Conference Book

Helsinki, Finland

TABLE OF CONTENTS

Production of the GPEN 2014 Conference Book sponsored by Allergan Inc.

GREETINGS FROM THE COMMITTEE

Dear Colleagues,

It is our great pleasure to welcome you here in Helsinki at GPEN 2014. About one year of organizing and planning lies behind us and finally the 10th biennial meeting of the Globalization of Pharmaceutics Education Network (GPEN) has arrived. It has been both a demanding and rewarding time and we are grateful to all the people involved in organizing GPEN 2014 and supporting the event as session chairs, judges, or by provision of guidance.

Over 270 attendees not only means that we repeat to achieve the high attendance of GPEN 2012, but is a clear sign of the quality and importance of GPEN as an institution. Furthermore, this year we are happy to feature 50 podium presentations, 108 poster presentations, and 8 short courses with expert speakers from academia and industry alike.

We are very excited to have Professor Jonathan Knowles from the Institute for Molecular Medicine Finland (FIMM, University of Helsinki) give the keynote lecture on curing serious diseases. In our opinion the wish to reach this aim and being able to help and serve humanity is one of strongest motivators when being involved with health sciences.

The Organizing Committee wishes great experiences during the scientific sessions, the career center, and the social events as well as new, interesting, and beneficial contacts to all of you. We also wish all the best to the organizers of the upcoming GPEN 2016 meeting in Kansas and encourage the doctoral students to seize these chances to organize, to interact, and to grow beyond the purely day-to-day academic settings whenever possible.

Enjoy your time in Finland and have a great time at GPEN 2014!

On behalf of the Organizing Committee,

Noora Sjöstedt
Co-Chair Student Organizing Committee
University of Helsinki

Björn Peters
Co-Chair Student Organizing Committee
University of Eastern Finland

Prof. Arto Urtti
Co-Chair Organizing Committee
University of Helsinki

Prof. Paavo Honkakoski
Co-Chair Organizing Committee
University of Eastern Finland

THE HOST UNIVERSITIES

The School of Pharmacy, University of Eastern Finland

The mission of the School of Pharmacy is to teach pharmaceutical subjects at the highest educational level, and to promote scientific drug research and its application for the benefit of society at large.

The School of Pharmacy at the University of Eastern Finland started in the present form in 2010, but was originally founded in 1973 in the University of Kuopio. The staff is about 150, and approximately 700 students aspire towards Bachelor and Master Degrees in six disciplines of Social Pharmacy, Pharmaceutical Chemistry, Biopharmacy, Pharmaceutical Technology, Pharmacology and Toxicology. Clinical Pharmacology is taught mainly for students of Medicine and Dentistry. We also run the Master's Degree Programme in General Toxicology and Environmental Health Risk Assessment. The School has about 100 post-graduate students, who conduct their advanced studies towards the Ph. D. degree.

The School also houses the Drug Research and Development Centre, which offers a dynamic and flexible model for industrial collaboration.

Faculty of Pharmacy, University of Helsinki

The history of the Faculty of Pharmacy began in 1897 when the Pharmaceutical Institute was founded in Helsinki. Later the Institute was turned into a Department of Pharmacy. It was first a part of the Faculty of Medicine and then the Faculty of Science. The Faculty of Pharmacy, situated on Viikki Campus, was founded in 2004.

The Faculty offers the Bachelor and Master of Science degrees in Pharmacy. The postgraduate degrees are the Doctor of Philosophy in Pharmacy and the Doctor of Philosophy. The Faculty also provides professional postgraduate education in industrial pharmacy and hospital pharmacy and plays an active part in the continuing education in the field. The Faculty is also nationally responsible for the Swedish language Master's degree programmes in pharmacy.

The Faculty of Pharmacy staff is about 183, and approximately 950 students, of which 205 are undergraduate and 127 are postgraduate students.

The Faculty complies 3 divisions that are running research in the fields of biopharmaceutics, pharmaceutical biology, pharmaceutical chemistry, pharmaceutical technology, pharmacology, and social pharmacy. Faculty hosts also Centre for Drug Research (CDR), a multidisciplinary research program that is carrying out research on drug discovery tools and pharmaceutical nanotechnologies, particularly in relation to natural products and biologicals.

THE LOCAL ORGANIZING COMMITTEE

UNIVERSITY OF EASTERN FINLAND

UNIVERSITY OF HELSINKI

Björn Peters
Hristo Zlatev
Laura Pelkonen
Piia Kokkonen
Sofia Sousa
Prof. Paavo Honkakoski

Noora Sjöstedt
Astrid Subrizi
Eva Ramsay
Otto Kari
Dr. Paul Bromman
Tatu Lajunen
Prof. Arto Urtti

Former members:

Henna Ylikangas
Dr. Pyry Välitälo

ACKNOWLEDGMENTS

The GPEN 2014 Local Organizing Committee expresses their upmost gratitude to all who contributed to the successful organization of the GPEN 2014 meeting. In particular a special thanks to:

- Professor Ron Borchardt and the GPEN Executive Committee and Board of Directors (Professors Kenneth Audus, Teruna Siahaan and Per Artursson)
- Ms Nancy Helm (University of Kansas)
- University of Helsinki staff (Professor Marjo Yliperttula, Ms Pia-Tuulia Sonck and Ms Katri Kiiliäinen)
- Special thanks to Dr. Paul Bromann of the University of Helsinki Faculty of Pharmacy, for valuable assistance in planning and organizing GPEN 2014
- University of Eastern Finland, School of Pharmacy staff (Ms Marja Lappalainen)
- Professor Jonathan K.C. Knowles (Keynote Speaker)
- All judges of oral and poster presentations
- All industry observers
- All short course coordinators
- All session chairs
- All faculty and industrial short course invited speakers
- All faculty and industrial career center participants
- All GPEN 2014 sponsors
- GPEN 2012 Organizing Committee (Monash University)

SPONSORS

The GPEN 2014 Local Organizing Committee would like to thank all the meeting sponsors, for their generous contribution and support in all forms.

admescope

UPM
The Biofore Company

BIOPREDIC INTERNATIONAL

AstraZeneca

Pfizer

FinPharma Doctoral Program

Lilly

WILEY

ORION PHARMA

創剤フォーラム
Forum for Pharmaceutical Technology Innovation

THE NAGAI FOUNDATION TOKYO 1986

SUOMEN FARMASEUTTINEN YHDISTYS
FARMACEUTISKA FÖRENINGEN I FINLAND
THE FINNISH PHARMACEUTICAL SOCIETY

Bristol-Myers Squibb
Together we can prevail.®

SPONSORS LIST

PLATINUM SPONSORS

Absorption Systems LP

FPDP Pharmacy

Genentech, Inc.

GOLD SPONSORS

AbbVie

Janssen Research & Development, A Division of Johnson & Johnson

F. Hoffmann-La Roche AG

Federation of Finnish Learned Societies

SILVER SPONSORS

Allergan Inc.

Biogen Idec

Boehringer-Ingelheim Pharmaceuticals Inc.

Bristol-Myers Squibb Co.

Doctoral Programme in Materials Research and Nanosciences University of Helsinki

The Nagai Foundation Tokyo

Pfizer Pharmaceutical Sciences

UPM

BRONZE SPONSORS

AstraZeneca

Charles River

Eisai, Inc.

Eli Lilly and Company

Gilead Sciences, Inc.

GlaxoSmithKline

Journal of Pharmaceutical Sciences (John Wiley & Sons)

Merck & Co.

Orion Corporation

Santen Oy

FRIENDS OF GPEN 2014

Admescope Ltd.

Biopredic International

Dainippon Sumitomo Pharma Co., Ltd

Novartis Institutes for Biomedical Research

Shionogi and Co., Ltd

The Forum for Pharmaceutical and Technology Innovation, Japan

The Finnish Pharmaceutical Society

KEYNOTE

Wednesday, August the 27th 2014

5:00 PM-6:00 PM
Main building of the University of Helsinki

How can we be more successful in creating cures for serious diseases?

Dr. Jonathan K.C. Knowles

Institute for Molecular Medicine Finland (FIMM),

University of Helsinki, Finland

Dr. Knowles attended Magdalen College School in Oxford and received a First Class Honours Degree in Molecular Genetics from the University of East Anglia in Norwich, England. He received his Ph.D. in Genetics of Mitochondria with Professor G. H. Beale F.R.S. from the University of Edinburgh in Scotland.

Dr. Knowles was Head of Group Research and Member of the Executive Committee at Roche up to the end of 2009. He was a member of the Genentech Board for the last 12 years and a member of the Chugai Board for seven years. Dr. Knowles was also the chairman of the Corporate Governance Committee of Genentech. Dr. Knowles focused the company on key disease biology areas of high medical need and in-depth understanding of molecular pathology of disease. Under his leadership, the company developed and implemented a strategy of highly effective therapies based on personalized healthcare and built one of the best pharma pipelines in the sector.

Prior to this he served as Director of the Glaxo Institute in Geneva for 10 years and as head of European Research for Glaxo Wellcome.

He was for 5 years the Chairman of the Research Directors' Group of EFPIA (European Federation of Pharmaceutical Industry Associations) and was the first chairman of the Board of the Innovative Medicines Initiative, a unique public-private partnership between 28 Pharmaceutical companies and the European Commission with a budget of more than 2 Billion Euros over five years.

Jonathan Knowles holds a Distinguished Professorship in Personalised medicine at FIMM (Institute for Molecular Medicine Finland FIMM) at the University of Helsinki, Emeritus Professorship of Translational Medicine at EPFL in Switzerland, and has been appointed to a Visiting chair at the University of Oxford. In addition, he is a Member of the European Molecular Biology Organization and a Visiting Fellow of Pembroke College Cambridge. In 2011, Jonathan Knowles was appointed as a Trustee of Cancer Research UK, one of the world's leading Cancer Research Organisations.

PROGRAM

GPEN 2014

SCHEDULE OVERVIEW

August 27 Wednesday	August 28 Thursday		August 29 Friday		August 30 Saturday		August 31 Sunday
Arrival	*8 am - 8.10 am* Opening Remarks	*9 am - 12 pm* Career Center	*8.30 am - 12 pm* Podium Presentations	*9 am - 12 pm* Career Center	*9 am - 12.30 pm* Short Courses	*9 am - 12 pm* Career Center	*10 am - 6 pm* Optional Social Activity: Porvoo Day Trip
	8.10 am - 12 pm Podium Presentations						
12 pm - 5 pm Registration	*12 pm - 1.30 pm* Lunch & Poster Session		*12 pm - 1.30 pm* Lunch & Poster Session		*12.30 pm - 1.30 pm* Lunch		
	1.30 pm - 5.20 pm Podium Presentations	*1.30 pm - 5.20 pm* Career Center	*1.30 pm - 5.20 pm* Podium Presentations	*1.30 pm - 5.20 pm* Career Center	*1.30 pm - 5 pm* Short Courses	*1.30 pm - 5 pm* Career Center	
5 pm - 6 pm Keynote					*5 pm - 5.30 pm* Award Ceremony & Conference Closing		
6.30 pm - 8 pm Welcome Reception	*6 pm - 9 pm* Social Get-Together		*7.30 pm - 11 pm* Conference Banquet				

Wednesday, August the 27th

Time	Location	
12:00 PM	Main Building of the University of Helsinki	Registration
5:00 PM	Main Building of the University of Helsinki	Keynote **How can we be more successful in creating cures for serious diseases?** *Jonathan Knowles (Institute for Molecular Medicine Finland FIMM, University of Helsinki, Finland)*
6:30 PM	Helsinki City Hall	Welcome Reception

PROGRAM PODIUM

Thursday, August the 28th, Biomedicum Helsinki

Sponsored by Biogen Idec and Boehringer-Ingelheim Pharmaceutical Inc

Time		
8:00 AM		Conference Opening
8:10 AM	**S1**	Development of Dendrimer Based Drug Delivery System for Anticancer Therapy Kıvılcım Öztürk (Hacettepe University)
8:30 AM	**S2**	CpG Conjugation to Model Tumour Antigen Ovalbumin Leads to Enhanced $CD8^+$ T-Cell Proliferation Katrin Kramer (University of Otago)
8:50 AM	**S3**	Quantitative Analysis of In Vivo Fate of Mouse Melanoma B16BL6-Derived Exosomes after Intravenous Injection into Mice by Development of a Labeling Method for Exosomes Masaki Morishita (Kyoto University)
9:10 AM	**S4**	The Insulin Receptor: An Achilles' Heel for Schistosome Vaccine Development Rachel Stephenson (University of Queensland)
9:30 AM	**S5**	Depth Controlled Dermal Immunization of Rats with Inactivated Polio Vaccine by using Hollow Microneedles Pim Schipper (Leiden University)
9:50 AM	**S6**	Challenges of Determining Intrinsic Viscosity in Strongly Interacting Monoclonal Antibody Solutions Mariya Pindrus (University of Connecticut)
10:10 AM		Coffee Break, sponsored by Santen Oy
10:40 AM	**S7**	The Use of Human Intestinal Fluids to Study the Solubility/Permeability Interplay: Effect of Food on Carvedilol Absorption Jef Stappaerts (Katholieke Universiteit Leuven)
11:00 AM	**S8**	Effect of Intestinal Hydrolysis of Olmesartan Medoxomil on its Absorption Takahiro Yonemitsu (Kumamoto University)
11:20 AM	**S9**	Assessment of Vancomycin AUC/MIC and Clinical Outcome in MRSA Patients Alexander Voelkner (University of Florida)
11:40 AM	**S10**	Characterization and Application of Highly Stable 2'-F RNA Aptamers Specifically Targeting a Novel Biomarker of Pancreatic Ductal Adenocarcinoma (PDAC) Sarah E. Claypool (University of North Carolina)

12:00 PM		Lunch & Poster Presentations
1:30 PM	**S11**	Development of Controlled-Released Bee Venom Nasal Spray for Immunity Improvement Cho-A Lee (Chungnam National University)
1:50 PM	**S12**	Quantitative Evaluation of Intracellular Distribution and Human Placental Transfer of Doxorubicin and its Liposomal Formulations Suvi K. Soininen (University of Eastern Finland)
2:10 PM	**S13**	Synthesis and Biological Evaluation of Lipophilic Pt(IV) Derivatives of Oxaliplatin Incorporated in Nano-Carrier with Distinct Improvement in Anti-Tumor Activity Aiman Abu-Ammar (Hebrew University of Jerusalem)
2:30 PM	**S14**	Stable Polymeric Micelles for Tumor-Targeted Delivery of Therapeutic and Diagnostic Agents after IV Injection Yang Shi (Utrecht University)
2:50 PM	**S15**	Development of ^{177}Lu-Labeled Cathepsin S Cleavable HPMA Copolymers for Targeted Pancreatic Tumor Imaging and Radiotherapy Wen Shi (University of Nebraska)
3:10 PM		Coffee Break, sponsored by Orion Corporation
3:40 PM	**S16**	Prediction of Drug Loading in Single Excipients and Lipid Based Formulations Linda C. Persson (Uppsala University)
4:00 PM	**S17**	Modeling the Gram Negative Bacterial Cell Envelope: A New Approach for Permeability Investigation of Anti-Infectives Florian Gräf (Saarland University)
4:20 PM	**S18**	The Dispersion Releaser: An Optimized Tool for Selection of Colloidal Dosage Forms During Development Christine Janas (University Frankfurt)
4:40 PM	**S19**	Film Thickness Distribution Impacts Sunscreen Efficacy Myriam Sohn (University of Basel)
5:00 PM	**S20**	Cardiotoxicity of Donepezil Due to the Drug-Drug Interaction on the Efflux Transporter in the Heart Ryota Takeuchi (Kanazawa University)

Tali Football Hall (transportation arranged from Biomedicum)

6:00 PM	Social Get Together

Friday, August the 29th , Biomedicum Helsinki

Sponsored by Janssen Research & Development, A Division of Johnson & Johnson and Pfizer Pharmaceutical Sciences

Time		
8:30 AM	**S21**	Genetic Variants in Transcription Factors Are Linked to the Pharmacokinetics and Pharmacodynamics of Metformin Srijib Goswami (University of California San Francisco)
8:50 AM	**S22**	Effects of Angiotensin (1-7) Treatment on Diabetic Complications Anna Papinksa (University of Southern California)
9:10 AM	**S23**	Fluorescent Particles for the Tracking of Metastatic Cells Athanasia Dasargyri (ETH-Zurich)
9:30 AM	**S24**	Overcoming Trastuzumab Resistance via Pharmacological Inhibition of MEOX1 in Breast Cancer Stem Cells Joseph Burnett (University of Michigan)

9:50 AM	**S25**	Investigation of the Mechanism of Anti-Tumor Action of Arsenic Trioxide in Ovarian Cancer Cell Lines Kamila Katarzyna Kaminska (National University of Singapore)
10:10 AM		Coffee Break, sponsored by AstraZeneca
10:40 AM	**S26**	Quantification of Factors Governing Drug Release Kinetics from Nanoparticles: A Combined Experimental and Mechanistic Modeling Approach Kyle D. Fugit (University of Kentucky)
11:00 AM	**S27**	Nanoparticles in Polymer Nanospheres to Stabilize Gelatin without Crosslinkers Saeed Ahmad Khan (Philipps-University Marburg)
11:20 AM	**S28**	Oligonucleotide Hybridization-Mediated Drug-Free Macromolecular Therapeutics Te-Wei Chu (University of Utah)
11:40 AM	**S29**	The Influence of Shape on Cellular Uptake and Magnetic Targeting of Iron Oxide Nanoparticles ZhiZhi Sun (University of Manitoba)
12:00 PM		Lunch & Poster Presentations
1:30 PM	**S30**	The Involvement of Fatty Acid-Binding Protein 5 in the Blood-Brain Barrier Transport of Docosahexaenoic Acid Yijun Pan (Monash University)
1:50 PM	**S31**	Palbociclib Efficacy in Glioblastoma is Limited by Efflux Pump Activity at the Blood-Brain Barrier Karen E. Parrish (University of Minnesota)
2:10 PM	**S32**	HepaRG Acellular Matrix for Hepatic Differentiation of Human Pluripotent Stem Cells Liisa Kanninen (University of Helsinki)
2:30 PM	**S33**	Improved Liver Function and Relieved Pruritus after 4-Phenylbutyrate Therapy in a Patient with Progressive Familial Intrahepatic Cholestasis Type 2 Sotaro Naoi (University of Tokyo)
2:50 PM	**S34**	Constitutive Androstane Receptor Modulates Hepatic Energy Homeostasis with Species Selectivity Caitlin Lynch (University of Maryland)
3:10 PM		Coffee Break, sponsored by Charles River
3:40 PM	**S35**	The Effect of Route of Administration on the Immunogenicity of Recombinant Murine Growth Hormone Protein Aggregates Merry Christie (University of Colorado)
4:00 PM	**S36**	Parathyroid Hormone Coupled to Cell Penetrating Peptides for Oral delivery Mie Kristensen (University of Copenhagen)
4:20 PM	**S37**	Soluble Antigen Arrays (SAgAs) Mitigate Experimental Autoimmune Encephalomyelitis (EAE) Sharadvi Thati (University of Kansas)
4:40 PM	**S38**	Liquid Crystalline Nanodispersion as a Topical Delivery System for siRNA: Development, Characterization and In Vivo Knockdown Study Livia Vieira Depieri (University of Sao Paulo)
5:00 PM	**S39**	Pharmacological Modulation of Intratesticular Retinoic Acid Concentration and its Therapeutic Potential Samuel Arnold (University of Washington)

Vanha Ylioppilastalo / “Old Student house”, Mannerheimintie 3, Helsinki

7:30 PM Conference Banquet

PROGRAM SHORT COURSES

Saturday, August the 30th Biomedicum Helsinki, Short Courses I-IV (9:00 AM - 12:30 PM)

I Targeted Therapeutics: Protein and Peptide Drug Conjugates

Coordinator: Jennifer S. Laurence(University of Kansas)

Time	**Lecture Hall 1**, sponsored by Bristol-Myers Squibb Co.
9:00 AM	Introduction to Therapeutic Conjugates Jennifer S. Laurence (University of Kansas)
9:10 AM	The Chemistry of Synthesizing Polypeptide Drug Conjugates Teruna J. Siahaan(University of Kansas)
9:45 AM S40	Investigation of the Aqueous- and Solid-State Stability of Oxytocin as a Function of pH Gemma Nassta (Monash University)
10:05 AM	Chemical Instabilities of Antibody Drug Conjugates Christian Schoneich (University of Kansas)
10:40 AM	Coffee Break
11:00 AM	Analysis of Therapeutic Conjugates John Stobaugh (University of Kansas)
11:35 AM S41	Calculating the Mass of Subvisible Particles with Improved Accuracy as Formed from Stressed IgG1 Monoclonal Antibody Solutions Using Microflow Imaging Data Cavan Kalonia (University of Kansas)
11:55 AM	Physical Stability of Protein Conjugates Jennifer S. Laurence (University of Kansas)
12:30 PM	Lunch, sponsored by Merck and Co

II Drug Metabolism and Metabolomics

Coordinator: Seppo Auriola (University of Eastern Finland)

Time	**Seminar Room 1-2**, sponsored by FPDP Pharmacy
9:00 AM	DMPK along the Value Chain in Drug Discovery Tommy Anderson (AstraZeneca)
9:45 AM	Drug Metabolism Induction and Inhibition Studies in Drug Discovery and Clinical Implications & Pharmacogenetic Considerations in Drug Metabolism Tommy Anderson (AstraZeneca)
10:30 AM	Coffee Break
11:00 AM	Case Studies on how to Discover Drug Candidates with Optional Metabolic Properties Bianca Liederer (Genentech)
11:35 AM	Introduction to Metabolomics Kati Hanhineva (University of Eastern Finland)
12:10 PM S42	Role of Carrier Mediated Efflux of Nobilin Conjugation Products and Plant Extract of *Anthemis nobilis* L. in Nobilin Absorption in the Caco-2 Model Ursula Thormann (University of Basel)
12:30 PM	Lunch, sponsored by Merck and Co

III Pharmacokinetic and Pharmacodynamic Modelling in Drug Development and Treatment Individualization

Coordinator: Catherijne Knibbe (University of Leiden)

Time	**Lecture Hall 3,** sponsored by FPDP Pharmacy
9:00 AM	Introduction to PK/PD Modelling Concepts Pyry Välitalo (University of Leiden)
9:30 AM	Modelling & Simulation as a Decision Making Tool in Oncology Drug Development Coen van Hasselt (University of Leiden)
10:00 AM	Application of Disease System Analysis in the Investigation of Postmenopausal Osteoporosis Jan Berkhout (University of Leiden)
10:30 AM	Coffee Break
11:00 AM	Population PKPD Modelling to Optimise Dosing in Special Patient Populations Catherijne Knibbe (University of Leiden)
11:45 AM	Time-To-Event Models in Clinical Drug Development Benjamin Weber (Boehringer Ingelheim)
12:15 PM	Plenary Discussion
12:30 PM	Lunch, sponsored by Merck and Co

IV Pharmacogenomics and Precision Medicine

Coordinator: Tim Wiltshire (University of North Carolina)

Time	**Seminar Room 3,** sponsored by FPDP Pharmacy
9:00 AM	Pharmacogenetics: the Promise, and the Delivery Tim Wiltshire (University of North Carolina)
9:45 AM	Pharmacogenomics of Transporters Mikko Niemi (University of Helsinki)
10:25 AM	Coffee Break
10:40 AM	Pharmacogenomics and Drug Metabolism Janne Backman (University of Helsinki)
11:20 AM	Drug Sensitivity Testing and Individualized Systems Medicine to Guide Cancer Treatment Krister Wennerberg (FIMM)
12:00 PM	Discussion and Closing Remarks Tim Wiltshire (University of North Carolina)
12:30 PM	Lunch, sponsored by Merck and Co

Saturday, August the 30th Biomedicum Helsinki, Short Courses V-VIII (1:30 PM - 5:00 PM)

V Nucleic Acid and Gene Delivery

Coordinator: Yoshinobu Takakura (Kyoto University)

Time		**Seminar Room 1-2,** sponsored by Federation of Finnish Learned Societies
1:30 PM		Overview: Current Status and Future Perspective of Nucleic Acid Therapeutics Yoshinobu Takakura (Kyoto University)
2:05 PM		Targeting of Backbone Modified Oligonucleotides by Covalent Conjugation Harri Lönnberg (University of Turku)
2:40 PM		Lipoplex-Based Gene Delivery Thomas Anchordoquy (University of Colorado)
3:15 PM		Coffee Break, sponsored by The Finnish Pharmaceutical Society
3:45 PM		Polymer and Peptide-Based Gene Delivery Systems Enrico Mastrobattista (University of Utrecht)
4:20 PM	**S43**	Selection of Highly Stable Aptamers from a 2'-Fully Modified RNA Library Adam D. Friedman (University of North Carolina)
4:40 PM	**S44**	Optimization of DNA Nanostructure for Delivery of Nucleic Acid Drugs to Immune Cells Shozo Ohtsuki (Kyoto University)

VI How we Can Deliver Drugs across the Barriers by Transporters

Coordinator: Hiroyuki Kusuhara (University of Tokyo)

Time		**Lecture Hall 3,** sponsored by Federation of Finnish Learned Societies
1:30 PM		How we Can Deliver Drugs across the Barriers by Transporters Hiroyuki Kusuhara (University of Tokyo)
1:50 PM		Proteomic Analysis of Drug Transporters in the Barriers. Tetsuya Terasaki (Tohoku University)
2:30 PM		Blood-Ocular Barriers Arto Urtti (University of Helsinki, University of Eastern Finland)
3:10 PM		Coffee Break, sponsored by The Finnish Pharmaceutical Society
3:40 PM		Blood-Brain Barrier Transporters: Challenges and Opportunities for CNS Therapy Björn Bauer (University of Kentucky)
4:20 PM	**S45**	Brain Drug Distribution of a Cassette of Compounds in Alpha-Synuclein Transgenic Mice Sofia Gustafsson (University of Uppsala)
4:40 PM	**S46**	Analysis of Secretory Transport of Drugs in the Small Intestine Using the Ussing Chamber Method Takeshi Nakayama (University of Tokyo)

VII Modern Approaches of Nanotechnology

Coordinator: Hamid Ghandehari (University of Utah)

Time		Lecture Hall 1, sponsored by Doctoral Programme in Materials Research and Nanosciences University of Helsinki
1:30 PM		Nanotechnology: an Introduction Relevant to Pharmaceutical Sciences Hamid Ghandehari (University of Utah)
1:40 PM		Cellular Uptake and Subcellular Trafficking of Nanoconstructs Sarah Hamm-Alvarez (University of Southern California)
2:10 PM	**S47**	The Effect of Liposomal Components on Pro-Survival Signals within Normal Prostate Cells Ryan C. Bates (University of Colorado)
2:30 PM		Nanoconstructs in Drug Delivery Stefano Salmaso (University of Padova)
3:00 PM		Coffee Break, sponsored by The Finnish Pharmaceutical Society
3:30 PM		Theranostic Nanosystems: Opportunities and Challenges Andrew MacKay (University of Southern California)
4:00 PM	**S48**	Targeted Liposomal Ultrasound Contrast Agents Eric Sasko (Marburg University)
4:20 PM		Nanotoxicology Hamid Ghandehari (University of Utah)

VIII New Emerging Cell Models

Coordinator: Marjo Yliperttula (University of Helsinki)

Time		Seminar Room 3, sponsored by UPM
1:30 PM		Opening
1:40 PM		Novel Applications of the Sandwich-Cultured Hepatocyte Model for Transporter Investigations Kim Brouwer(The University of North Carolina at Chapel Hill)
2:10 PM		New Cell Based Approaches for Better Predictions of Drug Target and Off Target Interactions Per Artursson (Uppsala University)
2:40 PM	**S49**	Improvement in Blood-Brain Barrier Tightness through a Direct Contact hCMEC/D3 and Human Astrocyte Coculture Model Chris Kulczar (Purdue University)
3:00 PM		Coffee Break, sponsored by The Finnish Pharmaceutical Society
3:30 PM	**S50**	All Hepatocytes Are Not Equal: Characterization of Variability in Cryopreserved Human Hepatocytes Magnus Ölander (Uppsala University)
3:50 PM		2D and 3D Cell Biomaterial Culture Yan-Ru Lou (University of Helsinki)
4:20 PM		Hydrogels and Nanofibrillar Cellulose Markus Nuopponen (UPM)
4:50 PM		Closing
5:00 PM		**Award Ceremony & Conference closing (Lecture Hall 1),** sponsored by Journal of Pharmaceutical Sciences(John Wiley & Sons)

Sunday, August the 31th Optional Trip to Porvoo

10:00 AM Depart from Helsinki Harbor, Pier "Linnanlaituri" (in front of the Presidential palace)
6:00 PM Arrival to Helsinki

PROGRAM POSTERS

Thursday, August the 28th , Biomedicum Helsinki

Sponsored by Eisai, Inc. and Eli Lilly and Company

12:00 PM - 1:30 PM

P1 Folic Acid Functionalized Insulin Loaded Stable Chitosan Nanoparticles: Influence on Stability, Cellular Uptake, Pharmacodynamics and Pharmacokinetics
Ashish Agrawal (NIPER)

P3 *In Silico* Prediction of Intravitreal Primary Pharmacokinetic Parameters and Drug Concentrations: Tool for Ocular Drug Development
Eva M. del Amo (University of Eastern Finland)

P5 Adjuvants Show Dramatically Different Interactions with Model Cell Membranes
Lorena Antúnez (University of Kansas)

P7 Curcumin Loaded Solid Lipid Nanoparticles Coated with N-Carboxymethyl Chitosan to Modify Release
Jong-Suep Baek (Chungnam National University)

P9 Paclitaxel Loaded SLNs Modified with HPCD with Low Renal Toxicity for Enhancing Bioavailability
Jong-Suep Baek (Chungnam National University)

P11 *In Vitro* Enzymatic Degradation of Lipidified Apomorphine
Nrupa Borkar (University of Copenhagen)

P13 Asymmetrical Flow Field-Flow Fractionation with On-Line Detection for Drug Transfer Studies: Investigating Transfer Kinetics of a Model Drug between Liposomal Bilayers
Martin Brandl (Southern Denmark University)

P15 Shape-Modified Nanocarriers for Intracellular Drug Delivery
Arianna Castoldi (Saarland University)

P17 Development of Surface Modified Matrix and Segmented Reservoir Intravaginal Ring Devices for Sustained Delivery of Hydroxychloroquine
Yufei Chen (University of Manitoba)

P19 Evidence for the Functional Dimerization of the Human Bile Acid Transporter ASBT (SLC10A2)
Paresh Chothe (University of Maryland)

P21 Coupling an Absorption Sink to the *In Vitro* Lipid Digestion Model Improves Understanding of Drug Absorption from Lipid-Based Formulations
Matthew F. Crum (Monash University)

P23 Preparation and Evaluation of Bioactivated Polyelectrolyte Nanocomplexes for Favoured Cell Migration
Tiziana Di Francesco (University of Geneva)

P25 Tetraether Lipid-Based Transfection Reagents for the Expression of Luciferase Plasmid (pCMV-luc) in Various Cell Lines
Konrad H. Engelhardt (Phillipps-University Marburg)

P27 Assessment of Antileishmanial Activity of Pyrazinamide by *In Vitro* Time-Kill Curve Experiments against *Leishmania (Leishmania) amazonensis*
Nivea Falcao (University of Florida)

P29 Formulation of Self-Assembling Polyamino Acid Nanoparticles for Site-Specific Targeting of the Tumor Microenvironment
Zoë Folchman-Wagner (University of Southern California)

P31 Therapeutic Profile *In Vivo* of Formulation Containing Ursolic Acid on Experimental Chagas' Disease
Júnior Furini (University of Sao Paulo)

P33 Impact of Freeze-Drying Cycle on the Solid-State Properties and Long-Term Protein Stability: The Case of rhGH
Pawel Grobelny (University of Connecticut)

P35 Exploring Gastrointestinal Drug Behavior of Fenofibrate after Oral Administration in Man: Nano- versus Microparticles
Bart Hens (Katholieke Universiteit Leuven)

P37 Extraction of Archaeal Membrane Lipids from *Sulfolobus islandicus*
Sara Munk Jensen (Southern Denmark University)

P39 Improvement of Poorly Water-Soluble Albendazole by using Mucosal Adhesion Polymeric Nanoparticles
Bong-Seok Kang (Chungnam National University)

P41 Solid State Properties of Suberin-Containing Electrospun Polymeric Nanofibers
Karin Kogermann (University of Tartu)

P43 SIRT3 Inhibitors by Virtual Screening
Piia Kokkonen (University of Eastern Finland)

P45 An Increase in the Natural GDNF Expression Enhances and Protects the Nigrostriatal Dopaminergic System
Jaakko Kopra (University of Helsinki)

P47 Mechanism and Responsible Component of Apple Juice for a Long-Lasting Inhibition of Intestinal Absorptive Transporter OATP2B1
Akira Kubota (Kanazawa University)

P49 Enhanced Generation, Characterisation and Pre-Clinical Evaluation of Co-Stimulatory Molecules Expressing Oncolytic Adenovirus for Cancer Treatment of Human Patients
Lukasz Kuryk (University of Helsinki)

P51 Controlled Release from Liposomes by Light Activation
Tatu Lajunen (University of Helsinki)

P53 Enhanced Physicochemical Properties of Docetaxel-Loaded Solid Lipid Nanoparticles
Sang-Eun Lee (Chungnam National University)

P55 Chemoprevention by Curcumin: A Prodrug Hypothesis
Garvey Liu (University of Minnesota)

P57 Nano Ceramic Assembly for Pulmonary Delivery of Protein and Peptides
Deborah Lowry (Aston University)

P59 Supersaturation of Zafirlukast in Fasted and Fed Intestinal Media Measured *In Situ* by UV/Vis Fiber-Optic Probes: Effectiveness of Precipitation Inhibitors
Cecilie Maria Madsen (University of Copenhagen)

P61 Transdermal Delivery of Flufenamic Acid from PLGA Nanoparticles by Iontophoresis
Kristina Malinovskaja (University of Helsinki)

P63 Inhibition of Proteolytic Cleavage by inserting the Metal-Bound *cla*MP Tag Adjacent to a Known Recognition Site
Michaela L. McNiff (University of Kansas)

P65 Development of Hyaluronic Acid based IgG-Loaded dissolving Microneedles for Intradermal Protein Delivery
Juha Mönkäre (Leiden University)

P67 Analysis of Secretory Transport of Drugs in the Small Intestine Using the Ussing Chamber Method
Takeshi Nakayama (University of Tokyo)

P69 Microcontainers as an Oral Drug Delivery System
Line Hagner Nielsen (University of Copenhagen)

P71 Synthesis and Characterization of Native and PEGylated Poly-L-Lysine Dendrimers
Yewon Joanna Pak (University of Maryland)

P73 *In Vitro* Device to Investigate Fate of Macromolecules Following Intravitreal Injection
Sulabh Patel (F. Hoffmann-La Roche Ltd.)

P75 Effects of Sucrose in Micro- and Pilot Scale Freeze-Drying on Secondary Protein Structures Assessed by FTIR-ATR
Björn-Hendrik Peters (University of Eastern Finland)

P77 Investigating the Whole Brain Distribution of Macromolecules Administered into the Cerebrospinal Fluid
Michelle E. Pizzo (University of Wisconsin)

P79 siRNA Delivery to the Retinal Pigment Epithelium
Eva Ramsay (University of Helsinki)

P81 The Anti-inflammatory Activity of a Lead Fused-Cyclopentenone Phosphonate Compound and its Potential in the Local Treatment of Experimental Colitis
Abraham Rubinstein (Hebrew University of Jerusalem)

P83 Effectiveness of Bulb Extracts of *Allium* Species on Some Selected Plant Pathogenic Fungi
Sahar Samadi (Phillipps-University Marburg)

P85 Stereoselective Anticonvulsant and Pharmacokinetic Analysis of Valnoctamide, a CNS-Active Derivative of Valproic Acid with Low Teratogenic Potential
Tawfeeq Shekh-Ahmad (Hebrew University of Jerusalem)

P87 Breast Cancer Tumour Associated Macrophages Modulation by Free or Liposome Encapsulated Zoledronate
Sofia Sousa (University of Eastern Finland)

P89 Inhalable Nanocomposites for Targeted Pulmonary Delivery and Applications in Lung Cancer Therapy
Nathanael A. Stocke (University of Kentucky)

P91 Effect of the Immunostimulatory Peptide EP67 on Immune Cells Critical for Adaptive Responses
Shailendra B. Tallapaka (University of Nebraska)

P93 The Advantage of Correlative Microscopy for Macrophage Uptake Studies with Non- Spherical Particles
Clemens Tscheka (Phillipps-University Marburg)

P95 Effect of Alzheimer's Disease on the Gene Expression of Drug Transporters and Tight Junction Proteins in Brain
Kati-Sisko Vellonen (University of Eastern Finland)

P97 A Novel Assay for Binding Studies of Antibody-Modified Polyplexes for Tumor Targeting
Doru Vornicescu (Phillipps-University Marburg)

P99 Characterization of Highly Supersaturated Solution of Enzalutamide: A Study of Liquid- Liquid Phase Separation of A Lipophilic Compound
Venecia Wilson (Purdue University)

P101 Investigation of Drug Release from Non-Ionic and Anionic Cellulose Beads
Emrah Yildir (Åbo Akademi University)

P103 Supramolecular Assemblies of Amphiphilic β-Cyclodextrin with Lipids
Leïla Zerkoune (Université Paris-Sud)

P105 Molecular Dynamics Study of Surface Structure of the Drug Delivery Liposome
Aniket Magarkar (University of Helsinki)

P107 Improved Delivery of Valproic Acid into the Brain by LAT1-Targeted Prodrugs
Kristiina Huttunen (University of Eastern Finland)

P109 In Vitro Kidney Model for Drug Transport and Toxicity Testing
Aishwarya Jayagopal (University of California at San Francisco)

Friday, August the 29th, Biomedicum Helsinki

Sponsored by Gilead Sciences Inc. and GlaxoSmithKline

12:00 PM - 1:30 PM

P2 Surface Modifications of Polyethylene Sinter Bodies for Serological Diagnosis of Borreliosis
Mohammed Alasel (Phillipps-University Marburg)

P4 Nanotechnology for Neurotrophic Protein Delivery
Angelina Angelova (Université Paris-Sud)

P6 Enhanced Percutaneous Permeation of Dehydroepiandosterone Loaded Nanocapsules
Amit Badihi (Hebrew University of Jerusalem)

P8 Tadalafil Loaded Nanostructured Lipid Carriers Using Permeability Enhancers for Transdermal Delivery
Jong-Suep Baek (Chungnam National University)

P10 Reversal of Multidrug Resistance in MCF-7/ADR Cells Using Dual Drug-Loaded Solid Lipid Nanoparticles with Double Targeting
Jong-Suep Baek (Chungnam National University)

P12 Alzheimer's Disease: Receptor-Targeted BACE-1 Inhibition
Davide Brambilla (ETH-Zurich)

P14 Evaluation of Oncolytic Adenoviruses as Adjuvants for MHC-I Restricted Peptides for a New Cancer Vaccine Platform
Cristian Capasso (University of Helsinki)

P16 Impact of CYP3A5 Genetic Polymorphism on Mechanism-Based Inactivation by Lapatinib
Eric Chun Yong Chan (National University of Singapore)

P18 Exploitation of the Anti-Inflammatory and Cytoprotective Properties of Electrophilic Bioactive Compounds for Potential Application in Cancer Prevention
Eng-Hui Chew (National University of Singapore)

P20 Predicting Safe and Effective Doses for Children: Integrating Adult Clinical Parameters with *In Vitro* Metabolism by Pediatric Tissues and Modeling
Nicole R. Zane (University of North Carolina)

P22 A Molecular Switch Using Genetically Engineered Polymers
Jugal Dhandhukia (University of Southern California)

P24 Remote Loading of GLP-1s in PLGA Microspheres for Treatment of Type 2 Diabetes
Amy C. Doty (University of Michigan)

P26 Optimization of Tumor-Targeted Nanoparticles for Paclitaxel Delivery with Folate-PEG Conjugated Amphiphilic Cyclodextrins
Nazlı Erdoğar (Hacettepe University)

P28 Glucuronidation Converts Clopidogrel to a Potent Metabolism-Dependent Inhibitor of CYP2C8
Anne M. Filppula (University of Helsinki)

P30 Hydrolysis of DTPA Prodrug by Skin Carboxylesterases in Human Epidermal Keratinocytes
Jing Fu (University of North Carolina)

P32 Erythrocytic Stage Targeted Liposome Vaccine Delivery System against Malaria
Ashwini Kumar Giddam (The University of Queensland)

P34 Multi-Parametric Evaluation of Therapeutic Protein Aggregation
Floriane Groell (University of Geneva)

P36 Comparison of Brain and Plasma Levels of R-Flurbiprofen in Rats Following Oral and Intra-Nasal Administration
Paul C. Ho (National University of Singapore)

P38 RP-HPLC Method Development and Validation for Simultaneous Analysis of Ibuprofen and Pamabrom
Hye-Suk Jun (Chungnam National University)

P40 Quantitative Analysis and Preformulation of Extracts from *Aesculus Hippocastamum*
Ju-Heon Kim (Chungnam National University)

P42 VEGF in Diabetic Retinopathy
Emmi Kokki (University of Eastern Finland)

P44 Encapsulated Cells for Long-Term Secretion of Soluble VEGF Receptor 1: Material Optimization and Simulation of Ocular Drug Response
Leena-Stiina Kontturi (University of Helsinki)

P46 Lipid-Bound Indocyanine Green Particles Enable High Resolution Near-Infrared Diagnostic Imaging of the Lymphatic System
John C. Kraft (University of Washington)

P48 Transporter Mediated Permeation of *p*-Amino Benzoic Acid on Basal Membrane in Caco-2 Cell Monolayer
Keisuke Kurokawa (Kumamoto University)

P50 Oral Delivery of Anticancer Drug: Doxorubicin Complex Lead to Chemotherapy Maintenance at Home with Low Toxicity
Seho Kweon (Seoul National University)

P52 Technetium-99m labelling of Nanofibrillar Cellulose for Small Animal SPECT/CT
Patrick Laurén (University of Helsinki)

P54 Discovery of Novel Diarylpyrimidines as Potent HIV NNRTIs via a Structure-Guided Core-Refining Approach
Xiao Li (Shandong University)

P56 Discovery of Piperidine-Linked Pyridine Analogues as Potent Non-Nucleoside HIV-1 Reverse Transcriptase Inhibitors
Xinyong Liu (Shandong University)

P58 Design, Synthesis and Biological Evaluation of Small-Molecule Fluorescent Ligands Based on Cyanine 5 for α_1-Adrenoceptor
Zhao Ma (Shandong University)

P60 An Orally Delivered Bile Acid Based Formulation of Carboplatin for Effective Maintenance Chemotherapy
Foyez Mahmud (Seoul National University)

P62 Temperature-Sensitive Hybrid Hydrogels Charged with PLGA Particle as Parenteral Extended Release System
Pierre Maudens (University of Geneva)

P64 Design and Therapeutic Use of a Mutant Coiled-Coil Peptide for BCR-ABL Inhibition
Geoffrey D. Miller (University of Utah)

P66 Differential Effect of Buffering Agents on the Crystallization of Gemcitabine Hydrochloride in Frozen Solutions
Bhushan Munjal (NIPER)

P68 Developing Therapeutic Vaccine Formulations for the Treatment of Melanoma
Silke Neumann (University of Otago)

P70 Co-Delivery of Autoantigen and B7 Pathway Modulators for the Treatment of a Murine Model of Multiple Sclerosis
Laura Northrup (University of Kansas)

P72 Deposition and Stabilisation of Nanosuspensions by Flexographic Printing
Mirja Palo (Åbo Akademi University)

P74 Water Vapor Barrier and Mechanical Properties of Lignified Cellulosic Thin Films
Anna Penkina (University of Tartu)

P76 Novel Lipopolyplexes for Gene Delivery and Gene knockdown
Shashank Reddy Pinnapireddy (Phillipps-University Marburg)

P78 Therapeutic Cancer Vaccine Based on Polymeric Nanoparticles Containing HPV Synthetic Long Peptide and Poly IC
Sima Rahimian (Utrecht University)

P80 Ocular Melanin Binding: *In Vitro* Binding Studies Combined to a Pharmacokinetic Model
Anna-Kaisa Rimpelä (University of Helsinki)

P82 Label-Free CARS Imaging of Intestinal Epithelial Cells
Jukka Saarinen (University of Helsinki)

P84 Influence of the Surface Modifications on the Properties of Nanoparticles (Not presented)
Jens Schäfer (Phillipps-University Marburg)

P86 Strategies to Improve the Absorption of Biopharmaceutical Classification (BCS) Class IV Drugs – A Paclitaxel Case Study
Ramesh Soundararajan (UCL College of Pharmacy)

P88 Impact of Human SGLT (SLC5A) Mediated Transport on Apparent Permeability Can Be Studied in the *In Vitro* DSMZ -Caco-2 Cell Model
Bente Steffansen (University of Copenhagen)

P90 Oxidative Stress Protection by Exogenous Delivery of rhHsp70 Chaperone to the Retinal Pigment Epithelium (RPE), a Possible Therapeutic Strategy against RPE Degeneration
Astrid Subrizi (University of Helsinki)

P92 Redispersible Microspheres Composed of Nanoparticles for Pulmonary Application
Afra Torge (Phillipps-University Marburg)

P94 Hyperspectral Imaging in Quality Control of Inkjet Printed Pharmaceutical Dosage Forms
Hossein Vakili (Åbo Akademi University)

P96 Non-Labeled *In Vitro* Approaches for Assessment of Targeting and Cell Uptake Efficacy of Liver Targeted Liposomes
Tapani Viitala (University of Helsinki)

P98 Investigation of Inkjet Printed Formulations to Improve the Dissolution Rate of Indomethacin
Henrika Wickström (Åbo Akademi University)

P100 Dynamic Combinatorial-Mass Spectrometry Leads to Potent and Selective Inhibition of Nucleic Acid Demethylase
Esther Woon (National University of Singapore)

P102 TRIM39 is a Novel Regulator of MOAP-1 in Mammalian Cells
Victor C. Yu (National University of Singapore)

P104 Predicting Target-Substrate Interaction Constants by Quantitative Sequence-Kinetic Constant Relationships for Facilitating Protein-Protein Interaction Inhibitor Discovery
Peng Zhang (National University of Singapore)

P106 Studies towards the Use of Immobilized Lipase for *In Vitro* Lipolysis Experiments
Stephanie Phan (Monash University)

P108 Fogging in Lyophilized Drug Products
Dominique Ditter (F. Hoffmann-La Roche Ltd.)

PODIUM ABSTRACTS

GPEN 2014

S1 Development of Dendrimer Based Drug Delivery System for Anticancer Therapy

Kıvılcım Öztürk[a], Mustafa Ulvi Gürbüz[b], Metin Tülü[b], Sema Çalış[a]

[a]Hacettepe University, Turkey
[b]Yildiz Technical University, Turkey

Objective. The aim of the work is to investigate efficiency of originally synthesized dendrimers as drug carrier system.

Methods. In poly(amidoamine) PAMAM dendrimer synthesis, after the successive alkylations, microwave assisted amidation were performed within 60–90 minutes. We have conducted amidation step by refluxing ester terminated half generation and excess ethylenediamine mixture in the presence of 4-6 mL methanol at the 120-130 °C. LPR method was used for purification. To analyze the gemcitabine HCl quantitatively a RP HPLC method was developed and validated. Chromatography was carried out on an C18 column (250×4.6 mm;5µ) using a mixture of methanol and phosphate buffer (40:60) as the mobile phase at a flow rate of 1.0 mL/min, at 270 nm. Different core types of dendrimers were used to evaluate encapsulation efficiency of drug. Encapsulated drug amount for per molar of dendrimer was analyzed using HPLC.

Results. The reaction progresses were monitored by ATR spectroscopy to observe disappearance of esteric peak (1733 cm-1) and appearance amide I and amide II peaks (1642 cm-1 and 1558 cm-1). The 1H NMR spectra pattern has changed significantly due to esterification of amines by appearance of several new signals. The 13C NMR data also tend to support ester formation by appearance of several new peaks, particularly for carboxyl (C=O) chemical shifts at approximately 174 ppm, typical for ester compounds. The retention time of the drug was found to be 3.18 min. The method produced linear responses in the concentration range of 1-100 µg/mL of gemcitabine HCl. The drug could be loaded at 1:1 and 2:1(drug:dendrimer) molar ratio to ethylenediamine and poly(ethylene glycol) core PAMAMs respectively.

