Interestingly, we found significantly higher uptake of liposomes by white pulp (WP) cells of the spleen as early as 2hrs after I.V compared to healthy mice. To understand the mechanism behind this selective splenic targeting, we firstly analyzed the structural changes to the spleen by using H&E and TEM imaging. The H&E images showed a clear reduction in the diameter of the WP and marked loss of hematopoietic elements 1-2 days after MCAo. Similarly, TEM images showed a clear disruption of the WP splenic conduit system suggesting a loss in extracellular matrix (ECM) components. To understand other factors that could allow accessibility of liposomes into the WP, we studied the changes in the ECM as well as blood vessel permeability of the spleen using immunohistochemistry (IHC) staining. Our IHC data showed a significant reduction in ECM molecules (Collagen-I, Collagen-III), integrins (integrin α 6 β 4), and endothelial cell marker, CD31 in the WP 1-2d post-stroke. We also observed enhanced phagocytic capacity (LAMP2) of macrophages and higher expression of ECM remodelling enzyme, lysyl oxidase in the WP 24-48h after MCAo. These results suggest that the alteration in the splenic architecture, ECM, vascularity as well as enhanced phagocytosis may contribute to the initial enchantment in liposomes infiltration into the WP and mediate significant uptake by splenic cells. This unique observation highlights the potential of utilising LNP as a selective tool for peripheral immunomodulation to accelerate the development of effective therapies for post-stroke immunological complications.

Keywords: spleen; targeting; ischemic stroke; nanomedicine; immunomodulation; innate immune response, infection.

NANOCARRIER SYSTEMS TARGETING THE TUMOR MICROENVIRONMENT: FROM PH-MODULATING NANOCAPSULES TO THE CHOICE OF ANTIBODY ATTACHMENT

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The immunosuppressive tumor microenvironment has a major impact on the anti-tumor immune responses in tumor patients. One approach to overcome this can be performed with immune checkpoint inhibitors. Nevertheless, only around 50 % of melanoma patients even react to checkpoint inhibitors in the long-term run, and often come with severe immune related adverse events. Therefore, more research has to be conducted to both, improve our understanding of the tumor microenvironment and to address immune escape mechanisms even better.

Nanoparticle carrier systems are actually being developed to address this purpose. Previous studies have found that the transmembrane protein glycoprotein A repetition predominant (GARP) is highly expressed on the surface of various tumor entities, including melanoma as well as on immune modulating cells in the tumor microenvironment, such as activated Treg and platelets. We could show that GARP suppresses anti-tumor immune responses by inducing peripheral Treg and, at the same time, inhibiting the proliferation and cytokine production of T effector cells - making it an ideal target structure for nanoparticles to address.

Tumor-associated macrophages are the major suppressor of the innate immune response. A second approach is to repolarize these anti-inflammatory M2 macrophages to pro-inflammatory M1 macrophages by increasing the pH value of the tumor's microenvironment (Fig. 1). A pH-modulating effect could be already shown by applying antibody-urease conjugates, that convert endogenous urea into ammonia *in situ* to induce cytotoxicity. However, a considerable limitation of these antibody-enzyme conjugates is the rapid enzymatic degradation, which effects delivery efficiency and inhibits a permanent pH-modulation.

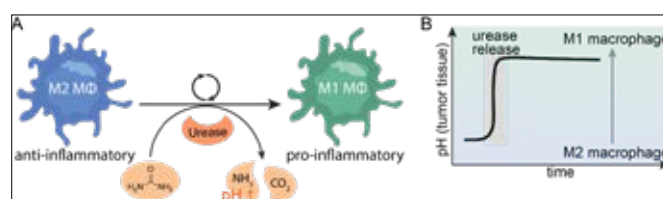


Fig. 1: Repolarization of anti-inflammatory M2 macrophages to pro-inflammatory M1 macrophages by increasing the tumor's pH. A Catalytic reaction of urea into ammonia and carbon dioxide using urease. B Graphical illustration of the pH value in time and repolarization of macrophages.

Here we show (i) improved selective targeting of nanocarriers to enable active accumulation in the tumor environment. We tested different liposomes with varying amounts of surface functionalization of PEG and/or biotin, which were found to be a good starting point for the attachment of an anti-GARP-antibody to increase selectivity. In addition, flow cytometry experiments revealed the uptake of model cargos (encapsulated inside liposomes and polymerosomes) in human melanoma cells (MaMel-19). Moreover, we discuss (ii) the preparation of urease-loaded nanocapsules and their application for the modulation of the tumor microenvironment.

PHYCOCYANIN SORAFENIB NANOFORMULATION WITH ENHANCED ORAL BIOAVAILABILITY AND ANTI-TUMOR EFFICACY AGAINST FLT3-ITD ACUTE MYELOID LEUKEMIA

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Sorafenib is a small molecule oral kinase inhibitor approved for the treatment of various cancer types including Renal Cell Carcinoma (RCC) and Hepatocellular Carcinoma (HCC). The molecule was identified to inhibit FMS-like tyrosine kinase 3 specifically with internal tandem duplication mutation (*FLT3-ITD*) which is the target of interest in our study. The choice of FLT3 as a target is due to the fact that it is most frequently mutated genes in hematological malignancies occurring in 25 to 30% of patients with Acute Myeloid Leukemia (AML). Patients with FLT3-ITD AML often present with more aggressive disease and have a significantly higher propensity for relapse after remission. The oral bioavailability of sorafenib is limited by the factors such as its aqueous insolubility, high rate of metabolism and limited gastrointestinal absorption. These factors result in poor pharmacokinetics necessitating higher dosing frequencies which can ultimately result in toxicity burden in the patients. Nanopharmacology emerged by designing biocompatible nanocarriers so as to enhance the bioavailability, improve the therapeutic efficacy and limit the toxicity of chemodrugs. The current study focuses on the development of oral sorafenib nanomedicines using a novel protein, phycocyanin as a carrier and this protein-sorafenib nanoformulation was evaluated for its potential in the treatment of AML. The nanoformulation consist of protein sorafenib nanoparticles surface coated with mucoadhesive and mucopenetrating polymers as a result of which the PK parameters were improved compared to that of the current clinical formulation (NATCO-Tablet). The tumor volume reduction in subcutaneous xenograft AML model in NOD-SCID mice infer to the *in vivo* antitumor potential of this oral protein-sorafenib nanoformulation compared to that of the clinical formulation.

ACKNOWLEDGEMENTS

Authors thank Nanobiotechnology Task Force: Department of Biotechnology (DBT), Government of India for financial support under the project "Translational Development of Protein Nanomedicine and Multifunctional Nano-Contrast Agents"; BT/PR7665/NNT/28/658/2013 and Amrita Vishwa Vidyapeetham for infrastructure support.

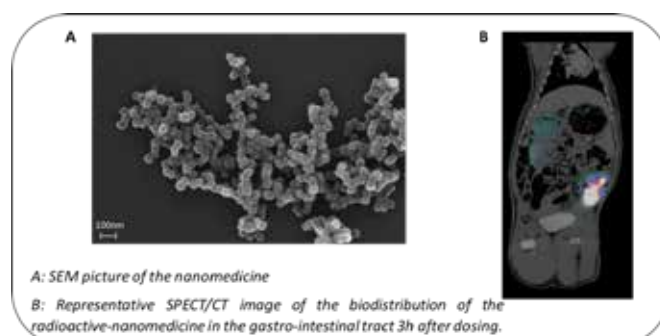
GUT-HOMING STABLE ENZYME ACTIVITIES TO DEVELOP EFFICIENT DIGESTIVE THERAPIES

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Background: Enzymes are the pillars of digestion, and responsible for the quality of nutrients ready for gastrointestinal uptake. Thus, enzymes have been developed for therapeutic purposes for those patients with faulty digestion or to control nutrient quality uptake. However, digestive diseases still represent a high unmet medical need as existing oral enzyme formulations show poor stability, susceptibility to gut conditions, lack of specific localization, and trigger variable intolerance profiles. Therefore, we have developed an oral therapeutic enzyme platform to coat the intestine with stabilized enzyme activity without triggering any inflammation. The platform is made of nanoparticles in which the enzyme is shielded and fully active while remaining unexposed to the harsh gut conditions and surrounding epithelium. The nanomedicine is further functionalized to provide mucoadhesive properties and coat the intestine with stabilized enzyme activity.

Aim: The objective was to generate a mucoadhesive nanomedicine and to demonstrate its gut-homing properties *in vivo*.

Methods: The nanomedicine was functionalized with mucus-binding moieties and its biocompatibility was assessed *in vitro* on a model of intestinal barrier, and *in vivo* on two animal species (mouse and Göttingen minipig). The affinity of the nanomedicine for the mucus was first evaluated *ex vivo* with a freshly collected pig intestine. The gastrointestinal localization of the nanomedicine was then investigated by a biodistribution study after a single oral administration of radiolabelled nanoparticles.

Results: We report here preclinical results showing that the enzyme nanomedicine exhibits long-lasting biocatalytic activity, and a markedly enhanced resistance to gut stresses (e.g., proteases). In addition, biocompatibility studies demonstrate that the nanomedicine is a suitable candidate for gastrointestinal applications as it does not induce any cytotoxicity or loss of integrity of the intestinal barrier. It is also perfectly well tolerated by animals. We demonstrate the direct influence of the surface functionalization of the nanomedicine on its interaction with intestinal mucus both *ex vivo* and *in vivo*. Indeed, the biodistribution studies highlight a significantly increased residence time of the mucoadhesive nanomedicine in the gastrointestinal tract compared to the residence time of the food bolus. The nanomedicine remains topical as it is not absorbed and is fully eliminated in the faeces along with the mucus.