Conclusions. These results are promising for further *in vitro* cell culture and i*n vivo* studies.

S2 CpG Conjugation to Model Tumour Antigen Ovalbumin Leads to Enhanced CD8+ T-Cell Proliferation

Kramer Katrin[a,b], Young Sarah[b], Walker Greg[a]

[a]University of Otago, School of Pharmacy, Dunedin, New Zealand
[b]University of Otago, Department of Pathology, Dunedin, New Zealand

Objective. Activation of cytotoxic CD8+ T-cells is crucial to developing an anti-tumour immune response. In order for T-cell activation to occur, tumour antigen needs to be cross-presented to activated dendritic cells. Dendritic cells cross-present tumour antigen on MHCI but need to be completely activated via danger signals to generate a potent CD8+ T-cell response through co-stimulatory molecules. Linking a vaccine adjuvant, CpG oligodeoxynucleotide (ODN) chemically to a tumour antigen enables endocytosis of both entities into the same cell. The CpG-ODN - tumour antigen conjugate activates dendritic cells via the toll-like receptor 9 in the endosome and the tumour antigen is cross-presented on MHCI.

Methods. The model tumour antigen ovalbumin was first purified via gel filtration and only the monomer was used for conjugation. The vaccine adjuvant CpG ODN was conjugated with a stable bis-arylhydrazone bond to the model tumour antigen ovalbumin. A molar ratio of 2.4:1 of CpG ODN to ovalbumin was determined by UV-absorption of the bis- arylhydrazone bond. Purification of the conjugates was performed by gel filtration. Size-exclusion chromatography identified conjugates with a range modification. Murine bone marrow derived dendritic cells (BMDC) were cultured for six days and pulsed for 24h with either a conjugate or a mixture of antigen and adjuvant. Carboxyfluorescein succinimidyl ester stained CD8+ T-cells were cocultured with stimulated murine BMDCs for 72h. BMDC activation and T-cell proliferation were analysed using flow cytometry.

Results. Effective activation of dendritic cells was shown by the upregulation of activation markers after stimulation with either the conjugate or the mixture of CpG ODN and ovalbumin. However the conjugate activated dendritic cells showed higher CD8+ T- cell proliferation compared to dendritic cells activated with the mixture.

Conclusions. Conjugating the vaccine adjuvant CpG ODN to the model tumour antigen ovalbumin using a stable bis-arylhydrazone bond leads to a higher degree of CD8+ T-cell proliferation compared to the CpG ODN antigen mixture.

S3 Quantitative Analysis of *In Vivo* Fate of Mouse Melanoma B16BL6-Derived Exosomes after Intravenous Injection into Mice by Development of a Labeling Method for Exosomes

Masaki Morishita[a], Yuki Takahashi[a], Takafumi Imai[a], Makiya Nishikawa[a], Yoshinobu Takakura[a]

[a]Graduate School of Pharmaceutical Sciences, Kyoto University, Japan

Objective. Exosomes are small membrane vesicles secreted from cells. Because exosomes work as a transporter for nucleic acids and proteins, exosomes are expected to become a new class of drug delivery

system (DDS) for these molecules. The information of *in vivo* fate of exogenously-administered exosomes is essential for the development of exosome-based DDS. However, *in vivo* fate of exosomes has been poorly investigated because of the lack of a method to quantitatively trace exosomes *in vivo*. In this study, we developed a method to label exosomes in order to analyze *in vivo* fate of exosomes.

Methods. We designed a fusion protein consisting of *Gaussia* luciferase and an exosome-tropic protein, lactadherin (gLuc-LA), and constructed a plasmid vector encoding gLuc-LA. B16BL6 murine melanoma cells were transfected with the plasmid, and exosomes secreted from the cells were collected. Immunoelectron microscopy was performed using an anti-gLuc antibody for the detection of gLuc-LA on the surface of exosomes. After intravenous injection of gLuc-LA-labeled B16BL6 exosomes into mice, the distribution of exosomes was evaluated by detecting the gLuc activity. Macrophage-depleted mice were prepared by using clodronate-containing liposomes.

Results. Immunoelectron microscopy revealed that gLuc-LA was present on the surface of the exosomes, indicating the successful labeling of exosomes with gLuc-LA. Intravenously injected gLuc-LA-labeled B16BL6 exosomes disappeared quickly from the blood circulation with a half-life of approximately 2 min. *In vivo* imaging demonstrated that gLuc-LA-labeled B16BL6 exosomes mainly distributed to the lung, spleen and liver. Moreover, the elimination of gLuc-LA-labeled B16BL6 exosomes from the blood circulation was markedly delayed in macrophage-depleted mice, indicating that macrophages significantly contribute to the *in vivo* fate of B16BL6 exosomes.

Conclusions. These results indicate that the labeling method is useful to understand the *in vivo* fate of exosomes. The present study also showed that macrophages play important roles in the clearance of exosomes.

S4 The Insulin Receptor: An Achilles' Heel for Schistosome Vaccine Development

Rachel Stephenson[a], Hong You[b], Donald McManus[b], Istvan Toth[a,c]

[a]School of Chemistry and Molecular Biosciences, The University of Queensland, Brisbane, Queensland, Australia
[b]Molecular Parasitology Laboratory, Infectious Diseases Division, QIMR Berghofer Medical Research Institute, Brisbane, Queensland, Australia
[c]School of Pharmacy, The University of Queensland, Brisbane, Queensland, Australia

Objective. Schistosomiasis remains one of the most insidious tropical parasitic diseases. Bovines are major animal reservoir hosts for Asiatic *Schistosoma japonicum*, responsible for up to 90% of environmental egg contamination. This feature underpins our efforts to develop a transmission blocking vaccine against *S. japonicum*. Schistosomes consume significant amounts of glucose daily but may depend on host insulin for its uptake. Chemotherapy with praziquantel has reduced morbidity rates but consequences of continuous re-infection in endemic areas remain unchanged. Vaccines represent the most attractive long-term alternative to invert this scenario. This project aims to further develop a safe, stable and more effective subunit vaccine based on insulin receptors from *S. japonicum* (SjIR1 and SjIR2).

Methods. On the basis of sequential analysis, together with the proteins predicted antigenic structure, nine peptide analogues from SjIR1 and eleven from SjIR2 were identified and synthesized. The positive control, from the alpha subunit of the HIR (α655-670), has been shown to exhibit specific binding activity towards radioiodinated insulin. Peptide synthesis was achieved using standard fluorenylmethyloxycarbonyl solid phase peptide synthesis and purified using RP-HPLC. *In vitro* human insulin binding studies have been achieved using Octet RED technology.

Results. Peptide epitopes from SjIR1 and SjIR2 have been successfully synthesized and screened for human insulin binding activity. Three insulin binding sites on SjIR1 have been identified and we found one peptide (SDYSLIIRHTKLKGIGLWKLKTLNSYPIALIDNPLMC) with stronger insulin binding ability, compared to the positive control, which is highly conserved in *S. japonicum*, *S. mansoni* and *S. haematobium*, but with low homology with the human IR. Six binding sites from SjIR2 have also been identified.

Conclusions. This data reinforces our hypothesis that SjIRs are potential transmission blocking vaccine candidates against S. japonicum and, being conserved, may also be suitable as clinical vaccine targets against African schistosomes, *S. mansoni* and *S. haematobium*.

S5 Depth Controlled Dermal Immunization of Rats with Inactivated Polio Vaccine by Using Hollow Microneedles

Pim Schipper[a], Koen van der Maaden[a], Stefan Romeijn[a], Gideon Kersten[b], Wim Jiskoot[b], Joke Bouwstra[a]

[a]Division of Drug Delivery Technology, Leiden Academic Centre for Drug Research (LACDR), Leiden University, Leiden, the Netherlands
[b]Institute for Translational Vaccinology (Intravacc), Bilthoven, the Netherlands

Objective. 1) Optimize the hollow microneedle applicator for depth controlled microinjections into skin. 2) Use the optimized applicator to investigate the effect of insertion depth and adjuvants on the immune responses *in vivo*.

Methods. Hollow microneedles were fabricated by etching fused silica capillaries (20 µm inner diameter) with hydrofluoric acid (49%). Previously, an applicator for hollow microneedles was developed that allows microinjections at a maximum injection rate of 2

µL/min into skin. To increase the injection rate, the applicator head was redesigned by replacing Luer Lock with high-pressure resistant CapTite™ connectors. The usability of the hollow microneedle/adapted applicator combination was evaluated by determining the delivered volume as a function of insertion depth, injection rate, and delivered volume into rat skin. Subsequently, the hollow microneedle/applicator combination was used to immunize rats with a polio vaccine at different depths (250-550 µm) and adjuvanted with CpG or Cholera toxin (CT) at an injection depth of 400 µm.

Results. Adapting the applicator with high-pressure resistant CapTite™ connectors increased the allowable injection rate up to 60 µL/min without observable leakage of the system. During microinjections into rat skin at different depths (50-900 µm), large volumes (100 µL), and a high flow speeds (60 µL/min) only minimal leakage was observed (0.5%, 2% and 4%, respectively). Polio-specific IgG responses were dependent on the insertion depth, i.e. superficial injections resulted in up to two fold increased responses compared to conventional intramuscular immunization. Moreover, CpG and CT adjuvanted polio microinjections led up to 9 and 6 fold, respectively, increased IgG responses.

Conclusions. The applicator for hollow microneedles was optimized, which resulted in a 30 fold increased injection rate with only minimal leakage. Moreover, this study shows that microneedle-based intradermal polio vaccination can lead to superior immune responses compared to conventional intramuscular immunization.

S6 Challenges of Determining Intrinsic Viscosity in Strongly Interacting Monoclonal Antibody Solutions

Mariya Pindrus[a], Sandeep Yadav[b], Steven J. Shire[b], Devendra S. Kalonia[a]

[a]Department of Pharmaceutical Sciences, University of Connecticut, Storrs, CT, USA
[b]Late State Pharmaceutical Development, Genentech, South San Francisco, CA, USA

Objective. To determine the effect of pH and ionic strength on intrinsic viscosity of several monoclonal antibodies (mAbs), and to investigate the limitations associated with intrinsic viscosity determination in strongly interacting systems.

Methods. Online viscosity detector attached to HPLC (Viscotek®) was used to determine intrinsic viscosity of three mAbs at pH 4, pH 6 and pH 8, ionic strengths ranging from ~0 to 150 mM. Molecular charge was determined from electrophoretic mobility data obtained on Malvern Zetasizer®. Interaction parameter, kD, was calculated from diffusion data obtained from Dynamic Light Scattering (DLS) measurements on Zetasizer®.

Results. Intrinsic viscosity of the mAbs varied from 5.5 to 6.4 mL/g with changes in pH at intermediate ionic strength conditions. Intrinsic viscosity varied significantly from 5.9 to 11.9 mL/g upon changes in ionic strength from ~0 mM to 150 mM at a given pH, with highest value observed at ~0 mM and lowest at 150 mM ionic strength conditions. Overall, similar intrinsic viscosity values (e.g. 5.8 to 5.9 mL/g) were observed for all mAbs tested at a given solution condition. Significant non-linearity in intrinsic viscosity tested at different protein concentrations was observed at ~0 mM. The cause was investigated by determining molecular charge and interactions. Correlation was found between charge and intrinsic viscosity. The magnitude of interactions was largest at ~0 mM ionic strength (kD on the order of 1000's mL/g), and correlated fairly well with intrinsic viscosity findings. Non-linearity observed in DLS profiles at ~0 mM was consistent with intrinsic viscosity data.

Conclusions. Underlying causes of non-ideality at ~0 mM conditions include significant unscreened charge and strong intermolecular repulsions. Non-ideality causes numerous assumptions to break down, thus imposing limitations on experimental techniques, and posing a concern that high intrinsic viscosity reported at ~0 mM conditions may be an artifact and not a true value.

S7 The Use of Human Intestinal Fluids to Study the Solubility/Permeability Interplay: Effect of Food on Carvedilol Absorption

J. Stappaerts[a], B. Wuyts[a], J. Tack[b], P. Annaert[a], P. Augustijns[a]

[a]Drug Delivery and Disposition, KU Leuven Department of Pharmaceutical and Pharmacological Sciences, Leuven, Belgium
[b]Department of Gastroenterology, University Hospitals Leuven, Belgium

Objective. The aim of this study was to determine the effect of food on the intestinal disposition of carvedilol by evaluating the solubility of and permeability for carvedilol in different media, including fasted and fed state simulated intestinal fluids (FaSSIF and FeSSIF) and human aspirated intestinal fluids of the fasted and fed state (FaHIF and FeHIF).

Methods. Carvedilol flux was assessed at a predetermined concentration (20 µM) in the different perfusion media using the *in situ* intestinal perfusion technique with mesenteric blood sampling in rat. Since carvedilol is a Biopharmaceutics Classification System class II compound, the thermodynamic solubility of carvedilol was determined in biorelevant media including FaSSIF, FeSSIF and HIF collected in the fasted and fed state. Finally, to simultaneously evaluate the effect of food on both solubility and permeability of carvedilol, intestinal perfusions were performed using saturated suspensions of carvedilol in the different perfusion media.

Results. The flux of carvedilol (20 µM) was found to be significantly higher in fasted state compared to fed state state conditions, both in simulated (13-fold) and in aspirated intestinal fluids (24-fold). In contrast, as compared to FaSSIF and FaHIF, solubility measurements revealed a significantly higher solubility in FeSSIF (4.2-fold) and FeHIF (2.6-fold), respectively. Upon intestinal perfusion with saturated suspensions of carvedilol, higher flux values in the fasted state compared to the fed state conditions were observed both in simulated (3.8-fold) and in aspirated (12.0-fold) fluids, despite the higher solubility of carvedilol in the fed state.

Conclusions. For lipophilic compounds such as carvedilol, fed state conditions generate strong micellar entrapment, which may limit the bioaccessible fraction, but improves solubility. Therefore, it is of utmost importance to consider the effect of food on both solubility and permeability. The use of human intestinal fluids in the intestinal perfusion set-up generates biorelevant experimental conditions, suitable to study the complex solubility/permeability interplay.

S8 Effect of Intestinal Hydrolysis of Olmesartan Medoxomil on its Absorption

Takahiro Yonemitsu[a], Keiichirou Tanaka[a], Teruko Imai[a]

[a]Graduate School of Pharmaceutical Sciences, Kumamoto University, Japan

Objective. Olmesartan medoxomil (OM) is a prodrug of olmesartan (OL: angiotensin Ⅱ blocker) for improving bioavailability. Membrane permeability of OL is improved by conjugation with medoxomil group (logP of OM: 1.0). However, the bioavailability of OM is around 20-25% after oral administration in rat and man. Such low bioavailability of OM is due to not only its efflux by MRP2 and BCRP, but also intestinal hydrolysis. The aim of this study is to evaluate the intestinal hydrolysis of OM on its process of mucosal membrane transport.

Methods. *In vitro* hydrolysis of OM was measured in 9000g supernatant (S9) fraction of rat jejunal mucosa. For *in situ* single-pass pefusion, rat jejunal loop (10 cm) and mesenteric vein were simultaneously perfused by MES buffer (pH 6.3) including 20µM OM at flow rate of 0.2 mL/min and KHBB buffer (pH 7.4) at 2.5 mL/min, respectively. The steady-state absorption parameters were calculated from the concentrations of OM and OL in both perfusates.

Results. The K_m, V_{max} and CL_{int} (V_{max}/K_m) for hydrolysis were calculated 54.8 µM, 3.53 nmol/min/mg protein and 66.7µl/min/mg protein, respectively. *In situ* experiment showed 5.45×10^{-5} cm/sec of permeability coefficient that predicted 100% absorption of OM. The metabolism clearance (28.8×10^{-3} µL/min) was 6.9 fold greater than the absorption clearance (4.20×10^{-3} µL/min). Consequently, 87% of OM taken up into mucosal cells was hydrolyzed. These data agreed with the hydrolysis rate (80%) predicted from the relationship between *in situ* absorption and *in vitro* CL_{int}. Furthermore, OL was transported four times faster in intestinal lumen than blood vessel. It was predicted that absorption of OM was 27% based on the total amount of OM and OL absorbed in blood vessel

Conclusions. The present data suggest that the intestinal hydrolysis of OM is major reason for its low bioavailability.

S9 Assessment of Vancomycin AUC/MIC and Clinical Outcome in MRSA Patients

Alexander Voelkner[a], Brian McCullough[b], Ken Klinker[b], Hartmut Derendorf[a]

[a]Pharmaceutics Department, University of Florida, USA
[b]UF Health Shands Hospital, USA

Objective. Clinical efficacy of vancomycin against MRSA infections is associated with an AUC/MIC≥400. The aim of this study was to evaluate the relationship

between vancomycin exposure, MIC and clinical outcome in patients with MRSA bacteremia.

Methods. A retrospective study at an 852-bed academic hospital was conducted from January 2010 to June 2011. Patients with MRSA bacteremia were included for analysis if they were ≥18 years old, received vancomycin for more than 24 hours and had at least one steady-state serum trough concentration measured. Clinical outcome was reported as 30-day mortality. Susceptibility of MRSA isolates against vancomycin was determined by Etest.

AUC/MIC ratios were computed based on the AUC and the infecting pathogen. AUC was calculated from dividing daily dose by CL; posthoc CL was estimated using a zero-order infusion, two compartment body model in NONMEM 7.2.

Statistical analysis was performed in R 3.0.1. ANOVA and Kruskal-Wallis tests were used for continuous data and categorical data was compared using χ^2 or Fisher's exact test. Logisitc regression was used if data had a dichotomous outcome.

Results. A total of 136 patients were identified for analysis and attributable mortality was 11% (overall mortality 14%). MIC values ≥1.5 mg/L were observed in 84.6% of the patients. AUC/MIC ratios in non-survivors tended to be higher (345 vs. 296), but no significant difference was found in comparison to survivors (p=0.6). Patients with acute renal failure (p=0.007) and elevated APACHEII score (p=0.004) were more likely to not-survive.

Conclusions. No significant relationship between vancomycin AUC/MIC and mortality was found. Predictors associated with a negative clinical outcome were acute renal failure and the APACHEII score.

S10 Characterization and Application of Highly Stable 2'-F RNA Aptamers Specifically Targeting a Novel Biomarker of Pancreatic Ductal Adenocarcinoma (PDAC)

Sarah E. Claypool[a], Hui Chen[a], Tracey Mu[a], Rihe Liu[a]

[a]The University of North Carolina at Chapel Hill, The United States of America

Objective. Pancreatic ductal adenocarcinoma (PDAC) is one of the leading causes of cancer death in the United States, with a 5 year survival of only 6%. This dismal outlook illustrates an urgent need for isolating and identifying novel biomarkers specific for PDAC, to enable the development of targeted therapies and diagnostic tools. This research aims to develop 2'-fluoro RNA aptamers that selectively bind to putative novel biomarkers on the cell-surface of PDAC cells without binding to non-pancreatic cancer cells; and that these aptamers can be targeting ligands for development of novel diagnostic and therapeutic tools for pancreatic cancer.

Methods. The series of unique 2'-F RNA ligands were identified by cell-SELEX (systematic evolution of ligands by exponential enrichment) using PDAC and HPNE (normal pancreas) cell lines. Confocal microscopy, immunohistochemisty, and flow cytometry were used for characterization. More than 15 variations of selected aptamer 1502, with 3' and 5' modifications, have been chemically synthesized and are currently being applied translationally.

Results. The optimized 15th round, selected 2'-F RNA aptamer, 1502, binds to PDAC specifically and efficiently (125 nM). Preliminary data demonstrates that 1502 can distinguish pancreatic cancer tissue from patient-derived xenografts (PDX). This targeting ligand has functionalized biocompatible poly (lactic-co-glycolic) acid nanoparticles, which carry the cytotoxic molecule, SN-38; enabling PDAC-specific cell killing. Additionally, a 1502-gold nanoparticle multivalent technology has been developed, allowing targeted hyperthermia and selective cell-killing with an 800 nm IR laser.

Conclusions. In summary, we are developing targeting ligands that are highly specific for PDAC but not normal pancreas or other cancer cells. These ligands have a high affinity to bind to PDX tissue samples and are undergoing further translational applications and multivalent technology development; currently being utilized to identify a potentially novel biomarker of PDAC. This biomarker identification will aid in future diagnostic tests that are of great need.

S11 Development of Controlled-Released Bee Venom Nasal Spray for Immunity Improvement

Cho-A Lee[a], Jong-Woon Jeon[b], Jin-kyu Park[b], Cheong-Weon Cho[a]

[a]College of Pharmacy and Institute of Drug Research and Development, Chungnam National University, South Korea
[b]Wissen Co., Ltd, #410 Bio Venture Town, 461-8, Daejeon 305-811, South Korea

Objective. Bee venom is encapsulated and coated using mucoadhesive polymers with nasal spray for convenient application and sustained-release.

Methods. Bee venom-loaded nanoparticle was prepared by double emulsion evaporation method. Firstly, bee venom of 15 mg was dissolved in 0.5 ml of aqueous solution. Simultaneously, PLGA was dissolved in 5 ml of dichloromethane. The bee venom solution and PLGA solution were emulsified with homogenizer at 20000 rpm for 5 min and a probe sonicator at amp. 20% for 2 min in ice bath to obtain w/o emulsion. Chitosan was dissolved at acetate buffer (pH 4.4) and then PVA was added into chitosan solution. The prepared W1/O emulsion was injected into external polyvinyl alcohol (PVA) phase previously prepared by conducting sonication. The obtained W1/O/W2

emulsion was stirred to evaporate organic solvent at room temperature overnight. The particle was isolated by centrifugation and washed with distilled water. Then the particle was lyophilized During the preparation of bee venom-loaded nanoparticle, various preparation procedures were modified to determine optimal condition. And then, SEM, encapsulation efficiency, zeta potential, size and PDI were evaluated. Also, release test was conducted.

Results. Preparation conditions of 20 ml of 0.5% PVA (23,000D), 0.7% chitosan as external aqueous phase, 100 rpm of evaporation speed and applying 35% sonicator power during 6 min were represented the integrity of the nanoparticle morphology, small particle size and the positive zeta potential.

Conclusions. It was suggested that this bee venom-loaded nanoparticle could be prepared by double emulsion evaporation method and will be evaluated for immunity improvement through nasal mucosa.

S12 Quantitative Evaluation of Intracellular Distribution and Human Placental Transfer of Doxorubicin and its Liposomal Formulations

Suvi K Soininen[a], Jenni K Repo[c], Vesa Karttunen[c], Heta Koivisto[a], Seppo Auriola[b], Kirsi H Vähäkangas[c], Marika Ruponen[a].

[a]Unit of Biopharmacy, School of Pharmacy, Faculty of Health Sciences, University of Eastern Finland, Kuopio campus, Finland
[b]Unit of Pharmaceutical Chemistry, School of Pharmacy, Faculty of Health Sciences, University of Eastern Finland, Kuopio campus, Finland
[c]Unit of Toxicology, School of Pharmacy, Faculty of Health Sciences, University of Eastern Finland, Kuopio campus, Finland

Objective. The intracellular target site of an anti-cancer drug doxorubicin (DOX) is nucleus. By encapsulating DOX into pegylated liposomes (CAELYX®) DOX related side-effects are significantly diminished. However, little is known about intracellular distribution and transfer through human placenta. The aim of this study was to analyze toxicity and intracellular distribution of DOX formulations in three cell lines *in vitro* and to evaluate transfer through human placenta.

Methods. The toxicity and the cellular and/or nuclear localization of free DOX, DOX encapsulated into pH-sensitive liposomes (L-DOX) and CAELYX® were analyzed in rat glioma (BT4C), human placental choriocarcinoma (BeWo) and human kidney clear cell carcinoma (Caki-2) cell lines. Transfer was studied in human placental 4h perfusion. Quantification of DOX was performed by a mass spectrometer.

Results. Free DOX and L-DOX were equally toxic in BT4C and BeWo cells (IC50 0.35–0.37 and 0.49–0.60 µM, respectively). However, the toxicity of CAELYX® was significantly lower (53–129 -fold) in both of the cell lines compared to free DOX and L-DOX.

In BT4C and Caki-2 cells, the cellular internalization of CAELYX® was 26–59 -fold lower at 2µM, and 35–102 -fold at 16 µM exposures compared to free DOX and L-DOX. Interestingly, at both concentrations all the internalized DOX, released from CAELYX®, was localized and accumulated in nuclei.

In human placental perfusions, CAELYX® was not transferred to the fetal circulation at all, whereas ~12% of free DOX and 6% L-DOX were. The amounts of DOX in the tissue were 38–70%, 15% and 1% of the dose in free DOX, L-DOX and CAELYX® perfusions, respectively.

Conclusions. Cellular uptake of CAELYX® is strongly reduced due to surface pegylation. This most likely explains low toxicity and inefficient permeation through human placenta of CAELYX® compared to free DOX and L-DOX. In future, longer perfusions will be performed.

S13 Synthesis and Biological Evaluation of Lipophilic Pt(IV) Derivatives of Oxaliplatin Incorporated in Nano-Carrier with Distinct Improvement in Anti-Tumor Activity

Aiman Abu-Ammar[a], Taher Nassar[a], Dan Gibson[a] and Simon Benita[a]

[a]The Institute for Drug Research of the School of Pharmacy, Faculty of Medicine, The Hebrew University of Jerusalem, POB 12065, Jerusalem, 91120, Israel

Objective. The aim of this study was to synthesize Pt(IV) lipophilic derivatives of oxaliplatin for improved drug performance and reduced toxicity in cancer therapy. Furthermore, the potential therapeutic advantage of incorporating such compounds in nanoparticles is being evaluated.

Methods. The Pt(IV) lipophilic derivatives of oxaliplatin were prepared, characterized and their biological activity evaluated in selected cancer cell lines. Nanoparticles (NPs) of OXA-PAL-ACT (OPA) were developed and physicochemical properties determined in terms of morphology, zeta potential, drug content, stability, cytotoxicity, cellular uptake and DNA platination. A preliminary *in vivo* efficacy study was carried out using an orthotopic intraperitoneal (i.p.) model of metastatic ovarian cancer in SCID-bg mice.

Results. OPA was selected as the lead compound of the synthetic Pt(IV) lipophilic derivatives of oxaliplatin, chemically identified using 1H-NMR, 195Pt-NMR, HPLC and elemental analysis. The experimental LogP value of 2.76 confirmed its lipophilic character. OPA NPs were prepared and characterized by TEM, SEM and AFM. Their mean diameter ranged between 150 and 230 nm. High encapsulation yields of OPA were obtained

(>95%), with a drug content in NPs varying from 21.5 to 22.7% w/w. The OPA NPs were successfully lyophilized and remained stable over 16 weeks at -20°C. Free oxaliplatin, OPA and OPA NPs were incubated over 120h with SKOV-3 and SKOV-3-luc cell lines to assess cytotoxicity. OPA and OPA NPs exhibited pronounced cytotoxic effect compared to oxaliplatin. It was observed that OPA and OPA NPs exhibited a greater tumor growth inhibition than oxalipaltin and respective controls in the metastatic ovarian cancer mouse model. OPA treatment reduction of average bioluminescence was significant at weeks 9 and 10 following tumor inoculation.

Conclusions. Potent Pt(IV) derivative of oxaliplatin was developed and characterized *in vitro* and *in vivo*. The preliminary results showed high potency of OPA and OPA-NPs over the native molecule. These encouraging results need to be further confirmed.

S14 Stable Polymeric Micelles for Tumor-targeted Delivery of Therapeutic and Diagnostic Agents after IV Injection

Yang Shi[a], Twan Lammers[a,b,c], Cornelus F. van Nostrum[a], Wim E. Hennink[a]

[a]Department of Pharmaceutics, Utrecht Institute for Pharmaceutical Sciences (UIPS), Utrecht University, Utrecht, The Netherlands
[b]Department of Experimental Molecular Imaging (ExMI), Helmholtz Institute for Biomedical Engineering, RWTH Aachen University Clinic, Aachen, Germany
[c]Department of Controlled Drug Delivery, MIRA Institute for Biomedical Technology and Technical Medicine, University of Twente, Enschede, The Netherlands

Objective. The aim of this study was to develop a π-π stacking interaction enabled platform technology for polymeric micelles that can solve problems such as insufficient stability and premature drug release in the blood circulation, and apply the technology for image-guided tumor-targeted delivery of chemo and photodynamic therapeutic agents.

Methods. Amphiphilic PEG-p(HPMAm) based polymers were synthesized with and without aromatic repeating units in the polymer chains. For image-guided drug delivery, the polymers were covalently labeled with a near-infrared probe. An anti-cancer drug (paclitaxel) or a novel photosensitizer (silicon phthalocyanine derivative) were physically loaded in the micelles. *In vitro* (photo)cytotoxicity studies were performed using different tumor cells. *In vivo* blood circulation kinetics and biodistribution of both the payloads and the micelles were studied using conventional HPLC methods and multimodal *in vivo* imaging techniques.

Results. The polymeric micelles with aromatic repeating units showed high loading capacity for the hydrophobic chemotherapeutic compounds tested and significantly prolonged circulation time in the blood stream. The *in vivo* imaging studies showed that the payloads and the polymeric micelles well co-localized in different organs. The drug-loaded micelles also showed high (photo)cytotoxicity.

Conclusions. The π-π stacking interactions enabled polymeric micelles to have high drug loading capacity and prolonged circulation time *in vivo* for tumor-targeted delivery of paclitaxel. This technology has also been applied for other hydrophobic compounds, like photosensitizers for photodynamic therapy. The therapeutic efficacy studies of paclitaxel-loaded micelles on different xenograft tumor models in mice are currently ongoing.

S15 Development of ^{177}Lu-labeled Cathepsin S cleavable HPMA Copolymers for Targeted Pancreatic Tumor Imaging and Radiotherapy

Wen Shi[a,b], Nilesh Wagh [a,b], Zhengyuan Zhou[a,b], Yinnong Jia[a,b], Susan K. Brusnahan[a,b], Jered C. Garrison[a,b]

[a]Department of Pharmaceutical Sciences, College of Pharmacy, University of Nebraska Medical Center, Omaha NE 68198, USA.
[b]Center for Drug Delivery and Nanomedicine, University of Nebraska Medical Center, 985830 Nebraska Medical Center, Omaha, NE 68198, United States

Objective. Many current approaches to develop better diagnostic and therapeutic tools have focused on the use of nanomaterials. For cancer diagnosis and therapy, the nanomaterials can passively accumulate in tumors due to the enhanced permeability and retention (EPR) effect. However, a major problem of many nanomaterials-based diagnostics and therapeutics is their opsonization and then fast uptake by macrophages (e.g., Kupffer cells) of the mononuclear phagocyte system (MPS), especially in the liver and spleen. So far, MPS accumulation remains an important obstacle in the clinical translation of many nanomedicines. Here we describe the application of 177Lu labeled HPMA copolymers incorporating different cathepsin S cleavable linkers (CSLs) for human pancreatic adenocarcinoma (PDAC) diagnostic imaging and radiotherapy. The cleavable copolymers are expected to enhance the diagnostic and therapeutic efficacy and reduce the MPS retention and toxicity.

Methods. Three cathepsin S cleavable peptide linkers with linking groups of different length (0,6 and 13 atoms) were utilized to link DOTA chelated ^{177}Lu to a Mw 109 kDa HPMA copolymers prepared by RAFT. These conjugates were evaluated by *in vitro* cleavage studies and *in vivo* biodistribution studies in mouse xenograft models of human pancreatic adenocarcinoma.

Results. *In vitro* enzymatic studies revealed that the longest linking group (13 atoms) led to faster cleavage by cathepsin S. The radiolabeled HPMA copolymers with CSLs incorporated demonstrated significantly higher levels of excretion and a significant decrease in

long-term hepatic and splenic retention of radioactivity, relative to the non-cleavable control. *In vivo* biodistribution studies showed that the length of the linking group had minimal impact on the non-target clearance. However, the HPMA copolymer with CSL bearing the null (0 atom) linking group demonstrated significantly higher levels of tumor retention relative to other CSLs.

Conclusions. Our results demonstrate that the CSLs can substantially reduce the non-target retention of radioactivity from ^{177}Lu-labeled HPMA copolymers thereby increasing their clinical potential.

S16 Prediction of Drug Loading in Single Excipients and Lipid Based Formulations

Linda C. Persson[a], Elena Diesch[a], William N. Charman[b], Christopher J. H. Porter[b] and Christel A.S. Bergström[a,b]

[a]Department of Pharmacy, Uppsala University, Sweden
[b]Drug Delivery, Disposition and Dynamics, Monash Institute of Pharmaceutical Sciences, Monash University, Australia

Objective. To develop tools that rationalize solubility measurements in lipid based formulations and hence, minimize the screening efforts undertaken during the process of formulation development.

Methods. Thermodynamic solubility was determined for 30 poorly water-soluble compounds (logP ≥ 2) in nine commonly used excipients and four lipid based formulations using a small scale shake-flask method at 37°C. Prior to sampling, the vials (n ≥ 3) were centrifuged at 37°C, 2800g for 30 minutes. Drug concentrations were determined by reverse phase HPLC or UV/FL plate reader. Equilibrium solubility was determined as the value when the solubility between two consecutive samples points (24h time difference) differed less than 10%. Formulation stability and self-emulsification in fasted state simulated intestinal fluid (pH 6.5, 37°C) was also investigated.

Results. The solvation capacity (mol/mol) of triglycerides was close to equal (R^2 0.98) and linearly correlated with the solubility in Capmul and Maisine 35:1 (mixed mono- diglycerides). Strong correlations were also found between polyethylene glycol 400 and several ethoxylated surfactants and co-solvents (R^2 0.85). Recent literature suggests that solubility in a formulation can be predicted by summing the amount possible to dissolve in each of the included excipients. As a proof of principle 7 of the compounds, including neutral acidic and basic drugs, were determined for the solubility in four formulations and the observed solubility was in good agreement with predicted values (R^2 0.96).

Conclusions. These findings prove that the extensive screening efforts and the amount of compound needed in development of lipid based formulations can be significantly reduced. Through determinations in key excipients, such as one triglyceride and one ethoxylated vehicle, the solubility in other excipients can be predicted. The concept shows potential to be extended further to estimation of loading capacity of complex formulations.

S17 Modeling the Gram Negative Bacterial Cell Envelope: A New Approach for Permeability Investigation of Anti-Infectives

Florian Gräf[a], Sarah Gordom[b], Claus-Michael Lehr[a,b]

[a]Saarland University, Germany
[b]Helmholtz-Institute for Pharmaceutical Research Saarland (HIPS), Germany

Objective. To develop an artificial model of the Gram negative bacterial cell envelope that can be used to investigate and quantify the permeability characteristics of anti-infectives or anti-infective delivery systems.

Methods. Separate models of the various bacterial cell envelope components will first be created; modelling of the bacterial cytoplasmic membrane was selected as a starting point. A permeation barrier consisting of the three major cytoplasmic membrane lipids of Gram negative bacteria (phosphatidylethanolamine, phosphatidylglycerol and cardiolipin) was prepared according to the methodology of the Phospholipid Vesicle-Based Permeation Assay (PVPA). In this respect, liposomes consisting of the above lipid components were prepared, and coated onto Transwell filter inserts via repeated centrifugation and drying steps. The coating process was optimized in order to ensure that the employed parameters led to the creation of an artificial layer with stable barrier function by carrying out simulated transport experiments. Therefore artificial lipid layers were incubated with Krebs Ringer Buffer for 5 h at 37°C with gentle agitation, and the transepithelial electrical resistance (TEER) of layers was measured at set time intervals. In a further step the layer integrity was confirmed using microscopy.

Results. Liposomes of a narrow size distribution were successfully prepared. An optimized liposome coating procedure was also established, with the finalized coating procedure showing stable and reproducible TEER values during the course of simulated transport experiments. The artificial membrane was also confirmed as being intact before as well as after the exposure to media, as evident from microscopy images.

Conclusions. A stable and reproducible model of the Gram negative bacterial cytoplasmic membrane was successfully developed by applying the PVPA and using bacteria-relevant lipids. First characterization of the coating layer was initiated. Further characterization of the developed model and feasibility studies form the focus of current and future work.

S18 The Dispersion Releaser: An Optimized Tool for Selection of Colloidal Dosage Forms During Development

Christine Janas[a], Jennifer Dressman[a], Matthias Wacker[a]

[a]Institute of Pharmaceutical Technology, Goethe University, Germany

Objective. Drug release testing is a standard procedure in the in-vitro characterization of new dosage forms during formulation development. Since colloidal drug carrier devices demand specific equipment and expertise for the assessment of release rate, many efforts have been made to address these issues. One example is the "Dispersion Releaser", an adapter for the USP apparatus 2, which in this work was applied to the selection of photosensitizer-loaded PLGA-nanoparticles for sustained release.

Methods. Temoporfin-loaded PLGA-nanoparticles were prepared by the diffusion emulsion evaporation method. Additionally, a polymeric coating agent was used to optimize surface properties of the generated particles. Selection of formulation candidates was conducted by monitoring colloid stability and release rate in a physiology-based model. Media were composed of phosphate buffered saline with a pH of 7.4 supplemented with fetal calf serum at 37 °C to simulate the parenteral route of administration.

Results. Particles ranging in size from 200 to 300 nm were tested. The zeta potential differs depending on preparation procedure between -20 and +50 mV. Colloid stability testing of formulations in release media revealed only slight changes in particle size and polydispersity index. A difference in release rate was observed for unmodified PLGA-nanoparticles compared to those with a polymeric coating when analyzed with the Dispersion Releaser technique. While a burst release from unmodified nanoparticles was observed, the coating enables release of the drug in a sustained manner.

Conclusions. Nanoparticles of high stability and with optimal release rate were selected by using the Dispersion Releaser technology. A rational formulation design based on the *in vitro* release studies was successfully implemented for formulation development.

S19 Film Thickness Distribution Impacts Sunscreen Efficacy

Myriam Sohn[a,b], Bernd Herzog[c], Georgios Imanidis[a,b]

[a]Department of Pharmaceutical Sciences, University of Basel, 4056 Basel, Switzerland
[b]School of Life Sciences, University of Applied Sciences & Arts Northwestern, Muttenz, Switzerland
[c]BASF Grenzach-Whylen, Germany

Objective. Sunscreen efficacy depends primarily on the absorbance properties of UV filters in the product. However, sun protection factor (SPF) frequently differs between sunscreens with the same filter composition depending on the vehicle and application procedure. In the present work, we tested the hypothesis that thickness and homogeneity of distribution of sun-screen film are also responsible for and can explain the measured SPF. We investigated the impact of application and of vehicle on the film thickness distribution based on topographical measurements and extracted quantitative data of the film.

Methods. Epidermal membrane of pig ears was used as biological substrate since pig skin is recognized to best simulate human skin. The film thickness distribution was obtained from the difference of topography before and after application of 2mg/cm² of sunscreen, providing a histogram of film thickness frequencies and the mean thickness, Smean. SPF *in vitro* was evaluated in the same samples by UV transmission measurements. Five sunscreen vehicles, two

application pressures and two spreading times were investigated.

Results. Smean was approximately 2 to 3µm which was smaller than the theoretical thickness, reflecting the remaining non-volatile compounds. We showed a significant influence of vehicle and application conditions on thickness and distribution of the film. High pressure, long spreading time and low formulation viscosity resulted in a smaller Smean. The same effect of the studied parameters was observed also for SPF *in vitro*. Smean correlated positively with SPF *in vitro* for all vehicles.

Conclusions. There is a strong influence of application procedure and vehicle on delivered UV protection of sunscreens. The differences in the film distribution provide an explanation of different SPFs observed between sunscreens containing the same UV filter composition. Therefore, characterizing the film residue on skin is fundamental to understand the behavior of sunscreens and optimize their performance.

S20 Cardiotoxicity of Donepezil Due to the Drug-Drug Interaction on the Efflux Transporter in the Heart

Ryota Takeuchi[a], and Ikumi Tamai[a]

[a]Faculty of Pharmaceutical Science, Institute of Medical, Pharmaceutical and Health Sciences, Kanazawa University, Japan

Objective. When donepezil, used for treatment of Alzheimers disease, was administered with cilostazol, an antiplatelet agent, the case of the heart toxicity presumably due to donepezil was reported. Such a cardiotoxicity of donepezil might be caused by an increase of local tissue concentration which cannot be monitored only from the change in blood concentration. We have previously shown that cilostazol is a substrate of P-glycoprotein (P-gp). In addition, expression of drug efflux transporters such as P-gp and BCRP in the heart tissues is reported. Therefore, we hypothesized that the donepezil-cilostazol interaction on the locally expressed transporter in heart is a cause of the heart toxicity. The purpose of the present study is to examine such a possibility of drug-drug interaction that may cause donepezil toxicity.

Methods. Cultured MDCK cells that are transfected with P-gp or BCRP gene were used to examine transport of donepezil by those efflux transporters. Heart tissue slices from rats were used for donepezil accumulation study.

Results. The results of donepezil transport in MDCK cells clearly demonstrated that BCRP accepts donepezil as a substrate, while it is not a good substrate of P-gp. Cilostazol inhibited BCRP-mediated donepezil transport in a concentration-dependent manner with an IC_{50} of 60 nM. Obtained IC_{50} of cilostazol is comparable with clinically achievable plasma free concentration. Donepezil uptake by heart tissue slices was increased in the presence of cilostazol and Ko143, a BCRP inhibitor.

Conclusions. Since donepezil is a good substrate of BCRP and BCRP is expressed in endothelial cells of heart, accumulation of donepezil in heart tissue might be controlled by BCRP, which could be inhibited by cilostazol clinically. Accordingly, when donepezil is coadministered with cilostazol, heart toxicity should be carefully monitored.

S21 Genetic Variants in Transcription Factors are Linked to the Pharmacokinetics and Pharmacodynamics of Metformin

Srijib Goswami[a], Sook Wah Yee[a], Sophie Stocker[a], Jonathan D. Mosley[b], Michiaki Kubo[c], Rich Castro[a], Joel A. Mefford[a], Christopher Wen[a], Xiaomin Liang[a], John Witte[a], Claire Brett[d], Shiro Maeda[c], Melissa D. Simpson[e], Monique M. Hedderson[f], Robert L. Davis[g], Dan M. Roden[b], Kathleen M. Giacomini[a], Radojka M Savic[a]

[a]Department of Bioengineering and Therapeutic Sciences, University of California San Francisco, San Francisco, CA
[b]Department of Pharmacology and Medicine, Vanderbilt University Medical Center, Nashville, Tennessee, USA
[c]Center of Genomic Medicine, RIKEN, Yokohama City, Japan
[d]Department of Anesthesiology, University of California, San Francisco, California, USA
[e]Marshfield Clinic Research Foundation, Marshfield, Wisconsin, USA
[f]Kaiser Permanente Northern California, Division of Research, Oakland, California, USA
[g]Kaiser Permanente Georgia, Center for Health Research Southeast, Atlanta, Georgia, USA

Objective. Although metformin is the most widely prescribed drug for the treatment of type 2 diabetes (T2D), 35% of patients fail to achieve therapeutic response. To date, most pharmacogenomic (PGX) studies have focused on genetic variants in transporter genes. Here, we investigate the effect of variants in transcription factor genes on metformin pharmacokinetics (PK) and pharmacodynamics (PD).

Methods. Data from 440 T2D patients on metformin were used to identify SNPs in transcription factors associated with glycemic response (PD: HbA1c levels). A PK mechanism was investigated using two approaches. 1) A multivariate regression analysis in 57 healthy subjects with metformin secretory clearance (CL) as the outcome. 2) Development of a population PK model using patient (n=133) and healthy subject (n=102) data to examine the role of transcription factor SNPs on metformin PK in relation to ethnicity and known transporter SNPs.

Results. Our top finding was in SP1, a transcription factor known to modulate expression levels of transporters involved in metformin PK. From our regression analysis, 5 variants in the SP1 region associated with Hba1c levels ($P<0.01$) and secretory CL ($P<0.05$). Our model validated 1 such variant, rs784888, on metformin apparent CL. Homozygous carriers of the minor allele are predicted to have a 24%

reduction in total CL. Independent of PK, SNPs in PPARA and HNF4A, e.g. rs149711321 (P=1E-05), associated with metformin PD. In addition to genetics, African Americans were observed to have a 26% increase in metformin CL relative to Caucasians.

Conclusions. This is the first PGX study to identify SNPs in transcription factors as determinants of variation in metformin PK and PD. The study also reveals lower metformin exposure levels in African Americans compared with other races.

S22 Effects of Angiotensin (1-7) Treatment on Diabetic Complications

Anna Papinska[a], Nicholas Mordwinkin[a],Christopher Meeks[a], Sachin Jadhav[a], Alick Tan[a], Maira Soto[a], Theresa Espinoza[a], Norma Roda[a], Jeannette Arangua[a], Tamar Cohen[a], Kevin Gaffney[a], and Kathleen Rodgers[a]

[a]University of Southern California, USA

Objective. The purpose of this study was to investigate the effects of Angiotensin (1-7) [A(1-7)] administration on type 2 diabetes [T2D] related complications; namely diabetic nephropathy and cardiomyopathy.

Methods. Leptin receptor knockout mice (db/db) were treated daily with 500ug/kg/day A(1-7) for 2-16 weeks to assess effects of prolonged treatment. Kidney function was evaluated by measuring proteinuria, glomerular hypertrophy and mesangial expansion. Non-invasive *in vivo* ultrasound imaging was used to assess hemodynamics of blood flow through the kidneys. Possible mechanisms of drug action were determined by measuring markers of inflammation and oxidative stress in tissues. Heart function was evaluated using echocardiography and histological analysis of cardiomyocyte hypertrophy.

Results. Glomerular damage was not detected in kidneys of diabetic animals in the earliest treatment group. However, at the same time point, A(1-7) reduced oxidative stress in kidney tissue that had been damaged by T2D. Levels of inflammatory markers were reduced in diabetic animals and further decreased with treatment. After 12 weeks of treatment there was a significant reduction in proteinuria, glomerular size and mesangial expansion in diabetic animals.

Mice treated for 16 weeks exhibited improved cardiac output and shortening fraction compared to non-treated diabetic mice. Treatment also reduced cardiomyocyte hypertrophy. No changes in the heart function were detected in db/db mice before 10 weeks of age.

Conclusions. Our results suggest that oxidative stress occurs early in the development of diabetic nephropathy, even before significant glomerular damage or inflammation can be detected. In these animals A(1-7) treatment prevented protein damage caused by increased oxidative stress. Long-term treatment (12 weeks) reduced glomerular damage in diabetic animals. Longer treatment also improved systolic function and contractility of the heart. A(1-7) reduced cardiomyocyte hypertrophy; suggesting one possible mechanism of A(1-7) action in the heart. Together, these results show that A(1-7) administration improves cardiorenal function in T2D animals.

S23 Fluorescent Particles for the Tracking of Metastatic Cells

Athanasia Dasargyri[a], Pablo Hervella[a], Ailsa Christiansen[a], Steven Proulx[a], Michael Detmar[a], Jean-Christophe Leroux[a]

[a]Department of Chemistry and Applied Biosciences, ETH Zurich, Switzerland

Objective. Although both lymphatic and blood vessels have been shown to play a role in metastatic dissemination, the relative significance of each vascular system for the development of distant organ metastases remains controversial. This project aims to develop an *in vivo* fluorescence approach to characterize the importance of lymphatic metastasis to cancer dissemination. The experimental strategy aimed to design fluorescently-labeled, non-biodegradable polymeric particles able to target metastatic cells within the lymph node (LN). The tumor-selective engulfment of labeled particles in the LNs will allow the tracking of the metastatic cells *in vivo* as they traffic to distal organs.

Methods. 1 µm fluorescently-labeled polystyrene particles were PEGylated and functionalized with anisamide, a small organic molecule reported to target the Sigma-1 receptor. The expression of Sigma-1 receptor on cancer cells would thereby enable tumor-selective particle uptake.

The *in vitro* internalization of the functionalized particles by B16F10 murine melanoma cells was evaluated by flow cytometry and confocal microscopy.

Silencing of the Sigma-1 receptor, followed by *in vitro* uptake experiments, was performed to assess the involvement of this receptor in the internalization process.

Results. The surface functionalization of the particles was confirmed by the alteration of the surface charge.

Particle uptake studies on B16F10 cells showed enhanced internalization of the anisamide-functionalized particles.

Sigma-1 receptor silencing on B16F10 cells did not alter the uptake of the particles, suggesting that the internalization is not mediated by this receptor.

Conclusions. Tumor-specific labeling particles were prepared. Although the role of the Sigma-1 receptor in the internalization process could not be proven, the

tumor selective uptake of particles enables their use in *in vivo* tumor cell tracking studies. Sentinel intranodal particle injection in mice bearing B16F10 tumors, and subsequent assessment of cells within metastatic sites will reveal involvement of the sentinel lymph node in the metastatic process.

Acknowledgements. This project is financially supported by Krebsliga Schweiz.

S24 Overcoming Trastuzumab Resistance via Pharmacological Inhibition of MEOX1 in Breast Cancer Stem Cells

Joseph Burnett[a], Lichao Sun[a], Mari Gasparyan[a], Hasan Korkaya[a], Hui Jiang[a], Yajing Liu[a], Max Wicha[a], Duxin Sun[a]

[a]University of Michigan, United States of America

Objective. Nearly 40% of HER2+ breast cancers harbor PTEN inactivating mutations, which promote acquired and de novo resistance to trastuzumab. The purpose of this study is to identify novel signal transduction pathways utilized by trastuzumab-resistant breast cancers which can be exploited for the development of new therapeutic agents.

Methods. Characterization of trastuzumab-sensitive and resistant cell lines was carried out using microscopy, flow cytometry, real time PCR, and ELISA assays *in vitro*. MTS proliferation assay was employed to identify the selectivity of model compound sulforaphane (SF) in cell lines. Global analysis of mRNA in cell lines treated with SF was performed using RNA sequencing. The effect of siRNA on proliferation and self-renewal in trastuzumab-resistant cell line was evaluated for candidate genes. Pharmacological inhibition of target genes were assessed *in vivo* using orthotopic tumor xenograft models. A tissue array containing 75 patients' tumor samples were utilized to assess the clinical impact of these findings.

Results. Trastuzumab resistance was generated by shPTEN and long term culture with trastuzumab (BT474-PTEN$^-$-LTT). BT474-PTEN$^-$-LTT cells underwent the epithelial-mesenchymal transition and reduced ERBB receptor family signaling. While SF had no effect in parental BT474 up to 50 μM, inhibition of BT474-PTEN$^-$-LTT by SF produced an IC_{50} of 8.96 μM. RNA sequencing revealed that SF reduced expression of 23 genes, preferentially expressed in BT474-PTEN$^-$-LTT, greater than 2-fold. MEOX1 was the only gene tested with siRNA capable of inhibiting proliferation and self-renewal. *In vivo*, SF reduced MEOX1 expression, inhibited primary tumor growth, and reduced CSC frequency. Immunostaining of primary breast cancers exhibited expression of MEOX1 primarily in triple negative tumors.

Conclusions. MEOX1 is a novel target within trastuzumab-resistant breast cancer, capable of regulating bulk proliferation and CSC self-renewal. Further, expression of MEOX1 in tissue from breast cancer patients suggests strong support for development of clinical therapies.

S25 Investigation of the Mechanism of Anti-Tumor Action of Arsenic Trioxide in Ovarian Cancer Cell Lines

Kamila Katarzyna Kaminska[a], John Leow Wee Peng[a], Pei-Shi Ong[a], Eng Hui Chew[a]

[a]Department of Pharmacy, National University of Singapore, Singapore

Objective. Arsenic trioxide (ATO) is currently approved for treatment of acute promyelocytic leukemia. There is also potential for ATO to be used for treatment of several solid tumors including ovarian cancers. However, while ATO has been shown to induce cytotoxicity in ovarian cancer cells, the precise mechanism remains unclear. In the light that inhibition of the redox protein thioredoxin reductase (TrxR) has been reported to be inhibited by ATO in breast cancer cells, we proposed that TrxR targeting was one underlying mechanism of anti-tumor action of ATO against ovarian cancer cells.

Methods. MTT cell viability assay was performed to evaluate the anti-proliferative activity of ATO in SKOV3 and OVCAR3 ovarian carcinoma cells. Biochemical assays were performed to measure cellular activities of TrxR, thioredoxin (Trx), glutathione reductase (GR) and lutathione peroxidase (GPx). Western blotting was carried out to examine the levels of Trx-apoptosis signal regulating kinase 1 (ASK1) complexes pulled down by Trx mmunoprecipitation, as well as the levels of various cellular proteins.

Results. ATO displayed potent anti-proliferative activities against SKOV3 and OVCAR3 cells. Furthermore, significant inhibition of TrxR was observed, whereas activities of other redox enzymes (Trx, GR and GPx) were less affected in the ATO-treated cells. In particular, treatment with ATO at 20 μM had resulted in decrease of TrxR activity by 64% in SKOV3 cells and by 86% in OVCAR3 cells. Furthermore, we demonstrated that ATO treatment resulted in a dosedependent decrease in the interaction of ASK1 with Trx, and a dose-dependent activation of ASK1-dependent p38 and c-Jun N-terminal kinase (JNK) mitogen-activated protein kinase (MAPK) signaling pathways.

Conclusions. Our study revealed an ATO-induced attenuation of TrxR activity in ovarian cancer cells, which suggested that TrxR inhibition played a role in ATO-induced cell death.

S26 Quantification of Factors Governing Drug Release Kinetics from Nanoparticles: A Combined Experimental and Mechanistic Modeling Approach

Kyle D. Fugit[a] and Bradley D. Anderson[a]

[a]Department of Pharmaceutical Sciences, The University of Kentucky, Lexington, KY 40536, USA

Objective. Advancements in nanoparticle drug delivery of anticancer agents require mathematical models capable of predicting *in vivo* formulation performance from *in vitro* characterization studies. Such models must identify and incorporate the physicochemical properties of the therapeutic agent and nanoparticle driving *in vivo* drug release. This work identifies the factors governing loading and release of liposomal formulations of the anticancer agent topotecan.

Methods. Physicochemical characterization of topotecan's ionization equilibrium (i.e. pKa) and its dimerization constant were determined spectrometrically while HPLC was used to monitor the interconversion kinetics of topotcean between it lactone and carboxylate forms. A non-sink ultrafiltration method was used to monitor release kinetics from passively loaded topotecan under various conditions (e.g. pH). Active loading of topotecan resulting from a lowered intravesicular pH due to ammonia transport was also studied by this method. Release from these actively-loaded formulations was monitored by alterations in topotecan's interconversion kinetics using the above-mentioned HPLC method. Mathematical modeling of the data obtained from these studies provided a mechanistic analysis of the factors governing topotecan transport in liposomal formulations.

Results. Based on the results of passive-loading studies, mechanistic modeling identified three permeable species in which the zwitterionic lactone form of topotecan was the most permeable. Ring-closing kinetics of topotecan from its carboxylate to lactone form were found to affect topotecan release in the neutral pH region. Further modeling of actively-loaded formulations identified drug self-association and ion-pairing affected its loading while increasing levels of extravesicular ammonia accelerated its release.

Conclusions. This work identifies several of the key parameters governing topotecan transport in liposomal formulations. This mechanistic understanding will provide a rational approach for optimizing future liposomal formulations of topotecan and an example for characterizing liposomal formulations of other drugs.

S27 Nanoparticles in Polymer Nanospheres to Stabilize Gelatin without Crosslinkers

Saeed Ahmad Khan[a,b], Marc Schneider[a]

[a]Department of Pharmaceutics and Biopharmacy, Philipps-University Marburg, Germany
[b]Department of Pharmacy, Kohat University of Science and Technology, Pakistan

Objective. One of the most important steps in preparation of gelatin nanoparticles (GNPs) is the crosslinking step. This step is mandatory to reach sufficient mechanical properties of the nanoparticle and for drug release. Crosslinkers connect particular gelatin chains and hence stabilize the particles. In addition to crosslinking of gelatin chains, crosslinkers can react with similar active sites of loaded proteins or peptides. Hence the particles cannot be used for encapsulation of protein- and peptide-based drugs. Therefore, the principal aim of this work was to put forward an alternative technique for stabilization of gelatin nanoparticles.

Methods. Our system is based on NiNOS (nanoparticles in nanospheres) concept. Where gelatin nanoparticles are entrapped in a pharmaceutical relevant polymer Eudragit®E 100 (E100), offering a pH-dependent behavior. We used a unique nanoprecipitation-emulsion solvent evaporation technique. Organic phase of the dissolved E100 containing dispersed GNPs was emulsified with aqueous phase containing PVA, followed by homogenization. Evaporation of organic phase yielded solidified nanospheres with entrapped GNPs. Gelatin release was used as a parameter to determine the effective entrapment of GNPs in nanospheres.

Results. Dynamic light scattering (DLS) studies showed that homogenization speed is the size determining step for nanospheres. Higher speed of homogenization produced smaller nanoparticles and vice versa. Nanospheres of 200-300 nm size with narrow distribution were produced with a homogenization speed of 15000 rpm. Scanning electron microscopy (SEM) revealed that E100 concentration was critical for morphology of the nanospheres. Lower E100 concentration showed spherical pores equal to the size of GNPs (i.e. 90nm) on the surface of nanospheres. The pores vanished with increase in E100 concentration, and thus gelatin entrapment was improved.

Conclusions. It can be concluded that gelatin nanoparticles can be stabilized by entrapping them in pH responsive polymer nanospheres. Though the use of E.100 has limitations in parenteral delivery, we see its potential for buccal route (work ongoing).

This study is the first of its kind to provide proof of concept for stabilization of gelatin nanoparticles without crosslinking.

S28 Oligonucleotide Hybridization-Mediated Drug-Free Macromolecular Therapeutics

Te-Wei Chu[a], Jiyuan Yang[a], Rui Zhang[a], Monika Sima[a], and Jindřich Kopeček[a,b]

[a]Department of Pharmaceutics & Pharmaceutical Chemistry, University of Utah, USA
[b]Department of Bioengineering, University of Utah, USA

Objective. A novel nanomedicine approach is proposed where hybrid materials are used as "bio-mimics" to trigger cellular events and result in new therapeutic effects. We designed a therapeutic platform that mimics the mechanism of immune effector cells to crosslink surface receptors of target cells and induce apoptosis. This platform was tested against B-cell lymphomas that highly express the CD20 antigen. The therapeutic system is composed of two nanoconjugates: (1) an anti-CD20 Fab' linked to a morpholino oligonucleotide (MORF1), and (2) a linear polymer (P) of N-(2-hydroxypropyl)methacrylamide (HPMA) grafted with multiple copies of complementary MORF2. The two conjugates self-assemble via MORF1-MORF2 hybridization at the surface of malignant B-cells, which crosslinks CD20 and initiates apoptosis. We named the system "drug-free macromolecular therapeutics" because it contains no small-molecule cytotoxic compounds.

Methods. Self-assembly of two nanoconjugates (Fab'-MORF1, P-MORF2) was characterized *in vitro* by DLS and CD spectroscopy. Confocal microscopy was used to study the cell surface biorecognition. Apoptosis of malignant B-cell lines was determined by caspase-3, annexin V, and TUNEL assays. *In vivo* anticancer efficacy was evaluated in mice bearing systemic B-cell lymphomas.

Results. *In vitro* characterization demonstrated a fast binding (<10 min) between two conjugates (Tm = 59 °C). Self-assembly of Fab'-MORF1 and P-MORF2 occurred at the surface of B-cells, with subsequent apoptosis induction. When tested in a mouse model of human non-Hodgkin lymphoma, the two conjugates (either administered consecutively or as a premixture) eradicated cancer cells and produced long-term survivors. No residual tumors were found in the surviving mice.

Conclusions. We demonstrated a new paradigm of therapeutics that is "drug-free" and "immune-independent". The proposed two-step (consecutive) treatment offers the opportunity of pretargeting, *e.g.*, the timing of administration of P-MORF2 can be optimized in individual patients based on the pharmacokinetics and biodistribution of Fab'-MORF1. This platform can be applied to crosslink any non-internalizing receptor and potentially treat other diseases.

S29 The Influence of Shape on Cellular Uptake and Magnetic Targeting of Iron Oxide Nanoparticles

Zhizhi Sun[a], Matthew Worden[b], James A. Thliveris[a], Torsten Hegmann[b], Donald W. Miller[a]

[a]University of Manitoba, Canada
[b]Kent State University, U.S.A.

Objective. Our laboratories are examining iron oxide nanoparticles (IONPs) as a potential platform for drug delivery to the brain. While much effort has focused on grafting ligand onto the IONP surface to enhance cellular uptake in brain endothelial cells, herein we report alteration of IONP shape can influence both the preferential cellular uptake in endothelial cells and the ability to augment cellular uptake with application of an external magnetic field.

Methods. Nanosphere and nanoplatelet shaped IONPs with the same overall size and negative surface charge were synthesized. Cellular uptake profiles of nanosphere and nanoplatelet IONPs were compared in brain and lung endothelial cells, as well as liver, intestine, and kidney epithelial cells. Quantitative determination of cellular IONP was performed using ferrozine assay. Transmission electron microscopy (TEM) was used to confirm the internalization of IONPs.

Results. Uptake of nanospheres was minimal in all cells tested with or without magnetic field exposure. In contrast, a 3-fold enhancement in internalized nanoplatelets was observed in endothelial cells compared to nanospheres. Application of an external magnetic field increased nanoplatelet uptake in endothelial cells by 10 fold. The uptake of nanoplatelets in epithelial cells was 3 times lower than endothelial cells. TEM confirmed preferential uptake of nanoplatelets in endothelial cells. No toxicity was observed in endothelial cells treated with nanoplatelets.

Conclusions. Nanoplatelets have improved cellular uptake profiles compared to nanospheres. The increased cellular uptake of the nanoplatelets was especially apparent in endothelial cells. The increased magnetic properties of the nanoplatelets result in enhanced uptake in the presence of an external magnetic field. These properties may allow for better cellular penetration across capillary beds such as the blood-brain barrier. Support provided by NSERC-CIHR Collaborative Health Research Project, the Ohio Third Frontier Program for Ohio Research Scholars, and an NSERC Student Fellowship.

S30 The Involvement of Fatty Acid-Binding Protein 5 in the Blood-Brain Barrier Transport of Docosahexaenoic Acid

Yijun Pan[a], Christopher JH Porter[a], Martin J Scanlon[a], Joseph A Nicolazzo[a]

[a]Monash Institute of Pharmaceutical Sciences, Monash University, Australia

Objective. To investigate if fatty acid-binding protein 5 (FABP5) mediates docosahexaenoic acid (DHA) transport across the blood-brain barrier (BBB) and whether the reduced brain levels of DHA observed in Alzheimer's disease (AD) are associated with attenuated FABP5 function at the BBB.

Methods. Human FAPB5 was expressed in *E.coli* and the binding affinity of DHA for human FABP5 was assessed using isothermal titration calorimetry. The uptake of ^{14}C-DHA was measured in human brain microvascular endothelial cells (hCMEC/D3) with and without FABP5 silencing and the BBB transport of ^{14}C-DHA assessed in wild-type and FABP5 deficient mice using a transcardiac perfusion technique. The BBB transport of ^{14}C-DHA (by transcardiac perfusion) and FABP5 expression in isolated cerebral microvessels (by Western blot) was compared between 8 month male wild-type and APP/PS1 mice (AD model).

Results. DHA bound to human FABP5 with an equilibrium dissociation constant of 155±8nM (mean ± SEM, n=15). FABP5 siRNA transfection decreased FABP5 mRNA by 53.2±5.5%, and this was associated with a 17.1±2.7% reduction in ^{14}C-DHA uptake in hCMEC/D3. DHA K_{in} decreased by 40.0±10.7% in FABP5 deficient mice. The BBB expression of FABP5 was decreased 18% in APP/PS1 and this was associated with a 42.1±12.6% reduction in ^{14}C-DHA BBB transport.

Conclusions. This study has demonstrated that FABP5 binds to DHA and that reduced FABP5 expression in the BBB occurs in tandem with decreases in DHA uptake into the brain. The data suggest that the reduced brain levels of DHA seen in AD may be a result of decreased FABP5 expression at the BBB.

S31 Palbociclib Efficacy in Glioblastoma is limited by Efflux Pump Activity at the Blood-Brain Barrier

Karen E. Parrish[a], Jenny Pokorny[b], Rajendar K. Mittapalli[a], Katrina Bakken[b], Jann N. Sarkaria[b], William F. Elmquist[a]

[a]Department of Pharmaceutics, Brain Barriers Research Center, University of Minnesota, MN 55455
[b]Radiation Oncology, Mayo Clinic, Rochester, MN 55905

Objective. Glioblastoma multiforme (GBM) is the most common primary brain tumor in adults and has a poor prognosis. Developing effective therapies for this disease is hampered by the blood-brain barrier (BBB), which limits delivery of anti-cancer agents to infiltrative tumor cells. The cyclin-dependent kinase 4 (Cdk4) pathway is hyperactivated in approximately 75% of GBM in association with homozygous deletion of p16 (52%) and amplification of Cdk4 (18%) and Cdk6 (1%). Palbociclib (PD0332991) is a potent Cdk4/6 inhibitor that has remarkable efficacy in treating non-brain tumors. The purpose of this study is to determine the mechanisms limiting efficacy of palbociclib therapy in an orthotopic xenograft model.

Methods. Brain distribution studies of palbociclib were conducted in FVB wild-type (WT), and triple knockout (TKO; Mdr1a/b$^{(-/-)}$Bcrp1$^{(-/-)}$) mice following an oral dose (10, 50, 100 or 150 mg/kg). Concentrations of palbociclib were determined by LC-MS/MS. Survival studies were conducted in patient-derived primary GBM22 xenograft model in nude mice.

Results. The brain exposure of palbociclib in TKO mice following a 10 mg/kg oral dose (AUCbrain-to-AUCplasma ratio) was 150-fold higher than WT mice [WT: .044; TKO: 6.24]. For survival studies, palbociclib was dosed at either 10 mg/kg/day or 150 mg/kg/day continuously. Consistent with sub-therapeutic delivery, palbociclib did not prolong the median survival of an orthotopic GBM22 xenograft model. Conversely, treatment of GBM22 xenografts at 150 mg/kg/day grown as flank tumors resulted in a significant (45 day) survival benefit. Additionally, the brain concentrations following a 150 mg/kg dose are comparable to the flank tumor concentrations following a 10 mg/kg dose [770 ± 230 ng/mL and 730

± 510 ng/mL, respectively], and both were sub-therapeutic.

Conclusions. These data suggest that the poor brain delivery of palbociclib may limit clinical efficacy of this Cdk4/6 inhibitor in the treatment of brain tumors such as glioblastoma.

S32 HepaRG Acellular Matrix for Hepatic Differentiation of Human Pluripotent Stem Cells

Liisa Kanninen[a], Pauliina Porola[a], Jonanna Niklander[a], Melina Malinen[a], Anne Corlu[b], Christiane GuGuen-Guillouzo[b], Arto Urtti[a], Marjo Yliperttula[a], Yan-Ru Lou[a]

[a]Division of Pharmaceutical Biosciences, Centre for Drug Research, Faculty of Pharmacy,University of Helsinki, Finland
[b]Inserm UMR991, Liver Metabolism and cancer, Université de Rennes 1, France

Objective. Hepatocytes, the main cell type of the liver, are needed in the drug discovery for prediction of biotransformation pathways, possible drug-drug interactions, and hepatotoxicity of drug candidates. Pluripotent stem cells offer valuable and limitless supply of hepatic cells with human origin for *in vitro* drug studies. In majority of the current differentiation protocols stem cell fate is guided towards hepatic-like cells with stepwise growth factor treatment. However, it is known that not only soluble factors but also cell-cell interactions and cell-matrix interactions play important role in the complex, multistage cell differentiation process. The objective of our research was to establish a novel hepatic cell derived acellular matrix (ACM) which can be used as a culture platform for human pluripotent stem cell differentiation towards hepatocytes.

Methods. Human embryonic stem (hES) cells WA07 and human induced pluripotent stem (hiPS) cells iPS(IMR90)-4 were first differentiated to definitive endoderm (DE) cells in standard Matrigel culture using optimized growth factor cocktail. Next, DE cells were seeded onto human liver progenitor HepaRG cell-derived ACM for further hepatic differentiation. After stepwise growth factor treatment, cells were analyzed with immunofluorescence staining, flow cytometry, and qPCR. Human primary hepatocytes and HepaRG cells were used as controls.

Results. DE cells expressed CXCR-4 and HNF3B both on mRNA and protein level. On day 10 cells were positive for liver progenitor markers AFP and CK19 and for HNF4A which controls the initiation of expression of several key hepatic transcription factors. On the end-point, day 17, cells showed hepatocyte-like morphology and expressed hepatic markers AAT and ALB in mRNA level but the expression level was remarkably lower compared to primary hepatocytes.

Conclusions. HepaRG cell-derived ACM supports hepatic differentiation of hES and iPS cells. However, differentiation needs to be further optimized to obtain mature hepatocytes which could be later used in drug studies.

S33 Improved Liver Function and Relieved Pruritus after 4-Phenylbutyrate Therapy in a Patient with Progressive Familial Intrahepatic Cholestasis Type 2

Sotaro Naoi[a], Hisamitsu Hayashi[a], Takeshi Inoue[b], Ken Tanikawa[c], Koji Igarashi[d], Hironori Nagasaka[e], Masayoshi Kage[c], Hajime Takikawa[f], Ayano Inui[g], Toshiro Nagai[b], Hiroyuki Kusuhara[a]

[a]The University of Tokyo, Japan,
[b]Dokkyo Medical University, Japan,
[c]Kurume University Hospital, Japan,
[d]TOSOH Corporation, Japan,
[e]Takarazuka City Hospital, Japan,
[f]Teikyo University School of Medicine, Japan

Objective. Progressive familial intrahepatic cholestasis type 2 (PFIC2) is caused by mutations in *ABCB11* encoding the bile salt export pump (BSEP) that mediates biliary excretion of bile salts across the canalicular membrane (CM) of hepatocytes. Currently, no medical therapy has been established for PFIC2. Previously, we found that 4-phenylbutyrate (4PB) was a potential therapeutic agent for PFIC2 patients who retain transport activity of BSEP. Here, we examined the effects of 4PB therapy in a patient with PFIC2.

Methods. All encoding exons and flanking areas of *ABCB11* were sequenced. *In vitro* studies with HEK293T cells, McA-RH7777 cells, and liver specimens were performed to characterize the influence of the identified mutation on BSEP and to examine the potential of 4PB treatment in the PFIC2 patient. 4PB was administered orally with gradually increasing dosage (200, 350, and 500 mg/kg/day) for 6 months, and biochemical, histological, and clinical data were collected.

Results. A homozygous c.3692G>A (p.R1231Q) mutation was identified in *ABCB11*. *In vitro* studies showed that this mutation decreased the cell surface expression level of BSEP, but not its transport activity, and that 4PB treatment at a clinically relevant dosage relieved the decreased expression. In the PFIC2 patient, 4PB therapy at a dosage of 500 mg/kg/day partially restored BSEP expression and significantly improved liver tests and pruritus.

Conclusions. 4PB therapy may be a new medication for PFIC2 patients who retain transport activity of BSEP per se.

S34 Constitutive Androstane Receptor Modulates Hepatic Energy Homeostasis with Species Selectivity

Caitlin Lynch[a], Yongmei Pan[a], Linhao Li[a], Peter W. Swaan[a], Hongbing Wang[a]

[a]University of Maryland, Baltimore, USA

Objective. The constitutive androstane receptor (CAR, NR1I3), predominantly expressed in the liver, is an important xenobiotic receptor. Recent studies revealed that CAR also influences hepatic energy metabolism and improves diabetes and obesity conditions in mice. Thus, identification of novel CAR activators may offer a potential therapeutic avenue for metabolic disorders.

Methods. Strategies incorporating computational-based virtual screening with biological approaches were employed to identify and validate novel human (h) CAR modulators. Luciferase reporter assays were performed by transfecting various hCAR expression and CYP2B6 reporter plasmids into HepG2 cells. Human primary hepatocytes were utilized to explore the effects of CAR activation on target gene expression and hepatic energy homeostasis.

Results. Two novel hCAR activators, UM104 and UM145, identified through virtual screening were subjected to biological evaluation of their role in energy metabolism. In human primary hepatocytes, the mRNA and protein expression of gluconeogenic enzymes, glucose-6-phosphatase and phosphoenolpyruvate carboxykinase were concentration- dependently repressed by UM104 and UM145. However, only negligible effects on genes associated with fatty acid synthesis and lipogenesis were observed. Further functional assays revealed that hCAR activation decreased the synthesis of glucose but not triglyceride in human hepatocytes. In contrast, activation of mouse (m) CAR significantly repressed the expression of typical genes associated with gluconeogenesis, lipogenesis and fatty acid synthesis in mouse primary hepatocytes, correlating well with previous *in vivo* observations in mouse.

Conclusions. This research identified an important species difference between hCAR and mCAR in energy metabolism. Unlike that of mCAR, activation of hCAR selectively inhibits gluconeogenesis without simultaneous suppressing fatty acid synthesis and lipogenesis. These findings warrant species specific caution when exploring CAR as a potential therapeutic target for metabolic disorders.

S35 The Effect of Route of Administration on the Immunogenicity of Recombinant Murine Growth Hormone Protein Aggregates

Merry Christie[a], Raul Torres[b], Ross Kedl[b], Theodore Randolph[c], and John Carpenter[a]

[a]University of Colorado Denver, USA
[b]National Jewish Health and the University of Colorado School of Medicine, USA
[c]University of Colorado, USA

Objective. The aim of the work was to determine the effect of the route of administration on the immunogenicity to recombinant murine growth hormone (rmGH) protein aggregates.

Methods. We created protein aggregates by subjecting rmGH to freeze-thaw stress and quantified the nano- and micro-sized particles via SEC, particle tracking analysis, resonant mass measurement and flow imaging. We also created a population of nano-sized particles by ultra-centrifuging the freeze-thawed protein at 110,000g for 1 hour at 4°C. Protein aggregates were injected on days 2 and 23 into CB6F1 mice via subcutaneous, intraperitoneal and intravenous (via tail vein) routes. Submandibular blood draws were performed on days 1, 22 and 36. The production of anti-rmGH IgG antibody isotypes was monitored with ELISA to determine immunogenicity.

Results. Compared to the ultra-centrifuged protein, the freeze-thaw protein injections contained much higher quantities of micro-sized protein particles. No major difference in immunogenicity could be ascertained between the freeze-thaw stressed and ultra-centrifuged rmGH protein injections. However, the intravenous route was the most immunogenic of the three tested; which is surprising considering the current popular viewpoint in vaccine immunology dictates the subcutaneous route to be more immunogenic than intravenous.

Conclusions. Both nano- and micro-sized protein aggregates have the capacity to elicit and immune response. The route of administration is a major factor impacting the immunogenicity of protein aggregates. Based on the results of this study, protein aggregates injected via the intravenous route elicit a more pronounced immune response compared to the subcutaneous and intraperitoneal routes, contrary to the prevalent beliefs on this issue.

S36 Parathyroid Hormone coupled to Cell Penetrating Peptides for Oral delivery

Mie Kristensen[a], Jens Berthelsen[b], Hanne Mørck Nielsen[a]

[a]Section for Biologics, Department of Pharmacy , Faculty of Health and Medicinal Sciences, University of Copenhagen, Universitetsparken 2, DK-2100 Copenhagen O, Denmark
[b]Department of International Health, Immunology and Microbiology, Faculty of Health and Medical Sciences, University of Copenhagen, Blegdamsvej 3, DK-2200 Copenhagen N, Denmark

Objective. Oral delivery of peptide drugs constitutes a number of challenges including poor membrane permeability. To improve this, the use of cell penetrating peptides (CPPs) is of great interest as they have shown potential in improving the transepithelial transport of therapeutic peptides upon co-administration or direct conjugation. Knowledge is however lacking regarding the effect on the cell penetrating propensity of the CPP, for which an α-helical content is important, as a result of direct conjugation.

Hence, the objective is to produce fusion-peptides comprising the biologically active part of parathyroid hormone (PTH(1-34)) coupled to different CPPs and characterize these according to secondary structure and ability to permeate an intestinal epithelium *in vitro*.

Methods. PTH(1-34)-CPP fusion-peptides were expressed in *E. coli* as inclusion bodies, solubilized and purified by affinity chromatography and RP-HPLC. The secondary structure was studied using circular dichroism (CD) spectroscopy and the delivery propensity was assessed employing the intestinal Caco-2 cell line.

Results. PTH(1-34) and different N- or C-terminally CPP-coupled PTH(1-34) were successfully produced. CD spectra revealed a disordered secondary structure in buffer, but in the presence of liposomes the α-helical content increased in some but not all fusion-peptides. The specific N- or C-terminal positioning of the CPP was shown to be important for the transepithelial permeability, with N-terminal CPP-coupling resulting in significantly improved transport when compared to C-terminally CPP-coupled PTH(1-34). However, only one of the fusion-peptides was able increase the permeability over Caco-2 cell monolayers when compared to PTH(1-34) administered alone or co-administered with the CPPs.

Conclusions. Direct coupling of a CPP to a therapeutic peptide was investigated with respect to overall secondary structure and permeation across an intestinal epithelium, showing that both the α-helical content and the transepithelial permeability were dependent on the specific CPP sequence and the specific N- or C-terminal CPP coupling.

S37 Soluble Antigen Arrays (SAgAs) Mitigate Experimental Autoimmune Encephalomyelitis (EAE)

Shara Thati[a], Joshua Sestak[a], Brittany Hartwell[b], Laura Northrup[a], Brad Sullivan[a], Laird Forrest[a], Teruna Siahaan[a], Cory Berkland[a,b,c]

[a]Department of Pharmaceutical Chemistry, University of Kansas
[b]Department of Bioengineering, University of Kansas
[c]Department of Chemical and Petroleum Engineering, University of Kansas

Objective. The aim is to monitor the transport of SAgAs varying in size and peptide loading to help in identifying the ideal route of administration, dosing schedule, dosing amount, and dosing volume.

Methods. The SAgAs were synthesized using oxime chemistry by conjugating peptides to hyaluronic acid (HA). SAgAs were purified by dialysis and then lyophilized. Peptide concentration on HA and relative molecular weight was determined by HPLC and SEC, respectively. *In vivo* efficacy was evaluated in the EAE mouse model by monitoring animal weight and clinical score. According to R. O. Weller, et al., MS is initially thought to be a systemic disease, but in later stages, is localized in the lymph nodes in the superior half of the body. In order to test this, the first study involved finding different routes of administration of SAgAs, including subcutaneous, intramuscular, and intraperitoneal injections to mice with EAE. Dosing schedules, volumes, and amounts were also varied to find an efficacious treatment method.

Results. Different routes of administration and dosing schedules showed little to no difference in disease outcomes. Results suggested a single subcutaneous dose approaches the clinical efficacy of three doses (200 nMol PLP). Decreasing the injection volume from 100 uL to 20 uL slightly reduced efficacy.

Conclusions. Varying the injection site does not seem to affect clinical scores, since the large injection volume will disperse easily throughout a mouse's body. Changing the volume slightly affected efficacy, but the drainage kinetics should be further studied. Since hyaluronic acid can be varied in size, SAgA can be designed to drain to local lymph nodes after a subcutaneous injection. The next steps will be to correlate the size of the SAgA, densities of peptides, and ratio of the two peptides (LABL:PLP) to compare local (i.e. lymph node) versus systemic administration (i.e. absorption into blood).

S38 Liquid Crystalline Nanodispersion as a Topical Delivery System for siRNA: Development, Characterization and *In Vivo* Knockdown Study

Lívia Vieira Depieri[a], Lívia N. Borgheti-Cardoso[a], Fabiana T. M. C. Vicentini[a], Márcia C. A.Fantini[b], M. Vitória L. B. Bentley[a]

[a]School of Pharmaceutical Sciences of Ribeirao Preto - University of Sao Paulo, Brazil
[b]Institute of Physics - University of Sao Paulo, Brazil

Objective. The goal of this study was the development, characterization and *in vivo* TNF-α silencing evaluation of liquid crystalline nanodispersion (LCN) carrying siRNA, aiming to introduce gene therapy as a new approach for the treatment of skin disorders.

Methods. LCN was developed using sonication to disperse the bulk gel phase composed of monoolein/oleic acid/polyethyleneimine, in excess water, containing Poloxamer. It was characterized by polarized light microscopy and small angle x-ray diffraction; mean diameter (z), polydispersity (Pdl) and zeta potential were measured by dynamic light scattering; LCN cytotoxicity was evaluated in mouse fibroblast (L929) cell lines by the MTT assay; complexing efficiency was evaluated by electrophoresis; cellular uptake was assessed by flow cytometry and fluorescence microscopy. *In vivo* TNF-α knockdown was evaluated in hairless mouse skin inflammation model, induced with TPA, through TNF-α measurements by ELISA, after topical application of LCN carrying TNF-α siRNA.

Results. LCN was characterized as hexagonal phase and presented z, PdI and zeta potential of 202.9 (± 5.0) nm; 0.261 (± 0.018); 1.2 ± 0.7 mV and 215.4 (± 7.9) nm; 0.273 (± 0.021); 0.7 ± 1.0 mV with and without siRNA at 10 μM, respectively. LNC was effective in complex the siRNA and presented high cell viability (~92%) and cell uptake (~90%). TPA induced a significant increase in TNF-α levels in skin mouse; however, pre-treatment with the LCN-siRNA TNF-α (10μM), significantly reduced TNF-α levels (~80%) compared with control groups.

Conclusions. LCN is a promising siRNA delivery system for topical application since presented reduced particle size, it was able to complex the siRNA, presented high cell viability and cellularuptake and it was able to promote TNF-α knockdown *in vivo.*

Acknowledgements. FAPESP and CNPq, Brazil.

S39 Pharmacological Modulation of Intratesticular Retinoic Acid Concentration and its Therapeutic Potential

Samuel Arnold[a], Travis Kent[b], Michael Griswold[b], Cathryn Hogarth[b], Nina Isoherranen[a]

[a]University of Washington, School of Pharmacy, USA
[b]Washington State University, School of Molecular Biosciences, USA

Objective. In mice, spermatogenesis requires intratesticular formation of retinoic acid (RA) by ALDH1A (1A1-1A3). Inhibition of ALDH1A by WIN 18,446 causes male infertility. The aim of the work was to determine the effect of WIN 18,446 on mouse tissue RA concentrations.

Methods. The inhibition of ALDH1A by WIN 18,446 was determined *in vitro*. Next, mice were treated with either single dose or multiple daily doses (7 days) of WIN 18,446. WIN 18,446 pharmacokinetics, RA concentrations, ALDH1A activity, and ALDH1A protein expression were quantified in testis, liver, and kidney as a function of time after WIN administration.

Results. *In vitro*, WIN 18,446 is a potent reversible inhibitor of ALDH1A1 and ALDH1A3 (IC_{50}= 102 and 187 nM) and it inactivates ALDH1A2 (k_{inact}= 27.8 hr^{-1} K_I=1,468 nM). After a single oral dose to mice, WIN 18,446 had a t_{max} of 1 hr, C_{max} of 1,200 nM, and $t_{1/2}$ of 3 hr. The tissue RA concentrations decreased > 60% following this dose of WIN 18,446. While liver and kidney RA concentrations returned to predose levels after 24 hours, testis and serum concentrations were still 75% lower than control. 24 hours after the 7th dose, the liver and kidney RA concentrations were similar to control mice, but intratesticular and serum RA were both decreased 50% demonstrating tissue specific inhibition of RA synthesis. ALDH1A activity was decreased (25% of control) in the testis, but not in the liver and kidney after WIN 18,446 treatment. The ALDH1A activity is in agreement with ALDH1A2 localization in the testes and inactivation by WIN 18,446.

Conclusions. WIN 18,446 specifically decreases ALDH1A activity and RA in the testis providing a unique strategy for male contraception.

SHORT COURSE ABSTRACTS

GPEN 2014

S40 Investigation of the Aqueous- and Solid-State Stability of Oxytocin as a Function of pH

Gemma C. Nassta[a], Michelle P. McIntosh[a], David A.V. Morton[a], Richard J. Prankerd[a]

[a]Monash Institute of Pharmaceutical Sciences, Monash University, Australia

Objective. Peptides and proteins containing disulphide bonds have been shown to undergo degradation via two consecutive mechanistic steps: (i) β-elimination, catalysed by hydroxide ions, followed by (ii) disulphide exchange. As the disulphide bond is cleaved, generation of free thiols is hypothesised to result.

As the degradation mechanisms of the disulphide bond-containing peptide, oxytocin, are currently not fully understood, the aim of this work was to first compare oxytocin degradation rates in aqueous solution at varying pH conditions, and between solid and aqueous states at a single pH, with heat stress. The second aim was to probe oxytocin degradation mechanisms by quantifying free thiols and identifying the resulting degradation products.

Methods. Solutions of oxytocin, buffered to pH 2, 4 and 8 and a spray-dried powder formulation, buffered to pH 8 prior to drying, were stored at 4, 40 and 50°C. Free thiol concentrations were measured periodically with Ellman's spectrophotometric assay; the remaining oxytocin concentrations were determined by HPLC; and degradation products were identified by mass spectrometry.

Results. For solution pH values either side of pH 4, oxytocin degraded faster – the fastest degradation rate being observed at pH 8. Free thiols were not detected at pH 2 during the course of the experiment and the identified degradation products included various deamidation products. At pH 8, a maximum of 14.6% free thiols (referred to oxytocin concentration) was generated by 24 days into the stability study, suggesting that β-elimination was initiated, subsequently producing the identified dimers and trisulphides as degradation products. The solid state degradation rate was 40 fold lower than that of the aqueous solution.

Conclusions. The results suggest that oxytocin solution degradation at basic pH undergoes β-elimination, followed by disulphide exchange, whereas degradation in acidic pH involves acid-catalysed deamidation. The degradation rate was significantly reduced when oxytocin was formulated as a solid product compared to aqueous solutions, due to the vast reduction of moisture content post spray drying.

S41 Calculating the Mass of Subvisible Particles with Improved Accuracy as Formed from Stressed IgG1 Monoclonal Antibody Solutions Using Microflow Imaging Data

Cavan Kalonia[a], Ozan Kumru[a], Roman Mathaes[b], Julia Engert[b], Shuxia Zhou[c], C. Russell Middaugh[a], David B. Volkin[a]

[a]Department of Pharmaceutical Chemistry, Macromolecule and Vaccine Stabilization Center, University of Kansas, Lawrence, KS, USA, 66047
[b]Department of Pharmacy, Pharmaceutical Technology & Biopharmaceutics, Ludwig-Maximillians-University Munich, Butenandstr. 5-13, D-81377 Munich, Germany
[c]Janssen Research & Development, LLC, Horsham, PA, USA, 19044

Objective. One major stability concern with protein therapeutics is the formation of proteinaceous subvisible particles (1-100 μm) during manufacturing and/or storage. Our objective was to develop a methodology to determine the mass of individual subvisible particles in solution and the total weight of these particles in a given volume of a formulation.

Methods. Microflow digital imaging (MFI) was used to monitor subvisible particles in solution under various conditions. Two different methodologies were developed to calculate particle mass using size, morphology, and transparency data obtained by MFI. These methodologies were tested using spherical and non-spherical polystyrene standards and then applied to subvisible particles of an IgG1. The total calculated mass of the proteinaceous particles was compared to the experimentally measured mass of particles in more extensively aggregated samples.

Results. The calculated mass using the methods developed showed good agreement with the known values for the polystyrene standards. For the IgG1 mAb samples, calculated results were also comparable to the experimentally measured mass of extensively aggregated samples. Using the same MFI datasets, we demonstrate that particle mass calculations using the assumptions of spherical geometry or particle density similar to monomeric protein density can be inaccurate. The calculated particle mass obtained by applying these assumptions to the extensively aggregated IgG1 mAb datasets exceeded the experimentally measured mass by an order of magnitude.

Conclusions. We demonstrate that the total mass of protein particles was not only better estimated using morphology parameters based on actual MFI data (i.e., non-spherical particles of varying size and shape) but also by assuming a particle density less than the density of monomeric protein.

S42 Role of Carrier Mediated Efflux of Nobilin Conjugation Products and Plant Extract of *Anthemis nobilis* L. in Nobilin Absorption in the Caco-2 Model

Ursula Thormann[a,b], Rahel Hänggi[a], Georgios Imanidis[a,b]

[a]Department of Pharmaceutical Sciences, University of Basel, Basel, Switzerland
[b]Institute of Pharma Technology, School of Life Sciences, University of Applied Sciences and Arts Northwestern Switzerland, Muttenz, Switzerland

Objective. The aim of the study was to investigate the role of bioconversion and carrier mediated efflux of conjugation products in the intestinal *in vitro* absorption of nobilin in the Caco-2 model and to elucidate the effect of the full plant extract of the flowers of *Anthemis nobilis* L. on bioconversion and efflux.

Methods. Nobilin and its bioconversion products were determined in permeation experiments in the Caco-2 cell monolayer. Inhibitors of P-gp, BCRP, and MRPs were used to detect efflux and its association to bioconversion. The effect of the extract on those processes was determined. Permeation and bioconversion parameter values were deduced by means of kinetic multi-compartment modeling and the relative fraction absorbed was calculated.

Results. Nobilin exhibited high permeability, low absorption, and fast bioconversion yielding conjugation products with glucuronic acid, cysteine, and glutathione that were substrates of apical MRP2 and basal MRP3 and possibly MRP1and, the glucuronide and cysteine conjugate, also of P-gp. Inhibition of carriers led to diminished bioconversion and improved absorption of nobilin. The extract increased absorption mainly by directly inhibiting bioconversion but also by reducing efflux.

Conclusions. The transport - bioconversion interplay is proposed to be a good possibility to increase absorption of a compound undergoing extensive intestinal bioconversion. Plant extracts may increase absorption by this interplay in addition to inhibition of metabolic enzymes.

Acknowledgement. This work was sponsored by Alpinia Laudanum Institute of Phytopharmaceutical Sciences AG.

S43 Selection of Highly Stable Aptamers from a 2'-Fully Modified RNA Library

Adam D. Friedman[a,b], Dongwook Kim[a,b], and Rihe Liu[a,b]

[a]Division of Chemical Biology and Medicinal Chemistry, UNC Eshelman School of Pharmacy, University of North Carolina, Chapel Hill, North Carolina 27599-7568, U.S.A.
[b]Carolina Center for Genome Sciences, University of North Carolina, Chapel Hill, North Carolina 27599-7264, U.S.A.

Objective. The aim of this study was to investigate the enhanced nuclease stability of 2' fully-modified (fm) RNA aptamers, perform a proof-of-concept fmRNA aptamer selection against *Staphylococcus aureus* Protein A (SpA), and demonstrate fmRNA aptamer biological relevance by specifically killing *S. aureus* cells via fmRNA aptamer-targeted silver nanoparticle (AgNP) delivery.

Methods. SpA-binding fmRNA aptamers were identified via *in vitro* selection against Protein A immobilized on magnetic sepharose beads. Confocal microscopy visualized aptamer binding to endogenous SpA on *S. aureus* cell surfaces. AgNP-aptamer functionalization was achieved via docking of aptamers to a capture ligand, itself functionalized with a dithiocarbamate for covalent immobilization on AgNP surfaces. Microbiological techniques were used for determination of specific *S. aureus* cell killing. fmRNA was characterized for nuclease stability via alkaline hydrolysis and serum incubation studies.

Results. Selected fmRNA aptamers, featuring an unexpected hydrophobicity, bound SpA with KDs ranging from 39 nM to 475 nM. Aptamers demonstrated SpA selectivity versus *S.aureus* Protein G (SasG) and *P. magnus* Protein L (PpL). One of these aptamers, fmA12, was found capable of binding endogenously expressed *S. aureus* SpA. fmA12 was able to functionalize, stabilize, and deliver aggregation-prone silver nanoparticles (AgNPs) to *S.aureus* with SpA-dependent antimicrobial effects. fmRNA exhibited superior stability in alkaline conditions (99.25% survival) and 10% mouse serum (76% survival after 24 h) compared to 2'-partially modified RNA variants.

Conclusions. fmRNA represents a novel aptamer class with considerable potential to improve the *in vivo* applicability of nucleic acid-based affinity molecules.

S44 Optimization of DNA Nanostructure for Delivery of Nucleic Acid Drugs to Immune Cells

Shozo Ohtsuki[a], Makiya Nishikawa[a], Noriyuki Matsuzaki[a], Kohta Mohri[a], Masayuki Endo[b], Kumi Hidaka[b], Hiroshi Sugiyama[b,c], Yuki Takahashi[a] and Yoshinobu Takakura[a]

[a]Graduate School of Pharmaceutical Sciences, Kyoto University, Japan
[b]Institute for Integrated Cell-Material Sciences (iCeMS), Kyoto University, Japan
[c]Graduate School of Science, Kyoto University, Japan

Objective. Previous studies have shown that several DNA nanostructures, including polypod-like structured DNA and tetrahedral DNA (tetrahedron), are effective systems to deliver immunostimulatory CpG DNA to immune cells. In the present study, we aimed to elucidate the optimal structure of nano-sized DNA assembly for such delivery.

Methods. Three different nano-sized DNA assemblies, *i.e.*, tetrapod-like structured DNA (tetrapodna), tetrahedron and tetragonal DNA (tetragon), were designed using four 55-mer oligodeoxynucleotides (ODNs) and compared in terms of physicochemical property and interaction with immune cells. The formation of DNA nanostructures were evaluated by electrophoresis and atomic force microscopy (AFM). The thermal stability was evaluated by measuring the melting temperature (Tm). Then, the uptake by mouse macrophage-like RAW264.7 cells was examined using DNA nanostructures prepared with Alexa Fluor 488-labeled ODN. An immunostimulatory CpG DNA was linked to these DNA nanostructures. The immunostimulatory activity of CpG DNA-containing DNA nanostructures was examined in RAW264.7 cells by measuring the release of tumor necrosis factor (TNF)-α.

Results. Tetrapodna was obtained with high efficiency and purity at a wide range of ODN concentrations, whereas tetrahedron formed oligomers at high ODN concentrations. The Tm value of tetrapodna was higher than those of tetrahedron and tetragon, indicating that tetrapodna is more thermally stable than the others. Circular dichroism spectral data of all the preparations were similar to one another and comparable to that of B-form DNA. Tetrahedron was most efficiently taken up by RAW264.7 cells compared with the other two. CpG DNA tetrahedron induced the largest amounts of TNF-α, followed by CpG DNA tetrapodna.

Conclusions. These results indicate that tetrapodna is the best assembly as delivery vehicles for immunostimulatory nucleic acid to immune cells, because it has the highest preparation yield and high delivery efficiency, and that tetrahedron can be another useful vehicle if its preparation yield is improved.

S45 Brain Drug Distribution of a Cassette of Compounds in Alpha-Synuclein Transgenic Mice

Sofia Gustafsson[a], Veronica Lindström[b], Stina Syvänen[b], Martin Ingelsson[b], Margareta Hammarlund-Udenaes[a]

[a]Department of Pharmaceutical Biosciences, Sweden
[b]Department of Public Health / Geriatrics, Sweden

Objective. Pathophysiological impairment of the neurovascular unit, including the blood-brain barrier (BBB), is considered both a cause and consequence of neurodegenerative diseases. Considerable research is dedicated to delineate whether and how these changes occur and affect disease. However, little is known about the impact this might have on systemic drug delivery to the brain. Hence, the aim of the current study was to investigate BBB drug transport in healthy animals and transgenic animals overexpressing human α-synuclein.

Methods. In an *in vivo* study, wild type C57BL/6 (WT) and (Thy-1)-h [A30P] α-synuclein transgenic mice were dosed with a cassette of compounds, including digoxin, levofloxacin, paliperidone, oxycodone, and diazepam (1mg/kg, s.c.). The animals were terminated at 0.5h ($n_{transgen}$= 4, n_{WT} = 3), 1h ($n_{transgen}$= 5, n_{WT}= 3), or 3h ($n_{transgen}$= 5, n_{WT}= 3) after dosage. Blood was collected through cardiocentesis and brains were collected following PBS perfusion. In a second pilot study, brain tissue binding of the cassette compounds in transgenic (n=6) and WT (n=6) mice was investigated using equilibrium dialysis (ED). Drug concentrations were measured in plasma, brain, and ED brain and buffer samples using LC-MS/MS. The total brain-to-plasma concentration ratio (K_p) and fraction of unbound drug in brain ($f_{u,brain}$) was calculated.

Results. No differences in K_p were observed for any of the compounds when comparing healthy and transgenic mice. However, an increased binding in brain was observed for levofloxacin, paliperidone and digoxin in transgenic compared to WT animals.

Conclusions. Given an unchanged K_p in combination with increased brain tissue binding of three of the investigated compounds, this study indicates changes in BBB drug transport in an animal model expressing α-synuclein pathology compared to WT animals. However, further studies on drug brain and plasma binding is required in order to fully evaluate possible changes in brain drug delivery in the current model.