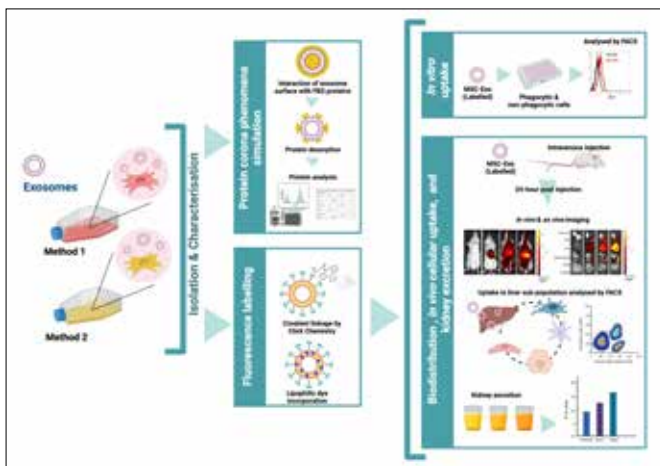
Conclusion: We demonstrate the capacity to coat the intestine with stabilized enzyme activity. This paves the way to the development of a series of novel oral drug compounds for digestive diseases.

CULTURING CONDITIONS OF MESENCHYMAL STEM CELLS DERIVED EXOSOMES ALTER THEIR PROTEIN CORONA FORMATION, CELLULAR UPTAKE AND IN VIVO ORGAN BIODISTRIBUTION

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Prolonged liver damages result in fibro-genesis leading to cirrhosis which can be cured only by liver transplantation. No efficient regenerative treatment option is currently available. There is an unmet need to discover new treatments. Therapeutic potentials of exosomes derived from mesenchymal stem cells (MSC-Exo) have been supported by several regenerative medicine/liver clinical trials and pre-clinical studies due to their immune-regulatory properties and low immunogenicity. We have previously shown that three-dimensional (3D) culture of MSCs produces exosomes with improved yield and enhanced therapeutic efficacy. In this study, we systematically study the effect of culturing conditions and media compositions on protein corona formation, *in vitro* uptake in phagocytic and non-phagocytic cells and *in vivo* organ biodistribution of the isolated exosomes in mouse model.

Scheme 1. Graphical abstract showing study of the effect of differential culturing conditions on protein corona formation and *in vivo* fate



METHODS

Conditioned culture media from different culturing methods (Method 1&2) were collected for exosomes isolation by ultracentrifugation on sucrose cushion. Size and number were measured by Nanoparticle Tracking Analysis (NTA), protein amount was determined by microBCA, and surface marker expression was conducted by dot-blot. The surface membrane of MSC-Exo was labelled using copper-free click chemistry or by incorporation of a lipophilic dye. Labelling stability was evaluated by measuring changes in fluorescence intensity over 24-hour fetal bovine serum (FBS) incubation. The interaction between MSC-Exo with biological proteins upon systemic delivery was simulated by incubating with FBS for 24 hours, followed by surface protein desorption to assess protein corona formation by SDS-PAGE and LC-MS. CD-1 mice were injected intravenously with labelled MSC-Exo and free dye. *In vivo*, *ex vivo* fluorescence imaging of organs, and urine clearance were evaluated at 24-hour post-injection. The uptake by liver cell subpopulations was determined by flow cytometry.

RESULTS

Different culture method could affect yield of protein content. No difference in labelling efficiency between two types of MSC-Exo labelled by both approaches was found. The distinct organ biodis-

tribution profiles could be also observed when comparing between MSC-Exo obtained by the two culturing methods; MSC-Exo derived from Method 1 and 2 showed higher rate of kidney excretion and liver accumulation, respectively. Both MSC-Exo types were taken up by liver cell subpopulations to different degrees. The protein corona profiles illustrated diversity in protein adsorption on MSC-Exo surface expectedly due to different types of proteins present in the culturing system.

CONCLUSIONS

Varying culturing methods and media compositions of MSCs affect the protein corona composition which could alter their cellular uptake pattern, organ tissue biodistribution and clearance. Future work will focus on further improving targeting to liver cell subset and their regenerative capacity in a liver mouse model.

ACKNOWLEDGEMENT

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BIOLOGICAL EFFECTS OF SYSTEMICALLY ADMINISTERED TLR7/8 AGONIST AND ANTIGEN-CONJUGATED NANOGELS ON IMMUNE CELL POPULATIONS IN THE LIVER AND IN THE SPLEEN

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Therapeutic vaccination against tumor antigens is of great interest regarding the variety of tumor types and individual immune condition for each patient. Vaccines for tumor therapy need to comprise tumor antigen, adjuvant and need to ensure passive or active targeting of antigen presenting cells in order to elicit tumor-specific adaptive immune responses. Different strategies, ranging from delivering antigen- encoding mRNAs to peptides or full antigens require immune-boosting adjuvants as well as carrier platforms to ensure cell type-specific uptake.

Here, we introduce a pH-responsive nanogel platform intended for antitumor vaccinations. The underlying chemical design allows for straightforward covalent attachment of an antigen as exemplified in this study for the model antigen (ovalbumin) and an immune adjuvant (imidazoquinoline-type TLR7/8 agonist) onto the same nanocarrier system.

It is known that this type of small-molecule TLR agonists when applied in soluble form cause systemic inflammation leading to severe side effects. We hypothesized that particulate application of the TLR7/8 agonist may largely prevent unwanted systemic inflammation. Here, we assessed the outcome of *in vivo* application of fluorescence-labeled polymeric nanocarrier conjugated with the

aforementioned TLR7/8 agonist, including biodistribution analysis of this nanocarrier by *in vivo* imaging and flow cytometry of organ-derived single cell suspensions, detection of liver damage markers and cytokines in serum and histopathologic studies of various organs for in depth analysis of potential cytotoxic effects.

We observed that polymeric nanocarriers when conjugated with TLR7/8 agonists accumulated in the liver and in the kidneys (Figure 1). Cytometric analysis of liver and spleen immune cell types confirmed uptake of nanocarriers into various antigen-presenting immune cell subpopulations at varying extent. Interestingly, co-delivery of IMDQ-functionalized particles with OVA-Protein worked best for the covalent conjugate in most of these immune cell types. We also monitored the cytokine profile of mice 24 h after *i.v.* injection and observed the highest levels of the pro-inflammatory cytokines TNF- α and INF- γ in case of application of the Dual-antigen/adjuvant loaded nanogel NP(IMDQ+OVA). Prompted by the strong accumulation of the nanogels in the liver, we assessed liver enzyme parameters in the blood but could not observe any differences. Moreover, histopathological analyses by hematoxylin–eosin staining of liver, spleen, kidney, heart, and lung tissue showed no histological anomalies after *i.v.* injection of the nanovaccine.

Regarding the versatility of opportunities and advantages, our nanogels, we consider this nanocarrier platform as promising for the development of highly customized and potent nanovaccines.

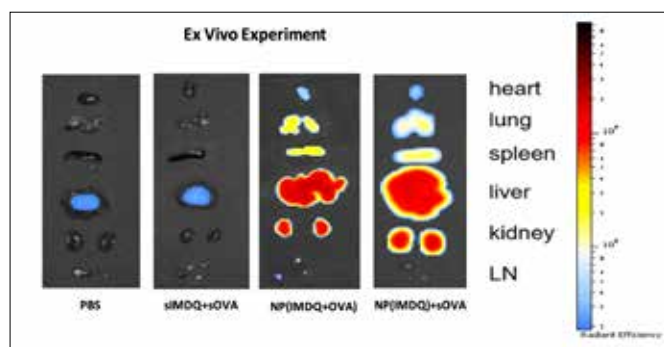


Figure 1. The extent of liver accumulation of systemically applied conjugation of TLR7/8 agonists polymeric nanocarriers after *i.v.* injection: fluorescence imaging of organs retrieved from mice treated with the various conjugations 24 hpi.

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NANODIAMOND MAGNETOMETRY FOR REAL-TIME MONITORING OF DRUG EFFICIENCY IN ARTHRITIS TREATMENT

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INTRODUCTION: Freeradicals (FRs) are omnipresent and one of the key players in the ageing process and various diseases development. Despite their relevance, information about FRs is sparse and therefore their use as clinical biomarkers is very limited. Since FRs are short-lived and reactive, it is challenging to detect them with the state-of-the-art methodology. In order to understand the multiple functions of free radicals in cell biology, our group has developed a new technique called diamond magnetometry². It allows us to detect free radical generation in real-time at the single cell level and in the biological fluids, with nanoscale resolution. We have been investigating FRs generation in samples from arthritis patients. Arthritis is a common disease which is characterized by a decline of cartilage in joints. It can lead to disabilities and diminished quality of life. The two most common types of arthritis are osteoarthritis (OA) where cartilage damage occurs in degenerative diseases and rheumatoid arthritis (RA) where the decline occurs during chronic inflammation of joints³. Here we demonstrate that diamond magnetometry can be used to differentiate between these forms of arthritis and can aid in a better understanding of the drugs working mechanism.

METHODS: In this study diamond magnetometry³ was applied to perform localised free radical detection in synovial fluid and cells obtained from arthritis patients. We have used the 70 nm fluorescent nanodiamonds (FNDs) as magnetometry probes. For every selected FND we have performed a sequence of T1 relaxometry measurements. First, we have measured T1s for FND in a dry state, then after we added a synovial fluid (SF) and in the last step piroxicam (2 μ g/ml). Similar T1 sequence was recorded for cells derived from the synovial fluid, cultured *in vitro* and treated with FNDs. To compare T1 relaxometry data with conventional FRs detection methods, we have performed a DCFDA assay.

RESULTS: T1 and DCFDA measurements (Fig. 1) have not shown any significant difference in the FRs concentration between the OA and RA samples. However, we have found that piroxicam decreased FRs generation in synovial fluid and cells from both OA and RA patients. The drug was more effective in OA samples.

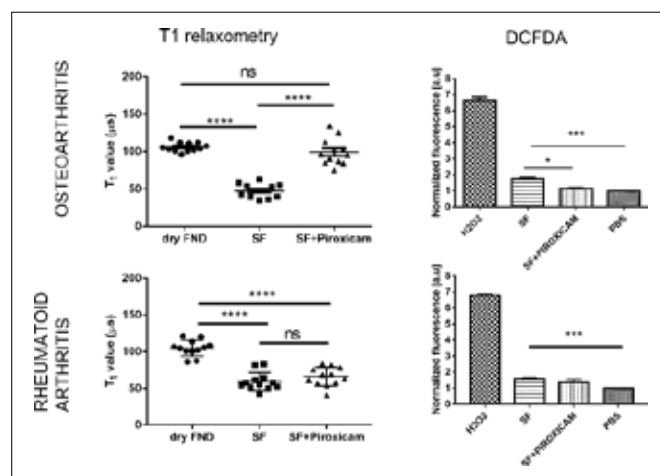


Fig. 1: FRs generation in synovial fluid from OA and RA patients. Shorter relaxation time (T1 value) or higher normalized fluorescence (DCFDA) correspond to increase in FRs concentration.

DISCUSSION & CONCLUSIONS: Our studies have shown for the first time that fluorescent nanodiamonds can detect free radical generation in samples from arthritis patients. This proof-of-concept exper-

imenthasalsoshownthatnanodiamond magnetometry enables to monitorefficiencyofanti-inflammatorytherapeuticsinreal-time.

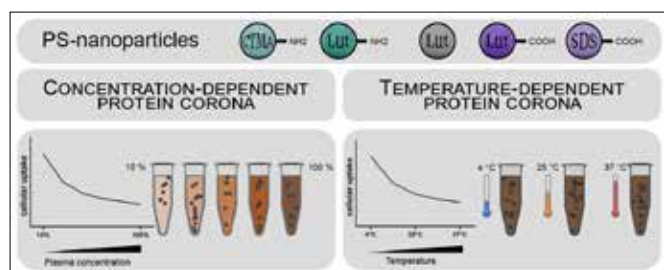
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TEMPERATURE, CONCENTRATION, AND SURFACE MODIFICATION INFLUENCE THE PROTEIN CORONA

JENNIFER OBERLÄNDER
member of the SFB1066

The composition of the protein corona varies according to multiple parameters and influences the cellular fate of the nanocarriers. Therefore, we investigated the influence of four key parameters (surfactant, surface charge, temperature, and plasma concentration) on the formation and composition of the protein corona and ultimately on the cellular uptake of pre-coated nanoparticles. At a fixed temperature and concentration, the surface charge, and surfactant influence the composition, with the greatest variation observed between surfactants for a fixed surface charge. We observed that the composition of the corona formed at low temperatures (4°C) differs from that formed at physiological temperatures (37 °C). At low plasma concentration (up to 25%), the corona tends to be more complex than at high plasma concentration (60% and above). Finally, we concluded that regardless of the formulation of the nanoparticles, the degree of uptake by cancer and endothelial cells of the nanoparticles decreased when pre-coated at increasing temperature or plasma concentration.



FCRN-TARGETED NANOMEDICINES FOR INTESTINAL DELIVERY OF AN ANTIDIABETIC PEPTIDE

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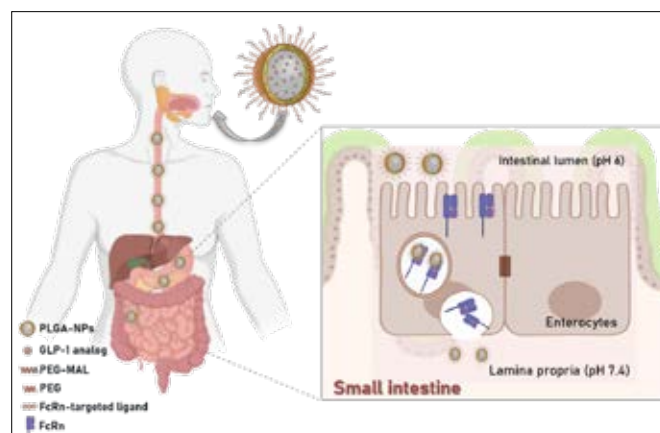
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Figure 1 – Mucodiffusive polymeric NPs encapsulating a GLP-1 analog, surface functionalized with FcRn-targeted ligands were produced by double emulsion technique. The nanosystem will be able to surpass the intestinal mucus layer and the intestinal epithelium by the presence of the mucopenetration polymer PEG and the FcRn-targeted ligands (peptide or

affibody) on the surface of the NPs, respectively. The binding of the NPs to the hFcRn occurs in a pH-dependent manner, meaning that the interaction is observed at acidic pH (intestinal lumen and endosomes pH) and the release at neutral pH (lamina propria pH).



Type 2 diabetes *mellitus* (T2DM) is a chronic and metabolic disorder characterized by insulin resistance and/or inadequate insulin secretion, causing hyperglycemia. Currently, the treatment embraces the exogenous administration of antidiabetic peptides, as glucagon-like peptide-1 (GLP-1) analogs, associated to nonpharmacological actions, as diet and exercise. GLP-1 analogs have become a promising approach in T2DM treatment, providing an effective glycemic control due to their resistance to the enzymatic degradation by dipeptidyl peptidase-4. However, GLP-1 analogs are mostly administered by invasive routes, which is associated to a poor patient compliance. Additionally, when orally administered, GLP-1 analogs are exposed to the physiological barriers of human body, namely the enzymatic activity and the acidic pH of the stomach, the mucus layer and the intestinal epithelial cells, which compromise their oral bioavailability. The main goal of this work is to improve the oral bioavailability of a GLP-1 analog by encapsulating into bioengineered nanoparticles (NPs), surface-decorated with ligands that target the intestinal neonatal Fc receptor (FcRn), responsible for the transcytosis of nanosystems through the enterocytes (**Figure 1**). Mucodiffusive PLGA-PEG NPs loaded with a GLP-1 analog were produced by double emulsion technique, resulting in a mean particle size close to 188 nm, a neutral surface charge and a GLP-1 analog association efficiency and loading of 53.5% and 1.3%, respectively. The GLP-1 analog release was tested in a medium that mimic the gastric and intestinal environment at 37 °C. The results demonstrated a slow sustained release after 8 hours of experiment. FcRn-targeted ligands were tested by Surface Plasmon Resonance (SPR) to demonstrate the pH-dependent binding to human FcRn (hFcRn). The affibody ZFcRn16 and the peptide CQRFTVGHFGGLYPANG (FcBP) displayed the best pH-dependent binding to hFcRn, binding more strongly at pH 6.0 than pH 7.4. Both ligands were chemically linked to the PLGA-PEG-MAL polymer through MAL-thiol chemistry, resulting in a conjugation efficiency of around 50%. Moreover, the successful functionalization of PLGA-PEG-MAL was also ensured by X-Ray Photoelectron Spectroscopy, where it was detected the presence of sulfur and an increase in the amount of nitrogen. Both functionalized polymers were used to produce polymeric NPs, resulting in an increase in the mean particle size, comparatively with non-functionalized NPs. Preliminary SPR studies were conducted with FcBP-conjugated NPs and affibody-conjugated NPs, showing a similar pH-dependent binding to hFcRn as the FcRn-targeted ligands alone. Additionally, the interaction between functionalized and non-functionalized NPs with Caco-2 cells, a cell line that endogenously express hFcRn, was studied, and a higher intestinal interaction was obtained for the functionalized NPs. Further, the intestinal permeability of both functionalized nanosystems will be tested using an *in vitro* 3D intestinal model, and the functionalized NPs with better permeability will be tested in *in vivo* models to validate their potential in enhancing the oral bioavailability of the GLP-1 analog.

DEVELOPMENT OF SELF-POWERED MULTIFUNCTIONAL PIEZOMAGNETIC NANOPARTICLES FOR NON-INVASIVE BBB MODULATION AND GLIOBLASTOMA TREATMENT

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Current therapies for diseases affecting the brain such as brain cancer and neurodegenerative disorders are limited to those which only reduce the symptoms of the disease but don't stop its progression or treat it¹. This is due to the presence of a tight barrier in the brain known as the blood brain barrier (BBB) which separates the brain tissue from the body's normal blood circulation. It acts as a protective barrier to the brain from unwanted chemicals but often presents a challenge in shuttling therapeutic cargoes into the brain². Here we report the development of a unique ultrasound (US) and magnetic field (MF) responsive nanoparticle system based on piezoelectric barium titanate and superparamagnetic iron oxide nanoparticles termed as PiezoMagnetic nanoparticles (PMNPs) for the non-invasive BBB modulation and glioblastoma treatment. These unique nanoparticles transduce US signals to electrical signals to permeate the tight junction formed by the endothelial cells in the BBB through nano-electroporation³. The BBB penetration capability of PMNPs was evaluated in self-assembled multicellular BBB spheroid models using the intrinsic Second Harmonic Generation (SHG) Imaging property of PMNPs. The anticancer drug cisplatin was further conjugated to PMNPs and exhibited superior anticancer properties as evaluated on 3D models of glioblastoma multiforme (GBM). Moreover, we examined the ability of PMNPs to transduce ultrasound signals to electrical signals for the effective penetration and delivery of Cisplatin in GBM spheroid models. We have therefore successfully developed an ultrasound responsive drug delivery system for the effective translocation into BBB and GBM treatment.