S46 Analysis of Secretory Transport of Drugs in the Small Intestine Using the Ussing Chamber Method

Takeshi Nakayama[a], Shinya Fukizawa[a], Kazuya Maeda[a], Takehito Ohtsubo[b], Toshio Kumai[b], Naoki Matsumoto[b], Paul A. Dawson[c], Yuichi Sugiyama[d], Hiroyuki Kusuhara[a].

[a]The University of Tokyo, Japan
[b]St. Marianna University School of Medicine, Japan
[c]Wake Forest University School of Medicine, USA
[d]RIKEN Innovation Center, Japan

Objective. Recent reports suggest that the excretion from the blood into the intestinal lumen contributes partly to the clearance of some drugs, but the underlying mechanisms have not been clarified. The aim of this research was to investigate the contribution of transporters to the secretory transport of drugs in the small intestine using the Ussing chamber method.

Methods. The secretory (basal-to-apical) transport of several drugs was measured in tissue sections from duodenum, jejunum, and ileum of mice in an Ussing chamber. The possible involvement of transporters in the secretory transport was assessed by observing the inhibitory effects of several compounds on their transport. The contribution of P-gp, Bcrp, and Ostα/β to intestinal secretion of drugs was investigated in ileum sections obtained from the corresponding knockout mice.

Results. The secretory transport of several drugs in the Ussing chamber was much higher in the ileum sections compared with the duodenum and jejunum sections. Their secretory transport in the ileum section was lower in Mdr1a/1b(−/−)/Bcrp(−/−) mice than that in wild-type mice. Several compounds (e.g., 200 μM fluvastatin, 100 μM apixaban) inhibited the secretory transport and drug accumulation in the ileum sections, suggesting the involvement of basal uptake transporter(s). By contrast, although Ostα/β is expressed predominantly on the basal side of the ileum along the small intestine, the secretory transport of several drugs in the ileum section was not decreased by knockout of Ostα.

Conclusions. The secretory transport of several drugs was almost selective in the ileum, implying that the ileum-specific expression of transporters contributes to the intestinal secretion. In secretory transport, P-gp and Bcrp contribute to the efflux from intestinal epithelial cells, whereas Ostα/β is not involved in the basal uptake of drugs. Clarification of the molecular mechanism underlying the basal transport is under way.

S47 The Effect of Liposomal Components on Pro-Survival Signals within Normal Prostate Cells

Ryan C. Bates[a], Jamie L. Betker[a], Thomas J. Anchordoquy[a]

[a]Skaggs School of Pharmacy and Pharmaceutical Sciences, University of Colorado Denver | Anschutz Medical Campus, USA

Objective. The aim of this work was to investigate the exposure of normal prostate cells (WPMY-1)to phosphatidylcholine (PC) and phosphatidic acid (PA) liposomes by assaying for their impact on pro-survival phospho-proteins (i.e. Akt-Ser473, Erk1/2-Thr202/Tyr204, GSK3β-Ser9). The ability to characterize differential phospho-protein profiles elicited by these bioactive lipids allows for liposome/nanoparticle design to be tailored based on delivery intent. Pro-survival drugs could be more effective if the delivery vector itself did not induce an attenuation of the pro-survival response. In the case of cancers, choosing among the most attenuating lipids could enhance a chemotherapeutic effect.

Methods. Phospho-proteins and endosomal trafficking intensities were collected by imaging with the LI-COR Odyssey dual-laser infrared imaging system or PerkinElmer Operetta high content imaging system.

Results. While bioactive lipids, such as PA, display an increase in pro-survival factors (i.e. Akt-Ser473), results with exogenously administered PC liposomes suggest this membrane component causes a significant decrease within pro-survival phospho-forms for the cell line tested within this study.

Conclusions. The exposure of WPMY-1 cells to a series of PC liposome concentrations suggest that while PC may constitute a large compositional percent of the plasma membrane, using it in high concentrations within liposomal/nanoparticle delivery may be depressing pro-survival factors. In contrast, PA liposomes were found capable of producing an increase in the pro-survival profile. This kind of information could have a dual-impact on designing therapeutic agents administered via liposomal technology: (1) Targeting high-content PC liposomes to tumor cells could aid in sensitizing to chemotherapeutic agents, (2) Targeting low/no-content PC liposomes with a pro-survival payload to non-cancerous tissues could prevent a potentially counteracting event (i.e. utilizing high PA content).

S48 Targeted Liposomal Ultrasound Contrast Agents

Eric Sasko[a]

[a]Institut for Pharmaceutical Technology & Biopharmacy (IPTB), University of Marburg, Germany

Objective. In this study we developed and analyzed nanoscaled liposomal ultrasound contrast agents which have stealth abilities. For model studies we attached Concanavalin A (ConA) to the Liposomes to give the ability to bind on mannan-coated surfaces.

Methods. The liposomes are composed of 1,2-Dipalmitoyl-sn-Glycero-3-Phosphocholin (DPPC), 1,2-dipalmitoyl-*sn*-glycero-3-phosphoethanolamine-N-(cyanur) and Cholesterol, prepared by lipid film hydration method. ConA attachment was made via cyanuric chloride linkage at pH 8.8. Fluorescence was obtained by the use of 1% (mol) 1-Palmitoyl-2-[6-[7-nitro-2-1,3-benzoxadiazol-4-yl)amino]hexanoyl]-sn-Glycero-3-Phosphoethanolamine. Size and zetapotential measurements were done by photon correlation spectroscopy (PCS) using a Malvern Nanosizer. Echogenicity was measured in an *in vitro* model composed of an agar-gel body with a tube in the middle to simulate a human blood vessel. By using a pump and a waterbath we created a constant current flow at 37°C with a pressure similar to the pressure to be found in the human blood system. A 2.5 MHz phased array ultrasonic probe and a sonographic unit were used to obtain pictures and videos of the model in greyscale. Mannan was coated on glass slides.

Results. Liposome size ranged from 70-130 nm with a zeta potential of -29 mV. The Liposomes show good echogenicity and long term stability under simple storage conditions (dispersed in water or buffer at 4°C). Fluorescence-marked Liposomes linked to ConA were able to attach to mannan-coated surfaces.

Conclusions. The possibility of attaching antibodies to stealth-liposomes that show good ultrasonic contrast offers a vast variety of new ways of diagnostic imaging. You could attach antibodies against specific cancer surface proteins or for example arteriosclerotic plaques.

S49 Improvement in Blood-Brain Barrier Tightness through a Direct Contact hCMEC/D3 and Human Astrocyte Coculture Model

Chris Kulczar[a], Sylvia Lefebvre[a], Donald Miller[b], Gregory Knipp[a]

[a]Department of Industrial and Physical Pharmacy, Purdue University, West Lafayette, IN
[b]Department of Pharmacology and Therapeutics, University of Manitoba, Winnipeg, CAN

Objective. As the number of NCEs targeted to the CNS increases, a better *in vitro* blood-brain barrier model is needed for more accurate high-throughput screening. Currently, much of the screening is performed across several different brain microvessel endothelial cell (BMEC) line monolayers which, while differing in tightness, are leaky in comparison to *in vivo* conditions. However, investigation of the neurovascular unit shows that BMEC direct contact with astrocytes may play an important role in forming a tighter blood-brain barrier (BBB). Here we look to determine if direct cell-cell contact improves BBB characteristics utilizing hCMEC/D3 cell co-culture on a monolayer of human astrocytes.

Methods. Human Astrocytes (ScienCell, Carlsbad, CA) were plated on poly-L-lysine coated Transwell inserts. Two days post seeding, hCMEC/D3 cells (courtesy of Dr. Couraud, Institut Cochin, Paris, France) were plated in direct contact above the human astrocytes. Transport studies were conducted seven days post hCMEC/D3 seeding. C^{14}-labelled paracellular markers of various sizes, such as Urea, Mannitol, Inulin, and PEG-5000, were used to determine leakiness of tight junctions.

Results. When co-culturing the hCMEC/D3 on human astrocyte monolayers, a significant decrease in apparent permeability of paracellular markers was observed independent of culture conditions comparative to either human astrocytes or hCMEC/D3 cells alone. In addition, transcellular routes of permeation including influx and efflux were unaffected.

Conclusions. The study indicates that direct contact between hCMEC/D3 cells and human astrocytes may serve as a more physiologically-relevant *in vitro* cell culture model for future high-throughput screening of CNS-targeted molecules.

S50 All Hepatocytes Are Not Equal: Characterization of Variability in Cryopreserved Human Hepatocytes

Magnus Ölander[a], Andrea Treyer[a], Fabienne Gaugaz[a], Erica Wong[a], Per Artursson[a]

[a]Department of Pharmacy, Uppsala University, Sweden

Objective. Primary human hepatocytes are widely used for studying hepatic metabolism and toxicity *in vitro*. Cryopreservation is a useful tool for alleviating the problem of fresh liver tissue scarcity. Unfortunately, hepatocyte quality after thawing is unpredictable, and a fraction of the cell batches do not adhere to collagen-coated surfaces, limiting their use. The objective of this study is to identify parameters that explain this batch-to-batch variability.

Methods. From our bank of 40 cryopreserved hepatocyte batches, 14 were selected, representative of plateable and non-plateable cells, respectively. Viability after thawing, along with mechanism of cell death (apoptosis or necrosis) and cell size distribution, is determined with image cytometry. Plateability is assessed by the degree of confluency after culturing cells on collagen-coated plates. In addition, functional analysis of important CYP450 enzymes and drug transporters is ongoing. Finally, global proteomic analysis and determination of the fraction of contaminating non-parenchymal cells via flow cytometry has been initiated.

Results. Hepatocyte isolations resulted in viable cell yields averaging 14×10^6 cells/g of tissue, with no statistically significant difference between plateable and non-plateable batches. Preliminary data indicates no significant difference in viability between the two groups (85% and 92%, respectively), but more detailed elucidation of cell death, as well as analysis of the presence of non-parenchymal cells, is in progress. Proteomic analysis and functional studies of drug-metabolizing enzymes and transporters are ongoing and will be presented.

Conclusions. Cryopreserved human hepatocyte batches show marked variability in plateability, which cannot be explained by differences in yield from isolation or cell viability. Therefore, deeper analysis of cell health and function is necessary, to shed more light on the underlying reasons for this unpredictable behavior.

POSTER ABSTRACTS

GPEN 2014

P1 Folic Acid Functionalized Insulin Loaded Stable Chitosan Nanoparticles: Influence on Stability, Cellular Uptake, Pharmacodynamics and Pharmacokinetics

Ashish Agrawal[a], and Sanyog Jain[a]

[a]Centre for Pharmaceutical Nanotechnology, Dept. of Pharmaceutics, National Institute of Pharmaceutical Education and Research (NIPER), SAS Nagar, Punjab, 160062, India

Objective. The aim of the present work was to develop and characterize folic acid functionalized stable chitosan nanoparticles (FA-Ch-NPs) for oral insulin delivery.

Methods. Different process variables were critically optimized and their effect on formulation parameters viz. particle size, PDI, zeta potential and entrapment efficiency was measured. Surface morphology was examined by using SEM and TEM. Chemical and conformational stability of the entrapped insulin was examined by gel electrophoresis and CD spectroscopy, respectively. Cellular uptake was determined in Caco-2 cell lines and *ex vivo* intestinal sections. Finally, *in vivo* glucose lowering effect and pharmacokinetic efficacy was determined in STZ induced diabetic rats.

Results. Extensive optimization resulted in the formation of particles with size of 284±3 nm, PDI of 0.236±0.005, ZP of 8.9±0.3 mV and EE of 89.5±0.9 %. Morphological evaluation revealed the formation of almost spherical particles. Entrapped insulin was stable chemically as well as conformationally throughout the process and the nanoparticles exhibited excellent stability in simulated biological fluids. Chitosan nanoparticles revealed controlled release of insulin over the period of 24 h following the higuchi model of drug release. Cellular uptake studies in Caco-2 cell lines demonstrated 2.98 fold higher uptake of folic acid functionalized nanoparticles (FA-Ch-NPs) in comparison with plain Ch-NPs. Furthermore, remarkably higher uptake was observed in ex vivo intestinal uptake studies. *In vivo* studies demonstrated 2.13 fold cumulative hypoglycemia and 21.8±2.4% relative bioavailability in comparison with subcutaneously administered insulin solution. Conclusively, the proposed strategy is expected to be a cost effective and easy to scalable strategy for oral insulin delivery.

Conclusions. FA-Ch-NPs demonstrated excellent stability in simulated biological fluids and maintenance of blood glucose level for extended period of time. Cost effectiveness, single step development and negligible regulatory hurdles can make the proposed formulation a clinical reality.

P2 Surface Modifications of Polyethylene Sinter Bodies for Serological Diagnosis of Borreliosis

Mohammed Alasel[a], Nina Dassinger[a], Doru Vornicescu[a], Michael Keusgen[a]

[a]Institute of Pharmaceutical Chemistry, Philipps University, Marburg, Germany

Objective. For the immobilization of biomolecules on solid polymer surfaces, the functionalization of these materials is necessary. One of the methods in photochemistry is by ultraviolet light (UV) to activate chemical bounds. This technique can be applied to polyethylene polymers, which later were utilized in serological diagnosis of Lyme Borreliosis disease.

Methods. Allyl alcohol was utilized as a monomer to introduce hydroxyl groups on the surface of 3D-polyethylene sinter bodies. Using UV photografting technique, allyl alcohol could be polymerized on the surface providing active hydroxyl groups that can be linked via (3-aminopropyl) triethoxy silane (APTES) to polysaccharides like mannan. In the next step, a fusion protein consisting of the lectin binding domain ConA and a *Borrelia* surface antigen has been immobilized by self-organization.

Results. Functionalization of the 3D-polyethylene surface has been performed utilizing the radical reaction of allyl alcohol with the polymer after exposure to UV light in the presence of an initiator (benzophenone). Surface modification has been confirmed by Fourier transform infrared spectroscopy (FTIR) and scanning electron microscopy (SEM) measurements. After surface modification, the hydroxyl groups were linked to self assembled monolayers of (3-aminopropyl) triethoxy silane (APTES) to provide the surface with amine groups. For amine coupling reaction, mannan was first activated using N,N-disuccinimidyl carbonate (DSC) followed by covalent immobilization via amide bounds. Using a fusion protein with one lectin (Concanavalin A; ConA) part and another antigen (lyme antigen) part, serological diagnosis of Lyme Borreliosis was possible.

Conclusions. Functionalization of the 3D-polyethylene has been successfully achieved using photografting technique. The final coating by the polysaccharide mannan allows fixation of genetically designed fusion proteins with a ConA moiety by self-organization. The latter step is a rather smooth procedure, which does not influence the biological functionality of the fusion protein in a negative manner.

P3 *In Silico* Prediction of Intravitreal Primary Pharmacokinetic Parameters and Drug Concentrations: Tool for Ocular Drug Development

Eva M. del Amo[a,b], Kati-Sisko Vellonen[b], Heidi Kidron[a], and Arto Urtti[a,b]

[a]Centre for Drug Research, Division of Pharmaceutical Biosciences, University of Helsinki, Helsinki, Finland
[b]School of Pharmacy, University of Eastern Finland, Kuopio, Finland

Objective. To build computational models to predict the intravitreal volume of distribution (V_{ss}) and clearance (CL) of new drug candidates and estimate the drug vitreous concentrations when administrated alone or in a drug delivery system.

Methods. Pharmacokinetic (PK) calculation of V_{ss} and CL from a curated database of intravitreal studies in rabbit eye. Multivariate analysis to build the V_{ss} and CL *in silico* models. PK simulations to predict drug concentrations in vitreous with the computational models. PK simulations when the drug is loaded in ocular delivery systems of first and zero order release.

Results. A simple and straightforward model for intravitreal CL was obtained with a Q^2 value of 0.62, predicting the internal and external test set within a mean fold error of 1.33. The relevant descriptors were $logD_{7.4}$ and hydrogen bond donor capacity. In 80% of the compounds intravitreal V_{ss} was between 1.18 ml and 2.28 ml. The implementation of the predicted parameters into STELLA models yielded good estimates of vitreous drug concentrations. Moreover, predicted CL is useful in the PK design of drug delivery systems.

Conclusions. The present work offers useful *in silico* tools to investigate a priori the intravitreal PK profiles after injection of drug candidates or the delivery systems.

P4 Nanotechnology for Neurotrophic Protein Delivery

Angelina Angelova[a], Borislav Angelov[b], Markus Drechsler[c] and Sylviane Lesieur[a]

[a]Université Paris-Sud, CNRS UMR8612 Institut Galien Paris-Sud, F-92290 Châtenay-Malabry, France
[b] Institute of Macromolecular Chemistry, Academy of Sciences of the Czech Republic, CZ-16206 Prague, Czech Republic

[c]Laboratory for Soft Matter Electron Microscopy, Bayreuth Institute of Macromolecular Research, University of Bayreuth, D-95440 Bayreuth, Germany

Objective. The purpose of our study is to design and structurally characterize liquid crystalline lipid nanoparticles suitable for delivery of neurotrophin BDNF molecules. Nanoparticles serve as reservoirs for controlled drug release and may influence the bioavailability of the administered protein. The nanoparticulate systems for neurotrophic factor delivery are expected to solve some of the challenges in neurodegenerative disease treatment.

Methods. The therapeutic use of neurotrophins is restrained by their fragility and rapid degradation in biological media after exogenous administration. Therefore, neurotrophin encapsulation in nano-sized vector systems was undertaken. The resulting nanoscale organizations were revealed by cryogenic transmission electron microscopy (cryo-TEM) and X-ray structural analysis (SAXS) in order to evaluate the ability of the investigated lipid particles for protein upload.

Results. We prepared nanoparticles from liquid crystalline self-assembled mixtures of nonlamellar lipids and neurotrophin BDNF. The nanoparticles were functionalized by a PEGylated amphiphilic derivative. The performed cryo-TEM and SAXS investigations established the formation of BDNF-loaded nanocarriers with multicompartment liquid crystalline organization. The interaction of the novel lipid nanocarriers with a differentiated human neuroblastoma SH-SY5Y cell line (a cellular model of neurodegeneration) was evidenced by confocal fluorescence microscopy imaging.

Conclusions. The obtained multicompartment liquid crystalline nanoparticles, encapsulating neurotrophic protein, may be anticipated to show therapeutic potential in repairing damaged neurons by regulation of the neuronal survival and plasticity.

P5 Adjuvants Show Dramatically Different Interactions with Model Cell Membranes

Lorena Antúnez[a], Prajna Dhar[b], Cory Berkland[a]

[a]Department of Pharmaceutical Chemistry, The University of Kansas, Lawrence, KS
[b]Department of Chemical and Petroleum Engineering, The University of Kansas, Lawrence, KS

Objective. Vaccines commonly contain antigens adsorbed to an adjuvant to enhance the immune response. Despite their prevalence, we do not fully understand how adjuvants stimulate immune cells. It is thought that receptors on antigen-presenting cells (APCs) recognize adjuvant-adsorbed antigen and both are phagocytized. However, a new theory suggests some adjuvants, specifically aluminum adjuvants, strongly interact with lipids within the cell membrane, especially those within lipid rafts. This adjuvant-lipid interaction disrupts the membrane, allowing antigen to enter the cell without the adjuvant. Understanding the interaction between adjuvants and APC membranes could help explain how adjuvants potentiate immune responses and could improve vaccine formulations. This work aims to characterize the interaction of bare and ovalbumin (OVA)-loaded Alhydrogel® and MF59

with cell membranes using representative lipid monolayers.

Methods. Alhydrogel® and MF59 were tested bare or with adsorbed OVA. Monolayers representing the bulk cell membrane contained equal parts DOPC and DPPC and monolayers representing lipid rafts contained equal parts DOPC, cholesterol, and sphingomyelin. Surface pressure was measured to monitor lipid-adjuvant interactions. Lipid domains were stained to observe their density and morphology in the presence of the different adjuvants.

Results. Alhydrogel® reduced the domain density and size while MF59 stabilized the monolayer and increased the domain size. Adsorption of OVA to Alhydrogel® reduced the surface pressure faster and depleted the monolayer more than the bare adjuvant. OVA-loaded MF59 reduced the surface pressure compared to MF59 alone, but remained constant over time.

Conclusions. The significant depletion of the monolayer by OVA-loaded Alhydrogel® indicates that it could directly disrupt the cell membrane. Conversely, the sustained interaction of MF59 with the monolayer implies it may have stable contact with cell membranes. The different behaviors of these adjuvants with model cell membranes suggest different mechanisms of antigen delivery, which may affect subsequent immune response.

P6 Enhanced Percutaneous Permeation of Dehydroepiandosterone Loaded Nanocapsules

Amit Badihi[a], Nir Debotton[a], Marina Frušić-Zlotkin[a], Yoram Soroka[a], Rami Neuman[b] and Simon Benita[a]

[a]Institute for Drug Research, School of Pharmacy, Faculty of Medicine, The Hebrew University of Jerusalem, Jerusalem 91120, Israel
[b]Department of Cosmetic Surgery, Hadassah Hospital Ein Kerem, Jerusalem 91120, Israel

Objective. In this study, we designed adequate DHEA preparations, in an attempt to enable local delivery of the active ingredient to the viable skin layers. In addition, the potential efficiency of DHEA NCs on dermal collagen synthesis was evaluated.

Methods. The Various PLGA nanocarriers were prepared using the solvent displacement method. The nanocarriers were characterized by size and zeta potential measurements and their morphological evaluation was assessed by TEM and Cryo-TEM. The physical state of the entrapped drug in the nanocarrier matrix was carried out using DSC. Site-localization experiments were performed on excised pig skin in Franz diffusion cells and collagen synthesis was evaluated upon human skin culture.

Results. Cryo-TEM observations and thermal analysis indicated that DHEA was successfully incorporated within a stable NCs based delivery system. Moreover, higher [^{3}H]-DHEA levels were recorded in the viable skin layers following different incubation periods of NCs on excised pig skin specimens as compared to DHEA oil solution (free molecules). Furthermore, significantly higher (4 fold) transdermal flux values were observed for the DHEA NCs as compared to the values elicited by the oil control solution. Finally, collagen synthesis in human skin organ culture, assessed by the incorporation of [^{3}H]-proline, was up to 50% higher for DHEA NCs 48 h post topical application than for the untreated specimens.

Conclusions. These results suggest that poly lactic-*co*-glycolic acid (PLGA) based NCs have promising potential to be used topically for various skin disorders.

P7 Curcumin Loaded Solid Lipid Nanoparticles Coated with N-Carboxymethyl Chitosan to Modify Release

Jong-Suep Baek[a] and Cheong-Weon Cho[a]

[a]College of Pharmacy, Chungnam National University, Daejeon 305-764, South Korea

Objective. Curcumin (CUR) has been reported to exhibit potent *in vitro* anticancer effects in a surfeit of human cancer cell lines and majorly in the carcinogenesis of GIT, in animals. However, its poor

solubility, bioavailability and stability limit its *in vivo* clinical efficacy for the cancer treatment. Solid lipid nanoparticles (SLNs) are promising delivery systems for the enhancement of solubility and bioavailability of hydrophobic drugs. However, initial burst release of drug from SLNs in acidic environment such as gastric milieu limits its usage as oral delivery system.

Methods. We prepared CUR-loaded SLNs coated with N-carboxymethyl chitosan (NCC) to protect the rapid release of CUR in acidic environment and enhance the bioavailability. These SLNs were characterized by determining particle size, zeta potential, encapsulation efficiency and polydispersity index. Furthermore, *in vitro* drug release was studied in simulated gastric and intestinal fluids.

Results. CUR-loaded SLNs coated with NCC exhibited 245.1 ± 5.4 nm of particle size, -10.4 ± 3.9 mV of zeta potential and 78.5 ± 3.1% of encapsulation efficiency. SEM images revealed a spherical architecture and correlated with the particle size results. The cumulative released % of CUR from CUR-loaded SLNs and CUR-loaded SLNs coated with NCC was conducted using a modified Franz diffusion cell. CUR-loaded SLNs exhibited 40.4 ± 5.4% release of CUR in 2 h in SGF. However, the release of CUR from SLN in SIF has shown controlled and sustained release of CUR from the SLN.

Conclusions. Based on these results, it can be concluded that this nanoparticles can be used to protect burst release in acidic environment and improve therapeutic efficacy of the drugs.

P8 Tadalafil Loaded Nanostructured Lipid Carriers Using Permeability Enhancers for Transdermal Delivery

Jong-Suep Baek[a] and Cheong-Weon Cho[a]

[a]College of Pharmacy, Chungnam National University, Daejeon 305-764, South Korea

Objective. The aim of this work was to investigate the transdermal nanostructured lipid carriers (NLCs) loaded with tadalafil (TAD), a practically insoluble selective phosphodiesterase-5 (PDE5) inhibitor in order to improve the solubility and skin permeability.

Methods. TAD-loaded NLC was prepared with glyceryl monostearate (GMS) as a solid lipid, oleic acid as a liquid lipid and Tween 80 as a surfactant. It was characterized by determining particle size, polydispersity index, zeta potential, encapsulation efficiency and TEM. *In vitro* skin permeation studies were carried out using Franz diffusion cells. Finally, the gel containing TAD-loaded NLCs was prepared.

Results. GMS and oleic acid were selected as solid and liquid lipid phase, because it allowed the highest solubility of TAD among tested lipids. For preparing NLCs, the lipid ratio was significant factor influencing on mean particle size, followed by the emulsifier concentration. The optimized NLC formulation showed 190.6 ± 5.1 nm of particle size and 89.6 ± 2.8 % of encapsulation efficiency. The TEM image of optimized NLC formulation showed typical spherical shapes. The increase of TAD skin permeability was occurred from all NLC formulations. Among them, TAD-loaded NLCs with limonene and ethanol as skin permeation enhancers exhibited the highest flux (about 4.8 folds) compared to saturated TAD solution. Furthermore, NLC gel with selected permeation enhancers exhibited tolerated toxicity in HaCaT cell line. In cellular uptake study, NLCs and NLC incorporated gel showed increasing tendency according time. However, NLC incorporated gel exhibited lower cellular uptake than that of NLCs only.

Conclusions. These results suggest that the NLC with limonene and ethanol as skin permeation enhancers could be a promising transdermal delivery carrier for TAD.

P9 Paclitaxel Loaded SLNs Modified with HPCD with Low Renal Toxicity for Enhancing Bioavailability

Jong-Suep Baek[a] and Cheong-Weon Cho[a]

[a]College of Pharmacy, Chungnam National University, Daejeon 305-764, South Korea

Objective. This study was to evaluate the potential of solid lipid nanoparticles (SLNs) of paclitaxel (PTX) modified with a 2-hydroxypropyl-beta-cyclodextrin (HPCD) system to enhance cellular accumulation of PTX into breast cancer cells via intravenous administration.

Methods. PTX-loaded SLNs (PS) or PS modified with HPCD (PSC) was prepared by hot-melted sonication. The anticancer activity of the PSC was evaluated in MCF-7 and MCF-7/ADR cells. Confocal microscopy was carried out to visualize the used to quantify cellular uptake. The pharmacokinetic study of PTX in PSC after intravenous administration and xenograft study were performed in rats. Also, the toxicity on kidney was determined by measuring size of kidney and creatinine level in plasma.

Results. PSC was successfully prepared by hot-melted sonication method with smaller size compared to PS. PSC exhibited higher anticancer activity and cellular uptake than that of PTX solution in MCF-7 cells. Furthermore, PSC showed not only higher bioavailability in rats after intravenous administration but also no significant difference of kidney toxicity compared PS or PTX solution. In xenograft study, the tumor volume in rat treated with PSC was smaller than those in rat treated with Taxol, indicated the PSC have better antitumor efficacy.

Conclusions. Based on these results, PSC could be a potentially useful delivery system for PTX with low

toxicity on kidney in breast cancer cells.

P10 Reversal of Multidrug Resistance in MCF-7/ADR Cells Using Dual Drug-Loaded Solid Lipid Nanoparticles with Double Targeting

Jong-Suep Baek[a] and Cheong-Weon Cho[a]

[a]College of Pharmacy, Chungnam National University, Daejeon 305-764, South Korea

Objective. Paclitaxel is used in the treatment of many types of cancer, but its intracellular uptake into multidrug resistant cells (MCF-7/ADR) is restricted by P-glycoprotein (P-gp) efflux. Curcumin has known to inhibit p-gp efflux pump. However, a potential drug-drug interaction exists between paclitaxel and curcumin. This study reports the preparation of multifunctional solid lipid nanoparticles (SLNs) encapsulated anticancer agent paclitaxel and p-gp inhibitor verapamil, intended for dual targeting of the drug to the tumor sites via a conjugation of folate and hyaluronic acid along with a reduced side effects.

Methods. Dual drug-loaded with dual targeting moiety SLNs (DD-SLNs) were prepared using solvent emulsification and evaporation method. Paclitaxel and curcumin were selected as drugs to incorporate in SLN. Specific amounts of curcumin and 2-hydroxypropyl-ß-cyclodextrin (HPCD) were mixed to obtain inclusion complexes, resulting in faster release than that of paclitaxel. These NPs were characterized for particle size, zeta potential, encapsulation efficiency, in vitro drug release, cytotoxicity, and cell uptake in breast cancer cell lines. To compare cytotoxicity, MTT assay was conducted using multidrug-resistant MCF-7/ADR cell line.

Results. After coated with hyaluronic acid and folic acid, particle size of SLNs (175.2 ± 14.2 nm) increased compared to non-coated SLNs (103.5 ± 4.1 nm). *In vitro* cytotoxic study, dual drug-loaded SLNs (DD-SLNs) exhibited significant higher cytotoxicity than those of non-coated SLNs.

Conclusions. Based on these results, it can be concluded that this novel nanoparticles were successfully prepared and strong cytotoxicity on multidrug resistance cell line MCF-7/ADR.

P11 *In Vitro* Enzymatic Degradation of Lipidified Apomorphine

Nrupa Borkar[a], Boyang Li[a], René Holm[b], Anders Håkansson[b], Anette Müllertz[a,c], Mingshi Yang[a] and Huiling Mu[a]

[a]University of Copenhagen, Denmark
[b]H. Lundbeck A/S, Denmark
[c]Bioneer: FARMA, Denmark

Objective. To overcome hepatic first-pass metabolism of apomorphine, a possible strategy may be to develop a lipid-based oral delivery system containing lipophilic prodrugs of apomorphine. Potentially, this may stimulate the lymphatic drug transport and thereby, avoid the hepatic first- pass metabolism. The aim of this study was to synthesize and purify lipophilic diesters of apomorphine and to investigate *in vitro* enzymatic degradation of the apomorphine diesters in simulated intestinal fluids by incorporating them into self-nanoemulsifying drug delivery systems (SNEDDS).

Methods. Apomorphine diesters were synthesized by treating apomorphine HCl with an excess of acid chloride upon heating for 6h. The crude product was purified by column chromatography and the purity was assessed by LC-MS and NMR.

Various compositions of SNEDDS preconcentrates were prepared by mixing oil (soybean oil, miglyol 812N or castor oil), co-surfactant (maisine 35-1), surfactant (cremophor-RH 40) and co- solvent (ethanol) in the incubator at 37°C for 12h. Miscible systems were used for further experiments.

The *in vitro* enzymatic diester degradation experiments were performed using aqueous medium containing bile salts, phospholipids, sodium chloride and maleate (pH 6.5). The degradation was initiated by addition of pancreatin with stirring at 37°C. Samples were collected before addition of enzyme, and 5, 10, 15, 20 and 30 min after; and further degradation of diester in samples was inhibited with an immediate dilution in organic solvent. The amount of diester was quantified by HPLC.

Results. About 78 %-100 % of the diester remained undegraded after 30 min when incorporated into SNEDDS, while the diester was fully degraded after 10 min when it was not administered with SNEDDS.

Conclusions. SNEDDS reduced enzymatic degradation of apomorphine diesters to a large extent in the presence of pancreatin. Thus, the apomorphine diester incorporated into SNEDDS may have a potential to be transported via lymphatic drug transport.

P12 Alzheimer's Disease: Receptor-Targeted BACE-1 Inhibition

Davide Brambilla[a*], Jong-Ah Kim[a*], Roxane Porchet[a] & Jean-Christophe Leroux[a]

[a]Department of Chemistry and Applied Biosciences, ETH Zurich, Switzerland

Objective. Despite the strong scientific effort, Alzheimer's disease, the most common form of dementia, remains a major challenge of medicine. Various studies highlighted a pivotal role for Aβ peptide aggregates in triggering the disease. Aβ originates from the cleavage of the amyloid precursor protein (APP) by the enzymatic action of the β-secretase (BACE1) a multi-functional enzyme. Accordingly, inhibition of BACE1 is considered a promising therapeutic strategy. Recent findings indicate that APP is endocytosed from the neuronal surface together with BACE1 *via* the LRP1 receptor and that most of the Aβ-producing cleavage takes place within the endosomal compartment (at acidic pH).

Our goal is to test a new drug targeting strategy by coupling a Secretase Inhibitor (SI) to the Angiopep-2 (ANG), a peptide currently in clinical trial for its ability to cross the blood brain barrier (BBB) and be internalized within neuronal cells *via* LRP1.

The resulting molecule bears two functional features: (*a*) transport of the SI across the BBB, and (*b*) LRP1 recognition, triggering the internalization of the SI, leading to its co-localization with BACE1 within the endosomes.

Methods. The influence of the ANG moiety on the activity of the inhibitor on BACE1 was tested by FRET. The ability of ANG to transport the inhibitor into the endosomes of human neuronal cells and the implication of LRP1 in the internalization was assessed by fluorescence microscopy and flow cytometry.

Results. The presence of ANG molecule only slightly modifies the inhibitory activity of the inhibitor on purified BACE1. On the contrary, it drastically increases the SI's internalization within human neurons, leading to clear endosomal localization.

Conclusions. The coupling of ANG can increase the specific endosomal localization of secretase inhibitors within human neuronal cells, possibly sparing other essential activities of BACE1.

Acknowledgements. D.B. and J-A.K. acknowledge support from the ETH Zurich Fellowship. This work is financially supported by *Carigest*.

P13 Asymmetrical Flow Field-Flow Fractionation with On-Line Detection for Drug Transfer Studies: Investigating Transfer Kinetics of a Model Drug between Liposomal Bilayers

Askell Hinna[a], Stefan Hupfeld[b], Judith Kuntsche[a] and Martin Brandl[a]

[a]Department of Physics, Chemistry and Pharmacy, University of Southern Denmark, Campusvej55, 5230 Odense, Denmark
[b]Aker BioPharma AS, Fjordallèen 16, N-0115 Oslo, Norway

Objective. To develop an *in vitro* method for prediction of premature loss of lipophilic/amphiphilic drug compounds from liposomal drug carriers via transfer to biological sinks upon intravenous administration. Here we report further refinement of a drug transfer assay, where liposomes are employed as a sink and its application to determine transfer kinetics of a lipophilic model drug, 5,10,15,20-tetrakis (4-hydroxyphenyl) 21H, 23h-porphine (*p-THPP*).

Methods. Small (*mean diameter ~70 nm*) p-THPP-loaded donor liposomes (DL) were incubated with large (*mean diameter ~270 nm*) drug-free acceptor liposomes (AL) at a total lipid concentration of 45 mg/mL with lipid mass ratio 1: 0.8 (DL: AL) at 37°C. Samples were taken at intervals for up to 48 h. Asymmetrical flow field-flow fractionation (AF4) was employed to separate donor- from acceptor-liposomes, and fractions were collected. p-THPP in both liposome types was quantified by on-line UV/VIS extinction measurements at 519 nm, under correction for turbidity using drug-free liposomes (blank). Methanolic solutions (1:10 v/v) of the collected fractions were analyzed offline by HPLC to validate on-line quantification.

Results. The amount of p-THPP in both donor- and acceptor liposomes could be determined by on-line UV/VIS extinction measurements at all time-points up to 48 h with unprecedented precision. The contribution of turbidity to the overall measured UV/VIS extinction of DL and AL were as small as ≤ 6% and 53% respectively at equilibrium. In consequence, on-line quantification of model drug was found reproducible (*rel* SD ≤ 5%), and in general in good agreement with off-line analysis by HPLC (no significant difference in DL fraction, ≤ 10% difference in AL fraction). The relative amount of p-THPP was plotted, and the transfer kinetics analyzed. Rate constants were ~0.0023 min^{-1} with half-lifes of ~300 min.

Conclusions. Our refined method was found suited to determine transfer kinetics of the model drug p-THPP between liposomal bilayers.

P14 Evaluation of Oncolytic Adenoviruses as Adjuvants for MHC-I Restricted Peptides for a New Cancer Vaccine Platform

Cristian Capasso[a], Mariangela Garofalo[a], Mari Hirvinen[a], Lukasz Kuryk[a], Dmitry Romanyuk[a], Maxim Antopolsky[a], Arto Urtti[b] and Vincenzo Cerullo[a]

[a]ImmunoViroTherapy Lab, Centre for Drug Research (CDR) and Division of Pharmaceutical Bioscience, Faculty of Pharmacy University of Helsinki, Finland Europe
[b]Drug delivery Group, Centre for Drug Research (CDR) and Division of Pharmaceutical Bioscience, Faculty of Pharmacy University of Helsinki, Finland Europe

Objective. The aim of this study is to evaluate the immunostimulating potential of MHC-I tumor-restricted peptides co-delivered with oncolytic adenoviruses (OAds).

Methods. MHC-I restricted peptides have been engineered in order to establish electrostatic interactions with the negative surface of OAds. We studied the resulting complexes by analyzing their electric charge (zeta potential) and aggregation profile (dynamic light scattering), and using surface plasmon resonance (SPR). Afterwards, we used different human-derived tumor cell lines to perform transduction and cell viability (MTS) assays. The uptake from antigen-presenting cells (APCs) has been evaluated by flow cytometer analysis. Then, we assessed cross presentation of SIINFEKL peptide *ex vivo* by pulsing splenocytes with the peptide and virus and then we analyzed the amount of peptide presented on MHC-I by flow cytometer.

Results. The analysis of the electric charge revealed that complexes formed using high amount of peptides are stable and positively charged (+20 mV). SPR analysis confirmed the interactions between positive peptides and the negative viral capsid. In addition, no significant aggregation has been observed in these conditions. Moreover, the biological activity (infectivity and tumor killing efficacy) of coated OAds is similar to naked OAds. Flow cytometer analysis revealed that antigen-presenting cells (APCs) are able to uptake the complex and that the presence of OAds is able to enhance the *ex vivo* cross-presentation of SIINFEKL peptide on MHC-I.

Conclusions. Our study clearly shows that the viral capsid can be used as scaffold to deliver immunogenic peptides without hindering the biology of oncolytic viruses. In addition, we report that APCs can uptake the complex *in vitro.* Finally, pulsing splenocytes *ex vivo* with peptide in presence of OAds seems to enhance the cross-presentation mechanism suggesting that our system might represent an important tool to develop more sophisticated cancer vaccine approaches.

P15 Shape-Modified Nanocarriers for Intracellular Drug Delivery

Arianna Castoldi[a,b], Petra Dersch[c], Sarah Gordon[a,b], Claus-Michael Lehr[a,b]

[a]Helmholtz Institute for Pharmaceutical Research Saarland (HIPS), Germany
[b]Saarland University, Germany
[c]Helmholtz Centre for Infection Research, Germany

Objective. To develop a polymeric nanocarrier that exhibits a non-spherical shape, and to investigate the impact and possible applications of such a nanocarrier in the field of drug delivery.

Methods. Poly(D,L lactic-co-glycolic) acid (PLGA) spherical nanoparticles were first prepared using a double emulsion/solvent diffusion method. Nanoparticles were characterized in terms of colloidal properties and imaged by scanning electron microscopy. Aspherical nanoparticles were then created by applying a distinct thermomechanical stimulus to the spherical nanoparticles, causing a modification of the primary shape (J.A. Champion et al., Proc. Natl. Acad. Sci. USA. 2007, 104, 602-9).This procedure was optimized, and produced aspherical particles were characterized with respect to their colloidal properties.

To investigate the feasibility of coupling a targeting moiety to the surface of produced particles, bovine serum albumin as a model protein was covalently conjugated to the surface of both spherical and aspherical nanoparticles. The efficiency of conjugation was calculated by quantifying the amount of bound protein, using the bicinchoninic acid assay.

Results. An optimized method for obtaining aspherical PLGA nanoparticles by applying a thermomechanical stimulus to spherical nanoparticles was successfully established. The change in particle shape was clearly evident from scanning electron microscopy images which demonstrated the presence of rod-shaped particles, and a correlation between the parameters of the applied stimulus and the resulting nanoparticle shape modification was established using different analytical techniques. The effect between the external conditions of both aspherical particle preparation and treatment and particle shape recovery was established. Furthermore, bovine serum albumin could be successfully coupled to the surface of both spherical and aspherical nanoparticles.

Conclusions. An optimized procedure for preparing a polymeric nanocarrier exhibiting a rod-shape was successfully developed. Current and future work focuses on drug loading of rod-shaped nanoparticles, as well as surface functionalization with specific targeting moieties followed by investigation of cellular uptake properties.

P16 Impact of CYP3A5 Genetic Polymorphism on Mechanism-Based Inactivation by Lapatinib

Eric Chun Yong Chan[a], James Chun Yip Chan[a] and Devester Yun Ming Choo[a]

[a]Department of Pharmacy, National University of Singapore, Singapore

Objective. Mechanism-based inactivation (MBI) of CYP450 enzymes results in irreversible loss of enzyme activity, increasing the risk of DDIs. Lapatinib, a dual tyrosine kinase inhibitor, exhibits idiosyncratic hepatotoxicity. Primarily metabolized by CYP3A4/5, lapatinib is oxidized to a reactive quinoneimine intermediate suggested to cause MBI of CYP3A5. CYP3A5 is polymorphically expressed, where *1/*1 carriers possess CYP3A5 levels up to 50% of total CYP3A content, while *3/*3 carriers have negligible CYP3A5. This study aims to explore the impact of CYP3A5 polymorphism on individual susceptibility to lapatinib-induced CYP3A4/5 inactivation.

Methods. *In vitro* inactivation of CYP3A4/5 by lapatinib was investigated in a panel of 12 CYP3A5-genotyped, single donor human liver microsomes (HLM). Lapatinib (0-75 μM) was pre-incubated with 0.6 mg/mL HLM for 0-30 minutes and residual enzyme activity determined using testosterone as the probe substrate to obtain the inactivation kinetics parameter k_{inact}/K_I. Reactive metabolite trapping studies using glutathione (GSH) as a trapping agent were performed in the same panel of HLM to determine the extent of quinoneimine formation.

Results. A two-fold higher k_{inact}/K_I ratio suggested higher potency of inactivation within CYP3A5 *1/*1 versus *3/*3 carriers (6.88 ± 2.09 and 3.57 ± 1.92 min^{-1} mM^{-1} respectively). Mean peak area ratios of lapatinib-derived GSH adduct were also two-fold higher in *1/*1 versus *3/*3 carriers (2.97 ± 1.11 and 1.27 ± 1.55 respectively, $p>0.05$). Both these parameters correlated positively with testosterone 6β-hydroxylation activity, confirming that these observations were mediated primarily by CYP3A4/5. Although not statistically significant, the results suggested that CYP3A5 *1/*1 carriers generate elevated levels of reactive metabolite and may experience a greater magnitude of lapatinib-induced inactivation.

Conclusions. This is the first attempt to elucidate the influence of CYP450 polymorphisms on modulating the susceptibility of an individual to CYP450 inactivation. Our results highlight the multidimensional impact of CYP450 polymorphisms beyond affecting the clinical efficacy of xenobiotics.

P17 Development of Surface Modified Matrix and Segmented Reservoir Intravaginal Ring Devices for Sustained Delivery of Hydroxychloroquine

Yufei Chen[a], Yannick Traore[a], Amanda Li[a], Emmanuel A. Ho[a]

[a]Laboratory for Drug Delivery and Biomaterials, Faculty of Pharmacy, University of Manitoba, Canada

Objective. The goal of this study was to develop novel intravaginal ring (IVR) drug delivery systems for the controlled, sustained (>14 days) release of hydroxychloroquine (HCQ) as a novel strategy for the prevention of HIV-1 infection within the female genital tract. HCQ has been reported to present anti-HIV activity and anti-inflammatory properties.

Methods. The polyether urethane matrix and reservoir IVR segments were fabricated by hot- melt injection molding. Matrix segments were either non-coated or coated with 10% polyvinylpyrrolidone (PVP) or 5% poly(vinyl alcohol) (PVA). Both matrix and reservoir segments were loaded with equivalent amounts of HCQ for release studies at 37°C in pH 4 release medium. HCQ was quantitated using reversed-phase high performance liquid chromatography. Accelerated stability studies were conducted at room temperature (RT) or at 40°C/75% relative humidity (RH) in an environmental chamber. *In vitro* cell viability and pro- inflammatory cytokine production of vaginal and ectocervical epithelial cells were evaluated by incubating the cells with IVR elution medium up to 30 days.

Results. Burst release of 41.08 ± 2.24% during the first 24h was observed in the non-coated matrix IVR segments, followed by decreasing release rate for 18 days. The burst effect was significantly ($P<0.05$) attenuated using PVP and PVA coating (34.63 ± 2.39% and 25.36 ± 2.62%, respectively). HCQ reservoir segments exhibited a near zero-order release profile with no burst release and average release rate of 195.59 ± 24.96 μg (4.67 ± 0.59%) per day. HCQ within both systems was stable under elevated conditions. No cellular cytotoxicity or significant pro-inflammatory cytokine production was observed.

Conclusions. This is the first study to fabricate and characterize surface modified matrix IVRs and segmented reservoir IVRs for the controlled, sustained release of HCQ over 14 days. Both systems demonstrated to be non-cytotoxic and may be a suitable platform for the prevention of HIV-1 transmission.

P18 Exploitation of the Anti-Inflammatory and Cytoprotective Properties of Electrophilic Bioactive Compounds for Potential Application in Cancer Prevention

Shridhivya A. Reddy[a], Amrita A. Nagle[a], Fei-Fei Gan[a], Truong-Minh Dang[b], Siew-Cheng Wong[b], Eng-Hui Chew[a]

[a]Department of Pharmacy, Faculty of Science, National University of Singapore, 18 Science Drive 4, Singapore 117543, Republic of Singapore
[b]Singapore Immunology Network (SIgN), Agency for Science, Technology and Research (ASTAR), 8A Biomedical Grove, Biopolis, Singapore 138648

Objective. Induction of the cytoprotective phase 2 enzymes through transcription factor Nrf2 is regarded as a cancer preventive strategy. Given the association of chronic inflammation with carcinogenesis, compounds eliciting anti-inflammatory and cytoprotective effects are promising candidates for chemoprevention. This study aimed to investigate the anti-inflammatory and cytoprotective activities of several structurally diversified compounds that contain a Michael acceptor or isothiocyanate moiety as electrophilic centres.

Methods. Compounds were assessed for their anti-inflammatory properties in LPS-induced mouse macrophage RAW264.7 cells and human monocytes isolated from donors. To assess the cytoprotective properties of these compounds, their ability to induce antioxidant response element (ARE)-mediated gene expression was assessed in HEK293 cells using the ARE luciferase reporter gene assay.

Results. The expression of inflammatory mediators including iNOS, COX-2, IL-1β, IL-6 and TNF-α were suppressed by the compounds. The NF-κB pathway was a molecular target, with cysteines in IKKβ found to be targeted by the compounds. Compounds identified to possess anti-inflammatory effects concomitantly produced induction of ARE transcription. The compounds showed induction of Nrf2 and phase 2 enzyme levels in $Keap1^{+}/^{+}$ MEFs that was abrogated in $Keap1^{-}/^{-}$ MEFs, underscoring the role of Keap1 in mediating these effects. Cysteines in Keap1 were indeed found to be targeted by the molecules.

Conclusions. This study had examined the anti-inflammatory and phase 2 enzyme inducing activities of electrophilic bioactive compounds, which may be further exploited as agents in cancer prevention.

P19 Evidence for the Functional Dimerization of the Human Bile Acid Transporter ASBT (SLC10A2)

Paresh Chothe[a], Robyn Moore[a] and Peter Swaan[a]

[a]University of Maryland, USA

Objective. ASBT (SLC10A2) is not only important for bile acid reclamation but also for cholesterol homeostasis which makes ASBT an excellent pharmacological target for the treatment of hypercholesterolemia. Previously, our lab has developed a seven transmembrane domain (7 TMD) model to understand ASBT topology. Although it is believed that ASBT acquires both monomer and dimeric forms there is no direct biochemical evidence in support of homodimerization of ASBT.