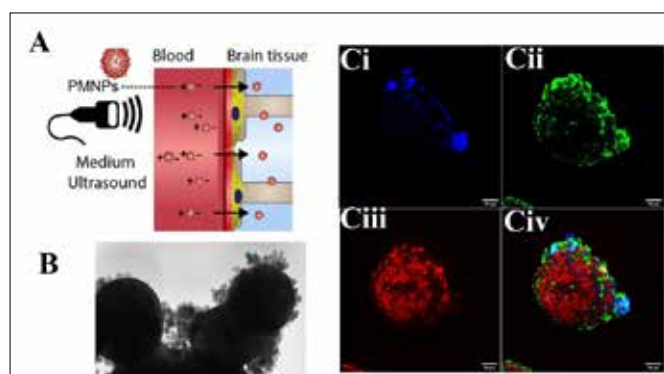


Figure 1. A) Schematic representation showing the mechanism of non-invasive BBB penetration by PMNPs using ultrasound (US), B) TEM image of the the PMNPs, Ci-iv) Confocal Laser scanning image showing the formation of BBB spheroids with human primary Brain Endothelial cells (Green, Cii) forming the surface monolayer to establish a tight cell-cell interaction with human primary pericytes (Blue, Ci) and human primary astrocytes (Red, Ciii) form the core of the spheroid mimicking the BBB structure, Civ) Merged image

ACKNOWLEDGEMENT

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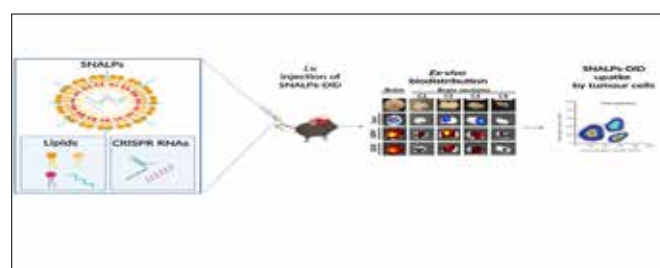
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IN VIVO APPLICATION OF CRISPR/CAS9 GENE EDITING USING LIPID NANOCARRIERS FOR THERAPEUTIC IMMUNE TARGET IDENTIFICATION IN GLIOBLASTOMA.

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INTRODUCTION

Despite advances in cancer immunotherapy, little progress has been accomplished in its clinical translation for glioblastoma (GBM) therapeutics. Failures are associated with multiple factors including the highly immunosuppressive tumour microenvironment (TME), the reduced number of circulating T cells, tumour heterogeneity, limited drug penetration and the presence of a subset of GBM-stem-like cells (GSCs) that activates different signalling pathways that further contribute to tumour growth and multi treatment resistance. Increasing evidence stresses the imperativeness of a multitarget approach that acts synergistically to (1) disrupt multiple pathogenic pathways in both GBM and GSC, (2) normalize the defective immunity in the TME and (3) enhance the antitumour immune response. CRISPR/Cas9 gene editing represents a powerful toolbox capable of correcting mutations and delete pathogenic genes, like onco-genes and tumour regulatory ones. However, it's *in vivo* application cannot take place unless subsidised with an appropriate delivery system.

METHODS

Single guide RNA (sgRNA) against reporter genes (green fluorescent protein (GFP), Luciferase (Luc)) and therapeutic immune targets have been designed and their specificity was validated using *in vitro* nuclease assay or flow cytometry. Stable nucleic acid-lipid particles (SNALPs) encapsulating Cas9 mRNA and sgRNA, were formulated and characterised utilizing dynamic light scattering (DLS). The nucleic acids encapsulation efficiency was measured QuantiTTM RiboGreenTM assay. SNALPs incubated in the presence of serum and nucleases were dissociated to extract the nucleic acids and determine the integrity of Cas9 mRNA and sgRNA using agarose gel electrophoresis. To understand the organ biodistribution profile of SNALPs, mice bearing orthotopic GSC tumours (Luc+/GFP+) received an intravenous injection of SNALPs labelled with Near Infrared (NIR) lipophilic fluorescent dye DiR (SNALPs-DiR) or far-red dye DiD (SNALPs-DiD). Twenty-four hours later the organs were harvested and imaged for Luc, GFP, DiR or DiD using IVIS Lumina Series III In Vivo Imaging System. Brain sections were dissociated into single cell suspension and assessed for SNALP uptake by flow cytometry. Next, we examined the SNALPs ability to mediate Cas9 mRNA delivery to GSC following intracranial administration. Twen-

ty-four hours post injection animals were euthanized, and tumour lysates were processed for western blot to detect expression of Cas9 protein.

RESULTS

In this study we validated sgRNA designed against the different target genes. Then, we formulated SNALPs characterized by an average size <150 nm, a neutral surface charge in physiologic conditions and a high encapsulation efficiency (>80%) of Cas9 mRNA and sgRNA. SNALPs could protect the nucleic acids from enzymatic degradation in the presence of serum and nucleases. SNALPs induced significant knockout of target genes in GSC with efficiency higher than commercially available transfection reagents. Accumulation of SNALPs in GSC tumours implanted intracranially in mice after intravenous injection was confirmed by optical imaging. Further optical and flow cytometry analysis suggested a higher uptake of SNALPs in cancerous areas compared to healthy brain tissues. Western blot analysis of GSC lysates confirmed successful expression of Cas9 protein following intracranial administration of SNALPs-mCas9.

CONCLUSIONS

The data suggests that SNALPs are a potent delivery system for CRISPR/Cas9 mediated gene editing of GSC. Ongoing work aims to validate the *in vivo* ability of CRISPR/Cas9 gene editing of GSC to identify at least one therapeutic combination of onco- and immune- targets.

MULTICOMPONENT ADJUVANTATION OF ANTI-GEN-BASED NANOCAPSULES USING SITE-DIRECTED CLICK CHEMISTRY CROSSLINKING FOR THE TREATMENT OF MELANOMA

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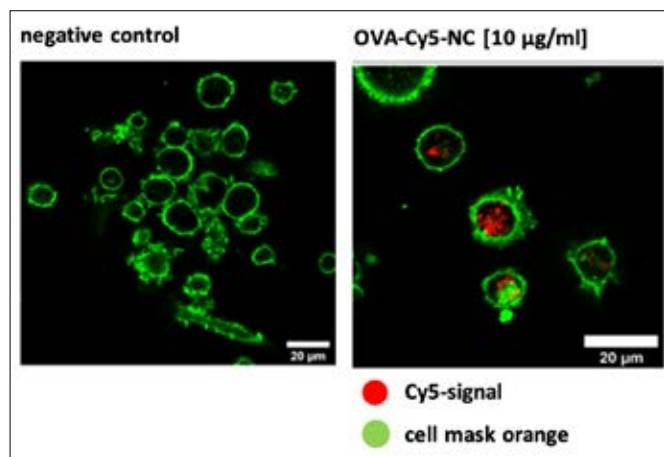


Fig. 1: Uptake of OVA-NC in BMDC

Nanocarrier-based antigen delivery is a promising vaccination approach in the context of tumor therapy. Formulating polymeric nanocapsules (NCs) out of tumor antigens in combination with vaccine adjuvants enables efficient targeting and maturation of dendritic cells (DCs), essential prerequisites for the induction of vigorous cellular immune responses. Aim of the present study was the synthesis of polymeric protein nanocapsules composed exclusively of vaccine antigens and encapsulated with combinations of adjuvants, as well as the evaluation of their potential to induce antigen-specific immune responses.

The NCs consisting of ovalbumin (OVA), were bioorthogonally crosslinked by copper-free azide-alkyne Click-Chemistry using the inverse miniemulsion technique. This method ensures integrity and processability of crosslinked antigens leading to effective epitope presentation by dendritic cells. The inverse miniemulsion approach led to polymeric nanocapsules with a spherical morphology that were efficiently ingested by DCs. In addition, a combination of the vaccine adjuvants Resiquimod (R848) and diABZI (STING agonist) were encapsulated to efficiently trigger strong DC activation analyzed by costimulatory surface marker expression and the secretion of pro-inflammatory cytokines. The induction of robust antigen-specific T cell proliferation was observed in DC-T cell co-cultures. The high biocompatibility and effectiveness of this vaccination platform was shown using the OVA-NC formulation as a therapeutic vaccine in the B16/OVA-Luc melanoma model.

In conclusion, multiadjuvant-functionalized protein nanocapsules are a promising delivery system for the combined delivery of antigens and vaccine adjuvants to dendritic cells promoting T cell-based immune responses. This novel anti-tumor vaccination strategy avoids the use of structural compounds, increases the antigen load of DCs and bears the potential to overcome tolerance and to induce vigorous antigen-specific anti-cancer immunity.

PH-DEPENDENT BEHAVIOR OF IONIZABLE CATIONIC LIPIDS IN MRNA-CARRYING LIPOPLEXES INVESTIGATED BY MOLECULAR DYNAMICS SIMULATIONS

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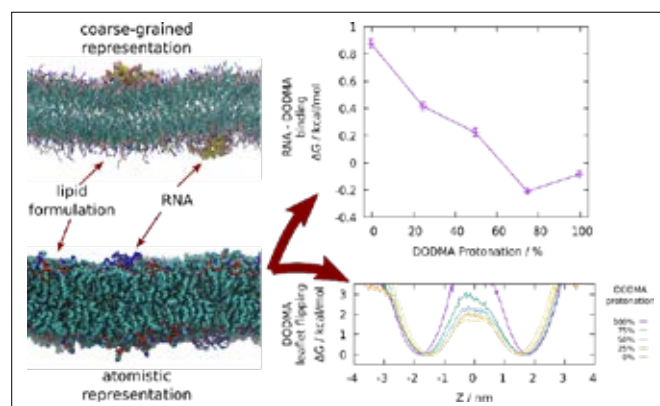


Figure 1. The pH-dependent structure of a lipoplex containing mRNA and ionizable lipids (DODMA) is explored using multi-scale molecular dynamics simulations. Top left: coarse-grained representation of the system used to sample long time scales; Bottom left: atomistic representation used to extract accurate structural properties of the system. Top right: Binding free energy of DODMA lipids with mRNA as a function of lipids protonated fraction (a proxy of pH). Bottom right: free energy of DODMA as a function of the position along the lipid bilayer. For low protonated fraction (high pH) the free energy barrier to leaflet flipping for DODMA becomes small. Adapted from ref.¹

Lipid-based nanoparticles and lipoplexes containing ionizable lipids in their formulation are among the most successful nanocarriers for mRNA-based therapies. At molecular level, the structure of these assemblies is still not fully understood, in particular regarding the role played by the ionizable lipid in the interactions with the RNA molecules. SAXS experiments have shown that lipoplexes based

on the ionizable lipid 2-dioleoyloxy-N,N-dimethyl-3-aminopropane (DODMA), under a certain range of conditions, have a lamellar structure, where lipid bilayers are separated by mRNA-rich layers, with an overall periodicity or spacing between 6.5 and 8.0 nm and a complex pH-dependence. Here^[1], a multiscale molecular dynamics simulation approach is used to investigate the structure and dynamics of these lipoplex formulations at varying pH level, as well as, the effects of the introduction of mRNA into the assemblies. It is observed that the interactions between DODMA and RNA is slightly attractive only at low pH levels, while it becomes effectively repulsive at high and intermediate pH levels. This results in a pH dependent relocation of the RNA inside the multilayers, from the lipid head groups at low pH to a more uniform distribution inside the hydrophilic slabs of the multilayers at high pH. We observe also that at high pH levels DODMA lipids undergo a gradual shift towards the hydrophobic part of the bilayer. This results in a significant increase of the leaflet-flipping rate of DODMA, a phenomenon which may ultimately affect the fusion process of the lipoplex with the endosomal membrane.

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TUMOR-PENETRATING UTORUBICIN POLYMERSOMES FOR CANCER THERAPY

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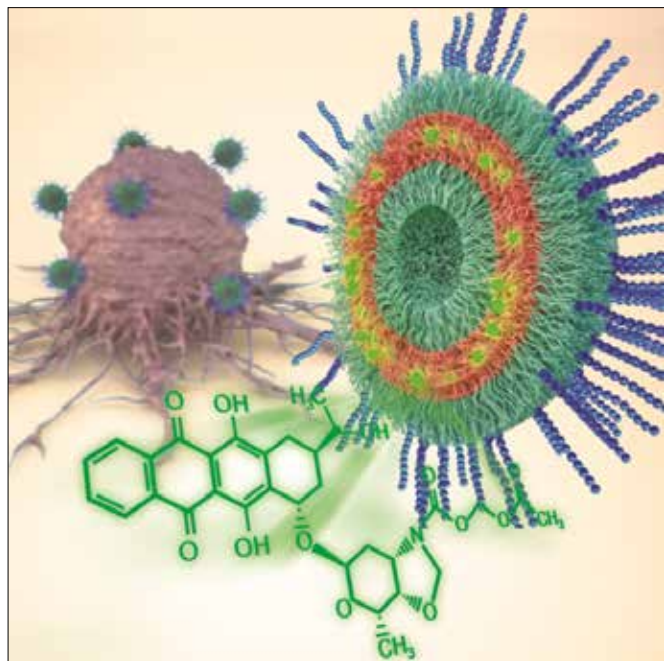


Figure 1. Novel anthracycline utorubicin encapsulated in peptide-targeted polymersomes for solid tumor targeting^[6]

Cancer is the second leading cause of death worldwide^[1] and mortality is expected to be rising in the future^[2]. Surgery and radiation therapy successfully eliminate the local and easily accessible tumors, but the disease spread all over the body can be controlled

only by systemic chemotherapy. However, for conventional chemotherapeutics, poor bioaccessibility, penetration, high off-target toxicity, and side effects due to exposure of normal cells to cytotoxic compounds remain unsolved challenges.

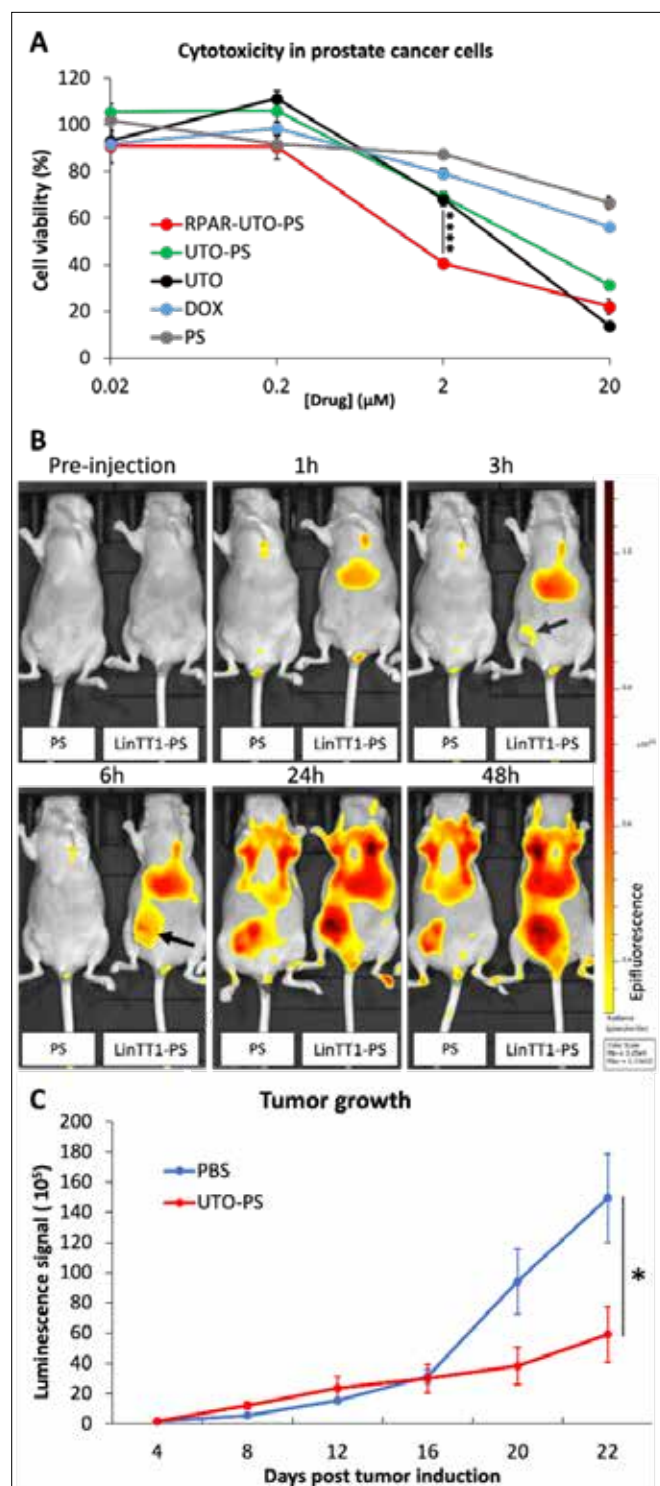


Figure 2. (A) *In vitro* viability of cultured prostate cancer cells (PCC-1, positive for expression of neuropilin-1, the receptor of RPAR homing peptide) after 30 min incubation with the indicated PS formulations at 0.02, 0.2, 2, and 20 μM of UTO, followed by 48 h follow up culture. (B) Peptide-guided polymersomes (LinTT1-PS) selectively accumulated in triple-negative breast tumor xenografts. PS: non-targeted polymersomes. (C) *In vivo* treatment with UTO-loaded PS decreased the progression of peritoneal carcinomatosis xenografts.

These problems can be alleviated by the development of drug-delivering nanoparticles (NPs) that specifically accumulate in tumors^[3]. Drug nanocarriers are engineered to have stealth properties to prolong the circulation half-life in the bloodstream to increase tumor exposure by drug-loaded nanocarriers^[4]. To enhance the selec-

tivity and accumulation of nanocarriers in tumor tissue, they can be functionalized with ligands having affinity to malignant tissue, such as antibodies and tumor-homing peptides^[5]. In addition, the NPs can be engineered to contain not only drugs and targeting ligands but also tracer agents that allow their diagnostic tracking^[3].

In collaboration with the Estonian company Toxinvent LLC, we developed a tumor-specific nanoplatform that specifically delivers a novel drug candidate utorubicin (UTO) to solid tumors (Fig. 1)^[6].

Free UTO was significantly more toxic to cultured tumor cell lines than the clinically used anthracycline, doxorubicin (Fig. 2A). Nano-formulated UTO encapsulated in biocompatible and biodegradable polymeric nanovesicles (polymersomes, PS) reduced the viability of cultured malignant cells and this effect was enhanced by functionalization with a tumor-penetrating peptide (TPP) (Fig. 2A). Systemic peptide-guided PS showed preferential accumulation in triple-negative breast tumor xenografts implanted in mice (Fig. 2B). Experimental treatment of mice bearing peritoneal carcinomatosis of gastric carcinoma origin (MKN45P) with UTO-PS resulted in efficient suppression of tumor growth (Fig. 2C). Our study suggests potential applications for UTO in the treatment of malignant diseases and encourages further preclinical and clinical studies on UTO as a nanocarrier payload for precision cancer therapy.