Methods. COS-1 cells were used for the transient transfection of WT and cysteineless ASBT. HA and Flag tags were introduced at C-terminal of WT and cysteineless ASBT by inverted PCR mutagenesis. Chemical cross-linking of WT ASBT was performed using two thiol-cleavable, lipophilic and bifunctional cross-linkers DSP and DTSSP. To study ASBT dimerization, immunoprecipitation experiments were carried out using HA and Flag tagged WT and cysteineless ASBT. Functional dimerization of ASBT was analyzed by co-expressing WT and cysteineless ASBT in COS-1 cells followed by [^{3}H]-TCA uptake and surface biotinylation. Saturation studies were performed to determine the effect of nonfunctional cysteineless ASBT on kinetic parameters (K_m and V_{max}) of WT ASBT.

Results. Chemical cross-linking with DSP and DTSSP showed that ASBT exists in both monomeric and dimeric forms and to some extent as higher order oligomeric forms. Immunoprecipitation experiments demonstrated physical interaction between HA and Flag tagged WT ASBT and Cysless ASBT suggesting that physical interaction between ASBT molecules does not require endogenous cysteines. Furthermore, co-expression of WT and Cysless ASBT revealed that WT ASBT dimerized with cysteineless ASBT in the membrane and cysless ASBT revealed a dominant negative effect on WT ASBT function which is further corroborated by kinetic analyses.

Conclusions. ASBT adapts a functional dimeric structure.

P20 Predicting Safe and Effective Doses for Children: Integrating Adult Clinical Parameters with *In Vitro* Metabolism by Pediatric Tissues and Modeling

Nicole R Zane[a], Souzan Yanni[a], Dhiren R Thakker[a]

[a]Division of Pharmacotherapy and Experimental Therapeutics, UNC Eshelman School of Pharmacy, The University of North Carolina at Chapel Hill, Chapel Hill, NC, 27599.

Objective. Voriconazole, a first-line antifungal, is cleared via oxidative metabolism. In children, clearance is 3-fold higher and oral bioavailability (F) approximately one-half compared to adults. *In vitro* metabolism of voriconazole was investigated to elucidate the molecular basis of these differences, and a physiologically based pharmacokinetic (PBPK) model was developed to predict safe and effective pediatric doses.

Methods. *In vitro* metabolism studies were conducted with adult and pediatric hepatic microsomes and singly expressed enzymes. An adult PBPK model (Simcyp; version 12.1) was developed based on hepatic *in vitro* metabolism data, and validated against adult clinical data. The adult model was modified by including pediatric hepatic *in vitro* metabolism data to develop a pediatric PBPK model.

Results. Voriconazole was metabolized by pediatric liver microsomes ~3-fold faster than by adult liver microsomes, reflecting higher clearance in children. Contribution of CYP3A4 toward metabolism was lower and that of CYP2C19 and flavin monooxygenase (FMO) was greater in children compared to adults. PBPK models accurately predicted adult and pediatric clearance of 2.4 and 5.0 mL/min/kg, respectively, and F of 83% in adults, but over-predicted the F in children by 2-fold. The over-prediction of F in children suggested intestinal first-pass intestinal metabolism, which was not considered in developing the model.

Conclusions. The greater contribution of CYP2C19 and FMO in children toward voriconazole metabolism may explain higher clearance since activity/expression of these enzymes is higher in children. PBPK models developed based on hepatic metabolism can predict *in vivo* clearance in children and adults. Failure of the pediatric model, but not of the adult model, to predict F suggested intestinal first-pass metabolism of voriconazole in children but not in adults. These studies show the value of *in vitro* metabolism and PBPK modeling studies in predicting PK parameters, and therefore predicting safe and effective doses in children.

P21 Coupling an Absorption Sink to the *In Vitro* Lipid Digestion Model Improves Understanding of Drug Absorption from Lipid-Based Formulations

Matthew F. Crum[a], Hywel D. Williams[a], Colin W. Pouton[a] and Christopher J.H. Porter[a]

[a]Monash Institute of Pharmaceutical Sciences, Monash University, Melbourne, Australia

Objective. One of the limitations to the more widespread use of lipid-based formulations in oral drug delivery is incomplete understanding of the drug properties that are most critical to *in vivo* performance. Here an *in vitro* model that incorporates both simulated lipid digestion and lipid/drug absorption was developed. Using this digestion-absorption model, the impact of drug formulation on formulation digestion, drug solubilisation and drug permeability was assessed *in vivo*.

Methods. An established *in vitro* model was used to simulate intestinal digestion. The addition of the absorptive sink was achieved using an isolated perfused rat jejunum. A peristaltic pump was used to connect the two systems, and digesting lipid formulations were continually perfused from the *in vitro* digestion apparatus through the jejunum.

Results. A range of lipid-based formulations, incorporating the model poorly water-soluble drug fenofibrate, were characterised by *in vitro* digestion. In general formulations containing higher quantities of co-solvent and surfactant (e.g. Lipid Formulation Classification Scheme (LFCS) Type IV formulation) resulted in higher supersaturation and more rapid drug precipitation when compared to those with proportionally higher quantities of lipid (e.g. LFCS Type IIIA formulation). In contrast, when the same formulations were examined using the coupled digestion-absorption model, drug flux into the mesenteric vein was similar regardless of formulation.

Conclusions. This work demonstrates the potential of a combined *in vitro* digestion and *in situ* permeability model to improve understanding of drug absorption from digesting lipid-based formulations *in vivo*. The data suggest that simple *in vitro* lipid digestion models may overestimate the potential for drug precipitation since they lack an absorption sink.

P22 A Molecular Switch Using Genetically Engineered Polymers

Jugal Dhandhukia[a], Isaac Weitzhandler[b], Wan Wang[a], John Andrew MacKay[a,c]

[a]Department of Pharmacology and Pharmaceutical Sciences
[b]Department of Chemical Engineering
[c]Department of Biomedical Engineering
University of Southern California, USA

Objective. Elastin-like polypeptides (ELPs) are environmentally responsive polymers that undergo phase separation in response to increased temperature, ionic strength and pH. What has been lacking is a strategy to design ELPs to respond to specific substrates. To address this deficiency, the aim was to investigate the hypothesis that ELP fusion proteins exhibit switchable solubility upon binding of specific small ligand molecules.

Methods. FKBP-ELP fusion constructs were genetically engineered and purified from *E.coli*. The sensitivity and stoichiometry of FKBP-ELP fusion proteins toward a small ligand molecule called CID (chemical inducer of dimerization) was tested by determining optical density profiles of fusion polymers using UV-Vis spectrometry. The reversible switching was further evaluated by determining the turbidity profiles of FKBP-ELP fusion polymers at a fixed temperature in presence of CID and the Rapamycin respectively. The binding affinity of CID towards FKBP-ELP was determined using Biolayer Interferometry. A mathematical model describing the mechanism of CID induced switching behavior of fusion protein was also developed.

Results. Stoichiometric addition of CID produced a decrease in transition temperature of FKBP-ELP fusion polymer indicating dimerization mediated increase in local length of appended ELPs. This observation was further confirmed by determining increase in optical density of fusion polymer at a fixed physiological temperature in addition of stoichiometric concentration of CID. The optical density returned to baseline levels in real time on stoichiometric addition of Rapamycin. This dimerization mediated reversible ELP phase separation was validated by a two-step mathematical model that successfully correlated the drop in transition temperatures observed on UV-Vis spectrometry with the hypothesized increase in ELP length produced by the CID.

Conclusions. Binding of small ligand molecules to FKBP-ELP successfully demonstrated reversible polypeptide switch. Due to the adaptability and specificity of this strategy, these fusion protein polymers may be evaluated for diverse applications in the detection of multimeric target substrates and treatment of specific diseases.

P23 Preparation and Evaluation of Bioactivated Polyelectrolyte Nanocomplexes for Favoured Cell Migration

Tiziana Di Francesco[a], Annasara Hansson[a], Olivier Jordan[a] and Gerrit Borchard[a]

[a]School of Pharmaceutical Sciences, University of Geneva, University of Lausanne, Switzerland

Objective. We aimed at creating polyelectrolyte nanocomplexes (PECs) linked to the tripeptide RGD, in order to favour wound healing when this process is impaired.

Methods. PECs were obtained in aqueous medium through coacervation, using chondroitin sulfate as a negatively charged polymer and RGD-carboxymethyl-N,N,N-trimethylchitosan (RGD-CM-TMC) as positively charged polymer. The latter was obtained by a three-step synthesis starting from commercially available chitosan.

PECs were characterized with different techniques such as dynamic light scattering, scanning electron microscopy (SEM) and electrophoretic mobility. The assays were carried out to determine the size, shape, charge, aggregation tendency, stability, storage and reproducibility of preparations. Moreover, interactions between skin cells (HaCat) and PECs were investigated in vitro using the Cell Proliferation Kit II®(XTT). Finally, the ability of the PECs to induce adhesion was investigated through the incubation of the nanoparticles with human dermal fibroblasts (HDF).

Results. Positively charged PECs showed a size in the 200 to 300 nm range, with no significant variations before and after centrifugation as well as before and after freeze-drying. The round shape of RGD-CM-TMC PECs was confirmed by SEM analysis.

After the contact of PECs with saline, PBS or physiological media, swelling was observed. The nanoparticles' stability in these media is nevertheless suggested, since swollen size remains constant over 1 day.

The XTT assay shows a cell viability of 90% after incubation with PECs at a concentration of 1000 µg/ml, hence the toxic effect can be excluded. Furthermore, PECs showed adhesion to HFD cells confirming their bioactivity.

Conclusions. We were able to create several batches of stable, storable and non-toxic nanoparticles, which could represent the basis for further applications in order to promote wound healing.

P24 Remote Loading of GLP-1s in PLGA Microspheres for Treatment of Type 2 Diabetes

Amy C. Doty[a,c], David G. Weinstein[a,c], Anna S. Schwendeman[b,c], Steven P. Schwendeman[a,c]

[a]Department of Pharmaceutical Sciences, University of Michigan, United States of America
[b]Department of Medicinal Chemistry, University of Michigan, United States of America
[c]The Biointerfaces Institute, University of Michigan, United States of America

Objective. The glucagon-like peptide 1 (GLP-1) peptide agonist liraglutide has become an important treatment option for management of type 2 diabetes. Due to its poor bioavailability, solution dosage forms of liraglutide must be administered by injection once-a-day. Based on our group's recent discovery that peptides can rapidly partition in low molecular weight acid-end-group poly(lactic-co-glycolic acid) (PLGA) for later controlled release, we sought to test a remote loading strategy for liraglutide. We demonstrate rapid microencapsulation of liraglutide at high loading and encapsulation efficiency in PLGA microspheres from aqueous solution for later controlled release.

Methods. Free acid terminated PLGA 50/50 (i.v.=0.34 dL/g) was used as received or suspended in 1M MgCl2 and shaken for 24 h at 25°C before washing with ddH2O and drying. Porous microspheres with or without suspended MgCO3 were prepared from untreated and MgCl2-treated PLGA using water-in-oil-in-water double emulsion solvent evaporation methods. Prepared microspheres were incubated in 1.0 mg/mL liraglutide aqueous solution for 24 h at 37°C. Loading was determined by loss of peptide from the loading solution, measured by ultra-performance liquid chromatography (UPLC) with UV detection. *In vitro* release was measured in HEPES buffered saline pH 7.4 at 37°C and peptide was measured by UPLC at 215nm.

Results. Treatment of PLGA with MgCl2 increased liraglutide loading from 1.8 ± 0.1% w/w to 6.5 ± 0.1% w/w (mean ± SEM n=3). Incorporation of MgCO3 further increased liraglutide loading to 9.6 ± 0.9% w/w (98 ± 1% encapsulation efficiency). Following low initial burst in the first week, peptide was released in a slow and continuous fashion for over 10 weeks.

Conclusions. Treatment of PLGA with MgCl2 improved loading and encapsulation efficiency of liraglutide. The aqueous-based loading strategy resulted in negligible initial burst of the GLP-1 agonist liraglutide, followed by sustained and long-term peptide release *in vitro*.

P25 Tetraether Lipid-Based Transfection Reagents for the Expression of Luciferase Plasmid (pCMV-luc) in Various Cell Lines

Konrad H. Engelhardt[a], Udo Bakowsky[a]

[a]Institute of Pharmaceutical Technology and Biopharmacy, Philipps-University Marburg, Germany

Objective. In this study, liposomal transfection reagents composed of various tetraether and conventional lipids were investigated. In contrast to monopolar lipids, archaeal tetraether lipids are equipped with C40, saturated methyl-branched biphytanyl chains, linked at both ends to polar head groups. Their unique structure makes them suitable for the oral delivery of sensitive substances like peptides and nucleic acids.

Methods. By using Soxhlet-apparatus, crude lipids with sugar headgroups were extracted from the freeze dried biomass of the Archaeal Sulfolobus acidocaldarius. Acid hydrolysis and subsequent purification steps resulted in pure GDNT (Glycerol Dialkyl Nonitol Tetraether) and GDGT (Glycerol Dialkyl Glycerol tetraether).

Preparation of liposomes was performed by using the thin-film hydration method. Tetraether lipids were mixed with conventional lipids like DPPC, EPC, Cholesterol, and DOTAP. The entrapment of pCMV-luc was checked by gel-retardation and EtBr exclusion assay.

Transfection reagents were characterized using AFM, Cryo-SEM and Zetasizer. Transfection efficiency was studied in A549, EA.hy926 and SKOV-3 cell lines.

Results. The yield of extracted crude lipid, hydrolyzed GDNT and GDGT was 6%, 1-2% and 0.5-1%, respectively.

Transfection reagents with up to 20 mol%, tetraether lipids could be prepared. Sizes of empty liposomes were in a range of 150 – 200 nm and zeta-potential was around +50 mV, depending on lipid composition. Sizes of lipoplexes increased to 250-400 nm and zeta potential drops to +10- 20 mV.

AFM and Cryo-SEM studies showed round shaped vesicles, with higher diameter compared with Zetasizer measurements.

Transfection efficiency increased with higher DOTAP content and was lower by adding conventional lipids like DPPC.

Conclusions. Stable liposomal transfection reagents with various amounts and types of purified archaeal tetraether lipids, could be prepared. They all showed good transfection efficiency in different cell lines, making them suitable as novel drug delivery systems.

P26 Optimization of Tumor-Targeted Nanoparticles for Paclitaxel Delivery with Folate-PEG Conjugated Amphiphilic Cyclodextrins

Nazlı Erdoğar[a], Levent Öner[a], Güneş Esendağlı[b], Thorbjorn T. Nielsen[c], Erem Bilensoy[a]

[a]Hacettepe University Faculty of Pharmacy, Department of Pharmaceutical Technology, 06100 Sıhhiye-Ankara, Turkey
[b]Hacettepe University, Cancer Institute, Department of Basic Oncology, 06100 Sıhhiye-Ankara, Turkey
[c]University of Aalborg, Faculty of Engineering and Science, Department of Biotechnology, Chemistry and Environmental Engineering, 9000, Aalborg, Denmark

Objective. The aim of the work was to develop and characterize paclitaxel loaded and actively targeted nanoparticles using a new folate-PEG-conjugated amphiphilic cyclodextrin derivative via factorial design. As a result of factorial design data, formulation that gave the desired characteristics was analysed in terms of anticancer efficacy in cell culture studies.

Methods. A new folated conjugated CD was characterized with H-NMR spectroscopy for structure and purity. Nanoparticles were prepared by the nanoprecipitation method with folate-conjugated cyclodextrin (X_1) and Pluronic F68 as surfactant (X_2). The formulations were determined in terms of particle size (Y_1), polydispersity index (Y_2), zeta potential (Y_3), encapsulation efficiency (Y_4) and the amount of paclitaxel entrapped in the nanoparticles (Y_5). Suface-response plots were drawn for these variables and in vitro release studies were performed for optimum formulations using the dialysis bag technique. Cell culture studies on folate positive MDA-MB-231 and MCF-7 cells were performed for anticancer efficacy of nanoparticles.

Results. It was found that nanoparticle size and drug loading were largely governed by cyclodextrin concentration in organic phase and that it was possible to obtain nanoparticles smaller than 100 nm without using surfactants. General size range for nanoparticles were between 80 to 100 nm with low PI values (<0.2). Zeta potential of nanoparticles were close to neutral (-4 mV) independent of formulation variables. This charge was a direct result of folate-PEG conjugated amphiphilic CD derivative. Paclitaxel displayed a high affinity to folate targeted cyclodextrin nanoparticles with loading capacity of more than 65%. MDA-MB-231 cell line and MCF 7 cell lines were used in cytotoxicity assays in order to discriminate the nanoparticles affinity towards folate receptors.

Conclusions. Folate conjugation of amphiphilic cyclodextrins that are able to form nanoparticulate carriers spontaneously can be a promising alternative for tumor targeted delivery of insoluble anticancer drugs such as paclitaxel.

P27 Assessment of Antileishmanial Activity of Pyrazinamide by *In Vitro* Time-Kill Curve Experiments against *Leishmania (Leishmania) amazonensis*

Nivea Falcao[a], Sebastian Morales[a], Cordula Heinrichs[a], Kenneth Hernandez[a], Peter Kima[a], Sherwin Sy[a], Hartmut Derendorf[a]

[a]University of Florida, United States

Objective. This study was designed to investigate the effect of PZA on parasitic killing by time-kill curve tests conducted with *Leishmania (Leishmania) amazonensis* specimen.

Methods. Promastigotes of *Leishmania (L.) amazonensis* on the stationary phase were cultured in Schneider supplemented with 20% heat-inactivated fetal bovine serum. 10 µg/mL of gentamicin was added and the parasite growth stimulation, performed at 23 °C. 5.2 x 107 cell forming units (CFU) per mL of promastigotes from axenic culture were seeded in each well of a 24-well plate. Wells were treated in triplicate with Schneider supplemented as a control or 1.9, 3.9, 7.8, 15.6, 31.25, 62.5, 125 µg/ml of PZA. Parasites for each well were counted every 48 hours for 10 days using a hemocytometer and microscope.

Results. In order to evaluate the PZA activity over time, studies made by Mendez *et al* (2009) demonstrated a decrease in cell proliferation of promastigotes of *Leishmania (Leishmania) major* after 48h. Comparatively, in our study the effect of PZA was determined using promastigotes of *Leishmania (Leishmania) amazonensis.* Our results displayed a decrease in promastigotes only after 192h. No significant differences among the tested concentrations were seen. The biggest difference in CFU/mL between control and PZA was observed at 192 h and amount of promastigotes decreased at day 10 in both control and PZA group.

Conclusions. Resistances could explain the differences demonstrated in our infection model. In summary, the data provided the grounds for further testing of PZA as a promising alternative therapy. Additionally, pharmacological and toxicological tests should be conducted to research the effects of PZA in other *Leishmania* infection models.

P28 Glucuronidation Converts Clopidogrel to a Potent Metabolism-Dependent Inhibitor of CYP2C8

Aleksi Tornio[a,b], Anne M. Filppula[a], Ollipekka Kailari[a], Mikko Neuvonen[a], Tommi H. Nyrönen[c], Tuija Tapaninen[a,b], Pertti J. Neuvonen[a,b], Mikko Niemi[a,b], Janne T. Backman[a,b]

[a]University of Helsinki, Department of Clinical Pharmacology, Helsinki, Finland
[b]HUSLAB, Helsinki University Central Hospital, Helsinki, Finland
[c]University of Helsinki, Center for Drug Research, Faculty of Pharmacy, Helsinki, Finland

Objective. This study aimed to examine the effect of clopidogrel on the CYP2C8 and OATP1B1 substrate repaglinide in humans, and explore the inhibition mechanism of clopidogrel on CYP2C8 *in vitro*.

Methods. Nine healthy volunteers received clopidogrel for three days (300 mg on day 1, followed by 75 mg daily) or placebo in a cross-over study. Repaglinide was administered 1 h after intake of clopidogrel on days 1 and 3, and after placebo. The inhibitory effects of clopidogrel and its metabolites 2-oxoclopidogrel, clopidogrel carboxylic acid, and clopidogrel acyl-β-D-glucuronide on CYP2C8 and CYP3A4 were studied in human liver microsomes. Based on the obtained data and literature, a physiologically-based pharmacokinetic model was constructed within Simcyp to elucidate the importance of different mechanisms in the clopidogrel-repaglinide interaction.

Results. In humans, the $AUC_{0-\infty}$ of repaglinide was increased 5.1- and 3.9-fold compared to control on days 1 and 3 of the clopidogrel treatment, respectively ($P<0.001$). The inhibition of CYP2C8 activity by clopidogrel acyl-β-D-glucuronide was NADPH-, preincubation time- and concentration-dependent, and the inhibitory effect could not be reversed by competitive inhibition or by dialysis. The CYP2C8-inactivation variables K_I and k_{inact} of clopidogrel acyl-β-D-glucuronide were 9.90 μM and 0.047 1/min. A physiologically-based pharmacokinetic model estimated that the clopidogrel-repaglinide interaction is mainly explained by mechanism-based inactivation of CYP2C8 by clopidogrel acyl-β-D-glucuronide and partially by OATP1B1 inhibition.

Conclusions. Clopidogrel markedly increases the plasma concentrations of repaglinide. This finding together with the *in vitro* data and physiologically-based pharmacokinetic simulations indicate that clopidogrel is a strong inhibitor of CYP2C8 due to its acyl-β-D-glucuronide, which is a mechanism-based inactivator of CYP2C8. According to the simulations, inhibition of OATP1B1 also contributes to the interaction at high clopidogrel doses. Clopidogrel use with repaglinide is best avoided, and care is warranted when clopidogrel is used concomitantly with CYP2C8 and OATP1B1 substrates.

P29 Formulation of Self-Assembling Polyamino Acid Nanoparticles for Site-Specific Targeting of the Tumor Microenvironment

Zoë Folchman-Wagner[a], Wei-Chiang Shen[a], Jennica L. Zaro[a]

[a]University of Southern California, Los Angeles, California

Objective. The aim of this work was to produce self-assembling, stabilized polyamino acid nanoparticles formulated and characterized for pH-dependent drug release in the mildly acidic tumor microenvironment. This mildly acidic (pH 6.5-7.0) tumor microenvironment has been well characterized, but has not been fully exploited in therapeutic targeting due largely to the difficulty of designing optimal drug carriers for the mildly acidic pH range.

Methods. The nanoparticles were characterized for size stability, and pH-sensitivity using dynamic light scattering analysis in aqueous solution. The drug (daunomycin) loading efficiency was measured by fluorescent and absorbance spectrophotometry.

Results. Precise control over nanoparticle characteristics was obtained by altering the ratio of charged polyamino acid components, as well as concentration of stabilizing agents. The nanoparticles had a reproducible and narrow size distribution (70 ± 3 nm), and were stable in aqueous solution for up to 72 hours at room temperature. Furthermore, the nanoparticles were capable of encapsulating the hydrophobic drug daunomycin, while maintaining their size and stability.

Conclusion. The nanoparticles described in this work consist of small, pH-sensitive peptide sequences that self-assemble to form stable and reproducible particles. This process enables a modular design, in which components could be modified individually or as a whole. The nanoparticles described in this work have potential as a breakthrough technology, and can be utilized in several different areas in the exploitation of the acidic microenvironments in diagnosis and treatment of disease.

P30 Hydrolysis of DTPA Prodrug by Skin Carboxylesterases in Human Epidermal Keratinocytes

Jing Fu[a] and Michael Jay[a]

[a]Division of Molecular Pharmaceutics, UNC Eshelman School of Pharmacy, University of North Carolina at Chapel Hill, Chapel Hill, NC 27599

Purpose. The pentaethyl ester prodrug of the chelating agent diethylene triamine pentaacetic acid (DTPA), referred to as C2E5, effectively decorporates transuranic radionuclides after transdermal delivery, an attractive route of administration for treating contaminated pediatric populations. Carboxylesterases (CESs) are important contributors to the metabolic pathways of xenobiotics, including prodrugs. Previous studies in our laboratory using human liver S9 fractions demonstrated that CESs play an important role in the hydrolysis of C2E5. The objective of the current work is to assess expression of CESs isoforms and enzymatic activity in human skin cell lines to determine if CESs in skin are responsible for C2E5 metabolism.

Methods. S9 fractions of an immortal human keratinocyte (HaCaT), human epidermal keratinocytes adult (HEKa) and human epidermal keratinocytes neonatal (HEKn) were collected. The gene and protein expression levels of CESs were evaluated by RT-PCR and Western blotting, respectively. The substrates p-nitrophenyl acetate and 4-nitrophenyl valerate were used to establish esterase activity in these samples. *In vitro* metabolism was measured by incubating [14C]-C2E5 with keratinocytes S9 fractions for 120 min at 37ºC, centrifuging and collecting the supernatants, and analyzing them by HPLC using a Flow Scintillation Analyzer.

Results. RT-PCR analysis revealed various levels of esterase expression in human skin samples. Western blotting analysis revealed CESs expression in human keratinocytes. Functional studies also showed that skin S9 fractions possessed certain CES activity by p-nitrophenyl acetate and 4-nitrophenyl valerate assay. Hydrolysis of C2E5 was detected and several de-esterified metabolites of C2E5 were produced.

Conclusion. Topical application of C2E5 can result in the systemic delivery of metabolites capable of chelating transuranic radionuclides in contaminated individuals. Understanding the metabolic mechanism of this DTPA prodrug in skin is critical in predicting its application in transdermal delivery in both pediatric and adult population following a nuclear terrorism event. The results of this study will aid in the selection of a safe recommended dose for potential human clinical trials.

P31 Therapeutic Profile *In Vivo* of Formulation Containing Ursolic Acid on Experimental Chagas' Disease

Júnior Furini[a], Juliana Barcellos[a], Juliana Marchetti[a], Cristiana Gonçalez[a], Sérgio Albuquerque[a]

[a]School of Pharmaceutical Science of Ribeirão Preto – USP, Brazil

Objective. The aim of this study was to evaluate the *in vivo* therapeutic action of ursolic acid and a derivative microencapsulated on blood trypomastigotes forms, as well as evaluating the survival of treated animals.

Methods. To perform our experiments, was used 24 male mice, divided into 3 groups and inoculated intraperitoneally with $1x10^4$ trypomastigotes forms of *Trypanosoma cruzi* CL Brener strain.

The drugs used in the trials were the Ursolic Acid (UA), previously active against this disease and its derivative Microencapsulated(ME) (8Polaxamer 470 : 1Sodium caprate : 1Ursolic Acid), thus containing 10% of active ingredient.

These groups of animals were treated for 20 days with UA–20mg/kg, ME–20mg/kg, respectively and the third group was untreated(UT). During this period, the blood trypomastigotes forms were counted by the method of Brenner. Furthermore, for 40 days the animals' survival was observed.

Results. After analyzing the results, we found that the concentration of blood trypomastigotes forms was decreased in animals that were given the test drugs.

The UT group showed, after 15 days, an average of $2.5x10^6$ parasites/mL, whereas the groups treated with UA and ME had the same period, $7.1x10^5$ and 9.0×10^5 parasites/mL, respectively. Moreover, survival of treated animals was increased by 80% when compared to untreated group.

Conclusions. These results indicate high trypanocidal activity of UA, and indicate that pharmacotechnical modifications potentiated the desired effect and this is, therefore, a good strategy against Chagas´ disease. It also shows that ME formulation, increased the survival of treated animals as well as the UA, although with only 10% of the active ingredient, which creates good prospects for clinical testing.

P32 Erythrocytic Stage Targeted Liposome Vaccine Delivery System against Malaria

Ashwini Kumar Giddam[a], Mehfuz Zaman[b], Jennifer M Reiman[b], Mariusz Skwarczynski[a], Michael F Good[b], and Istvan Toth[a,c]

[a]The University of Queensland, School of Chemistry and Molecular Biosciences, Australia
[b]Griffith University, Institute for Glycomics, Australia
[c]The University of Queensland, School of Pharmacy, Australia

Objective. The aim of the work was to investigate the applicability of mannosylated liposomes for the targeted delivery of attenuated blood stage whole malaria parasites to antigen presenting cells (APCs) to evoke a potent and long lasting immune response.

Methods. Various alkyne modified lipid core peptides (LCPs) were synthesized using standard solid phase peptide synthesis. LCPs were conjugated to mannose azide by alkyne-azide "click" reaction to produce mannosylated LCPs (MLCPs) with one or four mannose units. Liposomes encapsulating attenuated whole malaria parasites were formulated with or without MLCP in the formulation using lipid dehydration-rehydration method. Particle size of liposome formulations was determined using master sizer. In-vitro uptake and maturation marker expression studies were performed using APCs derived from mice spleen. Mice were immunized subcutaneously with liposome formulations and challenged with parasites.

Results. MLCPs varying in spacer lengths, lipid anchors, and mannose units were synthesized. Mannosylation of liposomes and parasite encapsulation was confirmed by confocal imaging and quantitatively determined by flow cytometer. The average particle sizes of all liposome formulations were found to be in the range of 10-12 µm. All MLCP-liposome vaccine formulations were taken-up efficiently by APCs when compared with the normal liposome vaccine formulation, and also enhanced the expressions of CD86 and MHC-II maturation markers significantly. After 2nd boost MLCP-liposomes generated enhanced levels of $CD4^+$ and $CD8^+$ T cell response compared to positive control.

Conclusions. Enhanced uptake by APCs was achieved with liposomes formulated with MLCPs when compared to normal liposomes. MLCP-liposomes also generated higher $CD4^+$ and $CD8^+$ T cell response compared to positive control.

P33 Impact of Freeze-drying Cycle on the Solid-State Properties and Long-Term Protein Stability: The Case of rhGH

Pawel Grobelny[a], Yemin Xu[b], Alexander von Allmen[c], Korben Knudson[c], John F. Carpenter[d], Theodore W. Randolph[b], Michael J. Pikal[a]

[a]Department of Pharmaceutical Sciences, School of Pharmacy, University of Connecticut, Storrs, Connecticut, 06269
[b]Center for Pharmaceutical Biotechnology, Department of Biochemistry, University of Colorado, Campus Box 596, Boulder, Colorado 80309
[c]Center for Pharmaceutical Biotechnology, Department of Chemical and Biological Engineering, University of Colorado, Campus Box 596, Boulder, Colorado 80309
[d]Center for Pharmaceutical Biotechnology, Department of Pharmaceutical Sciences, University of Colorado Denver, Anschutz Medical Campus, Aurora, Colorado 80045

Objective. The study was designed to investigate a correlation between the stability of a model protein - recombinant human growth hormone (rhGH) formulations and: (*i*) global dynamics, (*ii*) free volume, (*iii*) specific surface area (SSA), and (*v*) surface protein fraction.

Methods. Various freeze-dried formulations of rhGH were prepared using excipient combination of hydroxyethyl starch (HES), sucrose and trehalose in 2 mM sodium phosphate buffer at pH 7.4. The total excipient concentration in each formulation was kept constant at 5% (w/v). The formulations were lyophilized using five different cycles, specifically (*i*) standard lyophilization, (*ii*) pre-drying annealing lyophilization, (*iii*) post-drying annealing lyophilization, (*iv*) N2-immersion lyophilization and (*v*) N2-droplet-freezing lyophilization.

Results. All the lyophilization cycles yielded glassy solids with different solid-state characteristics. The density value of HES (1.4761 g/cm^3) was significantly lower when compared to the disaccharides. Densities of freeze-dried formulations containing sucrose and trehalose were essentially the same, 1.5113 g/cm^3 and 1.5055 g/cm^3, respectively regardless of freeze-drying cycle used. Post-drying annealing resulted in significantly improved stability of rhGH formulation with sucrose. For annealed formulations the stability increased in the order of HES < trehalose < sucrose, while T_g decreases in the same order. Regardless of the type of excipients used, samples prepared by two rapid freezing, liquid N2 treatment methods showed the highest amounts of protein on their surfaces, as a result of both larger SSAs and higher surface N percentages.

Conclusions. Monitoring the true density as well as the amount of protein on the solid-air interface can be utilized as a simple and quick predictor of stability.

P34 Multi-Parametric Evaluation of Therapeutic Protein Aggregation

Floriane Groell[a], Olivier Jordan[a] and Gerrit Borchard[a]

[a]School of Pharmaceutical Sciences, University of Geneva, University of Lausanne, Switzerland

Objective. During the last 30 years the biopharmaceutical industry has grown exponentially, now reaching 140 FDA approved recombinant proteins. However, therapeutic proteins may induce anti-drug antibodies after a few weeks of treatment. Stability problems and aggregate formation are thought to play a major role in this unwanted immunogenicity. Using interferon-α2b (IFNα2b) as a relevant example, we aimed to study its stability under various conditions and to decipher the role of therapeutic protein aggregates in the induction of immunogenicity.

Methods. Recombinant human IFNα2b gene was expressed in an *E.coli* BL21(DE3)pLysS-pET28b(+) system. Purification of the protein was done by IMAC and a bioactivity assay was performed with A549 cells infected with vesicular stomatitis virus. The protein structure was characterized in its native state as well as after forced aggregation by circular dichroism (CD), fluorescence spectroscopy and dynamic light scattering (DLS). Aggregation was induced by stirring, metal-catalyzed oxidation, pH modification, thermal stress and freeze-thawing cycles.

Results. Protein secondary-structure analysis by CD confirmed its high helical content. After forced degradation we observed a variation in the minimum ellipticity at 220 nm, reflecting structural denaturation of the protein. The two tryptophan residues in IFNα2b allowed us to monitor its structural modifications according to the stress applied. Maximum fluorescence emission shifted by 10 nm under thermal stress application, suggesting an increase in the environment polarity or a change in the protein folding. Anisotropy reveals the protein trend to form aggregates after denaturation. With DLS, we were able to estimate the size and subpopulations of aggregates generated. With native protein radii at around 5 nm, thermal stress for example led to big aggregates of up to 2 µm.

Conclusions. Stability studies revealed different aggregation patterns as a result of the stress treatment applied. Combined orthogonal methods gave us a more precise knowledge of the nature of the aggregates.

P35 Exploring Gastrointestinal Drug Behavior of Fenofibrate after Oral Administration in Man: Nano- versus Microparticles

Bart Hens[a], Joachim Brouwers[a], Maura Corsetti[b], Patrick Augustijns[a]

[a]Drug Delivery & Disposition, KU Leuven, Leuven, Belgium
[b]Translational Research Center for Gastrointestinal Disorders (TARGID), KU Leuven, Leuven, Belgium

Objective. The purpose of this study was to explore the intraluminal behavior of fenofibrate in man after oral intake of (i) Lipanthyl® (microparticles) or (ii) Lipanthyl Nano® (nanoparticles).

Methods. In a cross-over study, five healthy volunteers (3 men, 2 women; aged between 22-25 years) received a single oral dose of fenofibrate, either as microparticles (200 mg, 1 capsule of Lipanthyl®) or nanoparticles (145 mg, 1 tablet of Lipanthyl Nano®), together with 250 ml of water. Duodenal fluids were collected to determine intestinal concentration-time profiles of fenofibrate. In addition, blood samples were taken to assess the systemic exposure of fenofibric acid (active metabolite). In addition to fasting conditions, fed conditions were explored by coadministering fenofibrate with a liquid meal (400 ml of Ensure Plus®).

Results. For both formulations and in both nutritional states, no fenofibrate could be detected in the stomach, indicating poor dissolution in the absence of bile salts. In the duodenum, nanoparticles resulted in a 2.5-fold higher dose-corrected exposure to fenofibrate as compared to microparticles. Neither of the formulations induced fenofibrate supersaturation. Also the systemic exposure of fenofibric acid was significantly higher for the nanoparticles ($AUC_{0\text{-}8h}$: 31.65 µg.h/ml versus 13.56 µg.h/ml for the microparticles). Compared to the fasted state, fed conditions resulted in a 10-fold increase in duodenal exposure for the nanoparticles. In addition, both formulations showed an increase in mean duodenal C_{max}. However, this 10-fold increase was not accompanied with a significant increase in systemic exposure of fenofibric acid, suggesting a relative reduction in bio-accessible dose in fed state conditions due to micellar/vesicular entrapment.

Conclusions. This study demonstrates for the first time the intraluminal behavior of micro- and nanoparticles of fenofibrate in man. These *in vivo* results will serve as unique reference data for validation and/or optimization of different *in vitro*/*in silico* tools.

P36 Comparison of Brain and Plasma Levels of R-Flurbiprofen in Rats Following Oral and Intra-Nasal Administration

Si Min Au Yeong[a], Paul C. Ho[a]

[a]Department of Pharmacy, National University of Singapore, Singapore

Objective. R-flurbiprofen is a compound from the class of non-steroidal anti-inflammatory drugs (NSAIDs), but has minimal effects on cyclooxygenase (COX), contributing to its low toxicity. R-flurbiprofen has been associated with reduced amyloid beta (Aβ) peptides in Alzheimer's disease (AD). However, it was shown in Phase III clinical trial to give no significant improvement in functionalities in AD patients. A possible reason for the lack of clinical effect could be due to its low brain penetration. In this study, comparison between plasma and brain levels of R-flurbiprofen following 30 mg/kg oral and 3 mg/kg intra-nasal (IN) administration to male Sprague-Dawley rats was performed.

Methods. An appropriate volume of the R-flurbiprofen-HPβCD solution was administered orally to 3 male Sprague-Dawley rats at 30 mg/kg, intra-nasally to another 3 male Sprague-Dawley rats at 3 mg/kg. Blood samples were collected via jugular vein cannulation at 0.5, 1, 2, 4, 6, 8 hours after administration of R-flurbiprofen and stored at -20°C until analysis by HPLC.

Results. Very low brain level of R-flurbiprofen was observed as expected, with an average of 1.3% brain-to-plasma ratio obtained after both routes of administration. There was no significant difference in the ratios obtained from the respective route of administration. Even with the highest dose in humans (800 mg BD), which corresponds to a plasma concentration of approximately 185 μM, the amount in the brain as calculated using the ratio obtained would still much lower than the in-vitro tested therapeutic concentration of $IC_{50} \approx 300$ μM for AD. Though there was no significant improvement in brain penetration by IN administration, it was however found that IN administration resulted in much higher systemic exposure of the drug after dose normalization.

Conclusions. The finding indicated that IN administration could be applied to deliver lipophilic drugs with low oral bioavailability but with higher potency.

P37 Extraction of Archaeal Membrane Lipids from *Sulfolobus islandicus*

Sara Munk Jensen[a,b], Alexander H. Treusch[a], Christer S. Ejsing[c] and Martin Brandl[b]

[a]Department of Biology and Nordic Center for Earth Evolution (NordCEE), University of Southern Denmark, Odense, Denmark.
[b]Department of Physics, Chemistry and Pharmacy, University of Southern Denmark, Odense, Denmark.
[c]Department of Biochemistry and Molecular Biology, University of Southern Denmark, Odense, Denmark.

Objective. Archaea species live in extreme environments such as low pH, high salinity and high temperatures. Their membrane lipids show an unusual structure in order to survive at these conditions: there are ether linkages between the glycerol and carbon chains, as compared to the ester linkages of conventional phospholipids.

Oral drug delivery of macromolecules has been achieved by using liposome formulations although the stability of conventional liposomal in the GI-tract is poor. A proof of concept study has shown that these special tetraether lipids (TEL) can be used for improving the stability of the liposomes (Parmentier et al. 2011, Int J Pharm **415**(1-2): 150-157). With the intention of formulating liposomes with tetraether lipids a high yield extraction method is required. The aim of this study is to investigate different extraction methods for gaining high and reproducible amounts of tetraether lipids from archaea.

Methods. *Sulfolobus islandicus* was cultivated in 1.5 L DMSZ medium typically for 5 days. Extraction of the lipids was divided into a cell disruption step, extraction of the lipids by phase separation and analysis by shotgun lipidomics. Two different cell disruption methods were applied: probe sonication and dual asymmetric centrifugation (DAC). Subsequent the extraction of lipids followed a modified Bligh and Dyer protocol.

Results. The extraction methods were compared with respect to their yield of lipids and composition of the lipids in the extract as well as in terms of straightforwardness and time consumption. Our results indicate an increase in TEL-yield when using DAC as compared with probe sonication. Further the DAC-method is less time consuming, and thus promising of producing high amounts of TEL for future liposomal formulations.

Conclusions. An efficient extraction protocol for obtaining TEL from archaea-species was established.

P38 RP-HPLC Method Development and Validation for Simultaneous Analysis of Ibuprofen and Pamabrom

Hye-Suk Jun[a], Cheong-Weon Cho[a]

[a]College of Pharmacy, Chungnam National University, Daejeon 305-764, South Korea

Objective. A simple, selective, rapid, precise and economical reversed-phase high performance liquid chromatographic (RP-HPLC) method was developed for simultaneous analysis of ibuprofen (IBF) and pamabrom (PAMB) from in bulk and drug product.

Methods. The HPLC condition was on a C18 (250 x 4.6 mm, 5 µm) column with a mobile phase consisting of a mixture of water and acetonitrile (40:60). The retention time of IBF and PAMB was 2.6 and 8.0 min, respectively. The flow rate was 1.0 ml/ min. The elute was monitored at 254nm using a UV detector. The developed method was validated in terms of linearity, precision, accuracy, limit of detection (LOD) and limit of quantitation (LOQ). The method was validated according to the ICH guidelines.

Results. The linear regression showed a good linear relationship with a correlation coefficient (*R2*) value for IBF and PAMB of 0.9999 and 0.9998, respectively. The concentration range of IBF and PAMB was 125-2000 µg/ml and 63-100 µg/ml respectively. Intraday and interday precision were checked and less than 1% of RSD. Accuracy of all methods was determined by recovery studies and showed recovery between 98 to 100%.

Conclusions. The proposed method can be used for the analysis of fixed dose combination tablet of IBF and PAMB.

P39 Improvement of Poorly Water-Soluble Albendazole by Using Mucosal Adhesion Polymeric Nanoparticles

Bong-Seok Kang[a], Sang-Eun Lee[a], Choon-Lian Ng[a], Cheong-Weon Cho[a], Jeong-Sook Park[a]

[a]College of Pharmacy, Chungnam National University, Daejeon, 305-764, Korea

Objective. The purpose of this study was to develop oral dosage formulation of albendazole by using mucosal adhesion polymeric particles.

Methods. In order to improve the solubility of albendazole, mucosal adhesion polymeric nanoparticles (CS-PLGA NPs) were developed. Nanoparticles with PLGA: Poloxamer188 (ratio 50:50) were prepared by using modified solvent diffusion technique. PLGA and poloxamer188 were dissolved in acetone. Albendazole was mixed in organic solution by voltex agitation. Then, the obtained emulsion was poured into ethanol under moderate magnetic stirring, leading to immediate polymer precipitation in the form of nanoparticles. The formulations were diluted with water and the stirring was maintained for another 10 min. Then, the solvent was evaporated under 75°C. The CS coated PLGA NPs were suspended in chitosan in acetic acid solution under magnetic stirring for overnight at room temperature. Formulation of an albendazole inclusion complex was characterized using various techniques, including XRD, FT-IR, DSC, and SEM. Physicochemical properties including particle size, zeta potential, stability, encapsulation efficiency, drug loading efficiency, and *in vitro* release were investigated. Cytotoxicity of each formulation was measured by MTT assay.

Result. The results of XRD, FT-IR, and SEM showed the formation of albendazole-loaded nanoparticles. DSC results showed a thermal stability. The particle size was found to be approximately 300 nm, and zeta potential was -25 mV. The stability was maintained for 4 weeks. The encapsulation efficiency and drug loading efficiency was 50-60% and 10%, respectively. *In vitro* release studies showed sustained–release of albendazole. The CS-PLGA NPs formulation showed no cytotoxicity.

Conclusion. From the results, the CS-PLGA NPs could enhance the absorption of poorly water-soluble albendazole.

P40 Quantitative Analysis and Preformulation of Extracts from *Aesculus Hippocastamum*

Ju-Heon Kim[a], Jong-Suep Baek[a], Hye-Suk Jun[a], Cho-A Lee[a] and Cheong-Weon Cho[a]

[a]College of Pharmacy, Chungnam National University, Daejeon 305-764, South Korea

Objective. The aim of this study is to figure out the physicochemical characteristics of extracts of *Aesculus hippocastanum*.

Methods. The physicochemical characteristics such as scanning electron microscopy (SEM), particle size and zeta potential were conducted. Also, fourier-transform infrared spectroscopy (FT-IR) was conducted. The characteristics of powder such as angle of repose, bulk density and tapped density were measured. Cell viability study using MTT assay was conducted in Caco-2 cells. One hundred μl of cell culture medium containing $5x10^4$ cells was seeded in each well in a 96-well plate and incubated for 24 hr and 48 hr. The confluent wells were treated with various concentrations of extracts of *Aesculus hippocastanum* (1, 5, 10, 25, 50, 100, 250, 500 and 1000 ng/mL).

Results. The particle size was 324 ± 18.9 nm with 0.31 ± 0.02 of PDI and zeta potential was -27.0 mV. Angle of repose average was 47.5 ± 3.2 meaning poor flowability. Carr's index and Hausner ratio were calculated with bulk density and tapped density with 0.42 and 1.75, respectively, suggested that this extract had an approximately non-flow characteristics. From cell viability study using MTT assay, we confirmed that the extracts don't have cytotoxicity at various concentration of extracts. The morphology of extracts showed the irregular shape.

Conclusions. Based on powder characteristics, it was proposed that the extracts of *Aesculus hippocastanum* should be formulated by using appropriate pharmaceutical excipients.

P41 Solid State Properties of Suberin-Containing Electrospun Polymeric Nanofibers

Karin Kogermann[a], Liisi Rammo[a], Ivo Laidmäe[a], Kalle Kirsimäe[a], Natasa Skalko-Basnet[b], Jyrki Heinämäki[a], Peep Veski[a]

[a]University of Tartu, Estonia
[b]UiT, The artic University of Norway, Tromsø

Objective. The aim of the present study was to investigate the solid state properties of electrospun nanofibers containing a novel ingredient suberin (SUB), polyvinylpyrrolidone K90 (PVP), and chloramphenicol (CAP).

Methods. SUB:PVP in two different weight ratios (1:4 and 1:7) and in addition 5 mg of CAP were dissolved in 5 ml ethanol. Reference formulations containing PVP, SUB:PVP (1:4 and 1:7) and PVP+CAP were prepared by dissolving the solids in 5 ml of ethanol.

The nanofibers were prepared with the electrospinning equipment. Needle distance from the collector was set to 6 cm. The applied voltage between the needle tip and collector was 9.0 kV and injection rate was 2 ml/h.

The solid state properties of the samples were studied by XRPD. All samples were analyzed immediately after preparation. Scanning electron microscopy (SEM) was used for investigating the morphology of the nanofibers. Additionally, SUB:PVP+CAP containing formulations were analyzed after a short-term storage at ambient conditions.

Results. After electrospinning, initially crystalline SUB and CAP were transferred to amorphous state. A short-term storage at ambient conditions did not influence the solid state properties of the nanofibers. SEM revealed circular gross-section of the nanofibers with an average diameter ranging from approximately 300 to 500 nm. All electrospun samples exhibited a white color.

Conclusions. Electrospinning is a suitable technique to prepare amorphous SUB and CAP in a mixture with PVP. Amorphisation, however, was found to be only temporary. The nanofibers containing SUB:PVP in ratio 1:4 were selected for further studies.

Acknowledgements. Jaan Aruväli is acknowledged for performing the XRPD measurements. This study is part of the targeted financing project no SF0180042s09 and ETF grant project no 7980. The European Social Fund's Doctoral Studies and Internationalization Programme DoRa, and The Estonian Ministry of Education and Research are acknowledged for funding.

P42 VEGF in Diabetic Retinopathy

Emmi Kokki[a], Laura Alasaarela[a], Venla Olsson[a], Tommi Karttunen[b], Kati Kinnunen[b,c], Seppo Ylä-Herttuala[a,d]

[a]Department of Biotechnology and Molecular Medicine, A. I. Virtanen Institute for Molecular Sciences, University of Eastern Finland, Finland
[b]Department of Ophthalmology, Kuopio University Hospital, Finland
[c]Ophthalmology, Institute of Clinical Medicine, School of Medicine, University of Eastern Finland, Finland
[d]Gene Therapy Unit, Kuopio University Hospital, Finland

Objective. The aim of the study was to investigate the role of vascular endothelial growth factor (VEGF) in mouse model demonstrating diabetic retinopathy related alterations in the retina.

Methods. Retina alterations were induced to transgenic mice by subretinal injection with

adenovirus vector expressing Cre-gene (AdCre). Transgenic mice carry in their genome a loxP-STOP which can be excised using AdCre gene transfer leading to strong human VEGF-A165 expression.

The effect of VEGF in mouse retina was studied by optical coherence tomography before and after AdCre gene transfer at different time points. For histopathological studies serial sections were cut and used for hematoxylin-eosin and immunohistological stainings to study tissue vascularization.