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NOVEL INSIGHTS ON ENDOSOMAL ESCAPE OF LIPID NANOPARTICLES USING REFLECTOMETRY TECHNIQUES

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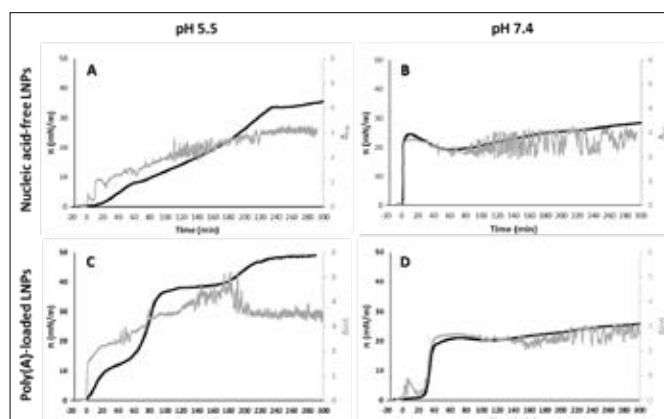


Figure 1. Change in surface pressure and Δ_{int} of nucleic acid-free LNPs (A and B) and Poly(A)-loaded LNPs (C and D) at pH 5.5 (A and C) and at pH 7.4 (B and D). LNPs Injection was performed at time 0. In presence of the endosomal membranes, at pH < 7.0 (Figure 2), a significant increase in π and in the baseline of Δ_{int} was recorded after injection of poly(A)-loaded LNPs. This is the result of the insertion of lipids and the delivery of ionizable lipid-nucleic acid complexes. On the other hand, at pH > 6.5 only minimal changes in π and Δ_{int} were recorded, except at pH 8.5 with EEM where fluctuations in Δ_{int} and larger domains with BAM were observed. As for LNPs alone, this is most likely the results of LNPs remaining as a whole at this pH, minimal LNP components integration into the monolayer and LNPs aggregation.

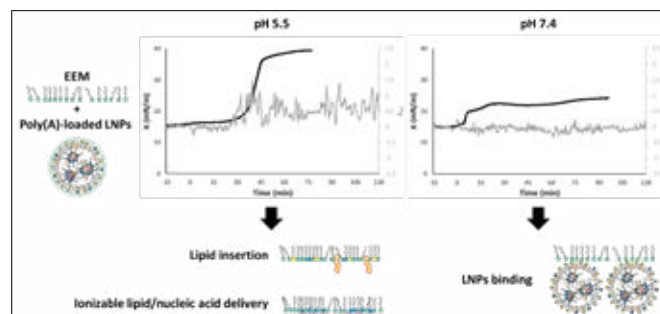


Figure 2. Change in surface pressure and Δ_{int} of EEM monolayer at pH 5.5 and pH 7.4 after injection of Poly(A)-loaded LNPs. LNPs Injection was performed at time 0.

BACKGROUND

Lipid nanoparticles (LNPs) are one of the most successful carriers for RNA delivery. In 2018, the U.S. Food and Drug Administration approved Onpattro, a LNP formulation containing siRNA targeting the transthyretin protein¹. Late in 2020, LNP vaccine formulations produced by Pfizer/BioNTech and Moderna containing nucleoside modified mRNA active against SARS-CoV-2, received marketing authorization in several parts of the world and these have now been administered to hundreds of millions of individuals^{2,3}. However, the limited understanding of endosomal escape mechanisms and the need to avoid lysosomal degradation, still represent a major bottleneck in the drive to significantly increase the amount of RNA cargo delivered to the cytosol (typically <3%) and thereby improve LNP efficacy, while reducing toxicity⁴. The proposed mechanism of LNP endosomal escape is that once within the acidic environment of the endosome, the ionizable lipid contained in the LNPs becomes cationic and interacts with the anionic lipids present in the inner side of the endosomal membrane forming hexagonal structures that can ultimately disrupt the membrane and allow the release of its nucleic acid cargo into the cytosol⁵.

AIM

Here, reflectometry techniques are used for the first time to study the interactions of nucleic acid-loaded LNPs injected below Langmuir monolayers as models of early and late endosomal membranes (EEM and LEM) at different pHs. The aim is to understand the physicochemical processes occurring at this stage of the endocytic pathway and to establish a robust physical basis to be used as a reference for future studies to develop more efficient LNPs.

RESULTS

In the present study, surface pressure (π) and ellipsometry (Δ_{int}) data highlighted different types of processes occurring at the air/water interface, such as the insertion of lipids, binding of whole LNPs and ionizable lipid-nucleic acid complexes delivery. Brewster angle microscopy (BAM) helped highlighting the presence of lipid domains of different phases in the monolayers.

Firstly, in absence of endosomal monolayers, higher π and Δ_{int} were reached at pH < 7.4 (Figure 1, A and C). We interpret this as components from LNPs translocating to the air/water interface. In fact, in acidic conditions, the ionizable lipids within LNPs become cationic

and LNPs unstable. Poly(A)-loaded LNPs reached higher π and Δ_{int} compared to nucleic acid-free LNPs, but only at pH 5.5 (Figure 1C), evidencing that the ionizable lipid, once positively charged, and nucleic acid form a complex that translocates at the air/water interface. At pHs > 7.0 fluctuations in Δ_{int} were recorded (Figure 1B and D), which are the result of larger/thicker domain formation as observed with BAM. We interpret this as LNPs remaining intact and, after PEG-shedding, tending to form aggregates at the air/water interface.

CONCLUSION

For the first time reflectometry techniques were used to investigate interactions between LNPs and model endosomal membranes. It was possible to interpret data obtained from π , Δ_{int} and BAM as clear, distinct phenomena occurring during endosomal escape. Such new information represents the basis for future studies on different LNPs formulations and, ideally, can be used to tune and design delivery systems with improved nucleic acid delivery capacities.

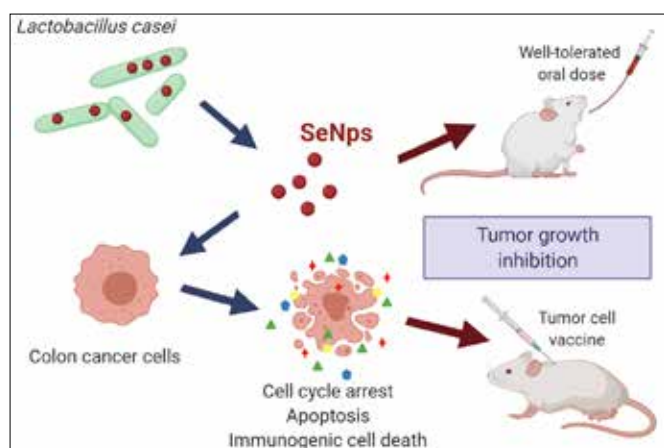
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DIVERSE BIOACTIVITIES OF BIOGENIC SENPS SPUR CANCER CELL-BASED VACCINE POTENTIAL

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Selenium (Se) is an essential trace element that exerts multiple and complex bioactivities. Regulation of immune responses and cancer cell growth inhibition are among the most interesting health-promoting effects that have been associated with Se. Noteworthy, there is a great demand for Se-based dietary supplements. However, Se has a narrow therapeutic index, as higher doses are associated with adverse toxic effects. Selenium nanoparticles (SeNPs) though, are more biosafe and more bioavailable Se forms.

We employed the probiotic strain *Lactobacillus casei* ATCC 393 to synthesize biogenic SeNPs. The nanoparticles were extracted from the bacteria, characterised, and their antitumor effects against colon cancer were assessed in cancer cell lines, in murine tumor models and in human biopsies.

We have previously reported that SeNPs induce apoptosis and immunogenic cell death in colon cancer cells. In this work, besides cell death, we also examined whether SeNPs regulate cell cycle. Moreover, we proceeded to investigate SeNPs' potential cancer-specific cytotoxicity by estimating the selectivity index in healthy primary and colon cancer cells and comparatively examined the growth inhibition induced by SeNPs to a non-nanoparticle source of Se (NaHSeO_3). Finally, by using SeNPs-treated CT26 cells that had underwent immunogenic cell death, we prepared a tumor cell vaccine that was administered in mice prior challenging the animals with live cancer cells of the same tumor cell line.

The round, 360 nm in diameter SeNPs were found to be decorated with bacteria-derived biomolecules, evident by the μ -FTIR analysis. Moreover, the nanoparticles were found to induce a G0/G1 cell cycle arrest in colon cancer cells analysed by flow cytometry, an observation which is in agreement with the higher protein levels of p21 and p27 that were also detected in SeNPs-treated cells. Significantly, the nanoparticles were not found to be toxic either in healthy primary cells or in mice upon oral administration in which they inhibited colorectal CT26 tumor growth by at least 50% as we had previously reported. Moreover, in the same CT26 tumor model in mice employed in a different experimental setup, the SeNPs-CT26 tumor cell vaccine delayed tumor growth and increased survival time of tumor bearing mice.

Our results indicate that the biogenic SeNPs derived from *L. casei* ATCC 393 exert cancer-specific growth inhibitory effects and induce apoptosis, cell cycle arrest and immunogenic cell death in colon cancer cells. These biogenic nanoparticles seem to hold a great potential as a novel anti-tumor agent that induces immunogenic cell death. Our research highlights the diverse bioactivities of the SeNPs that could be linked to the various bacteria-derived biomolecules associated with the nanoparticles. Future research should focus on the detailed characterisation of this coating and its potential contribution in the bioactivities attributed to SeNPs. Moreover, the immune responses raised by the SeNPs-treated cancer cells should be examined along with the reported ability of SeNPs to exert immunomodulatory effects. We believe that these biogenic SeNPs, could pose the basis for the development of novel combined-modality treatment approaches against colon cancer.

EBRAINS

MARTIN TELEFONT

EBRAINS is an AISBL registered in Belgium. It serves as the central hub of a distributed Research Infrastructure tasked with continuing efforts to build and develop a Brain Research Infrastructure. This effort was started in 2013 with the start of the EC-FET Flagship Human Brain Project and has seen contributions from 120+ partners over the decade of work it has active.

EBRAINS has been successful in its application for admittance to the ESFRI Roadmap 2021 and is actively preparing for the transition from project based development and support to research infrastructure development and support.

This poster gives a short overview of services hosted on EBRAINS which could be of interest to members of the nanomedicine community and advances researchers have achieved leveraging it in various settings (e.g. neurology, neurosurgery) and contexts (academia, clinical).



Clinical Topics: neurology, neurosurgery

Technology Topics: diagnostics, nano- imaging, GMP and quality assurance, diagnosis and therapy in the field of personalized medicine data from laboratory and imaging evaluation to diagnose and treat illnesses

Regulatory and Societal Affairs, Networking and Financing Topics: Cutting-Edge EU- Project Presentations

ENGINEERING NANOGELS FOR DRUG DELIVERY TO PATHOGENIC ASPERGILLUS FUMIGATUS

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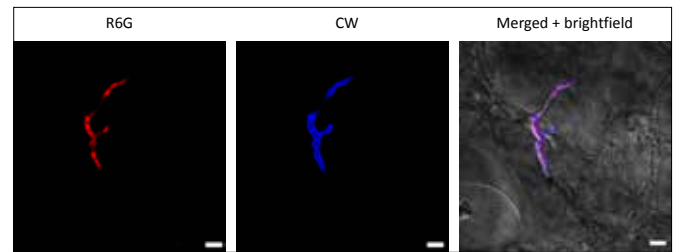
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The opportunistic mold *Aspergillus fumigatus* is one of the main fungal pathogens causing invasive infections in immunocompromised humans. Unfortunately, conventional antifungal agents are associated with low therapeutic efficacy and/or severe side effects.

[1] A promising alternative treatment approach is nanoparticle-based antifungal therapy. It leads to increased drug bioavailability and reduced toxicity, both boosting treatment efficacy^[2]. We previously showed that poly(glycidol)-(PG)-based nanogels have great potential when being used as vehicle for antifungals but need improvement when being co-incubated with fungus in the presence of serum^[3]. In this study, the nanogels (NGs) were modified to increase their affinity towards the positively charged cell wall of fungal hyphae by tuning their surface charge to more negative values. To form nanogels PG was modified with thiol groups as cross-linking moieties. Thiolated PG was synthesized via a three-step procedure beginning with an anionic ring opening polymerization followed by STEGLICH esterification for introduction of thiol groups. PG-based nanogels were prepared using an inverse nanoprecipitation technique as described before^[3]. The polymer was precipitated in acetone succeeded by oxidation leading to formation of hydrogel nanoparticles. To modify the nanogel surface charge an additional quenching step was introduced after oxidation. Furthermore, these NGs were loaded with different dyes as cargos using the “breathing-in” technique. To mimic the *in vivo* behavior of quenched NGs and their cargo, a precision-cut lung slices (PCLS) model, using lungs from healthy BALB/c mice, was employed for investigating dye-loaded, quenched NGs. The PCLS were infected with *Aspergillus fumigatus* spores and simultaneously co-incubated with NGs. We observed that, e.g., NGs loaded with an amphiphilic dye Rhodamine 6 G (R6G) were well taken up by the fungus (Figure 1: Confocal microscopy images of *A.fumigatus*-infected PCLS co-incubated with NGs (scale bars = 10µm). Strong uptake of R6G-loaded NGs by fungal hyphae can be observed. Hyphal cell wall was stained with calcofluor white (CW).), and only slightly taken up by the PCLS.

Figure 1: Confocal microscopy images of *A.fumigatus*-infected PCLS co-incubated with NGs (scale bars = 10µm). Strong uptake of R6G-loaded NGs by fungal hyphae can be observed. Hyphal cell wall was stained with calcofluor white (CW).



These results encourage future studies to further investigate the interaction of nanogels with fungus as well as the functionalization of particles with different targeting moieties and loading with antifungal agents.

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A POLYOXOMETALATE INCORPORATING, INJECTABLE HYDROGEL WITH PH- AND NIR-RESPONSIVENESS FOR CHEMO-PHOTOTHERMAL THERAPY

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Introduction: Polyoxometalates (POMs) are an emerging class of metal-oxide molecular clusters. Recent studies showed POMs have promising applications in the biomedical fields, such as ROS scavengers, anti-tumor therapeutics, and photo/radio sensitizers.^[1,2] In particular, {Mo154} is a promising photothermal agent due to its intervalence charge transfer transitions.^[3] However, its toxicity hinders the systemic administration. Herein, we developed an injectable and self-healing hydrogel (Mo154Gel) of easy preparation and administration, incorporating both {Mo154} and doxorubicin (DOX) for synergistic photothermal and chemotherapy (PCT). We chose a di-benzaldehyde functionalized polyethylene glycol (DF-PEG) and a thermo-responsive derivative from chitosan (CS-g-PNIPAAm) to generate the hydrogel network by imine bond, and a secondary crosslinking by the electrostatic interactions between the anionic {Mo154} and the cationic CS-g-PNIPAAm (Figure 1). Both crosslinking strategies are dynamic and reversible, meaning the whole hydrogel network could re-generate after mechanical break.

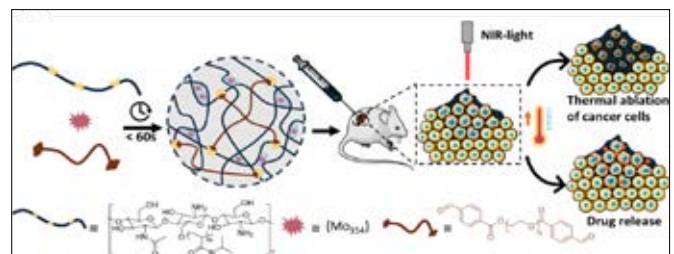


Figure 1 Overall scheme showing the design, preparation, and synergistic effects of PTT and NIR-triggered DOX release from Mo154Gel. Reproduced from *Advanced Materials*, 2007761. <https://doi.org/10.1002/adma.202007761>

Methods: Mo154Gel was prepared by simple mixing all the components at room temperature, and characterized by different techniques, to confirm the successful dual-crosslinking network. The rheological behaviour was also studied. Afterwards, we investigated the photothermal properties of Mo154Gel, and the controlled release of DOX. Finally, we evaluated the tumor ablation effects *in vitro* and *in vivo*, using DOX loaded Mo154Gel, combined with near-infrared (NIR) laser irradiation.

Results: Mo154Gel formed in 1 min upon mixing (Figure 2a). The hydrogel was homogenous, with uniform {Mo154} distribution. Rheological analysis confirmed the double-crosslinking network, and the fast recovery from damage, indicating the self-healing properties (Figure 2b and c). After NIR laser irradiation, Mo154Gel showed significant temperature increase of 35 °C within 10 min (Figure 2b and c). with good photothermal stability. DOX loaded Mo154Gel showed both pH- and NIR-triggered release due to the pH-sensitivity from Schiff-based bond and the thermo-responsiveness from CS-g-PNIPAAm (Figure 2e). *In vitro* cytotoxicity suggested Mo154Gel had negligible toxicity, but after NIR-laser irradiation melanoma cells were successfully suppressed (Figure 2f). A proof-of-concept *in vivo* study showed that Mo154Gel is safe and biodegradable within 2 weeks (Figure 3a and b). Further application of PCT led to complete tumor eradication in 5 mice (Figure 3c). The combination therapy of PCT led to better therapeutic outcomes compared with either chemotherapy or photothermal therapy alone (Figure 3c).

Figure 2. (a) and (b) Rheological studies of the hydrogel. Gel means the control hydrogel made of polymers without {Mo154}, while Mo154Gel means hydrogel incorporating {Mo154}. (a) Time sweep rheological analysis of the Gel (black) and Mo154Gel (green) performed at 25 °C and 1% of strain. (b) The G' and G'' in continuous step strain measurements (cycles of 1% and 100% of strain at 4 min for each cycle). (c) Self-healing and injectability: photo of the hydrogel right after punching a hole (~0.8 cm in diameter) (i) and 4 min after (ii). (iii) Photo of the hydrogel extruded using a syringe to write letters "UH". (d) Photothermal images of Gel and Mo154Gel after laser irradiation at 808 nm for 10 min at 0.8 W cm⁻². (e) NIR laser (0.8 W cm⁻²) triggered release of DOX from Mo154Gel at pH 7.4 and 6.2 at 37 °C. The dashed line corresponds to the release in the absence of the laser cycles (pH 7.4). Results are presented as mean ± standard deviation (N=3). Adapted from *Advanced Materials*, 2007761. <https://doi.org/10.1002/adma.202007761>