Results. OCT imaging shows intraretinal proliferation in mouse eye. Alterations are located at the site of subretinal injections.

Conclusions. The mouse model expressing human VEGF-A after AdCre induction, is suitable to study new treatments for human diabetic retinopathy.

P43 SIRT3 Inhibitors by Virtual Screening

Piia Kokkonen[a], Tarja Kokkola[a], Elina Jarho[a], Antti Poso[a], Maija Lahtela-Kakkonen[a]

[a]School of Pharmacy, Department of Health Sciences, University of Eastern Finland, Kuopio, Finland

Objective. The aim of the work is to identify novel inhibitors for epigenetic histone deacetylase enzyme, SIRT3. There are not many published inhibitors available for SIRT3 or other sirtuins, even though this NAD^+ dependent enzyme family offers unique possibilities to target the epigenetic regulation. SIRT3 is the most effective mitochondrial deacetylase enzyme which defends the mitochondrial DNA from oxidative damage. SIRT3 inhibition could be useful in the treatment of node positive breast cancer.

Methods. The structure of SIRT3 is known and it can be used in structure-based virtual screening. In virtual screening the molecules from a database are combined with the drug target structure in order to check if they can bind to it. This method is fast and cheap and reduces the amount of compounds in *in vitro* studies.

In this study, the 16 million drug-like compounds containing ZINC database was virtual screened with the SIRT3 structure and six hit compounds were tested with a fluorogenic assay. Two of these compounds inhibited more than 50% of SIRT3 activity. The active inhibitor structures were then used as queries in similarity searches from the ZINC database, resulting in more structures to be tested *in vitro*. The most potent inhibitors were also subjected to breast cancer cell proliferation tests.

Results. Four out of the 36 tested compounds could inhibit more than 50% of SIRT3 activity. The inhibitors contain chemical structures not present in previously published sirtuin inhibitors. The inhibitors also restrain breast cancer cell proliferation.

Conclusions. The virtual screening found SIRT3 inhibitors containing novel chemical structures which can inhibit breast cancer cell proliferation.

P44 Encapsulated Cells for Long-term Secretion of Soluble VEGF Receptor 1: Material Optimization and Simulation of Ocular Drug Response

Leena-Stiina Kontturi[a] , Estelle C Collin[b], Lasse Murtomäki[c], Abhay S Pandit[b], Marjo Yliperttula[a], Arto Urtti[a]

[a]University of Helsinki, Finland
[b]National University of Ireland, Ireland
[c]Aalto University, Finland

Objective. Therapies with vascular endothelial growth factor (VEGF) inhibiting factors are effective treatment options for retinal neovascular diseases, but these proteins can only be delivered as intravitreal injections. The objective of this study was to investigate cell encapsulation as a delivery system for prolonged anti-VEGF treatment of retinal neovascularization. Genetically engineered ARPE-19 cells secreting soluble vascular endothelial growth factor receptor 1 (sVEGFR1) were encapsulated in a collagen/hyaluronic acid (HA) hydrogel. The matrix composition and cell density were optimized, and long-term cell viability and protein secretion measurements were performed. To simulate the ocular response after intravitreal sVEGFR1 delivery, a pharmacokinetic/pharmacodynamic (PK/PD) model was developed.

Methods. The encapsulation material was type I collagen cross-linked with poly(ethylene glycol) ether tetrasuccinimidyl glutarate (4SPEG) and supplemented with HA. Viability of the encapsulated cells was studied using alamarBlue metabolic test and LIVE/DEAD staining with confocal imaging. Secretion of the sVEGFR1 protein was measured using ELISA method. PK/PD modeling and simulations were done with Matlab software.

Results. The system suitable for long-term protein delivery was 20 million cells/ml encapsulated in 5 mg/ml collagen cross-linked with 1 mM 4SPEG without HA. The cells remained viable and secreted sVEGFR1 at a constant rate for at least 50 days. Based on PK/PD modeling, delivery of sVEGFR1 from this cell encapsulation system leads to only modest VEGF inhibition, but improvements of the protein structure and/or secretion rate result in a strong and prolonged therapeutic effect.

Conclusions. The collagen/(HA)/4SPEG hydrogel is a suitable encapsulation matrix for sVEGFR1 ARPE-19 cells; the material supported the survival and protein secretion of the encapsulated cells for prolonged periods. The developed PK/PD model can be used to guide the design of intravitreal delivery systems before *in vivo* experiments. The anti-angiogenic protein delivery by encapsulated cells may offer a potential

alternative for the treatment of neovascular retinal diseases.

P45 An Increase in the Natural GDNF Expression Enhances and Protects the Nigrostriatal Dopaminergic System

Jaakko Kopra[a], Jaan-Olle Andressoo[b], Carolina Vilenius[b], Jelena Mijatovic[a], Nita Pulkkinen[a], T. Petteri Piepponen[a], Mart Saarma[b]

[a]Division of Pharmacology and Pharmacotherapy, Faculty of Pharmacy, University of Helsinki, Finland
[b]Institute of Biotechnology, Viikki Biocenter, University of Helsinki, Finland

Objective. Glial cell line-Derived Neurotrophic Factor (GDNF) protects and promotes the survival of dopamine (DA) neurons, when administered to cell cultures *in vitro* or to neurotoxin based *in vivo* models of Parkinson's disease. Despite this well established role for exogenous GDNF, the exact role of endogenous, physiological GDNF remains largely unknown, mainly due to the lack of proper animal models. For studying the role of endogenous GDNF in the development and function of nigrostriatal DA system, we created a knock-in, hypermorphic mouse model, where natural GDNF expression is increased, but still under control of native promoter and enhancers.

Methods. GDNF mRNA levels were measured with qPCR. We immunostained mouse brain sections from substantia nigra (SN) for DAergic markers TH and VMAT2 to stereologically estimate the number of labeled cells. We collected striatal tissue and microdialysis samples for HPLC DA measurements. We lesioned SN with unilateral injection of proteasome inhibitor lactacystin. We also studied performance in accelerating rotarod and corridor tests and measured responses to amphetamine stimulation.

Results. Heterozygous (hetz) and homozygous (homoz) hypermorphic mice have 30% and 70% increased brain GDNF mRNA levels. Hypermorphic mice had a 15% increase in SN DA cell number in the age groups of 7,5 days, 3-4 months and 17 months. Similarly striatal tissue DA levels were increased 20% in the same age groups. Consequently amphetamine-induced locomotor activity and striatal DA response were augmented by about 30%. The young Hetz mice demonstrated enhanced motor performance, whereas the old (15-17 m) improved motor learning in accelerating rotarod test. Finally, we saw a protection from lactacystin-induced toxicity.

Conclusions. In conclusion, our results demonstrate that already a modest increase in the natural GDNF expression functionally enhances and protects the nigrostriatal DAergic system with persistent changes until old age.

P46 Lipid-Bound Indocyanine Green Particles Enable High Resolution Near-Infrared Diagnostic Imaging of the Lymphatic System

John C. Kraft[a], Rodney J. Y. Ho[a]

[a]University of Washington, United States

Objective. Indocyanine green (ICG), the only near-infrared (NIR) fluorophore approved for human use, is unstable and its fluorescence decays readily in solution. To overcome these limitations, ICG-lipid interactions were elucidated to develop ICG-lipid nanoparticles (LNPs) that stabilize ICG, increase its NIR fluorescence intensity, and enhance image resolution of the lymphatic system.

Methods. ICG-LNPs and liposomes without ICG were prepared by mixing lipids [DSPC:DSPE-mPEG$_{2000}$ (9:1 m/m)] and ICG in organic solvent, drying under vacuum, and rehydrating in buffer. Particle size was reduced by sonication. Fluorescence and photon correlation spectroscopy were used to measure changes in particle size and fluorescence. Mice were given the free or LNP form of ICG (1 nmol ICG/mouse). NIR imaging was performed with an IVIS Lumina II.

Results. Maximal fluorescence intensity was detected at a lipid:ICG molar ratio of 250:1 (4.5 times that of free ICG). The fluorescence of ICG bound to LNPs was detectable at 1.5 cm (0.5 for free ICG) tissue depth; resistance to light and improved storage stability were observed. Compared to those treated with free ICG, mice subcutaneously administered with ICG-LNPs exhibited higher fluorescence intensity, NIR image resolution, and enhanced resolution of lymph nodes and lymphatic vessels.

Conclusions. ICG-bound LNPs exhibited nearly 100% incorporation and improved ICG image resolution in lymph nodes and vessels. The enhanced fluorescence intensity and extended image analysis time may allow detection of lymphatic abnormalities due to vessel restrictions, nodule growths, or obstructions.

Acknowledgements. Supported by NIH grants AI77390, RR00166, and RR025014.

P47 Mechanism and Responsible Component of Apple Juice for a Long-Lasting Inhibition of Intestinal Absorptive Transporter OATP2B1

Akira Kubota[a], Yukiko Murata[a], Takanori Mori[a], Keisuke Sekiya[a], Kyoko Goto[a], Takeo Nakanishi[a], and Ikumi Tamai[a]

[a]Faculty of Pharmaceutical Science, Institute of Medical, Pharmaceutical and Health Sciences, Kanazawa University, Japan

Objective. Apple juice (AJ) decreases drug absorption by inhibiting intestinal uptake transporter OATP2B1. We have also shown that AJ causes a long-lasting inhibition of OATP2B1, while mechanism and responsible compound are unclear. The aim of the present study is to clarify the mechanism and the compound responsible for a long-lasting inhibitory effect of AJ on OATP2B1.

Methods. AJ was applied to a HP-20 column and fractionated with water and various concentrations of methanol. After separating AJ components chromatographically, the responsible compound was identified by NMR and LC-MS/MS analysis. AJ and AJ component effects were studied in HEK293 cells stably expressing OATP2B1 or fusion protein of OATP2B1 with EGFP.

Results. By exposing OATP2B1-expressing cells to AJ prior to uptake measurement, significant reduction of OATP2B1 activity was observed and the uptake activity was recovered gradually within about 12 hrs. Membrane surface expression of OATP2B1 protein was decreased by exposing to AJ. In addition, intracellular signals of OATP2B1-EGFP fusion protein was increased by exposing the cells with AJ. These results suggest that a long-lasting inhibition of AJ is caused by internalization of OATP2B1.To identify responsible component of a long-lasting effect of AJ, AJ was isolated by applied to a HP-20 column and fractionated on the basis of difference in the lipophilicity and reversed phase-HPLC method. The isolated compound exhibited a long-lasting effect on OATP2B1 at the concentration in AJ.

Conclusions. It was suggested that AJ may inhibit OATP2B1 activity by decreasing membrane surface expression of OATP2B1, which leads to a long-lasting inhibition. Furthermore, we succeeded to identify a responsible compound in AJ for such a long-lasting effect. Although other components may also have a similar inhibitory activity on OATP2B1, it was demonstrated that OATP2B1-mediated drug absorption might be lowered by taking with AJ by both of simultaneous and long-lasting inhibition.

P48 Transporter Mediated Permeation of *p*-Amino Benzoic Acid on Basal Membrane in Caco-2 Cell Monolayer

Keisuke Kurokawa[a], Keiichiro Tanaka[a], Teruko Imai[a]

[a]School of Pharmacy, Kumamoto University

Objective. Previously, we have studied about relationship between intestinal absorption and hydrolysis of *p*-amino benzoic acid (PABA) derivatives using *in situ* single-pass perfusion of rat jejunum. The intestinal hydrolysis causes decrease of transport of not only PABA derivatives but also their metabolite, PABA. In case of procaine, it was slowly hydrolyzed to PABA, and PABA formed in mucosal cell was predominantly transported into mesenteric vein. Therefore, both procaine and PABA was absorbed into vein, resulting in superior absorption. In contrast, butyl-PABA was fastly hydrolyzed in mucosal cells. Therefore, only 18.5% of butyl-PABA was transported into vein and a large number of PABA was mainly transported into luminal side rather than vein due to passive diffusion. These data indicated that PABA was transported by certain transporter on basal membrane at low concentration. In this study, we investigated the transport and hydrolysis of PABA derivatives in Caco-2 cell monolayer. Furthermore, the transport of PABA was fully studied.

Methods. *In vitro* hydrolysis rate of butyl-PABA and ethyl-PABA was determined in 9000g supernatant (S9) of Caco-2 cell. Transport experiment was performed by Transwell® which was cultured 3~4 weeks after seeded with Caco-2 cell. PABA derivatives and [^{3}H]-PABA were used as a substrate.

Results. Hydrolysis clearance of ethyl-PABA was greater than that of butyl-PABA. These hydrolysis was inhibited by diisopropylfluorophospate and bis-*p*-nitrophenylphosphate, indicating the major enzyme is carboxylesterase. PABA derivatives were hydrolyzed during transport across Caco-2 cell monolayer. PABA formed in Caco-2 cell was transported into both AP and BL sides. In transport experiment of [^{3}H]PABA, apical(AP) to BL side transport was larger than opposite direction.

Conclusions. PABA derivatives are hydrolyzed during transport across Caco-2 cell monolayers and PABA formed in the cells is transported by certain transporter on basal membrane.

P49 Enhanced Generation, Characterisation and Pre-Clinical Evaluation of Co-Stimulatory Molecules Expressing Oncolytic Adenovirus for Cancer Treatment of Human Patients

Lukasz Kuryk[a, b], Lotta Vassilev[b], Tuuli Ranki[b], Sari Pesonen[b], Antti Vuolanto[b], MariangelamGarofalo[a], Mari Hirvinen[a], Cristian Capasso[a], Dima Romanyuk[a] and Vincenzo Cerullo[a]

[a]Laboratory of Immunovirotherapy, Division of Pharmaceutical Biosciences & CDR, Faculty of Pharmacy, University of Helsinki, Finland
[b]Oncos Therapeutics Ltd., Finland

Objective. Our aim was to i) engineer, characterize and produce oncolytic adenoviruses (oADV) coding for molecules exhibiting antineoplastic and co-stimulatory properties against cancer ii) evaluate toxicity and efficacy of our viruses.

Methods. Viruses were generated and amplified with adenovirus preparation techniques. Optimization studies on production process of viruses were performed in T-175 flask and subsequently up-scaled with multilayer flasks. Cell density at the time of infection, harvesting time, multiplicity of infection and harvesting method were optimized. The virus titering was based on spectrophotometric measurement. The potency assessment was based on i) the infectious titer measured with either tissue culture infectious dose or immunocytochemistry assay, and ii) assessment of the functionality and quantity of the co-stimulatory molecules expressed by viruses. The verification of the virus identity was done by i) restriction enzyme assay to show the genomic integrity and ii) PCR demonstrating the presence of the modifications in the genome. Antineoplastic properties were evaluated by *in vitro* and *in vivo* studies.

Results. We have constructed a double targeted, chimeric oncolytic adenoviruses, expressing a co-stimulatory molecules (CD40L, GM-CSF). ONCOS-102 is an engineered adenovirus (Ad5/3) that codes for granulocyte-macrophage colony-stimulating factor (GM-CSF). GM- CSF mediates anti-tumour effects by mobilizing and maturing dendritic cells as well as increasing the activity of cytotoxic T cells. In turn ONCOS-401 is a modified adenovirus (Ad5/3) that codes for the CD40 ligand (CD40L). CD40L serves as a local co-stimulatory signal greatly enhancing cytotoxic immune activities, inducing direct tumor cell apoptosis (programmed cell death) and down-regulating immunosuppressive T-cells recruited by cancer cells. Our viruses have been safely used in animal studies showing safety and antitumor efficacy.

Conclusions. We have generated oncolytic adenoviruses coding biologically active co- stimulatory molecules with antineoplastic properties and tested their efficacy and toxicity *in vitro* and *in vivo*.

P50 Oral Delivery of Anticancer Drug: Doxorubicin Complex Lead to Chemotherapy Maintenance at Home with Low Toxicity

Seho Kweon[a], Youngro Byun[a]

[a]Department of Molecular Medicine and Biopharmaceutical Science, WCU, Seoul National University, Korea

Objective. Although most of the anticancer drugs are only available through parenteral route and it shows several disadvantages such as low patient's compliance, higher costs and side effects. Doxorubicin (DOX) is a p-gp mediated anthracyclin drug and shows oral bioavailability below 5% due to rapidly efflux from intestinal cells and also metabolized in the liver. But negatively charged deoxycholic acid (DOCA), is secreted from the bile duct and re-absorbed through apical sodium dependent bile acid transporter on intestine, can form physicochemical complex with positive charge of DOX.

Methods. DOX complex was confirmed by differential scanning calorimetry. Permeability and absorption experiments were conducted using caco-2 cell line *in vitro*. DOX complex administered per oral to SD rat for pharmacokinetic study and C3H and Balb/c Nu/Nu mouse for therapeutic effects *in vivo*. Histological and hematological toxicities also were evaluated in same model.

Results. The formation of DOX complex is reversibly dependent on pH and buffer concentration, showing absorption via ASBT. Oral bioavailability of the DOX complex was showing ~30% which was 6 times higher than that of doxorubicin. 5 mg/kg dose of oral DOX complex group had therapeutic effect as much as 1 mg/kg doxorubicin IV group. Toxicities of DOX complex on intestine and liver were not observed and there were no difference with control group. Furthermore, since low doses of doxorubicin complex lead tumor cell to delay or arrest on cell cycle, co-administration with chk1 inhibitor (MK-8776) result in apoptosis or mitotic catastrophe on tumor cells. 1.25 mg/kg and 2.5 mg/kg dose of DOX complex increased their antitumor effects with chk1 inhibitor.

Conclusions. DOX/DOCA complex with chk1 inhibitor make anticancer drug less toxic and more convenient for patients, leading oral chemotherapy at home.

P51 Controlled Release from Liposomes by Light Activation

Tatu Lajunen[a], Timo Laaksonen[a], Tapani Viitala[a], Arto Urtti[ab]

[a]University of Helsinki, Finland
[b]University of Eastern Finland, Finland

Objective. This study aimed at controlled light triggered drug release from liposomal drug carriers.

Liposomal structure enables targeted delivery to specific cell type and light activation allows place and time controlled release of the contents from the drug carrier. This method should enhance the treatment of difficult diseases in the eye, skin and gastro-intestinal tract with reduced side effects.

Methods. The drug is encapsulated inside liposomes with targeting ligands on the lipid surface. The liposomes also include gold nanoparticles that emit heat due to a plasmon resonance phenomenon when irradiated with light of specific wavelength. The temperature in the liposomes rises above the phospholipid transition temperature and drug is released due to the increased fluidity of the lipid bilayer. By focusing the light on the diseased tissue at specified time, control over the treatment schedule can be achieved. Liposomes with pH sensitivity were also investigated for increased drug release within the cells after endocytosis and reduced drug release outside the cells.

The liposomes were produced with reverse evaporation method with a model drug (calcein) and gold nanoparticles within the aqueous core. Gold nanoparticles with different sizes and shapes were utilized to absorb light at various wavelengths and convert it into heat. Light activation was done with a LED light source with selectable wavelength combined to a plate handling robot system for repeatable experiments. The release of calcein was detected with fluorescent methods. The change in bilayer structure was measured with Langmuir/BAM measurements. The images of the liposomes were captured with Cryo-TEM equipment.

Results. Temperature and pH sensitive liposome formulations were developed. Up to 75% of the contents were released during 30 minutes of light activation compared to less than 20% of the control samples.

Conclusions. This formulation is an attractive option for targeted delivery and controlled release.

P52 Technetium-99m Labelling of Nanofibrillar Cellulose for Small Animal SPECT/CT

Patrick Laurén[a], Yan-Ru Lou[a], Mari Raki[b], Arto Urtti[b], Kim Bergström[b], Marjo Yliperttula[a]

[a]Division of Pharmaceutical Biosciences, Faculty of Pharmacy, University of Helsinki, Finland
[b]Centre for Drug Research, Faculty of Pharmacy, University of Helsinki, Finland

Objective. Investigate the properties of plant-derived nanofibrillar cellulose (NFC) hydrogel as an injectable platform for drug release. Evaluate NFC as a potential biomedical device and labeling properties.

Methods. A stannous chloride reduction method was used and optimized with ^{99m}Tc to label NFC for imaging purposes. Study compounds ^{123}I-NaI, ^{123}I-β-CIT and human serum albumin were mixed with the hydrogel and injected subcutaneously into 20 female BALB/c inbred mice. The release and distribution of study compounds were examined with a multimodality imaging device SPECT/CT. The drug release profiles were simulated by 1-compartmental Deconvolution and Loo-Riegelman pharmacokinetic models.

Results. The optimized ^{99m}Tc-NFC labeling was fast and reliable with well over 95 % labeling efficiency. The NFC hydrogel remained intact at the injection site during the 24 hour study. Study compounds were more concentrated at the injection site when administered with the NFC hydrogel compared with control saline and study compound mixtures. The NFC hydrogel reduced the elimination rate of a large compound, technetium-99m-labelled human serum albumin by 2 folds, but did not alter the release rate of the smaller compounds.

Conclusions. We have demonstrated a reliable and efficient method of ^{99m}Tc-NFC labeling that is easily prepared and administered. NFC did not disintegrate or migrate during the study despite the activity of the study animals while awake between image acquisitions. Potential local delivery or long-term controlled release treating chronic diseases, especially in areas accessible with injections such as the skin, could be possible with injectable wood pulp NFC hydrogels.

P53 Enhanced Physicochemical Properties of Docetaxel-Loaded Solid Lipid Nanoparticles

Sang-Eun Lee[a], Bong-Seok Kang[a], Choon-Lian Ng[a], Cheong-Weon Cho[a], Jeong-Sook Park[a]

[a]College of Pharmacy, Chungnam National University, Daejeon, 305-764, Korea

Objective. The aim of this study was to develop an efficient intravenous formulation from the study of physicochemical optimization of docetaxel-loaded solid lipid nanoparticles (SLN).

Methods. SLN were prepared by modified solvent-evaporation method. To optimize process, the thermal behavior of lipids was studied. Docetaxel-loaded SLN (DSLN) were composed of stearyl amine, egg phosphatidyl choline, polysorbate 80, and docetaxel. To study the physicochemical characterization of DSLN, particle size, polydispersity index, and zeta potentials were measured. The stability of DSLN was investigated for 2 months at 4°C. The morphology of DSLN was observed by using TEM. *In vitro* drug release, cytotoxicity, and cellular uptake were determined.

Results. From the DSC thermograms of stearyl amine, it is proved that the preparation temperature is a

critical parameter to prepare reproducibly DSLN in our experimental condition. The particle size and polydispersity index of DSL were about 200nm and 0.2, respectively. TEM images confirmed the results of DLS data and showed that most of DSLN had an identical-sized and spherical form. Docetaxel was incorporated in SLN with above 90% encapsulation efficiency. When stored at 4°C, DSLN were maintained stable for 2 months. *In vitro* drug release showed sustained-release pattern. MTT assay and cellular uptake results indicated that DSLN had better efficiency with low cytotoxicity.

Conclusions. From the results, the DSLN can be a promising and efficient formulation of docetaxel for intravenous administration.

P54 Discovery of Novel Diarylpyrimidines as Potent HIV NNRTIs via a Structure-Guided Core-Refining Approach

Xiao Li[a] Wenmin Chen[a], Ye Tian[a], Huiqing Liu[b], Peng Zhan[a], Erik De Clercq[c], Christophe Pannecouque[c], Jan Balzarini[c], and Xinyong Liu[a]

[a]Department of Medicinal Chemistry, Key laboratory of Chemical Biology (Ministry of Education), School of Pharmaceutical Sciences, Shandong University, 44, West Culture Road, 250012, Jinan, Shandong, P.R.China
[b]Institute of Pharmacology, School of Medicine, Shandong University, 44, West Culture Road,250012, Jinan, Shandong, P.R.China
[c]Rega Institute for Medical Research, KU Leuven, Minderbroedersstraat 10, B-3000 Leuven, Belgium

Objective. The aim of the work is to further explore untapped chemical space in NNIBP and obtain more potent back-up series of DAPY derivatives.

Methods. Guided by crystal structures of HIV-1 RT/DAPY complex and molecular modeling studies, new DAPY analogues were designed and synthesized in which the positions of nitrogen atoms in the central pyrimidine ring were changed, and nitro, amino and other nitrogen-containing groups were introduced at the primary position. Meanwhile, the two phenyl rings in the left and right wings and the NH linker were maintained in view of their paramount importance in parent drugs. Thus, this study will highlight the synthesis, biological evaluation of novel DAPY derivatives, and also, SARs will be discussed according to the antiviral activity and molecular docking results.

Results. 16 compounds significantly inhibited HIV-1 IIIB replication with EC_{50} values lower than 66 nM. Particularly, compound 7a was the most potent inhibitor against HIV-1 wild-type and double RT mutant HIV-1 strain K103N/Y181C, with an EC_{50} value of 2.5 nM (SI = 13740) and 0.33 μM (SI = 107), respectively compound 8c was found to show moderate anti-HIV-2 potency (EC_{50} = 5.57 μM). Preliminary structure-activity relationships (SARs) and molecular modeling of these new analogues were also discussed in detail.

Conclusions. Through structure-guided core-refining and side-chain optimization of the approved drug TMC125, a series of novel DAPY derivatives were designed, synthesized and evaluated for their antiviral activity. A brief discussion of the SARs expatiated that the nature of the substituent on left ring of the central pyrimidine ring had a significant impact on the antiviral activity. Meanwhile, molecular docking results were used to rationalize these biological data, which will be beneficial to further design more potent anti-HIV-1 agents.

P55 Chemoprevention by Curcumin: A Prodrug Hypothesis

Garvey Liu[a], Jayanth Panyam[a]

[a]University of Minnesota – Twin Cities, U.S.A.

Objective. Curcumin, a naturally occurring polyphenol, has demonstrated anti-cancer and anti- inflammatory potential in epidemiological studies. However, curcumin has poor bioavailability (<1%) due to low absorption and rapid metabolism. We propose a prodrug hypothesis to explain this paradox: the inactive glucuronide metabolites are naturally occurring prodrugs of curcumin that are selectively activated only in the tumor site to generate the active parent compound. Previous studies have shown that some tumors overexpress β-glucuronidase, an enzyme that hydrolyzes the glycosidic bond of glucuronides. Thus, the glucuronide metabolites likely generate the active agent 'on demand' at the required sites of action.

Methods. To test the prodrug hypothesis, we determined the correlation between β- glucuronidase expression and activity in different tumor types with the *in vivo* chemopreventive efficacy of curcumin. β-glucuronidase activity was determined in mammary tumor tissues with HER-2+ (BALB-neuT, TuBo) and triple negative (4T1, MDA-MB-231) phenotypes. Immunohistochemistry (IHC) studies on primary human breast tumor tissue and Western blotting with BALB-neuT mammary tumor tissue were conducted to measure β-glucuronidase expression. Chemopreventive potential of curcumin was evaluated in BALB/c mice bearing orthotopic JC (triple negative subtype) tumors.

Results. β-glucuronidase activity assays showed that the highest enzyme activity was in HER-2+ tumors. Similarly, IHC studies revealed β-glucuronidase expression levels to be highest in HER-2+ breast cancer compared to other subtypes. Normal and benign stages of tumor showed the lowest levels of β-glucuronidase while the invasive/metastatic stage showed the highest levels. Significant tumor growth inhibition was observed in BALB/c mice bearing JC tumors when dosed with an oral curcumin SMEDDS (self-microemulsifying drug delivery system) formulation.

Conclusions. Our results convincingly demonstrate the presence of β-glucuronidase in mammary tumors and point to a potential mechanism of action for natural chemopreventives such as curcumin that have poor oral bioavailability but have potent chemopreventive activity after oral administration.

P56 Discovery of Piperidine-Linked Pyridine Analogues as Potent Non-Nucleoside HIV-1 Reverse Transcriptase Inhibitors

Xinyong Liu[a], Xuwang Chen[a], Yuanyuan Li[a], Shufang Ding[a], Jan Balzarini[b], Christophe Pannecouque[b], Erik De Clercq[b], Huiqing Liu[c]

[a]Department of Medicinal Chemistry, Key laboratory of Chemical Biology (Ministry of Education), School of Pharmaceutical Sciences, Shandong University, 44, West Culture Road, 250012, Jinan, Shandong, P.R.China
[b]Institute of Pharmacology, School of Medicine, Shandong University, 44, West Culture Road,250012, Jinan, Shandong, P.R.China
[c]Rega Institute for Medical Research, KU Leuven, Minderbroedersstraat 10, B-3000 Leuven, Belgium

Objective. The aim of the work was to discover more active and less toxic HIV-1 non-nucleoside reverse transcriptase inhibitors based on our previous efforts.

Methods. Encouraged by our previous results and continued to pursue our studies, we undertook a new round of structural modification of diarylpyrimidine and piperidine-substituted triazine derivatives, in which, the crucial N atom in center cycle were retained to build hydrogen bond interactions with amino acid residues inside the binding pocket of HIV-1 NNRT. In addition, polar hydrophilic substituents and heterocycle were introduced to the right wing that is oriented directly into the exterior water through a rather small opening window in the protein/solvent interface. Herein, we detailedly report the synthesis, anti-HIV evaluation, preliminary structure–activity relationship (SAR) and molecular modeling of the newly designed piperidine-linked amino-triazine analogs.

Results. A series of piperidine-linked pyridine analogues were designed and synthesized. The title compounds were evaluated for activity against wild-type and resistant mutant strains of HIV-1 as well as HIV-2 in MT-4 cells. Excitingly, the highly potent compound BD-c1 (EC_{50} = 10 nM, CC50 ≥ 146 μM, SI ≥ 14126) displayed lower cytotoxicity and higher selectivity than etravirine (EC_{50} = 2.2 nM, CC_{50} = 28 μM, SI = 12884) against wild-type HIV-1. Compound BD-e2 (EC_{50} = 5.1 nM) showed better antiviral efficacy than the four reference drugs nevirapine, delavirdine, efavirenz and zidovudine against wild-type HIV-1. Many compounds were also active against the frequently observed drug-resistant double mutant HIV-1 (K103N+Y181C) strain.

Conclusions. A series of piperidine-linked pyridine analogues were designed and synthesized to continue our research into the discovery of more active and less toxic HIV-1 NNRTIs. The design, synthesis, anti-HIV evaluation, preliminary structure-activity relationship and molecular simulation of novel piperidine-linked pyridine analogues are represented.

P57 Nano Ceramic Assembly for Pulmonary Delivery of Protein and Peptides

Deborah Lowry[a], Fadi A. Abdulrazzaq[a], Lindsay J. Marshall[a], Mandeep Marwah[a] and Yvonne Perrie[a]

[a]Aston University, UK

Objective. The aim of the present research is to investigate the release properties of aquasomes in a DPI formulation.

Methods. 100 mg of hydroxyapatite was added to 10 mL of 0.15 M solution of trehalose under stirring at 4oC for 1.5 hours. The sample was then centrifuged, washed and freeze-dried. 15 mL of BSA (1 mg/mL) was added to the freeze-dried sample under stirring for 1.5 hours at 4oC. The sample was then centrifuged, washed and freeze-dried. The aerosolisation properties of BSA loaded aquasomes were investigated using a NGI. A quantity of 20 mg in a size 3 capsule was introduced to the 1-7 stages of the impactor (flow rate 60 L/min). The samples were redistributed in 10 mL of simulated lung fluid and placed in a shaking water bath at 37oC and 100 rpm. A quantity of 0.3 mL was taken for analysis at hourly time points up to 6 hours.

Results. The aquasomes had an average size of 1.5 ± 0.96 μm. Zeta potential values were calculated after the coating (trehalose, -11.6 ± 1) and loading (BSA, -1 ± 0.5) stages to ensure the aquasomes consisted of the three layers. The deposition of BSA loaded DPI aquasomes at each stage of the NGI at the time of manufacturing and after 6 months at 25^0C/60% RH (n = 3) were investigated. Approximately 70% of the delivered dose was found to have a cut-off diameter of 2.82 μm. *In vitro* release testing found that BSA release is controlled over the 6 hour time period.

Conclusions. Aquasomes with an aerodynamic diameter of 0.94 μm released 430 μg of BSA. This is very encouraging for potential protein/peptide delivery using aquasomes via the pulmonary route. Cell culture studies will be performed to complement the study using human bronchial epithelial cell lines.

P58 Design, Synthesis and Biological Evaluation of Small-Molecule Fluorescent Ligands Based on Cyanine 5 for α_1-Adrenoceptor

Zhao Ma[a], Lupei Du [a], Minyong Li[a]

[a]Department of Medicinal Chemistry, Key Laboratory of Chemical Biology (MOE), School of Pharmacy, Shandong University, Jinan, Shandong 250012, China

Objective. Within the large family of G-protein coupled receptors (GPCRs), α1-adrenergic receptors (ARs) mediate many crucial physiological effects in human body. Our current understanding of the molecular pharmacology of the α1-AR is mainly dependent on the use of radioligand binding techniques and GFP-based fluorescent techniques, but these methods are limited, as they are unsafe and laborious procedures. Small-molecule fluorescent ligands are powerful tools to study GPCRs since they can be employed in various experiments to understand receptor structure and function. In this current research, a series of small molecules based on cyanine 5(Cy5) are designed and synthesized to study α1-ARs.

Methods. Two pharmacophores of α1-AR antagonists, quinazoline and phenylpiperazine, are conjugated to cyanine 5 by click reactions. The radioligand binding assay is used to determine the affinity for receptors. MTT assay is used to the cytotoxicity. Absorbance and fluorescence spectra are recorded by microplate reader, and fluorescence imaging is performed using the Zeiss fluorescence microscope.

Results. Nine compounds are designed and synthesized, and all of them show high affinity for α1-adrenoceptor. Their largest emission peaks are at about 660nm. Especially, the fluorescence of compound Q4, which shows highest affinity for α1-AR, changes according its surroundings and its fluorescence is quenched greatly in 50mM PBS. This environment-sensitive property makes Q4 a good candidate in receptor- or cell- based drug screening and fluorescence imaging of intact cells.

Conclusions. We designed and synthesized nine compounds based on Cy5 to develop fluorescent ligands for α1-AR. From them, Q4 drew a lot of attention for its environment- sensitive property. Q4 shows huge potential in the study of α1-AR.

P59 Supersaturation of Zafirlukast in Fasted and Fed Intestinal Media Measured *In Situ* by UV/Vis Fiber-Optic Probes: Effectiveness of Precipitation Inhibitors

Cecilie Maria Madsen[a], Anette Müllertz[a] and Thomas Rades[a]

[a]University of Copenhagen, Faculty of Health and Medical Sciences, Department of Pharmacy, Denmark.

Objective. The aim of this study was to examine the effectiveness of precipitation inhibitors (PI) on the supersaturation of Zafirlukast *in vitro* and compare these results with *in vivo* behavior.

Zafirlukast (ZA) is a leukotriene antagonist marketed for treatment of asthma (Accolate®). Oral administration of ZA with food can reduce the bioavailability by 40%. ZA is poorly water soluble, and formulated in its amorphous form (aZA).

aZA has a solubility and dissolution advantage compared to the crystalline hydrate form. It has been shown that aZA will supersaturate upon dissolution with respect to its crystalline form, and thus in theory the bioavailability of ZA increases upon amorphisation. However, due to the unstable nature of a supersaturated system, ZA precipitates as the hydrate form and the concentration decreases accordingly. The precipitation can be inhibited, and the supersaturation period prolonged by addition of polymers such as hydroxypropylmethylcellulose (HPMC) and polyvinylpyrrolidone (PVP). HPMC and PVP are excipients in Accolate®.

Methods. The level and duration of supersaturation was examined by powder-dissolution with *in situ* measurements of absorbance with an UV/Vis probe in simulated intestinal media *in vitro*. Preliminary studies suggested the method to be beneficial as a screening tool, but not as a precise prediction tool for *in vivo* behavior.

Results. A prolonged duration of supersaturation of aZA was demonstrated in presence of HPMC and PVP (w/w, aZA:PI, 1:1) *in vitro*. PVP also raised the level of supersaturation. The duration of supersaturation was shorter in fed than in fasted state simulated intestinal media. The effectiveness of the precipitation inhibitors was lowered in fed state intestinal medium *in vitro*. These results are in accordance with the observed *in vivo* behavior of ZA.

Conclusions. This study indicates that dissolution experiments *in vitro* can be used to examine supersaturation, effectiveness of PI and potential food effects on these.

P60 An Orally Delivered Bile Acid Based Formulation of Carboplatin for Effective Maintenance Chemotherapy

Foyez Mahmud[a], Youngro Byun[a]

[a]Department of Molecular Medicine and Biopharmaceutical Sciences, and College of Pharmacy, Seoul National University, Seoul 151-742, South Korea

Objective. At present, mostly the anticancer drugs are given parenterally, showed drug concentration above the maximum tolerable concentration (MTC) and then rapidly eliminate from the plasma causing enhanced side effects. In contrast, oral maintenance therapy may increase therapeutic efficacy by increasing the exposure time to cancer cells and also reduce the side effects by maintaining the drug concentration below MTC level. Carboplatin is a member of platinum family and active against various cancer types. However, a major limitation of carboplatin is the poor oral bioavailability which imposes IV administration. In this context, we have developed a physical complex between carboplatin with deoxycholic acid (DOCA) that can increase the lipophilicity of drug formulation and may facilitate the uptake in GI tracts through bile acid transporters.

Methods. The pharmacokinetics studies of carboplatin/DOCA (mole ratio 1:2) were done in SD rats and analyzed by ICP-MS. The pharmacodynamics studies were evaluated in SCC7 and A549 tumor bearing mice models. Apoptosis in tumor and GI tracts were assessed by TUNEL and H&E staining. Three weeks toxicity studies have been conducted in C3H mice model following 10 mg/kg/daily oral dose.

Results. The X-ray diffraction patterns showed the complex formation due to disappearance of crystalline peaks from carboplatin. The results indicated that orally administered complex was effectively absorbed into SD rats and the oral bioavailability was found 25% compared to IV group. In antitumor study, low dose continuous oral therapy showed the substantial tumor inhibition in mice and also increase the number of TUNEL positive cells in SCC7 and A549 tumor section. The three weeks toxicity studies of 10 mg/kg oral dose did not produce any abnormalities in hematological and serological parameters in C3H mice having intact morphology of GI tracts.

Conclusions. Our studies offer safe and effective oral maintenance chemotherapy of carboplatin for cancer patients at home.

P61 Transdermal Delivery of Flufenamic Acid from PLGA Nanoparticles by Iontophoresis

K. Malinovskaja[a], H. Labouta[b], M. Schneider[c], T. Laaksonen[d], J. Hirvonen[a]

[a]Division of Pharmaceutical Chemistry and Technology, University of Helsinki, Finland
[b]Department of Chemistry, University of Calgary, Canada
[c]Institute of Pharmaceutical Technology and Biopharmacy, Philipps University of Marburg, Germany
[d]Division of Pharmaceutical Biosciences, Centre for Drug Research, University of Helsinki, Finland

Objective. The aim of the study was to investigate the influence of the combination of nanoencapsulation and iontophoresis on the permeation of the lipophilic model drug flufenamic acid (FFA) across skin using poly(lactide-co-glycolide) (PLGA) as carrier polymer.

Methods. Nanoparticles from PLGA labelled with fluoresceinamine (FA-PLGA) loaded with FFA were prepared using solvent extraction method. The particles were characterized by encapsulation efficiency, size, ζ-potential and morphology using Zetasizer and SEM. The stability of nanoparticles was as evaluated as a change in hydrodynamic diameter and PDI after 8 h of iontophoresis or 24 h contact with buffer/skin. *In vitro* permeation studies were performed in Franz diffusion cells at 32°C across human epidermis and full-thickness porcine skin. FFA was iontophoresed from the cathode with either 100% constant current, 75%on/25% off pulsed current or 50%+:50%- alternating current (current density 0.5 mA/cm^2; frequency 500 Hz). The penetration depth of nanoparticles in skin was evaluated by confocal LSM.

Results. FFA was loaded into spherical FA-PLGA nanoparticles with an average diameter of 174.2 nm, ζ-potential of -8.5 mV and drug loading of 5.6 w/w%. Particles showed no changes in stability after 8 h of any iontophoretic treatment and 24 h of contact with human epidermis or full-thickness porcine skin. About 50% of the total drug amount was gradually released from nanoparticles in 24 hours and the application of iontophoretic current had no effect on the release. Transdermal fluxes from nanoparticle formulation remained lower compared to respective solution due to limited release of drug. From nanoparticle formulation pulsed current profile resulted in comparable or higher flux compared to constant current profile.

Conclusions. Charged PLGA nanoparticles can be successfully used in combination with iontophoresis for the controlled delivery of therapeutic agents across the skin. Pulsed current can be applied to increase transdermal permeation of drugs from nanoencapsulated formulations.

P62 Temperature-Sensitive Hybrid Hydrogels Charged with PLGA Particle as Parenteral Extended Release System

P. Maudens[a], O. Jordan[a], and E. Allémann[a]

[a]School Pharmaceutical Sciences, University of Geneva, University of Lausanne, Switzerland

Objective. To develop and evaluate chitosan-hyaluronate hybrid thermosetting hydrogels charged with microparticles.

Methods. Thermosensitive solutions were formulated with chitosan-hyaluronate and a gelling agent.

Drug-loaded PLGA particles were dispersed into the thermosensitive solutions. They were prepared according to a process involving the emulsification of an intrinsically fluorescent drug, followed by solvent evaporation, washing, and freeze-drying. Particle size measurements were performed using laser-light diffraction. Scanning electron microscopy was performed in order to determine the surface characteristics of the particles/hydrogels.

Stability of hydrogel solutions were determined by formulation appearance at 4 ºC and at room temperature over time. Stability of particle distribution inside the hybrid gel was investigated using a confocal scanning light microscope.

The injectability of the systems was investigated with a 1-mL syringe fitted with 18G to 29G needles. The injection force was assessed using a texture analyser. The gelation point was determined at 37ºC into a vial filled with PBS at pH 7.4. Viscosity and elasticity were investigated by dynamic rheometry as a function of temperature.

Results. Varying the chitosan and hyaluronate concentration as well as the concentration of the gelling-agent, various gelation times and stabilities could be observed. Rheology and texture analysis confirmed the sol-gel transition at body temperature and the ease of injectability, respectively. Chitosan-hyaluronate hybrid hydrogels carrying drug-loaded microparticles could be a promising temperature-dependent parenteral drug delivery system.

Conclusions. Chitosan-Hyaluronate hydrogels is an attractive system for PLGA particle drug delivery and tissue engineering that combine biodegradability, biocompatibility and the ability to form *in situ* gel-like implants.

P63 Inhibition of Proteolytic Cleavage by Inserting the Metal-Bound *cla*MP Tag Adjacent to a Known Recognition Site

Michaela L. McNiff[a], Brittney J. Mills[b], Jennifer S. Laurence[a]

[a]Department of Pharmaceutical Chemistry, University of Kansas, United States
[b]Department of Chemistry, University of Kansas, United States

Objective. The idea of chemically conjugating a small drug molecule to a targeting moiety such as an antibody has provided opportunity to mitigate the toxic effects associated with anticancer treatments. A new approach to conjugation involves the discovery of a novel tripeptide with metal binding capabilities. The *cla*MP Tag is composed of the amino acid sequence asparagine-cysteine-cysteine (NCC) and can be genetically engineered into a protein. Recombinant proteins are often expressed with encoded tags to facilitate folding, solubility, and/or purification. Once produced, it is desirable to remove these tags using a protease such as thrombin or Factor Xa. The influence on the rate and fidelity of cleavage by the enzyme of placing the *cla*MP Tag next to the proteolytic cleavage sites is explored here using different proteins.

Methods. The process of inline conjugation of the *cla*MP tag was accomplished through genetic engineering and standard bimolecular techniques. Several proteins were used as model systems. Recombinant protein was expressed and purified using chromatography. Factor Xa was used to cleave the tag adjacent to the *cla*MP site and samples for SDS-PAGE were collected over time. A typhoon imager was used for densitometry analysis.

Results. Through densitometry analysis, the *cla*MP tag was determined to affect the cleaving efficiency of Factor Xa.

Conclusions. The investigation of the influence of the ^{cla}MP tag on the cleavage site provides insight into the surrounding environment. The way the tag binds metal creates a square planar geometry allowing for a possible steric effect occluding the cleavage site. Also, the tag carries a 2^- charge, which may exhibit electrostatic effects.

P64 Design and Therapeutic Use of a Mutant Coiled-Coil Peptide for BCR-ABL Inhibition

Geoffrey D. Miller[a] and Carol S. Lim[a]

[a]University of Utah, Department of Pharmaceutics and Pharmaceutical Chemistry, Salt Lake City, UT, USA

Objective. The aim of this work was to design a peptide capable of inhibiting BCR-ABL oligomerization that could be used as a therapeutic in CML and Ph^+ ALL.

Methods. The oncoprotein tyrosine kinase BCR-ABL is the causative agent and driving force of chronic myeloid leukemia (CML). In order to activate signaling characteristic of this disease, BCR-ABL must homo-oligomerize via an N-terminal coiled-coil domain. Using rational design and computational modeling, we have designed a construct (termed CC^{mut3}) capable of inhibiting BCR-ABL oligomerization, which is based on the structure of the endogenous BCR-ABL coiled-coil (CC) domain. This modified α-helical construct includes mutations into the endogenous CC domain (C38A, K39E, S41R, L45D, E48R, Q60E) that lead to non-self-recognizing properties of the construct, favoring hetero-oligomerization between CC^{mut3} and BCR-ABL while disfavoring homo-oligomerization between two CC^{mut3} molecules.

Results. Delivering this CC^{mut3} construct as a gene, we have shown that CC^{mut3} favors hetero-oligomerization with and co-localizes with BCR-ABL. Next, we have demonstrated the capability of CC^{mut3} to inhibit BCR-ABL auto-phosphorylation and downstream signaling as well as reduce proliferation, oncogenic potential, and induce apoptosis in CML cells. In addition, combining CC^{mut3} with the most recently FDA-approved tyrosine kinase inhibitor, ponatinib, allows a dose reduction of ponatinib and shows increased therapeutic efficacy *in vitro*. Finally, CC^{mut3}, both alone and in combination with ponatinib, showed similar anti-proliferative and apoptotic results when tested on CML cells containing BCR-ABL with the elusive kinase domain mutation T315I.

Conclusions. CC^{mut3} has shown promising *in vitro* results that could potentially lead to its use as an effective therapeutic either alone or in combination for CML or Ph^+ ALL. A safe, effective involving the addition of a hydrocarbon backbone (staple) to the peptide, is currently under development.

P65 Development of Hyaluronic Acid Based IgG-Loaded Dissolving Microneedles for Intradermal Protein Delivery

Juha Mönkäre[a], M. Reza Nejadnik[a], Khalil Baccouche[a], Wim Jiskoot[a], Joke A. Bouwstra[a]

[a]Drug Delivery Technology, Leiden Academic Centre for Drug Research, Leiden University, Leiden, The Netherlands

Objective. Dissolving microneedles were developed for intradermal protein delivery, based on hyaluronic acid and using immunoglobulin G (IgG) as a model protein. Hyaluronic acid was chosen since it is a biocompatible, FDA-approved polyanionic polysaccharide that is naturally present in the skin.

Methods. Hyaluronic acid (Mw 150 kDa) was dissolved at different concentrations in phosphate buffer (pH 7.0, 10 mM). In the case of IgG loaded microneedles, IgG was added (2-10 % (w/w) of total mass of hyaluronic acid). The hyaluronic acid/IgG solution was applied onto a PDMS mold to create microneedles and by using vacuum and centrifugation efficient distribution of the solution into the microholes was ensured. Subsequently, the solution was dried overnight at 37°C before peeling off the obtained microneedle array from the mold. Microneedles were analyzed with light, fluorescence and scanning electron microscopy. *Ex vivo* human skin was used to study the skin penetration of microneedles by trypan blue staining and the dissolution of microneedles in the skin by measuring microneedle length after the application.

Results. IgG-loaded (at least up to 10% (w/w)) microneedles were successfully produced and the hyaluronic acid concentration of 5 % (w/v) was found to be optimal for the preparation. Microneedles were sharp and their length was on average over 290 μm that corresponded well the length of the microneedles (300 μm) of the original master array. Skin penetration studies showed that microneedles were able to penetrate the stratum corneum of the *ex vivo* human skin and reach the epidermis. After the penetration into the skin, microneedles started to dissolve immediately and after 10 min they were nearly completely dissolved.

Conclusions. IgG-loaded dissolving microneedles for intradermal delivery were prepared successfully from hyaluronic acid. Microneedles penetrated human skin, and after their penetration they dissolved quickly in the skin.

Acknowledgments. The research leading to these results has received support from the Innovative Medicines Initiative Joint Undertaking under grant agreement n° 115363 resources of which are composed of financial contribution from the European Union's Seventh Framework Programme (FP7/2007-2013) and EFPIA companies' in kind contribution. Dr. Conor O'Mahony (Tyndall National Institute, University College Cork, Ireland) is acknowledged for providing master microneedle arrays for the preparation of PDMS molds.

P66 Differential Effect of Buffering Agents on the Crystallization of Gemcitabine Hydrochloride in Frozen Solutions

Bhushan Munjal[a], Mehulkumar Patel[a], Arvind K Bansal[a]

[a]Department of Pharmaceutics, National Institute of Pharmaceutical Education and Research (NIPER), SAS Nagar, Punjab 160062, India

Objective. The purpose of this study was to evaluate the differential effect of buffering agents on the crystallization of a model drug, gemcitabine hydrochloride (GHCl) in frozen solutions.

Methods. Four buffering agents, viz. citric acid (CA), malic acid (MA), succinic acid (SA) and tartaric acid (TA) were selected. Aqueous GHCl solutions (30 mg/mL) containing various buffering agent

concentrations (25 – 200 mM) were frozen *in situ* in DSC and XRD and analyzed during the cooling and heating runs. Onset of GHCl crystallization during heating run in DSC was measured to compare the differential effect of buffering agents. T_g', unfrozen water content in the freeze concentrate and crystallization propensity of the buffering agents was also determined for mechanistic understanding of the underlying effects. Solutions containing GHCl (30 mg/mL) and buffering agents (100 mM) were freeze dried and examined for cake integrity.

Results. CA and MA inhibited while SA facilitated crystallization of GHCl even at 25 mM concentration. Increasing the concentration enhanced their effect. However, TA inhibited GHCl crystallization at concentrations < 100 mM and facilitated it at concentrations ≥ 100 mM. Lyophilization of GHCl with either SA or TA yielded elegant cakes, while CA and MA caused collapse. T_g' failed to explain the inhibitory effects of CA, MA and TA as all buffering agents lowered the T_g' of the system. Differential effect of buffering agents on GHCl crystallization could be explained by consideration of two opposing factors: (i) their own crystallization tendency and (ii) unfrozen water content in the freeze concentrate.

Conclusions. It was established that API crystallization in frozen solution is affected by the type and concentration of the buffering agents

P67 Analysis of Secretory Transport of Drugs in the Small Intestine Using the Ussing Chamber Method

Takeshi Nakayama[a], Shinya Fukizawa[a], Kazuya Maeda[a], Takehito Ohtsubo[b], Toshio Kumai[b], Naoki Matsumoto[b], Paul A. Dawson[c], Yuichi Sugiyama[d], Hiroyuki Kusuhara[a].

[a]The University of Tokyo, Japan
[b]St. Marianna University School of Medicine, Japan
[c]Wake Forest University School of Medicine, USA
[d]RIKEN Innovation Center, Japan

Objective. Recent reports suggest that the excretion from the blood into the intestinal lumen contributes partly to the clearance of some drugs, but the underlying mechanisms have not been clarified. The aim of this research was to investigate the contribution of transporters to the secretory transport of drugs in the small intestine using the Ussing chamber method.

Methods. The secretory (basal-to-apical) transport of several drugs was measured in tissue sections from duodenum, jejunum, and ileum of mice in an Ussing chamber. The possible involvement of transporters in the secretory transport was assessed by observing the inhibitory effects of several compounds on their transport. The contribution of P-gp, Bcrp, and Ostα/β to intestinal secretion of drugs was investigated in ileum sections obtained from the corresponding knockout mice.

Results. The secretory transport of several drugs in the Ussing chamber was much higher in the ileum sections compared with the duodenum and jejunum sections. Their secretory transport in the ileum section was lower in Mdr1a/1b(−/−)/Bcrp(−/−) mice than that in wild-type mice. Several compounds (e.g., 200 μM fluvastatin, 100 μM apixaban) inhibited the secretory transport and drug accumulation in the ileum sections, suggesting the involvement of basal uptake transporter(s). By contrast, although Ostα/β is expressed predominantly on the basal side of the ileum along the small intestine, the secretory transport of several drugs in the ileum section was not decreased by knockout of Ostα.

Conclusions. The secretory transport of several drugs was almost selective in the ileum, implying that the ileum-specific expression of transporters contributes to the intestinal secretion. In secretory transport, P-gp and Bcrp contribute to the efflux from intestinal epithelial cells, whereas Ostα/β is not involved in the basal uptake of drugs. Clarification of the molecular mechanism underlying the basal transport is under way.

P68 Developing Therapeutic Vaccine Formulations for the Treatment of Melanoma

Silke Neumann[a], Roslyn Kemp[b], Rod Dunbar[c], Thomas Rades[d], Sarah Hook[a]

[a]School of Pharmacy, University of Otago, Dunedin, New Zealand
[b]Department of Microbiology and Immunology, University of Otago, Dunedin, New Zealand
[c]School of Biological Sciences, University of Auckland, Auckland, New Zealand
[d]Department of Pharmacy, University of Copenhagen, Denmark

Objective. Anti-inflammatory drugs such as inhibitors of cyclooxygenase-2 (COX-2) are considered promising candidates to enhance therapeutic cancer vaccination. The beneficial effects of these drugs rely on the inhibition of immune-suppressive cell populations, such as myeloid-derived suppressor cells (MDSCs), in the tumour microenvironment. MDSCs suppress desired T-cell responses and thereby limit the efficacy of cancer vaccines. Here we investigated the effect of licofelone, a dual COX-2 and 5-lipoxygenase inhibitor, to improve anti-tumour responses *in vivo* when administered in combination with a cancer vaccine.

Methods. Melanoma-bearing mice were intravenously injected on day 6 with a long peptide vaccine, formulated into cationic liposomes containing the adjuvant α-galactosylceramide (α-GalCer) and either licofelone or celecoxib. Simultaneously, mice received subcutaneous injections of either licofelone or celecoxib formulated into cationic liposomes every other day for 14 days after tumour implantation. Changes in body weight and tumour size were recorded

and mice were culled when the tumours exceeded a size of 200 mm^2.

Results. Immunisation with the long peptide vaccine in combination with licofelone significantly prolonged survival in melanoma-bearing mice whereas treatment with licofelone in cationic liposomes only did not improve anti-tumour effects as compared to the control group. In contrast, combination of celecoxib and the long peptide vaccine did not prolong survival as compared to the vaccine group.

Conclusions. These results suggest that the dual COX-2/5-LO inhibitor licofelone is a powerful tool to improve therapeutic vaccination strategies in melanoma.

P69 Microcontainers as an Oral Drug Delivery System

Line Hagner Nielsen[a,b], Stephan Sylvest Keller[b], Jette Jacobsen[a], Thomas Rades[a], Anja Boisen[b]Anette Müllertz[a,c]

[a]Department of Pharmacy, Faculty of Health and Medical Sciences, University of Copenhagen, Copenhagen, Denmark
[b]Department of Micro- and Nanotechnology, Technical University of Denmark, Kongens Lyngby, Denmark
[c]Bioneer:FARMA, Department of Pharmacy, Faculty of Health and Medical Sciences, University of Copenhagen, Copenhagen, Denmark

Objective. The purpose of this study was to evaluate microcontainers *in vitro* and *in vivo* as an innovative oral drug delivery system for the poorly water-soluble drug, furosemide.

Methods. Microcontainers, with an inner diameter of 223 µm, were fabricated in SU-8 through two steps of photolithography. The microcontainers were filled with amorphous sodium salt of furosemide (prepared by spray drying). Subsequently, a 10 µm layer of Eudragit® L100 was spray coated on the cavity of the drug-filled microcontainers. The release of the drug from the microcontainers was evaluated in a biorelevant gastric medium (pH 1.6) and a biorelevant intestinal medium at pH 6.5. The intestinal permeability of the amorphous furosemide salt loaded into the microcontainers was evaluated using a Caco-2 cell model. Furthermore, drug-filled and Eudragit-coated microcontainers were dosed orally to rats and blood samples were taken over 24 h.

Results. The release experiments revealed that the Eudragit® layer prevented drug release in the gastric medium, while an immediate release of the amorphous furosemide salt was seen in the intestinal medium. The Caco-2 cell studies showed a fast permeability of the amorphous furosemide salt with no significant differences between the microcontainers (P_{app} $1.79 \cdot 10^{-5} \pm 0.068 \cdot 10^{-5}$ cm/s, mean±SD, n=11) and powder of amorphous furosemide salt (P_{app} $1.62 \cdot 10^{-5} \pm 1.04 \cdot 10^{-5}$ cm/s, mean±SD, n=11). The rat study demonstrated that the amorphous furosemide salt dosed in microcontainers showed an oral relative bioavailability of 220.2±43.2% (mean±SEM, n=6) compared to amorphous furosemide salt filled into capsules and coated with Eudragit® L100.

Conclusions. Microcontainers show considerable potential as a future oral drug delivery system.

P70 Co-Delivery of Autoantigen and B7 Pathway Modulators for the Treatment of a Murine Model of Multiple Sclerosis

Laura Northrup[a], Joshua Sestak[a], Bradley Sullivan[a], Sharadvi Thati[a], Teruna Siahaan[a], Charlotte Vines[b], and Cory Berkland[a]

[a]The University of Kansas, Lawrence, Kansas, USA
[b]University of Texas at El Paso, El Paso, Texas, USA

Objective. Peptides targeting the B7 immune signaling pathway were co-delivered with autoantigen using a novel soluble antigen array (SAgA) for the therapeutic treatment of a murine model of multiple sclerosis.

Methods. The B7 signaling pathway was targeted using adaptations of SAgA technology achieved by covalently linking B7-binding peptides and disease causing autoantigen (proteolipid peptide; PLP) to hyaluronic acid (HA). Three independent B7-targeted SAgAs were created containing peptides to either inhibit or potentially stimulate the B7 signaling pathway. The SAgAs were then characterized by high performance liquid chromatography, gel permeation chromatography, and microflow imaging. Through the use of a murine model of multiple sclerosis, experimental autoimmune encephalomyelitis (EAE), the B7-targeted SAgAs were tested for their ability to suppress disease symptoms. Following the study, primary splenocytes were isolated from EAE mice treated with B7-targeted SAgA and their cytokine profiles were investigated in response to re-stimulation with autoantigen.

Results. Surprisingly, all SAgAs were found to suppress EAE disease symptoms. Altered pro-inflammatory cytokine expression was observed in primary splenocytes isolated from SAgA-treated mice, indicating that SAgAs with different B7-binding peptides may suppress EAE through different immunological mechanisms.

Conclusions. This study demonstrates that SAgAs can successfully suppress a murine model of multiple sclerosis, EAE, through co-delivery of autoantigen and peptides targeting with the B7 signaling pathway.

P71 Synthesis and Characterization of Native and PEGylated Poly-L-Lysine Dendrimers

Yewon Joanna Pak[a], Peter Swaan[a]

[a]Department of Pharmaceutical Sciences, Center for Nanomedicine and Cellular Delivery, University of Maryland, Baltimore, MD 21201, USA

Objective. Recent exploration of dendrimers has shown that this "tree-like" polymer can be used as a drug delivery system for improved targeting to solid tumors. Commercially available poly (amidoamine) (PAMAM) dendrimers have the potential to cause toxicity in vivo due to non-biodegradability at sites of accumulation. Poly-L-Lysine (PLL) dendrimers are another class of dendrimers that possess a biodegradable structure. However, since PLL dendrimers are cytotoxic due to cationic surface charges, potential advancement of PEGylated PLL dendrimers were investigated. PEGylation of dendrimers improves retention in the blood circulation without causing toxicity to healthy cells while maintaining the ability to deliver chemotherapeutic drugs to a targeted internalization pathway in human breast cancer cells.

Methods. PLL dendrimers were synthesized through a controlled, step-wise synthesis, which included the addition of lysine groups by protecting and deprotecting BOC groups on the surface of the dendrimer. BOC-protected lysine was added in a repeated manner until the Generation 3 PLL dendrimer was synthesized. The product was PEGylated using polyethylene glycol (PEG) 2000 in order to mask the charges on the outer surface of the dendrimer. Both the PLL dendrimer and PEGylated PLL dendrimer were characterized using NMR, mass spectrometry, and size exclusion chromatography. In order to assess cytotoxicity of the PLL dendrimer and PEGylated PLL dendrimer, MCF-7 cells were used. MCF-7 cells were treated with varying concentrations of the dendrimers, and the IC_{50} for both dendrimers was measured using a WST-1 cell viability assay.

Results. Successful synthesis of PLL dendrimers and PEGylated PLL dendrimers was confirmed using NMR and mass spectrometry. The polydispersity of the two compounds showed that both dendrimers were monodisperse with polydispersity index of 1.03 and 1.04. Compared to PLL dendrimers, PEGylated PLL dendrimers show higher cell viability of almost 100% cell viability even at high concentration.

Conclusions. PEGylated PLL dendrimers can be used as an effective chemotherapeutic delivery system with no inherent cell cytotoxicity.

P72 Deposition and Stabilisation of Nanosuspensions by Flexographic Printing

Mirja Palo[a,b], Ruzica Kolakovic[a], Timo Laaksonen[c], Daniela Fors[a]
Anni Määttänen[a], Natalja Genina[a], Jouko Peltonen[a], Niklas Sandler[a]

[a]Åbo Akademi University, Finland,
[b]University of Tartu, Estonia
[c]University of Helsinki, Finland

Objective. The aim of this study was to investigate the use of flexographic printing as a deposition technique to stabilize nanosuspensions.

Methods. Aqueous nanosuspensions with indomethacin (IND) and itraconzole (ITR) were made by ball-milling (Pulverisette 7 Premium, Fritsch GmbH, Germany). Poloxamer 407 (60 wt% of the drug amount) was used as a stabilizer.

The nanosuspensions were printed on 3 different substrates – polyethylene terephthalate (PET) film, Easybake® rice sheet and Blue Dragon® rice paper. A laboratory scale printability tester (IGT Global Standard Tester 2, IGT Testing system, The Netherlands) was used to prepare formulations with the size of 0.5 cm^2.

The printed formulations were characterized with X-ray diffractometry (XRD) and scanning electron microscopy (SEM). Content analysis and dissolution studies for IND and ITR were performed with UV/Vis spectrophotometer and high performance liquid chromatography (HPLC), respectively.

Results. Nanosuspensions with IND (145.1 mg/ml) and ITR (112.2 mg/ml) were successfully produced by wet ball-milling. At least partially crystalline APIs were identified in printed formulations with XRD. Nanosuspensions were evenly distributed on the substrates after the printing. The SEM images of the flexographically fabricated samples did not reveal any agglomeration of the nanosuspensions.

The dissolution rate of the APIs from nanosuspensions and the printed samples increased compared to the dissolution rate from the raw drug substance. Printed formulations of IND and ITR on edible substrates (Easybake® rice sheet and Blue Dragon® rice paper) showed slower dissolution profiles compared to the samples printed on PET film.

Conclusions. Samples from nanosuspensions were successfully produced by flexographic printing. The printed formulations contained solid particles from nanosuspensions without any observed agglomeration. In addition, the dissolution rate of the drug from the printed formulations increased compared to the pure API. The results indicate that the approach taken can be considered as a promising fabrication method for nanoparticulate systems.

P73 *In Vitro* Device to Investigate Fate of Macromolecules Following Intravitreal Injection

Sulabh Patel[a], Jan Olaf Stracke[a], Ulrike Altenburger[a], Hanns-Christian Mahler[a], Joerg Huwyler[b], Dhananjay Jere[a]

[a]Pharmaceutical Development & Supplies, Pharma Technical Development Biologics Europe, F. Hoffman-La Roche Ltd., Basel, Switzerland.
[b]Department of Pharmaceutical Sciences, Division of Pharmaceutical Technology, University of Basel, Basel, Switzerland.

Objective. Intravitreal drug delivery is critical for the treatment of posterior segment ocular diseases such as wet-AMD, diabetic retinopathy, diabetic edema etc. Following intravitreal injections, drug distribution in the back of the eye tissues is mainly influenced by physicochemical parameters of the therapeutic agent and formulation components, and their interactions with the components of vitreous humor (VH). Not much attention has been paid to investigate these interactions, primarily due to unavailability of a reliable and representative *in vitro* model for the *in vivo* conditions. In a current set of studies, we were able to establish a novel *in vitro* non-cell based model to mimic intraocular conditions. This new tool enables us to study the fate of formulation components following intravitreal injection.

Methods. A customized multi-chamber device designed for controlled diffusion to mimic intraocular conditions like VH gel diffusion, and systemic clearance (choroidal blood flow). Diffusion control membrane cut-off was optimized to achieve desired flux, and permeability.

Results. The optimal device showed the desired retention and diffusion profile, which mimic intraocular flux, stability and permeability.

Conclusions. The *in vitro* model can act as a cost effective high throughput alternative to study *in vivo* fate of macromolecules and/or excipients following intravitreal injection.

P74 Water Vapor Barrier and Mechanical Properties of Lignified Cellulosic Thin Films

Anna Penkina[a], Anirudra Parajuli[b], Sara Fontiveros Martin[c], Osmo Antikainen[b], Maija Hakola[b], Sirpa Vuorinen[b], Timo Repo[b], Jouko Yliruusi[b], Peep Veski[a], Karin Kogermann[a] and Jyrki Heinämäki[a]

[a]University of Tartu, Estonia
[b]University of Helsinki, Finland
[c]Complutense University of Madrid, Spain

Objective. To investigate the film formation of externally lignified aqueous hydroxypropyl methylcellulose (HPMC) and evaluate the effect of lignin on water vapor permeation (WVP) and mechanical stress-strain properties of films.

Methods. Industrial softwood kraft lignin (Indulin AT) and catalytic pretreated softwood lignin (CPSL) were used for lignifying HPMC films plasticized with polyethylene glycol (PEG 400). Free films were prepared by a casting/solvent evaporation method.

The WVP of films was determined using the thin films (150-200 μm) cut to a suitable size, fixed onto the anhydrous calcium chloride ($CaCl_2$) containing glass vials and immediately tightly sealed with a thin elastic band and Parafilm® M barrier film. The glass vials were held at 23 ± 2°C and 80 ± 2% RH, and the increase in weight was measured at regular intervals.

The mechanical properties of the plasticized and lignified free films were studied by using a Lloyd LRX materials tester. Tensile strength, elongation (strain) at break, modulus of elasticity (Young´s modulus) and work done were calculated from the stress-strain curve. Glass transition temperature (T_g) of the films was determined by using a differential scanning calorimeter (DSC).

Results. The type and amount of lignin affected the WVP and mechanical stress-strain properties of HPMC films but did not change the thermal properties (T_g 97-118°C) of the films. The lignified HPMC films containing Indulin AT were mechanically stronger, tougher and more elongated than the respective films containing CPSL or the reference non-lignified films.

Conclusions. External lignification of cellulosic thin films improves water vapor barrier and mechanical properties of films.

Acknowledgements. This study is part of the targeted financing project no SF0180042s09 and ETF grant project no 7980. The work is also supported by the European Social Fund's Doctoral Studies and Internationalization Programme DoRa and the Estonian Ministry of Education and Research.

P75 Effects of Sucrose in Micro- and Pilot Scale Freeze-Drying on Secondary Protein Structures Assessed by FTIR-ATR

Björn-Hendrik Peters[a], Ferdinand Molnár[a], Jarkko Ketolainen[a]

[a]School of Pharmacy, University of Eastern Finland, Finland

Objective. Microscale freeze-drying offers strongly needed short process cycles for early stage formulation development. To investigate scale dependent effects of sucrose as a stabilizing agent, secondary structures of lysozyme formulations were assessed in comparison to common vial freeze-drying at pilot scale equipment.

Methods. The model formulations consisted of 1% lysozyme (Dalian Greensnow Egg Products, China) with sucrose (Sigma-Aldrich, USA) concentrations of 0%, 0.5%, and 1% (all w/w), respectively. All formulations were prepared with Milli-Q-Water (EMD

Millipore, USA). Microscale freeze-drying was performed on a THMS350V heating stage (Linkam Scientific, UK). For pilot scale freeze-drying a Lyostar II system (SP Scientific, USA) was utilized. Sample spectra were collected using a Nicolet 8700 FT-IR spectrometer (Thermo Fisher Scientific, USA) and consist of 256 scans at a resolution of 2 cm^{-1}. Principle component analysis (PCA) of smoothed 2nd derivative amide I spectra was performed utilizing SIMCA-P+ (Umetrics AB, Sweden).

Results. A sucrose concentration dependent shift from β-sheet (peaks at 1641.15 cm^{-1}, 1630.55 cm^{-1}) and β-turn (peak at 1689.36 cm^{-1}) structures to α-helices (peaks at 1653.69 cm^{-1}, 1652.72 cm^{-1}) was observed. Furthermore, reduced variation between micro- and pilot scale samples at increased sucrose content was indicated. Smaller differences between sample bottom and top were seen with increasing excipient content especially for pilot scale samples. Annealed samples revealed an overall high similarity to samples of the same formulation prepared by the regular freeze-drying cycle although a tendency to increased variation at the bottom part of annealed samples was noticed.

Conclusions. The results of the lysozyme model formulations indicate that stabilization efforts undertaken in the microscale environment could be transferred to pilot scale vial freeze-drying.

P76 Novel Lipopolyplexes for Gene Delivery and Gene knockdown

Shashank Reddy Pinnapireddy[a], Udo Bakowsky[a]

[a]Institute for Pharmaceutical Technology and Biopharmacy, Philipps University - Marburg, Germany.

Objective. The aim of this study was to formulate polymer and nucleic acid complexes which were further encapsulated in a lipid layer to form unique nanostructures called Lipopolyplexes. Lipopolyplexes are nanostructures intended for transfection i.e. delivery of nucleic acids coding either for specific reporter genes such as GFP (Green Fluorescence protein) or mediating knockdown. In the current study the *in-vitro* efficiency of PEI (Polyethylenimine) based polymers and lipid combinations of DOPE (1, 2-dioleoyl-sn-glycero-3-phosphoethanolamine), DPPC (1, 2-dipalmitoyl-sn-glycero-3-phosphocholine) and Cholesterol was investigated.

Methods. The Lipopolyplexes in this study were analysed for their physical characteristics, complex stability, transfection efficiency and toxicity. The characterisation of the lipid nanostructures for this study was done by Photon Correlation Spectroscopy and Laser Doppler Micro-electrophoresis. Complex stability was performed by Gel-Retardation assay and Heparin Competition Assay. Transfection efficiency of the Lipopolyplexes was evaluated using Luciferase reporter gene assay, GFP expression and siRNA mediated knockdown in various cell lines. Toxicity of the complexes was assessed by the MTT assay.

Results. The Lipopolyplexes intended for the gene delivery and knockdown were in the size range of 205 ± 5 nm and had a zeta potential of 5 mV. The results from the Gel-Retardation assay have shown that the complexes were stable and disintegrated in the presence of Heparin concentrations slightly higher than that present in the human body. Transfection efficiency reported by the reporter gene assay was around 450,000 RLU's (Relative Luminescence Units). The toxicity of the Lipopolyplexes was found to be lower in comparison with other transfection reagents used.

Conclusions. Lipopolyplexes are seen to be by far more viable transfection agents with a better toxicity profile than that of PEI and cationic Lipoplexes. Apart from a better toxicity profile they also happen to have an increased transfection efficiency which can partly be attributed to their relatively low toxicity and are also stable over a longer period of time.

P77 Investigating the Whole Brain Distribution of Macromolecules Administered into the Cerebrospinal Fluid

Michelle E. Pizzo[a,b], Daniel J. Wolak[a], Robert G Thorne[a,b,c,d,e]

[a]Division of Pharmaceutical Sciences, University of Wisconsin-Madison School of Pharmacy, USA
[b]Clinical Neuroengineering Training Program,
[c]Neuroscience Training Program,
[d]Cellular and Molecular Pathology Graduate Training Program, & the
[e]Institute for Clinical and Translational Research, University of Wisconsin-Madison, USA

Objective. The aim of this work is to investigate the whole brain distribution and mechanisms of distribution of potentially therapeutic macromolecules administered into the cerebrospinal fluid.

Methods. Fluorescently labeled macromolecules of various sizes were infused into the cisterna magna of anesthetized rats for approximately one hour. Ex vivo fluorescence was used to determine distribution in the whole brain and in slices. Intensity profiles normal to the brain surface were used to estimate effective diffusion coefficients (D^*) for each molecule in brain and were compared to measurements using integrative optical imaging (IOI), a highly validated method for determining D^* in brain tissue after intraparenchymal pressure injection.

Results. The olfactory bulbs and perivascular space (PVS) of large caliber surface arteries showed high fluorescence for all molecules. However, smaller molecules (3 kDa dextran, 50 kDa antibody fragment) showed diffuse signal around vessels, while larger molecules (e.g., 150 kDa antibody) appeared confined to the PVS. Slices showed apparent diffusion across the brain surface and PVS signal even in deep brain

regions. Diffusion analysis of surface slice data yielded D^* estimates similar to IOI values for smaller molecules, but larger molecules diffused more slowly than expected from IOI values.

Conclusions. Diffusion into the brain varied between areas, possibly due to regional differences in barrier properties of the pia mater and glia limitans at the brain surface. Our diffusion analysis of slice data suggests that larger molecules may be more hindered by these barriers than small molecules. Our results also confirm that fast, convective flow within the PVS plays a significant role in whole brain distribution. Larger molecules appeared more confined to the PVS, suggesting size selectivity of the interface between the PVS and adjacent parenchyma. Our findings suggest a complex combination of diffusive and convective transport determines macromolecule distribution in the brain following central input.

P78 Therapeutic Cancer Vaccine Based on Polymeric Nanoparticles Containing HPV Synthetic Long Peptide and Poly IC

Sima Rahimian[a], Marieke F. Fransen[b], Jan Willem Kleinovink[b], Jonathan Riis Christensen[a], Maryam Amidi[a], Ferry Ossendorp[b] and Wim E. Hennink[a]

[a]Department of Pharmaceutics, Utrecht Institute for Pharmaceutical Sciences (UIPS), Utrecht University, The Netherlands
[b]Department of Immunohematology and Blood Transfusion, Leiden University Medical Center, The Netherlands

Objective. The aim of current study is to design a cancer vaccine against human papillomavirus (HPV)-associated cancer by using polylactide-co-hydroxymethylglycolide (pLHMGA) nanoparticles (NPs) containing a synthetic long peptide (SLP) derived from HPV16 oncoprotein and a TLR3 ligand (poly IC), as an adjuvant.

Methods. HPV SLP ± poly IC loaded NPs were prepared by double emulsion solvent evaporation technique. Particle size and morphology were characterized by DLS and TEM. Loading efficiency of HPV SLP and Poly IC were measured by HPLC and Quantifluor RNA assay, respectively. The therapeutic efficacy of NP vaccine was evaluated in tumor bearing mice. Upon vaccinations, the level of HPV specific CD8+ T cells in the systemic circulation was determined using tetramer staining assay and the tumor size was monitored in time.

Results. NPs were spherical with a size ranging from 400-500 nm. Loading efficiency of HPV SLP and poly IC in the nanoparticles was around 50%. HPV SLP + poly IC NPs and HPV SLP NPs + soluble poly IC substantially suppressed the tumor growth in mice. HPV specific CD8+ T cells were readily detectable in mice immunized with any combination of HPV NP and poly IC, regardless of poly IC being in the same particle (co-encapsulated) or in soluble form, while HPV SLP in soluble form or in IFA ± Poly IC exhibited significantly lower levels of CD8+ T cells.

Conclusions. HPV SLP nanoparticles could enhance the tumor specific T-cell response when combined with poly-IC, as compared to soluble SLP + poly IC. Tumor growth was significantly suppressed using NPs + poly IC. This suggests that pLHMGA nanoparticles are suitable candidates as cancer vaccines.

Acknowledgement. This research was performed within the framework of Cancer Vaccine Tracking project (#030-302), center for translational molecular medicine (CTMM).

P79 siRNA Delivery to the Retinal Pigment Epithelium

Eva Ramsay[a], Sarah Hehir[b], Sander Groenen[b], Timo Stukenkemper[b], Sanjay Sarkhel[a], Marika Ruponen[c], Neil Cameron[b] and Arto Urtti [a,c]

[a]Centre for Drug Research, University of Helsinki, Finland
[b]Department of Chemistry, Durham University, UK
[c]School of Pharmacy, University of Eastern Finland, Finland

Objective. Inhibition of IL-6 secretion, by small interfering RNA (siRNA) seems as a promising strategy for the treatment of inflammation related retinal diseases. However, drug delivery systems are needed to improve poor biopharmaceutical properties of siRNA. A novel block copolymer, based on poly(benzyl-l-glutamate) and poly-l-lysine (PLE_{30}-b-PK_{30}) was studied for siRNA delivery to the retinal pigment epithelium (RPE).

Methods. Polyplexes of PLE_{30}-b-PK_{30} and siRNA were formed at varying charge ratio (N/P) and siRNA binding was determined by an agarose electrophoresis gel. The size and zeta potential of the polyplexes were measured by DLS and zetasizer, respectively. The IL-6 silencing effect was studied by exposing ARPE-19 cells for polyplexes for 4-5 hours and IL-6 concentrations were measured after further incubation of 24-72 hours by an ELISA kit. The cytotoxicity of the polyplexes was analyzed by the MTT assay. The cellular uptake efficiency of the polyplexes, using fluorescently labelled siRNA, was studied after a 4 hour exposure by employing flow cytometer analysis. Lipofectamine was used as a control carrier.

Results. For transfection studies polyplexes of charge ratio 6/1-9/1, resulting positive surface charge and a mean size of around 50 nm were used. After 48-72 hours the polyplexes silenced IL-6 expression up to 30% in comparison to 50% silencing effect of Lipofectamine. The polyplexes did not cause cell death and they were found to be taken up by the cells efficiently.

Conclusions. The PLE_{30}-b-PK_{30} block copolymer seems as a promising carrier for siRNA and for the delivery to the RPE. Further modifications of the carrier are needed, in order to improve the siRNA delivery

properties.

P80 Ocular Melanin Binding: *In Vitro* Binding Studies Combined to a Pharmacokinetic Model

Anna-Kaisa Rimpelä[a], Heidi Kidron[a], Arto Urtti[a]

[a]Centre for Drug Research, Division of Pharmaceutical Biosciences, University of Helsinki, Finland

Objective. As melanin binding affects a drug's pharmacokinetics and effect in ocular tissues, it should be considered in ocular drug delivery. The aim was to study melanin binding of drugs *in vitro* and cellular kinetics of the drugs in pigmented retinal pigment epithelial (RPE) cells. A kinetic model of melanin binding and cellular kinetics was built to get a better understanding of pigment binding in cellular drug delivery.

Methods. The *in vitro* melanin binding of drugs was studied using isolated melanin from porcine RPE and choroid. The studies were done at pH 7.4 and pH 5 to mimic the acidic environment of the cellular melanosomes. The compounds chosen for the study; nadolol, timolol, chloroquine, methotrexate, carboxydichlorofluorescein (CDCF) and dexamethasone, are small molecules with diverse physicochemical properties (octanol/water partitioning coefficient (logP), pK_a, acid/base status). Primary porcine RPE cells were used to study the amount of uptake of the set of compounds. The kinetic model was constructed and simulated with STELLA® software.

Results. All the basic compounds bound to melanin *in vitro*. The acidic compounds did not seem to bind at pH 7.4 but did bind at pH 5. Chloroquine, as expected, showed the highest binding levels. The cellular uptake of chloroquine was significant, at least partly due to melanin binding, whereas the other compounds were taken into the cells to a much smaller extent. The kinetic simulations with the binding parameters resulted in a good match with the experimental results of the cell study.

Conclusions. The results of the *in vitro* studies and the simulation model give a good idea of the importance of melanin binding in ocular drug delivery. The model can be used in the future as a base for more comprehensive models of the effect of melanin binding on ocular pharmacokinetics.

P81 The Anti-Inflammatory Activity of a Lead Fused-Cyclopentenone Phosphonate Compound and its Potential in the Local Treatment of Experimental Colitis

Dorit Moradov[a], Helena Shifrin[a], Efrat Harel[a], Mirela Nadler-Milbauer[a], Morris Srebnik[a] and Abraham Rubinstein[a]

[a]The Hebrew University of Jerusalem, Faculty of Medicine, School of Pharmacy Institute for Drug Research, P.O. Box 12065, Jerusalem 91120, Israel

Purpose. To screen a series of previously reported fused-cyclopentenone phosphonates in order to identify a lead compound and to explore its possible mode of action.

Methods. The ability of the compounds to reduce secreted TNFα levels by LPS-activated mouse peritoneal macrophages, as well as their cytotoxicity (MTT) in increasing concentrations was compared. A lead compound, namely, diethyl 3-nonyl-5-oxo-3,5,6,6a-tetrahydro-1H-cyclopenta [c]furan-4-ylphosphonate ("P-5") was identified. P-5 effect on IL-6, INFγ, MCP-1, IL-1α, MIP-1α and RANTES levels was tested in vitro, followed by an *in vivo* analysis in a colitis-induced rat model. Inflammation severity quantification was assessed macroscopically and by measuring tissue MPO and iNOS activity and TNFα and IL-1β levels. The levels of p38, IκBα and ERK and their phosphorylation products p-p38, p-ERK was compared by Western blot.

Results. The longer the aliphatic side chains on the furan ring of the tested fused-cyclopentenone phosphonates, the better their anti-TNFα activity. P-5 reduced TNFα, IL-6, IL-1α, INFγ, MCP-1, MIP-1α and RANTES in LPS-activated macrophages. Moreover, it was effective in the local treatment of experimental colitis in the rat, where it inhibited mucosal MPO activity, reduced expression of iNOS and decreased mucosal levels of TNFα and IL-1β.

Conclusions. Although not having an inhibitory effect on human recombinant TACE, P-5 could be used for the spatial therapy of IBD (e.g. by colon-specific drug platforms). Its mode of action involves MAPKs through the ERK pathway but not through p38 and with no effect on IκBα, probably, with no effect on the expression of the NF-κB transcription factor.

P82 Label-free CARS Imaging of Intestinal Epithelial Cells

Jukka Saarinen[a], Antti Isomäki[b], Erkan Sözeri[a], Hélder Santos[a], Clare J Strachan[a]

[a]Division of Pharmaceutical Chemistry and Technology, Faculty of Pharmacy, University of Helsinki, Finland
[b]Biomedicum Imaging Unit, Faculty of Medicine, University of Helsinki, Finland

Objective. To investigate the potential of CARS microscopy for live imaging of cells that are used to mimic the intestinal epithelium.

Methods. Human adenocarcinoma Caco-2 and HT-29 cells were cultured in 25 cm^2 culture flasks. The medium used was high glucose Dulbecco's Modified Eagle Medium (DMEM) with 10% of heat inactivated fetal bovine serum, 1% penicillin and streptomycin, 1% non-essential aminoacids and 1% L-glutamine.

For imaging cells were seeded on collagen-coated PTFE Transwell® inserts with a density of 20000 cells / 0.33 cm^2 per insert.

Cells were imaged using a Leica TCS SP8 CARS microscope. It consists of a Leica DMI inverted microscope with two forward- and two epi-directed PMT detectors and a picoEMERALD solid-state-laser light source (APE GmbH). A heating chamber with controlled temperature and CO_2 was also integrated into the system. PTFE Transwell® inserts were placed on a coverslip while imaging using an HCX IR APO L 25×/0.95 W water immersion microscope objective. CARS signals between 2700 and 3000 cm^{-1} were recorded.

Results. Structures in both Caco-2 and HT-29 cells were visible. The strongest signal at 2860 cm^{-1}, representing CH_2 stretching, revealed lipid droplets and cell membranes in both cell types. The lipid droplet size and distribution were different between cell types.

Conclusions. CARS microscopy is well suitable for label-free live imaging of cell cultures used to mimic the intestinal epithelium, which could in the future be extended to label-free *in situ* imaging for studying cell-nanoparticle interactions.

P83 Effectiveness of Bulb Extracts of *Allium* Species on Some Selected Plant Pathogenic Fungi

Sahar Samadi[a], Michael Keusgen[a]

[a]Institute of Pharmaceutical Chemistry, Philipps-Universität Marburg, Germany

Objective. *Allium* volatile compounds like allicin, further thiosulphinates and their transformation products of selected species from Southwest and Middel Asia were subjects of this investigation. Antifungal activities (MIC) of *A. ampeloprasum* L., *A. atroviolaceum* Boiss., *A. cepa* L., *A. cepa* L. var. *aggregatum* G. Don, *A. darwasicum* Regel, *A. hollandicum* R.M.Fritsch, *A. jesdianum* Boiss. & Buhse, *A. jesdianum* Boiss. & Buhse subsp. *angustitepalum* (Wendelbo) F.O.Khass. & R.M.Fritsch, *A. karataviense* Regel, *A. macleanii* Baker, *A. moly* L., *A. nevskianum* Vved., *A. oschaninii* B.Fedtsch., *A. rosenorum* R.M.Fritsch, *A. rotundum* L., *A. sativum* L., *A. scorzonerifolium* Redouté, *A. stipitatum* Regel, *A. talassicum* Regel and miconazole (positive control) on *Aspergillus flavus*, *A. niger*, *Penicillium digitatum*, *P. italicum* and *Mucor hiemalis* were investigated.

Methods. Dilution series of ethyl acetate extracts obtained from *Allium* bulbs were tested on all the above mentioned fungi using PDA micro-dilution susceptibility testing method, disk diffusion method and double-dish chamber.

Results. Extracts of *A. stpitatum* showed the highest antimicrobial effect against all the tested fungi (MIC ≥ 0.53g/ml; related to the fresh bulb weight) followed by *A. sativum* (MIC ≥ 0,54g/ml), *A. ampeloprasum* (MIC ≥ 0,85g/ml) and then *A. karataviense* (MIC ≥ 1,53g/ml). The MIC of miconazole as a control was ≥0.27mg/ml. From the fungal point of view, *P. italicum* showed the highest susceptibility, while *M. hiemalis* and *A. flavus* demonstrated more resistancy towards *Allium* extracts and miconazole.

Conclusions. The results indicate that extractions of *Allium* spp. have antifungal activity and might be promising, at least, in 'biological' treatment of fungal-associated plant diseases. Raw extracts were investigated. Identification of active substances is ongoing.

P84 Influence of the Surface Modifications on the Properties of Nanoparticles

Jens Schäfer[a], Udo Bakowsky[a].

[a]Department of Pharmaceutics and Biopharmacy, Philipps University Marburg, Germany

Objective. The modification of carrier systems with polyethylene glycol chains is a well-known tool to protect them from macrophages. This so called "stealth"-effect as a surface modification is one example for changing the surface properties of nanoparticles or other systems. Additionally the modification of the surface with cationic molecules can change the surface charge for optimizing the complexation properties for nucleic acid (NA) whereas functionalizing of the systems can be achieved with proteins or sugars

Therefore, we focus on the development of new drug delivery systems with surface modifications and the characterization of nanoscaled systems.

Methods. Biodegradable PLGA/chitosan nanoparticles were prepared by a emulsion- diffusion–evaporation technique and modified with different lipid

compositions. The particles were physicochemical characterized with DLS, LDA and AFM and tested *in-vitro.*

Results. Surface modification of biodegradable particles with different lipid compositions change the properties like zeta potential as well as the lipophilic behavior of the developed systems. Depending on the surface modifications, these systems showed new qualities compared to the parent system.

Conclusions. The modification of biodegradable nanoparticles or other system with lipids can help us to identify the needed surface properties for specific drug delivery systems.

P85 Stereoselective Anticonvulsant and Pharmacokinetic Analysis of Valnoctamide, a CNS-Active Derivative of Valproic Acid with Low Teratogenic Potential

Tawfeeq Shekh-Ahmad[a], Naama Hen[a], Boris Yagen[a], John H. McDonough[b], Richard H. Finnell[c], Bogdan J. Wlodarczyk[c], Meir Bialer[c]

[a]School of Pharmacy, Institute for Drug Research, The Hebrew University of Jerusalem, Israel.
[b]Pharmacology Branch, Research Division, US Army Medical Research Institute of Chemical Defense, Maryland, USA.
[c]Department of Nutritional Sciences, Dell Pediatric Research Institute, The University of Texas at Austin, USA

Objective. Valnoctamide-(VCD), a CNS-active chiral constitutional isomer of valpromide (VPD), the corresponding amide of valproic acid (VPA), exhibits stereoselective pharmacokinetics-(PK) in animals and humans. The current study comparatively evaluated the pharmacodynamics (PD; anticonvulsant activity and teratogenicity) and PK of VCD four individual stereoisomers.

Methods. The anticonvulsant activity of VCD individual stereoisomers was evaluated in several rodent anticonvulsant models including: maximal electroshock, 6Hz psychomotor, subcutaneous metrazol and the pilocarpine and soman-induced status epilepticus (SE). The PK-PD relationship of VCD stereoisomers was evaluated following ip administration-(70mg/kg) to rats.

Results. VCD had a stereoselective PK with (2S,3S)-VCD exhibiting the lowest clearance and consequently, a twice-higher plasma exposure than all other stereoisomers. However, there was less stereoselectivity in VCD anticonvulsant activity and each stereoisomer had similar ED_{50} values in most models. Nevertheless, in the MES (Rat-ip) model, 2R,3S)-VCD and (2R,3R)-VCD (ED_{50}= 43 mg/kg) were found to be more active than the other two stereoisomers and the racemate (ED_{50}= 55-73 mg/kg). In the scMet (Rat-po) model, (2R,3S)-VCD was the most active compound (ED_{50}= 11 mg/kg) compared to the other three individual stereoisomers and the racemate. VCD stereoisomers (258 or 389 mg/kg) did not cause NTD. These doses are 3-12 times higher than VCD-anticonvulsant-ED_{50} values.

Conclusions. VCD displayed stereoselective PK that did not lead to significant stereoselective activity in various anticonvulsant rodent models. If VCD exerted its broad-spectrum anticonvulsant activity using a single mechanism of action (MOA) it is likely that it would exhibit a stereoselective PD. The fact that there was no significant difference between racemic-VCD and its individual stereoisomers, suggests that VCD's anticonvulsant activity is due to multiple MOA.

P86 Strategies to Improve the Absorption of Biopharmaceutical Classification (BCS) Class IV Drugs – A Paclitaxel Case Study

Ramesh Soundararajan[a], Kenji Sasaki[a], Andreas Schatzlein[a,b], Ijeoma Uchegbu[a,b].

[a]UCL School of Pharmacy, United Kingdom
[b]Nanomerics, United Kingdom

Objective. To examine the effect of both drug dissolution and the P-glycoprotein (P-gp) efflux pump on the oral pharmacokinetics of a hydrophobic drug.

Methods. The poorly aqueous soluble model drug, paclitaxel, was encapsulated within N-(2-phenoxyacetamide)-6-O-glycolchitosan (GCPh) nanoparticles. Male MF1 mice (22-35g) were administered paclitaxel formulations, at low (6.66mgkg^{-1}, 10 mgkg^{-1}) and/ or high doses (20 mgkg^{-1}) by oral gavage either in the form of high dissolving formulations (GCPh paclitaxel nanoparticles or Taxol) or as poorly dissolving formulations (paclitaxel fine nanocrystals, paclitaxel large nanocrystals or paclitaxel solid dispersion formulations). Paclitaxel was administered in the absence or presence of the P-gp efflux pump inhibitor verapamil (40mgkg^{-1}). Plasma samples were analysed for paclitaxel content by high performance liquid chromatography.

Results. Our results show a dose-dependent saturation of the P-gp pump by paclitaxel from Taxol, while the absorption from low doses of Taxol were influenced by verapamil, absorption from high doses of Taxol were unaffected by verapamil. With the paclitaxel nanocrystals and solid dispersion formulations there were low levels of absorption in the presence and absence of verapamil indicating that a low level of *in vivo* dissolution is the major factor hindering the oral absorption of BCS Class IV P-gp substrates such as Paclitaxel. Drug absorption from GCPh nanoparticles was not affected by verapamil at any dose, suggesting that alternative pathways of absorption exist when paclitaxel is formulated as GCPh micelles.

Conclusions. Paclitaxel's oral absorption is hampered to a greater extent by its poor *in vivo* dissolution than by the activity of the P-gp efflux pump and with suitable dissolution enhancers, the P-gp efflux pump may be saturated with a high dose of the drug, leading to favourable absorption kinetics. Additionally a chitosan based nanoparticle is also able to promote paclitaxel absorption by using alternative uptake mechanisms that bypass the P-gp pump.

P87 Breast Cancer Tumour Associated Macrophages Modulation by Free or Liposome Encapsulated Zoledronate

Sofia Sousa[a], Seppo Auriola[a], Jukka Mönkkönen[a], Jorma Määttä[a,b]

[a]School of Pharmacy University of Eastern Finland, Finland
[b]Institute of Biomedicine University of Turku, Finland

Objective. The current study aimed to target breast cancer (BC) tumour associated macrophages (TAM) with free and liposome encapsulated zoledronate (respectively ZOL and ZOL-LIP), in order to modulate their polarization status.

Methods. The *in vitro* polarization of J774 murine macrophages was done upon culture in 4T1 breast cancer cell-conditioned medium (4T1CM) and stimulation with LPS and ZOL or ZOL-LIP were tested prior to LPS stimulus. At the cell culture assay endpoints key macrophage M1/M2 polarization markers were analysed by qPCR, multiplex ELISA, zymography and Griess assay.

Results. In this setting 4T1CM reduced the pro-inflammatory activation of macrophages and increased the secretion of matrix metalloproteinases (MMPs). A non-cytotoxic dose ZOL-LIP enhanced the expression of iNOS, a marker of M1 activation, without diminishing the expression of M2-type markers.

Conclusions. Bone is a common site of BC metastasis, mostly because of the vicious cycle between osteoclasts and circulating BC cells which promotes osteolysis and tumour growth. Therefore, anti-resorptives like the bisphosphonate ZOL, have been used to manage BC bone metastasis. According with the tumour type, 5-50% of the tumour mass consists of TAM and increased intratumoral macrophage density correlates with poor prognosis. The majority of TAMs resemble macrophages of the M2 polarization state, generally assisting survival and progression of the tumour cells and suppressing adaptive immune responses. The classically activated M1 macrophages lead to inflammation. Without malignant mutations and being genetically stable, TAMs are less likely to develop drug resistance and are therefore good therapeutic targets.

We propose that ZOL-LIP may be suitable for altering the activation status of TAM, favouring cytotoxic immune responses. Studies with the orthotopic 4T1.luc2 Balb/c mouse model are being conducted, using ZOL and ZOL-LIP in a neoadjuvant context.

P88 Impact of Human SGLT (SLC5A) mediated Transport on Apparent Permeability Can Be Studied in the *In Vitro* DSMZ -Caco-2 Cell Model

Bente Steffansen[a], Abel Malek Laghmoch[a], Carsten Uhd Nielsen[a]

[a]Drug Transporters in ADME, Faculty of Health and Medical Sciences, University of Copenhagen, Denmark

Objective. The aim was to investigate expression and function of sodium glucose transporter(s) (SGLT) in Caco-2 cells obtained from two different cell banks.

Methods. In Caco-2 cells obtained from DSMZ and ATCC, influx of 1.6 or 3.2 µM [^{14}C] methyl-α-D-glucopyranoside ([^{14}C]α-MDG) was studied with or without Na^+ and 0.1mM phlorizin (SGLT1 inhibitor) at pH 7.4. In DSMZ, concentration dependent influx and bidirectional transepithelial transport of α-MDG was also studied. All studies were made on confluent cells grown for 19-21 days at either the bottom of plastic wells or on Transwel™ polycarbonate filter inserts. SGLT expression was investigated using RT-PCR. mRNA isolated from DSMZ cells, using specific primers towards SGLT1, SGLT2, and GAPDH.

Results. Influx of the SGLT substrate α-MDG was sodium-dependent and phlorizin-inhibitable and >100 times higher in Caco-2 cells obtained from DSMZ than ATCC, i.e. $1.48 \cdot 10^{-10}$mol·cm^{-2}·min^{-1} and $1.07 \cdot 10^{-12}$, respectively. Concentration-dependent SGLT-mediated α-MDG influx in Caco-2 cells from DSMZ was characterized by an apparent K_m–value of 1.8±0.16mM with a V_{max} of 1.4±0.46nmol·cm^{-2}·min^{-1}. The transepithelial transport of α-MDG was highly polarized with apparent A-B and B-A permeabilities of $1.5 \cdot 10^{-5} \pm 8.1 \cdot 10^{-7}$ and $1.7 \cdot 10^{-7} \pm 1.5 \cdot 10^{-8}$cm·s^{-1}, respectively. In presence of 1mM phlorizin, the A-B permeability of α-MDG was reduced to $3.2 \cdot 10^{-7} \pm 2.9 \cdot 10^{-8}$cm·s^{-1}. SGLT1/2 were both expressed at the mRNA level in Caco-2 cells from DSMZ.