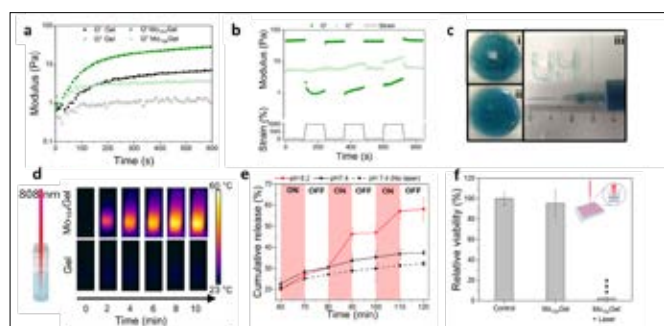
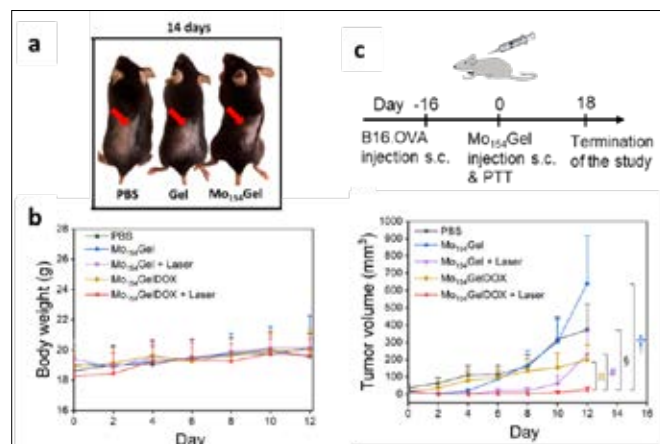


Figure 3. *In vivo* results. (a) Hydrogel degradation *in vivo* in healthy C57BL/6J mice. The red arrows indicate the location of the injection. (b) and (c) The proof-of-concept of the efficacy and safety of Mo154Gel on C57BL/6J mice with B16.OVA melanoma. (b) Body-weight and (c) tumor growth curve of mice treated with PBS, Mo154Gel, Mo154Gel and subsequent laser irradiation (0.8 W cm⁻², 10 min), DOX loaded Mo154Gel and DOX loaded Mo154Gel followed by laser irradiation (0.8 W cm⁻², 10 min). Results are expressed as mean ± standard error of the mean (N=7). The statistical test was performed by two-way ANOVA followed by Tukey's post-test. αp

< 0.0001 , # $p = 0.053$, § $p < 0.0001$, † $p < 0.001$, compared with Mo154GelDOX + Laser group. Adapted from *Advanced Materials*, 2007761. <https://doi.org/10.1002/adma.202007761>



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For more figures and detailed results, please check: *Advanced Materials*, 2007761. <https://doi.org/10.1002/adma.202007761>

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COMPARATIVE ANALYSIS OF NUCLEIC ACID-BASED ADJUVANTS FOR THE ACTIVATION OF DENDRITIC CELLS TO IMPROVE NANO-VACCINES

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DCs (dendritic cells) are a primary target of nano-vaccines due to their potency to induce even primary T cell responses at activated state. Protein and peptide antigen of exogenous origin is presented primarily via MHCII only, yielding predominantly activation of antigen-specific CD4+ T helper cells. The usage of antigen-encoding mRNA has the major advantage that transfected DC will present antigen-derived peptides in parallel also via MHCI, stimulating CD8+ T cells to differentiate to tumor cell killing cytotoxic T lymphocytes.^[1]

It is conceivable that *in vivo* only a limited number of nano-vaccines will transfect a given DC, which means that the amount of co-delivered adjuvant will be very limited. In order to yield maximal DC stimulation, it may be beneficial to co-deliver besides antigen-encoding mRNA that may trigger as well e.g. TLR3 also different types of stimulatory nucleic acid-based adjuvants, which activate distinct danger receptors and signaling pathways, but converge on the level of gene expression. Therefore, co-administration of mRNA plus various types of stimulatory nucleic acid-based adjuvants may yield synergistic effects in terms of DC activation. We have started to screen various CpG-containing oligodesoxynucleotides (CpG oligos) known to trigger TLR9 as well as DNA oligos known to engage cytoplasmic DNA sensors to identify for both types of adjuvants which within their group yield maximal DC activation.^[2,3] In a second round of experiments these adjuvants are co-applied, with each component at suboptimal concentration to monitor synergistic effects. For this, we screen on the one hand expression of surface ac-

tivation markers required for antigen presentation (MHC I, MHC II), co-stimulators (CD80, CD86) as well as pro-inflammatory cytokines like IFN α , IL-12 and TNF α , all required for optimal T cell stimulation. In addition, we also monitor expression of co-inhibitory receptors (PD-L1, PD-L2) and anti-inflammatory cytokines (e.g. IL-10), which may be upregulated as well, since the ratio of costimulatory and co-inhibitory signals determines the extent of T cell activation and their polarization. We perform these tests using murine bone marrow derived DC, differentiated from bone marrow with either GM-CSF, yielding a rather homogenous population of inflammatory (inf)DC, or FLT3L giving rise to a heterogeneous composition of DC subpopulations (cDC1, cDC2, pDC).

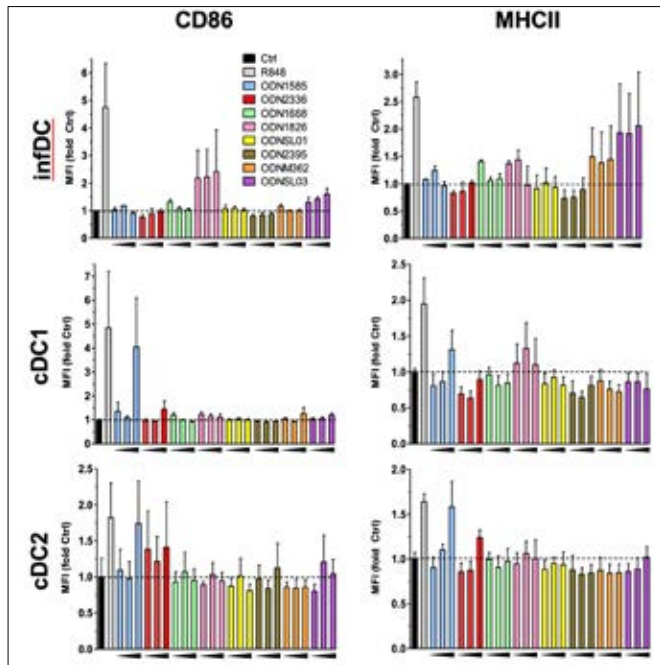
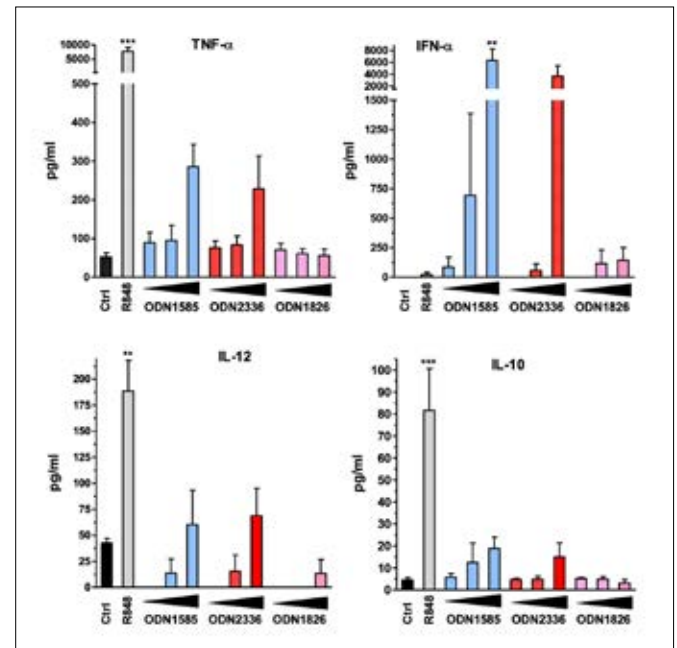


Figure 1. Expression of MHCII and the costimulatory receptor CD86 by DC populations. DC were incubated with different concentrations of CpG oligos (50, 100 or 250 ng/ml) or R848 (1 μ g/ml). On the next day, expression of MHCII and CD86 by inflammatory (inf) DC and cDC1/2 was assessed by flow cytometric analysis. Graphs denote the fluorescence intensities (MFI) (mean \pm SEM of 3-4 experiments) of marker expression. Preliminary data revealed striking differences in the stimulatory activity of the different CpG oligos in the different DC subsets analyzed (Figure 1). The TLR7/8 agonist R848 was included as a positive control. infDC showed enhanced levels of CD86 expression after overnight incubation with ODN1826 only, whereas ODNLS03 was most potent to confer upregulation of MHCII. Further, both cDC1 and cDC2 responded strongest to treatment with ODN1585 by upregulating of CD86 and of MHCII especially in case of cDC2.

After the evaluation of DC activation markers, cytokine analysis of FLT-3L differentiated BMDC was performed to investigate the efficacy of those CpG oligos which upregulate surface activation markers of cDC1/2 on the level of cytokine release (Figure 2). ODN1585 as well as ODN2336 in a dose-dependent manner exerted comparable stimulatory effects on all cytokines monitored, including type-I interferon IFN- α which induces strong anti-viral effects. In addition, pro-inflammatory IL-12 as well as TNF- α , required to differentiate/polarize T cells to exert anti-tumor effects. The preliminary results show the DC population-specific efficacy of the respective CpG oligos which are the base for further adjuvant analysis. Ongoing experiments are dedicated to evaluate the suitability of virus-derived DNA oligos (HSV60, ISD, VAVC70) which trigger cytoplasmic DNA sensors such as STING and MDA5 for DC stimulation.^[3] As a next step potential synergistic effects of both types of nucleic acid-based adjuvants will be evaluated.

Figure 2. CpG oligos enhance pro-inflammatory cytokine release of DC. FLT3L-differentiated DC were stimulated and incubated as

described (Fig. 1) and supernatants were retrieved before subjecting cells to surface marker analysis. Cytokine concentrations were assayed by CBA. Data denote the mean \pm SD of 2-3 experiments. Statistical differences versus *Ctrl are indicated (one way ANOVA, Tukey test). ** p <0.01, *** p <0.001.



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