Conclusions. For studying SGLT-mediated transepithelial transport and influx of drug candidates Caco-2 cells obtained from DSMZ seems to provide a good *in vitro* model for intestinal transport.

P89 Inhalable Nanocomposites for Targeted Pulmonary Delivery and Applications in Lung Cancer Therapy

Nathanael A. Stocke[a] , Heidi M. Mansour[b], Susanne M. Arnold[a], Dr. Meenakshi Upreti[a], J. Zach Hilt[a]

[a]University of Kentucky, United States of America
[b]University of Arizona, United States of America

Objective. The aim of this work is to develop a more effective treatment modality for non-small cell lung cancer patients by physically targeting the treatment to the lungs by pulmonary delivery through inhalation of dry powder nanocomposites. Here we report the synthesis of novel nanocomponents, formulation of aerodynamically adequate dry powder composites, physicochemical characterization of the materials, and *in vitro* cell studies for preliminary proof- of-concept.

Methods. Iron oxide (Fe_3O_4) magnetic nanoparticles (MNPs) were synthesized through aqueous co-precipitation of ferric and ferrous iron salts at a 2:1 molar ratio. Hydrogel nanoparticles were synthesize via thermally initiated radical polymerization in the presence of sodium dodecyl sulfate (SDS) and imbibed with cisplatin (CDDP) in an acetic acid buffer at a pH of 4.0. Spray- drying was used to formulate dry powders containing mixtures of these nanomaterials, and free CDDP, with the excipient D-mannitol. A thorough physicochemical characterization of incorporated nanocomponents as well as resulting dry powder composites was carried out using a variety of techniques. Cascade impactor studies with the Next Generation Impactor (NGI) were carried out on powders in order to examine their aerosol performance and *in vitro* cell studies were used to examine cytotoxic profiles of formulated materials on representative human lung cancer cell lines.

Results. MNPs were successfully synthesized with a hydrodynamic diameter of ~145 nm and HNPs were synthesized within a range of 50-150 nm (depending on SDS concentration). These nanocomponents were incorporated into dry powder composites through spray drying and showed excellent aerosol performance. *In vitro* cell studies showed limited toxicity of nanocomponents as well as retained cytotoxicity of CDDP.

Conclusions. These results highlight the potential of inhalable nanocomposites as an improved treatment modality in lung cancer therapy.

P90 Oxidative Stress Protection by Exogenous Delivery of rhHsp70 Chaperone to the Retinal Pigment Epithelium (RPE), a Possible Therapeutic Strategy Against RPE Degeneration

Astrid Subrizi[a], Elisa Toropainen[b], Eva Ramsay[a], Anu J. Airaksinen[c], Kai Kaarniranta[b,d], and Arto Urtti[a,e]

[a]Centre for Drug Research, University of Helsinki, Finland
[b]Institute of Clinical Medicine, University of Eastern Finland, Finland
[c]Laboratory of Radiochemistry, University of Helsinki, Finland
[d]Department of Ophthalmology, Kuopio University Hospital, Finland
[e]School of Pharmacy, University of Eastern Finland, Finland

Objective. To measure the cytoprotective effects of recombinant human Heat shock protein 70 kDa (rhHsp70) against oxidative stress and study its cellular uptake, intracellular and intraocular distribution in the retinal pigment epithelium.

Methods. Human retinal pigment epithelial cells (ARPE-19) were pre-treated with rhHsp70 for 24 h and 48 h before being exposed to 1.25 mM hydrogen peroxide. Non-treated cells served as control. We analyzed interleukin 6 secretion, cell viability, and cytolysis. Uptake and intracellular distribution of fluorescently labeled rhHsp70 were investigated with flow cytometry and confocal microscopy, respectively. Ocular distribution of radioactively labeled rhHsp70

was followed ex vivo in porcine eyes by micro SPECT/CT.

Results. After exposure to hydrogen peroxide, IL-6 secretion decreased by 36-39% when ARPE-19 cells were pre-treated with rhHsp70. Cell viability increased by 16-32%, and cell lysis, measured by the release of lactate dehydrogenase, decreased by 33-43%. ARPE-19 cells endocytosed rhHsp70 added to the culture medium and the protein was localized in late endosomes and lysosomes. Following intravitreal injection into isolated porcine eyes, we found 20% rhHsp70 in the RPE.

Conclusions. Recombinant hHsp70 protein offers protection against oxidative stress. RPE cells take up the exogenously delivered rhHsp70 and localize it in late endosomes and lysosomes. This work provides the basis for a therapeutic strategy to target aggregate-associated neurodegeneration in AMD.

P91 Effect of the Immunostimulatory Peptide EP67 on Immune Cells Critical for Adaptive Responses

Shailendra B. Tallapaka[a], Vamsi K. Karuturi[a], Pravin Yeapuri[a], Sam D. Sanderson[a], Joseph A. Vetro[a,*]

[a]Department of Pharmaceutical Sciences, University of Nebraska Medical Center, U.S.A

Objective. EP67, a novel immunostimulatory peptide derived from human complement component C5a, generates long-term humoral and cell-mediated immune responses against several immunogens but its mechanism of action is incompletely understood. Thus, the objective of this study is to more fully understand the effect EP67 on critical immune cells that are present and/or recruited to the immunization site.

Methods. Immature dendritic cells (iDC), pro-inflammatory (Mϕ-1) and anti-inflammatory (Mϕ-2) macrophages were generated by culturing human monocytes for 7 days in the presence of GM- CSF/IL-4, GM-CSF, or M-CSF respectively. The effect of EP67 on the activation/maturation and differentiation of these cells was determined by comparing the expression of activation/maturation cell surface markers by flow cytometry and the levels of chemokines and cytokines in cell culture supernatants by multiplex assay. The effect of EP67 on monocyte apoptosis/necrosis was also determined by comparing FITC-Annexin V/propidium iodide staining by flow cytometry.

Results. Compared to untreated and inactive scrambled EP67 (scEP67), EP67 did not change the expression of activation/maturation markers on iDCs, Mϕ-1, or Mϕ-2 macrophages. In contrast, EP67 induced higher levels of several immune cell chemoattractants and pro-inflammatory cytokines by iDCs and monocytes but had no effect on Mϕ-1 or Mϕ-2 macrophages. EP67 also increased the survival of monocytes, accelerated the differentiation of monocytes into iDCs and Mϕ-1. Mϕ-2 macrophages generated in the presence of EP67, compared to untreated and scEP67, expressed higher levels of CD163 (scavenger receptor) and CCR7 (lymph node homing marker) suggesting an increased potential for antigen clearance/transport to lymph nodes.

Conclusions. These results suggest that EP67 initiates adaptive immune responses by initially stimulating iDCs present at the immunization site to induce the release of chemokines/cytokines that recruit PMNs, monocytes, macrophages, and additional DC, leading to the formation of an immunostimulatory microenvironment that amplifies immune responses against the immunogen.

P92 Redispersible Microspheres Composed of Nanoparticles for Pulmonary Application

Afra Torge[a], Julia Rinas[a], Marc Schneider[a]

[a]Pharmaceutics and Biopharmacy, Philipps University Marburg, Germany

Objective. The inhalative antibiotic treatment of *Pseudomonas aeruginosa* infections in cystic fibrosis patients is still limited by the barrier formed by mucus and bacterial biofilm. With the use of nanoparticles a better penetration into these barriers and a higher effectiveness of the antibiotics is expected.

Easy application of the nanoparticles via dry powder inhaler is envisaged by embedding nanoparticles into redispersible microparticles for controlled deposition.

Methods. PLGA was used as biodegradable and biocompatible material for the nanoparticles, which were prepared by single emulsion method. For fluorescence detection they were labelled with Coumarin-6. Prior to spray drying, the nanosuspension was added to a mannitol solution containing Rhodamine-B. By spray drying (Nano B-90 spray dryer) with different parameters, the PLGA nanoparticles were successfully embedded in a mannitol matrix, forming microspheres. By SEM and CLSM imaging the morphology of those particles and the distribution of the nanoparticles within the microparticles was analyzed. Redispersibility of the microspheres was investigated by SEM imaging after exposure to 100 % relative humidity and 37 °C, simulating conditions in human lung.

Results. The spray dried microspheres exhibited smooth surfaces and homogeneously distributed nanoparticles. Furthermore, the particles turned out to be hollow or at least not completely filled. The microparticles were shown to disintegrate in nanoparticles when exposed to 100 % relative humidity.

Conclusions. By spray drying, nanoparticles can be embedded in microspheres, which can easily disintegrate in nanoparticles again in lung conditions.

By encapsulation of antibiotics into the nanoparticles, these microspheres might be used as a drug delivery system improving inhalative treatment of *Pseudomonas aeruginosa* infections of cystic fibrosis patients.

Acknowledgements. The BMBF is thanked for funding the FiDel-project (Fibrose Delivery, FKZ: 13N12530).

P93 The Advantage of Correlative Microscopy for Macrophage Uptake Studies with Non- Spherical Particles

Clemens Tscheka[a], Marius Hittinger[a], Claus-Michael Lehr[b], Nicole Daum[b], Marc Schneider[a]

[a]Pharmaceutics and Biopharmacy, Philipps University Marburg, Germany.
[b]Helmholtz Institute for Pharmaceutical Research Saarland (HIPS), Helmholtz Center for Infection Research (HZI), Germany

Objective. Phagocytosis is a vital mechanism for clearance of foreign material and senescent cells from the body; their geometry influences the mode of internalization and the residence time. Precise analytical techniques are required in order to gain a sound understanding of phagocytosis. Drug carrier systems face phagocytosis as a clearance mechanism; macrophage uptake is the most relevant mechanism in the deep lung, for instance. Usually different populations of macrophages need to be used for qualitative and quantitative analysis, because different techniques are applied. Correlative light and electron microscopy (CLEM) synergistically combines fluorescence light microscopy (FLM) and scanning electron microscopy (SEM) on the very same position of the sample to analyze identical cells, both qualitatively and quantitatively. We applied cylindrical particles that have the dimension to proceed to the deep lung and quantified the macrophage uptake. The cylindrical particles serve as a model for a fibrous drug delivery system.

Methods. Cylindrical particles composed of 500 nm silica beads (blue fluorescence) were interconnected with agarose, conserving the shape of the template pores (2x10 µm). Track- etched membranes with their homogeneous, cylindrical pore in high abundance serve as templates. The system Shuttle & Find™ by Carl Zeiss™ was used for correlative microscopy.

Results. The shape dependent uptake mechanism of cylindrical particles by macrophages could be confirmed; solely uptake from the pointy side could be observed. CLEM has proven to be a precise and fast technique for the analysis of particulate uptake. In comparison to the techniques on which it is based (SEM and FLM) a considerable time-dependent misinterpretation could be corrected.

Conclusions. CLEM represents a valuable addition to the toolbox of analytical techniques used for uptake studies, combining the advantages of FLM and SEM. Identical cells are analyzed, increasing the security of the findings. In conventional approaches different populations of cells are statistically compared.

P94 Hyperspectral Imaging in Quality Control of Inkjet Printed Pharmaceutical Dosage Forms

Hossein Vakili[a], Ruzica Kolakovic[a], Natalja Genina[a], Niklas Sandler[a], Mathieu Marmion[b], Harri Salo[b]

[a]Pharmaceutical Sciences Laboratory, Department of Biosciences, Åbo Akademi University, Finland
[b]Specim Spectral Imaging Ltd., Oulu, Finland

Objective. The aim of the study was to investigate the dosing accuracy of inkjet printing in deposition of a pharmaceutical ink solution as a function of number of printed layers and further to investigate NIR hyperspectral imaging method in quality control of such printed dosage forms.

Methods. *Inkjet printing:* A Canon iP3600 printer cartridge was modified by replacing the commercial ink with a solution of anhydrous theophylline in glycerol:water:ethanol (10:45:45 vol%). Dose escalation samples were prepared printing 1-32 layers on top of a planar paper susbtrate. *Assay by UV spectrophotometry:* All measurements were performed at 213 nm wavelength using a Cary 50 UV-Vis Spectrophotometer (Varian Medical Systems, Inc., Palo Alto, California, USA). *NIR Hyperspectral Imaging Measurements:* All the measurements were performed using a SisuCHEMA hyperspectral imaging instrument (SPECIM, Spectral Imaging Ltd., Oulu, Finland).

Results. The data indicated the ability of inkjet printing to deposit precise doses of 7.52 µg cm^{-2} of API onto the paper substrate under each printing pass. The predicted API content of by NIR hyperspectral imaging method resulted in less than 5% error of prediction as compared to the real content measured by UV spectrophotometry for samples with higher than 5 passes under the print head (35 µg cm^{-2}) and less than 15% error of prediction for samples with lower API content. The larger error was a result from the background noise from the substrate.

Conclusions. From a drug manufacturing perspective the flexible and continuous manufacturing process of inkjet printing opens up interesting possibilities for accurate dosing of API for individualized therapeutic needs of patients such as in pediatrics and geriatrics. It was also shown that NIR hyperspectral imaging can be used as a rapid and non-destructive method to quantify the drug content and spatial distribution of the API on planar printed samples.

P95 Effect of Alzheimer's Disease on the Gene Expression of Drug Transporters and Tight Junction Proteins in Brain

Kati-Sisko Vellonen[a], Katja Kanninen[b], Anthony R. White[c], Tarja Malm[b], Jari Koistinaho[b], Marika Ruponen[a]

[a]School of Pharmacy, Faculty of Health Sciences, University of Eastern Finland, Kuopio Finland
[b]A.I.Virtanen Institute for Molecular Sciences, University of Eastern Finland, Kuopio, Finland
[c]Department of Pathology, The University of Melbourne, Parkville, Victoria, Australia

Objective. The proper functioning of the capillary endothelium of the blood-brain barrier (BBB) is essential for maintaining brain homeostasis and protection against penetration of harmful agents. In neurodegenerative diseases such as Alzheimer's disease (AD) the tightness and expression of transporters on the BBB may be impaired and thus alter the access of compounds to the brain. The aim of this study was to quantify the expression of genes that are important in brain pharmacokinetics in transgenic mouse models of AD.

Methods. Gene expression of 20 drug transporters and four tight junction proteins was measured by RT-qPCR in brain tissues (hippocampus, cortex and cerebellum) of aged APP/PS1, ApdE9 and Tg2675 mice (12-16 months) and in brain microvessels isolated from 3-10 week old APdE9 mice and their wild type (wt) controls.

Results. The differences in mRNA expression of drug transporters and tight junction proteins between AD samples and wt controls were minor in the brain tissues. However, the preliminary results from isolated brain microvessels showed that expression of influx transporters Slc22a8 and Slco1a4, efflux transporters Abcb1a, Abcc4 and Abcg2, and tight junction proteins Ocln and Cldn5 were over 2-fold lower in AD mice in comparison to wt controls. Interestingly, the same reduction was seen also for endothelial cell marker Pecam1, which expression in AD samples was only a third of the control. There were also evident differences (>2-fold) in the expression levels of various transporters and tight junction proteins between brain regions of healthy mice.

Conclusions. The brain microvessels of very young AD mice have deviations in the gene expression pattern of drug transporters and tight junction proteins. Reduced expression of these proteins may alter the passage of drugs into the AD brain. Further studies are needed to confirm the changes both at protein and functional level.

P96 Investigation of Inkjet Printed Formulations to Improve the Dissolution Rate of Indomethacin

Henrika Wickström[a], Ruzica Kolakovic[a], Anni Määttänen[c], Kristian Gunnelius[c], Petri Ihalainen[c], Natalja Genina[a] Korbinian Löbmann[b], Thomas Rades[b], Niklas Sandler[a]

[a]Pharmaceutical Sciences Laboratory, Åbo Akademi University, Finland,
[b]Department of Pharmacy, University of Copenhagen, Denmark
[c]Laboratory of Physical Chemistry, Åbo Akademi University, Finland

Objective. One purpose of this study was to investigate inkjet-printing behavior of different indomethacin (IMC) formulations. The main aim was to investigate the potential to create co- amorphous systems using inkjet technology as a means to increase the dissolution rate of the poorly soluble model drug.

Methods. A piezoelectric inkjet printer (PixDro LP 50) was used to print 1x1 cm^2 squares onto a paper substrate with the inks containing IMC and other formulation components such as l- arginine or polyvinylpyrrolidone (PVP). Dissolution properties and the dose accuracy were studied. IR spectroscopic analysis and scanning electron microscopy imaging were also done to identify the solid state of the drug.

Results. It was possible to identify optimal parameters for printing that affected the droplet formation and the printing quality to allow high dose accuracy. Increased dissolution rates were found for all printed samples as compared to the dissolution rate of the raw material. However, it still remained unclear if the increased drug release was due to the even and spatially accurately separated distribution of the drug substance on the substrate alone, the conversion of crystalline IMC to the amorphous counterpart or interactions between components of the printed system. The drug was identified in all the samples by spectroscopic methods but the accurate determination of solid state form of the drug was not possible due to the interference with the spectra of the substrate.

Conclusions. Formulations containing the poorly soluble drug IMC were successfully prepared and distributed on a substrate by the piezoelectric inkjet printing technology. The printed drug formulations showed high content uniformity and increased drug dissolution rates. The approach taken is promising in formulation of poorly soluble drugs. Further studies are needed to understand the properties and the interactions between the components of the printed system.

P97 Non-labeled *In Vitro* Approaches for Assessment of Targeting and Cell Uptake Efficacy of Liver targeted Liposomes

Tapani Viitala[a], Mohammed Aburaia[a,b], Alma Kartal-Hodzic[a], Timo Oksanen[a], Marjo Yliperttula[a]

[a]University of Helsinki, Faculty of Pharmacy, Department of Pharmaceutical Biosciences, Centre for Drug Research, Helsinki, Finland
[b]Al-Azhar University, Faculty of Pharmacy (Boys), Department of Pharmaceutics and Ind. Pharmacy, Cairo, Egypt

Objective. The aim of the work was to utilize two surface-sensitive label-free techniques in combination with cell model membranes and cell monolayers for developing new *in vitro* methods for assessing the targetability and cell uptake efficacy of silymarin loaded liver targeted liposomes.

Methods. The quartz crystal microbalance (QCM) technique was used to study cell surface interactions between silymarin loaded liposomes with both synthetic supported lipid bilayer membrane models and lipid bilayers containing a mixture of synthetic lipids and extracted HepG2 cell membranes. The surface plasmon resonance technique (SPR) in combination with living cell sensing was used for studying the real-time interaction and cell uptake kinetics of silymarin loaded liposomes by intact HepG2 cells.

Results. QCM measurements revealed that the targeting formulations adsorbed to a larger extent to the biomimetic membrane models containing either a terminal lactosyl group or the extracted HepG2 cell membrane when compared to the non-targeted formulations. The real-time SPR responses measured during the interaction of liposome formulations with HepG2 cell monolayers could be fitted to a kinetic model describing the cell uptake of the liposome formulations. Both the QCM and SPR results correlated well with *in vitro* cell uptake studies performed in well plates. The *in vitro* cell uptake, QCM and SPR studies allowed us to choose the most promising candidate as a liver targeting liposome for a pilot *in vivo* pharmacokinetic study, which showed that the most promising candidate formulation was removed from blood circulation within 4 hrs while the reference formulation remained in plasma for up to 48 hrs.

Conclusions. Our results demonstrates that the developed QCM and SPR based *in vitro* approaches are promising tools for assessing targeting and cell uptake efficacy during the development of NP based drug delivery systems, as well as for screening of the most promising candidates for *in vivo* studies.

P98 Characterization of Highly Supersaturated Solution of Enzalutamide: A Study of Liquid- Liquid Phase Separation of A Lipophilic Compound

Venecia Wilson[a], Kevin J. Edgar[b], Lynne S. Taylor[a]

[a]Purdue University, United States of America
[b]Virginia Polytechnic Institute and State University, United States of America

Objective. Liquid-Liquid Phase Separation (LLPS) is a phenomenon that is observed when a highly supersaturated solution forms aggregates of a drug-rich phase in a drug-lean phase when a certain concentration is exceeded. Addition of more drug will not result in higher concentration of free drug, but rather facilitate the formation of more drug-rich aggregates. The objective of this study was to characterize the phase behavior of a highly lipophilic drug ,enzalutamide, and investigate the use of additives to inhibit the crystallization from supersaturated solutions.

Methods. The theoretical amorphous solubility was estimated using the Hoffman equation, the melting properties of the drug, and the crystalline solubility. The crystalline solubility was determined using high performance liquid chromatography (HPLC.) The concentration where drug-rich aggregates form was investigated using fluorescence, ultraviolet (UV) spectroscopy, and nuclear magnetic resonance (NMR). Crystallization induction time experiments were performed using UV spectroscopy.

Results. The amorphous solubility of enzalutamide was estimated to be around 25 times higher than the crystal solubility. The concentration where drug-rich aggregates were formed was found to be in close agreement with the estimated amorphous solubility value. In the absence of additives, enzalutamide was observed to crystallize within about 10 minutes. Cellulose derivatives were found to be effective crystallization inhibitors.

Conclusions. The phase behavior of highly supersaturated enzalutamide solutions was found to be complex. At moderate supersaturations, only crystallization occurred, while at higher supersaturations, beyond the amorphous solubility, the system first formed drug-rich aggregates through the process of liquid liquid phase separation and then crystallized. Polymers were found to influence the kinetics of the crystallization process.

P99 Dynamic Combinatorial-Mass Spectrometry Leads to Potent and Selective Inhibition of Nucleic Acid Demethylase

Esther Woon[a], Joel Toh[b], Lingyi Sun[a], Lisa Lau[a], Joanne Low[a], Colin Tang[a], Yong-gui Gao[b]

[a]Department of Pharmacy, National University of Singapore, 18 Science Drive 4, 117543, Singapore
[b]Institute of Molecular and Cell Biology, 61 Biopolis Drive, 138673,Singapore

Objective. N-Methylation of DNA/RNA is of significant biological and clinical interest, and can be found in the genomes of diverse organisms. Some of these modifications are damaging lesions which can lead to mutations, others are enzyme catalyzed, and have critical roles in cell biology. N-methylation of nucleic acid can be directly reversed by the AlkB subfamily of enzymes, of which the *Escherichia coli* AlkB is the first to be identified. Nine human homologues (ALKBH1-8 and FTO) have since been reported, many of which employed Fe(II) and 2-oxoglutarate (2OG) to bring about demethylation of N-methylated DNA/RNA substrates.

Despite some physiological connections with these nucleic acid demethylases, notably for FTO, which is unequivocally linked to obesity, their regulatory roles remain unclear. We are therefore interested in the development of small molecule probes that target their catalytic domains to enable mechanistic and functional studies.

Methods. We applied a combined approach employing dynamic combinatorial chemistry and non-denaturing ESI-mass spectrometry (DCMS), which led to the identification of inhibitors that are selective for specific AlkB subfamilies. The compounds were then evaluated using thermal shift binding assays, biochemical assays, and crystallographic analyses.

Results. Our DCMS approach led to the rapid identification of several selective compounds with IC_{50}s in the low micromolar range, hence demonstrating that subfamily selective inhibition of the AlkB enzymes is possible. Some of these inhibitor were also shown to be selective over other Fe(II)-and 2OG-dependent oxygenases. This work further validates the use of the DCMS method for rapid lead generation, where good correlation between ESI-MS-based binding results, IC_{50} values, and thermal shift assays were observed.

Conclusions. The combined results, including crystallographic analyses, should provide a basis for the development of potent and selective probe against other nucleic acid demethylases, suitable for use as functional probes and, in the longer term, possibly for clinical use.

P100 A Novel Assay for Binding Studies of Antibody-Modified Polyplexes for Tumor Targeting

Doru Vornicescu[a], Sabrina Höbel[b], Achim Aigner[b], Michael Keusgen[a]

[a]Institute of Pharmaceutical Chemistry, Philipps-University Marburg, Germany
[b]Rudolf-Boehm-Institute for Pharmacology and Toxicology, University of Leipzig, Germany

Objective. The present work deals with the delivery of nucleic acids for tumor-specific gene therapy approaches. The specific binding of cetuximab-modified PEG-PEI (polyethylene glycol-polyethyleneimine) complexes, which can be loaded with nucleic acids, to the epidermal growth factor receptor (EGFR) is characterized by Surface Plasmon Resonance (SPR).

Methods. Nanoparticles obtained by chemical coupling of EGFR specific antibody cetuximab to the low molecular weight PEI via PEG-spacer in order to reduce non-specific interactions and subsequent complexation with small interfering RNA (siRNA) provide the basis for the setup of the novel SPR assay. SPR measurements to evaluate the binding specificity of the cetuximab-modified polymers to an immobilized EGFR-Fc fusion protein were performed. Furthermore, the formed complex/polyplex mediating the cellular uptake, allows PEI/nucleic acid complexes to interact with RISC (RNA-induced silencing complex) which enables gene knock-out or gene silencing RNA interference (RNAi). Confocal microscopy studies on complexed fluorescently labeled siRNA were performed to confirm the binding of the whole complex to the immobilized receptor.

Results. First, the binding of the cetuximab-modified polymer to an immobilized EGFR-Fc fusion protein was investigated. The immobilization of this fusion protein was performed on a hydrophobic C18-functionalized gold surface loaded with Protein A. Trastuzumab-modified PEI was chosen as negative control. Free Fc binding sites were blocked by subsequent addition of human IgG. No specific binding of PEI as well as modified PEI-PEG on a Protein A sensor surface was observed. Furthermore, confocal microscopy of the SPR prism clearly revealed a fluorescence signal in the case of the polyplexes containing labelled siRNA.

Conclusions. The specific binding of cetuximab-modified PEG-PEI as well as cetuximab- modified PEI/siRNA to immobilized EGFR factor was successfully characterized by SPR. It could be demonstrated that SPR is a suitable tool for studying the binding of nanoparticles intended for tumor-specific gene therapy approaches.

P101 Investigation of Drug Release from Non-Ionic and Anionic Cellulose Beads

Emrah Yildir[a], Ruzica Kolakovic[a], Jani Trygg[b], Pedro Fardim[b], Niklas Sandler[a]

[a]Pharmaceutical Sciences Laboratory, Department of Biosciences, Åbo Akademi University, Biocity, 3rd floor, Artillerigatan 6, FI-20520 Finland
[b]Laboratory of Fibre and Cellulose Technology, Åbo Akademi University, Porthansgatan 3, FI-20500 Finland

Objective. The project aim was to investigate the ability of new type of porous cellulose beads (CBs) to entrap and release active pharmaceutical ingredients (APIs) and as such be used as spherical matrix systems for controlled drug delivery. The more specific objective was to investigate the effect of preparation conditions and properties of CBs on release of freely and poorly water soluble drugs.

Methods. Two types of CBs were tested as potential drug carriers – nonionic and anionic CBs. Ranitidine hydrochloride and quinine sulfate were used as positively charged model APIs for drug loading of anionic CBs while piroxicam and griseofulvin were used as poorly water soluble model APIs for loading of nonionic CBs. The drug loading was performed by immersing swollen CBs in a solution of drug in suitable solvent followed by drying at the room temperature. The morphology of empty and drug

loaded CBs, and distribution of APIs within the beads was examined using FE-SEM. The solid-state characterization of was performed by FTIR. Content analysis and *in vitro* drug release studies of loaded CBs were carried out with UV/Vis Spectroscopy and suitable mathematical models were investigated to explain drug release kinetics.

Results. FE-SEM imaging analysis showed that the drug was located on the surface and also evenly distributed inside of the porous CBs. Solid-state characterization and SEM analysis indicated that griseofulvin and piroxicam existed in crystalline form. The *in vitro* drug release studies showed that anionic charged CBs were able to entrap higher amount of drug than nonionic ones and that release profiles were affected by the charge of the beads.

Conclusions. This study shows that porous CBs produced by precipitation of dissolved cellulose can be successfully used as drug carriers. Tailoring their properties (e.g. charge) it is possible to incorporate drugs with different physicochemical properties (charge, water solubility) and achieve controlled drug delivery.

P102 TRIM39 is a Novel Regulator of MOAP-1 in Mammalian Cells

Victor C. Yu[a], Ran Tao[a], Chong Teik Tan[a], Yu-Chin Su[a], Ang-Yu Liu[a] and Xiaohong Zhang[a]

[a]Department of Pharmacy, Faculty of Science, National University of Singapore, Singapore

Objective. MOAP1, first isolated in a screen for Bax-associating proteins, interacts with Bax upon apoptotic induction. It plays an effector role in mediating Bax function in the mitochondria. MOAP1 is a short-lived protein ($t_{1/2} \approx 25$min) that is degraded by the ubiquitin-proteosome system, but is stabilized by apoptotic signals. The aim of this study is to identify regulator of protein stability of MOAP1 using the yeast two hybrid screen.

Methods. hMOAP-1 was cloned in-frame with the Gal-4 DNA binding domain in pAS2 (HA) vector and used to screen a yeast two hybrid library derived from adult human brain (CLONTECH, USA). Four independent clones encoding TRIM39 were identified as binding partner of MOAP-1 from the screen. Screening and subsequent protein-protein interactions were carried out in yeast Y190 reporter strain.

Results. TRIM39 was isolated as a binding partner of MOAP1 and it belongs to a family of proteins, characterized by a Tripartite Motif (TRIM), consisting of a RING domain, a B-box and a coiled coil domain. The presence of the RING domain makes TRIM39 a possible candidate for an E3 ligase, since a subset of E3 ligases contains the RING domain. TRIM39 co-localizes well with MOAP1 in the cytosol. Surprisingly, in contrast to degrading MOAP1 as an E3 ligase would, transient co-expression of TRIM39 confers stability to MOAP1. TRIM39 interacts with the centre portion of MOAP1 which is required for interacting with the ubiquitin-proteosome system. Moreover, TRIM39 inhibits the ubiquitination of MOAP1, which could then result in reduced degradation of MOAP1. Furthermore, elevated levels of TRIM39 sensitize cells to apoptosis induced by staurosporine and etoposide.

Conclusions. Our data suggests that TRIM39 could regulate the function of MOAP1 in apoptosis by enhancing its stability.

P103 Supramolecular Assemblies of Amphiphilic β-Cyclodextrin with Lipids

Leïla Zerkoune[a], Angelina Angelova[a], Luc Choisnard[b], Annabelle Gèze[b], Denis Wouessidjewe[b] and Sylviane Lesieur[a]

[a]Université Paris-Sud, IGPS, UMR CNRS 8612, 92290 Châtenay-Malabry, France
[b]Université Joseph Fourier, DPM, UMR CNRS 5063, 38000 Grenoble, France

Objective. The aim of the present work was to prepare and characterize self-assembled nano-objects incorporating a novel, water-insoluble β-cyclodextrin derivative (βCD-C10), which possesses multiple (n=7-8) hydrophobic chains (C10) in the presence of two different classes of lipids.

Methods. In the first time**,** a nonionic surfactant, like polyoxyethylene oleyl ether (Brij 98) was used to form PEGylated nanoparticles of an amphiphilic CD derivative. These nano-objects are prepared by hydration of a lyophilized film of βCD-C10 by micellar solution of brij 98. Quasi-elastic light scattering (QELS) and microscopy were employed for nanoparticles characterization. The inclusion complexation between a model drug compound and βCD-C10 incorporated in nanostructured assemblies was also investigated by fluorescence microscopy.

Then, the ability of βCD-C10 to form mixed structures with dimyristoylphosphatidylcholine (DMPC) has been studied. Miscibility of the two amphiphiles is examined in fully hydrated mixtures by differential scanning calorimetry (DSC) and X-ray diffraction at small and wide angles.

Results. The results obtained by the different experiments showed that the interaction between βCD-C10 and surfactant micelles leads to formation of mixed dispersed objects of nanometer sizes. The analysis of the fluorescence spectroscopy results gives evidence for the incorporation of the studied model drug in more hydrophobic environment provided by the cavities or hydrophobic chains of the amphiphilic CD-C_{10}. The physical-chemical characterization of DMPC/βCD-C10 systems demonstrates that the β-cyclodextrin derivative is partially miscible to the phospholipids.

Conclusions. Further to the nanoprecipitation method for fabrication of nanoparticles from hydrophobic cyclodextrin derivatives, this work demonstrates that novel cyclodextrin materials can be prepared by self-assembly and hydration of mixed lyophilized films using PEGylating surfactants as stabilizers of the generated dispersed nanoparticles. The formation of a βCD-C10/DMPC mixed lamellar phase occurs until at βCD-C10 molar ratios close to 4 mol% permitting by dispersal to obtain supramolecular assemblies under form liposomes.

P104 Predicting Target-Substrate Interaction Constants by Quantitative Sequence-Kinetic Constant Relationships for Facilitating Protein-Protein Interaction Inhibitor Discovery

Zhang Peng[a], Chen Yuzong[a]

[a]BioInformatics & Drug Design Group, Department of Pharmacy, National University of Singapore

Objective. Drug potency highly depends on the competitive advantages over the substrates of drug targets, but in some cases it is less clear about what potencies are needed to ensure the drug competitive. An example is the therapeutic intervention through disrupting protein-protein interactions, suggesting the significance of binding affinity between protein and its substrate. Therefore, there is a need to predict protein-protein interaction kinetic constants so as to determine the needed potency for drugs against specific protein-protein interactions.

This work aims to provide a proof of concept study on Quantitative Sequence-Kinetic Constant Relationship (QSKR) through profiling the protein feature from primary sequence, without adopting 3D structure.

Methods. We collected a highly diverse interaction kinetics dataset (867 K_d data, 112 K_{off} data, 127 K_{on} data), and PROFEAT protein features were generated to represent the interacting protein pairs. The three sets of kinetic data were modeled using ε-SVR method with parameter optimization, and ten-fold-cross-validation was applied for performance evaluation. Maximum-Relevance-Minimum-Redundancy (mRMR) algorithm was adopted for feature selection, and new ε-SVR models were constructed based on the highly ranked features.

Results. Being the very first such QSKR study, we achieved (R^2_{train} =0.9821; R^2_{test} =0.4925) for K_d dataset, (R^2_{train} =0.9501; R^2_{test} =0.4598) for K_{off} dataset, and (R^2_{train} =0.9797; R^2_{test} =0.5561) for K_{on} dataset. mRMR feature selection had reduced half of the data dimensions, giving a comparably good predictive performance.

Conclusions. As a proof of concept, we had break through some limitations in current protein-protein-interaction kinetics research: 1) no PDB structure was used; 2) no commercial software was employed; 3) highly diverse kinetic dataset was collected; 4) SVR algorithm was first-time applied for QKSR.

This study demonstrated a general quantitative relationship between the features retrieved from protein primary sequences and the interaction kinetics constants, thus the prediction of the binding constants may help efficient selection of

bioactive compound with sufficient potency.

P105 Molecular Dynamics Study of Surface Structure of the Drug Delivery Liposome

Aniket Magarkar[a], Esra Karakas[a], Tomasz Rog[b], Alex Bunker[a]

[a]Centre for Drug Research University of Helsinki, Finland
[b]Tampere Institute of Technology, Tampere, Finland

Objective. Surface charge of the DDL is one of the important properties responsible for its interactions with the proteins in bloodstream. The aim of this work was to investigate the surface charge of drug delivery liposome by molecular dynamics simulations.

Methods. We used all atom molecular dynamics simulations investigate the interactions of DSPC/Cholesterol liposome membrane bilayer and synthetic lipid formulated bilayer with Na^+, K^+, Ca^{2+} ions at physiological concentration. We varied the concentration of cholesterol in DSPC liposome to understand its effect of surface charge of liposome as well. The computational simulations were performed in parallel with *in vitro* ζ potential measurements, which is the indicative of surface charge.

Results. With our MD simulation studies we show that, the presence of cholesterol results in a decrease in Na+ binding for the typical neutral (zwitterionic) phospholipid membranes (DSPC and POPC). The ζ-potential measurements carried out in parallel with our simulations showed decrease in its surface charge with increase in its cholesterol content. While cholesterol has been shown to alter several properties of the phospholipid membrane, this specific effect of altering the membrane charge is novel and has both biological and pharmaceutical relevance.

In the case of liposomes formulated with synthetic lipids CPe, when their physicochemical properties were compared to the PC formulated ones, CPe formulated liposomes differed in their surface charge and release profile of the encapsulated molecules. This difference in electrostatic potential of the PC and CPe formulated membrane bilayer is due difference in the membrane-cation interactions.

Conclusions. The computational modeling and *in vitro* experiments showed that, effective surface charge of the liposome is altered due to formulation component, cholesterol. Also in the case of synthetic lipid formulated liposome, the surface charge is markedly different when compared to its natural analogues due difference in the interactions with the salt ions present in the blood stream.

P106 Studies towards the Use of Immobilized Lipase for *In Vitro* Lipolysis Experiments

Stephanie Phan[a], Stefan Salentinig[a], Adrian Hawley [b], Ben J. Boyd[a]

[a]Monash Institute of Pharmaceutical Sciences, Monash, 381 Royal Parade, Parkville, VIC 3052, Australia
[b]SAXS/WAXS beamline, Australian Synchrotron, 800 Blackburn Rd, Clayton, VIC 3168, Australia

Objective. *In vitro* lipolysis experiments are used to examine drug disposition, solubilisation and precipitation during digestion of lipid-based drug formulations. The current method from a structural and analytical perspective utilizes porcine pancreatic extract, however there are many proteins in the extract which contribute to high background scattering when using small angle X-ray scattering (SAXS) and dynamic light scattering (DLS) to understand the structural evolution in these systems. These may obscure scattering from colloidal species produced on digestion, and complicate resolution of structures that are present. Thus the aim of this work was to investigate the possibility of using an immobilized lipase, to improve the current method for studying structures during *in vitro* lipolysis.

Methods. Immobilised lipase was a recombinant enzyme from lipase B from *Candida antartctica* covalently bound to macroporous polyacrylate resin. An *in vitro* digestion model was used for lipolysis experiments: lipids were added to simulated intestinal fluid at pH 6.5 in the thermostatted digestion vessel controlled to 37 °C. Lipase was added to initiate digestion and the liberation of fatty acid was titrated with NaOH to maintain the system at constant pH. SAXS and cryogenic-transmission electron microscopy were used to determine colloidal structure.

Results. After background subtraction, SAXS measurements showed that a dispersion of the immobilized lipase compared to the porcine pancreatic lipase in digestion buffer had significantly lower background scattering. Digestion of a medium chain triglyceride using both lipases resulted in the production of a lamellar phase, indicating that structure formation was not affected by the lipase used, and the former showed reduced background scattering. However longer digestion time was required at the same activity to achieve the same extent of digestion.

Conclusions. Immobilised lipase is suitable for use *in vitro* lipolysis experiments, and the background is reduced with immobilized lipase, rendering it a better lipase for structural resolution during lipolysis.

P107 Improved Delivery of Valproic Acid into the Brain by LAT1-Targeted Prodrugs

Kristiina M. Huttunen[a], Lauri Peura[a], Monika Vernerova[a], Mikko Gynther[a], Jarkko Rautio[a]

[a]School of Pharmacy, Faculty of Health Sciences, University of Eastern Finland, P.O. Box 1627, FI-70211 Kuopio, Finland

Objective. Most brain disorders and diseases lack effective drug therapies, mainly due to inability of drugs to cross the blood-brain barrier (BBB). L-type amino acid transporter (LAT1) is selectively expressed at the BBB carrying large, neutral amino acids as well as amino acidmimetic drugs and prodrugs into the brain. In the present study, four prodrugs of valproic acid, in which non-natural amino acids were attached as promoieties via hydrolysable ester and amide bonds, were designed, synthesized and evaluated as LAT1-targeted prodrugs to improve the transport of valproic acid into the brain.

Methods. Hydrolysis rates of the prodrugs were determined in rat and human liver S9 fractions and plasma as well as in 10% rat brain homogenate. The affinity of prodrugs to bind to LAT1 was evaluated with the *in situ* rat brain perfusion technique as competitive binding of [14C]-L-leucine. Furthermore, the brain uptake of amide prodrugs was studied *in vivo* in rats after intravenous bolus injection (IV).

Results. The ester prodrugs were bioconverted to valproic acid more efficiently in rat-based media relative to humans, while the corresponding amide prodrugs showed remarkably higher stability. LAT1-mediated brain uptake of [14C]-L-leucine was inhibited by 90-95% in the presence of the prodrugs. After IV administration, the amide prodrugs were able to cross the BBB and remained a longer period of time in the brain than valproic acid itself. Therefore, the brain:plasma ratios of the prodrugs were significantly higher (0.08-0.19) than with valproic acid (0.05).

Conclusions. Decreased uptake of [14C]-L-leucine indicates that the prodrugs have high affinity for LAT1. Furthermore, the amide prodrugs were transported into the brain more efficiently than valproic acid after IV administration. Although the corresponding ester prodrugs are too unstable to be studied in rats they may serve as potential LAT1-targeted prodrugs for humans, according to the *in vitro* bioconversion rates.

P108 Fogging in Lyophilized Drug Products

Dominique Ditter[a,b,c], Holger Röhl[a], Michael Adler[a], Hanns-Christian Mahler[a,c]

[a]Late Stage Pharmaceutical and Processing Development, Pharmaceutical Technical Development Biologics Europe, F. Hoffmann-La Roche Ltd., Basel, Switzerland
[b]Department of Pharmaceutical Sciences, Division of Pharmaceutical Technology, University of Basel, Basel, Switzerland
[c]Institute of Pharmaceutical Technology, University of Frankfurt, Frankfurt/Main, Germany

Objective. Vial fogging is a phenomenon observed after lyophilization due to drug product solution creeping upwards along the inner vial surface, being a cosmetic product defect. The main factor to control fogging is primarily the inner vial surface hydrophilicity/hydrophobicity. The usage of vials with a hydrophobic surface offers a solution for fogging. However, surface hydrophobicity may also possibly be modified due to processing, such as the lyophilization process itself. The purpose of this work was to investigate the influence of the lyophilization process parameters on the extent of fogging.

Methods. A protein formulation containing a monoclonal antibody (mAb) was freeze-dried in both hydrophilic vials (untreated) and hydrophobic vials (hydrophobic coating). Vials were washed and depyrogenized in-house prior to filling and freeze-drying. In addition, a fluorescein dye test was performed to verify the fogging effect on the raw material.

Results. Lyophilization process parameters had a minor impact on the extent of fogging. Nevertheless, fogging could not be eliminated by optimizing lyophilization process parameters. The usage of hydrophobic vials, however, showed reproducible results resulting in no fogging at all.

Conclusions. Fogging can be avoided when using vials with a hydrophobic coating, where the variations of the lyophilization process play a minor role.

P109 In Vitro Kidney Model for Drug Transport and Toxicity Testing

Aishwarya Jayagopal[a], Peter Soler[a], Nicholas Ferrell[b], Paul Brakeman[c], Deanna Kroetz[a], William Fissell[b], Shuvo Roy[a]

[a]Bioengineering & Therapeutic Sciences, UCSF
[b]Nephrology, Vanderbilt Univ.
[c]Pediatrics, UCSF

Background. Active transport by the renal proximal tubules plays a significant role in human drug disposition and is therefore important to model when developing drugs. However, current *in vitro* drug testing methods generally fail to mimic important physiological cues such as shear stress across the tubular cells. Here, design for a scaled microfluidic *in vitro* model that can accurately reflect the filtration functionality, cellular environment and active transport capacity of the human kidney is proposed. Preliminary results on the effect of shear stress on MDCK cells transfected with organic cation transporters are presented.

Methods. The proposed kidney model dimensions were selected based on physiological parameters and functionality of available materials. The device will be composed of silicon nanopore and porous polymer membranes as well as proximal tubule cells integrated into a polydimethylsiloxane scaffold.

Preliminary shear stress experiments were performed in an existing parallel plate bioreactor. MDCK cells transfected with a pair of uptake and efflux transporters (hOCT2/hMATE1) were grown on under static conditions until confluence and then placed in the bioreactor. Then, uptake of 4-(4-dimethylamino)styryl-N-methylpyridinium (ASP+), a fluorescent OCT2/MATE1 substrate, by cells under shear stress was measured for 1 hour with or without pretreatment (30 minutes) with Imipramine, an OCT2/MATE1 inhibitor.

Results. Cells cultured under shear stress showed an increase in ASP+ uptake when compared to cells cultured under static conditions.

Conclusions. These preliminary results indicate that shear stress induces uptake of the cationic active transport substrate ASP+ when compared to static growth conditions.

PRACTICAL INFO

GPEN 2014

PARTICIPANTS LIST

University participants list

Name	Email	Affliation
Abu-Ammar Aiman	aimana@ekmd.huji.ac.il	Hebrew University of Jerusalem
Agrawal Ashish	ashishagrawalpharma@gmail.com	NIPER
Alasel Mohammed	maaalasel@yahoo.com	Philipps-University Marburg
Albuquerque Sergio	salbuquerque@usp.br	University of Sao Paulo
Anchordoquy Tom	tom.anchordoquy@ucdenver.edu	University of Colorado
Angelova Angelina	angelina.angelova@u-psud.fr	Université Paris-Sud
Antunez Lorena	lorena.antunez@ku.edu	University of Kansas
Arnold Samuel	slarnold@uw.edu	University of Washington
Artursson Per	per.artursson@farmaci.uu.se	Uppsala University
Audus Kenneth	audus@ku.edu	University of Kansas
Augustijns Patrick	patrick.augustijns@pharm.kuleuven.be	Katholieke Universiteit Leuven
Auriola Seppo	seppo.auriola@uef.fi	University of Eastern Finland
Badihi Amit	amit.badihi@gmail.com	Hebrew University of Jerusalem
Baek Jongsuep	sen8408@naver.com	Chungnam National University
Bates Ryan	bates@proteinfolding.net	University of Colorado
Bauer Bjoern	bjoern.bauer@uky.edu	University of Kentucky
Bauer-Brandl Annette	annette.bauer@sdu.dk	Southern Denmark University
Bentley Maria Vitoria	vbentley@usp.br	University of Sao Paulo
Bergström Christel	christel.bergstrom@farmaci.uu.se	Uppsala University
Berkhout Jan	jan.berkhout@erasmusmc.nl	Leiden University
Bogner Robin	robin.h.bogner@gmail.com	University of Connecticut
Borchard Gerrit	gerrit.borchard@unige.ch	University of Geneva
Borchardt Ronald	rborchardt@ku.edu	University of Kansas
Borkar Nrupa	nrupa.borkar@sund.ku.dk	University of Copenhagen
Born Gerlinde	g.born@em.uni-frankfurt.de	University of Frankfurt
Boyd Ben	ben.boyd@monash.edu	Monash University
Brambilla Davide	davide.brambilla@pharma.ethz.ch	ETH-Zürich
Brandl Martin	mmb@sdu.dk	Southern Denmark University
Brodin Birger	birger.brodin@sund.ku.dk	University of Copenhagen
Bromann Paul	paul.bromann@helsinki.fi	University of Helsinki
Brouwer Kim	kbrouwer@unc.edu	University of North Carolina
Bunker Alex	alex.bunker@helsinki.fi	University of Helsinki
Burnett Joseph	jpburnet@umich.edu	University of Michigan
Byun Youngro	yrbyun@snu.ac.kr	Seoul National University
Capasso Cristian	cristian.capasso@helsinki.fi	University of Helsinki
Casteleijn Marco	marco.casteleijn@helsinki.fi	University of Helsinki
Castoldi Arianna	arianna.castoldi@uni-saarland.de	Saarland University
Chadwick Keith	chadwick@purdue.edu	Purdue University
Chan Eric Chun Yong	phaccye@nus.edu.sg	National University of Singapore
Chen Yufei	umche355@myumanitoba.ca	University of Manitoba
Chew Eng-Hui	phaceh@nus.edu.sg	National University of Singapore
Cho Cheong-Weon	chocw@cnu.ac.kr	Chungnam National University
Chothe Paresh	dmurray@rx.umaryland.edu	University of Maryland

University participants list (cont.)

Name	Email	Affliation
Christie Merry	merry.christie@ucdenver.edu	University of Colorado
Chu Te-Wei	tewei.chu@utah.edu	University of Utah
Claypool Sarah	sarah.e.claypool@gmail.com	University of North Carolina
Crum Matt	matthew.crum@monash.edu	Monash University
Dasargyri Athanasia	athanasia.dasargyri@pharma.ethz.ch	ETH-Zürich
del Amo Eva	eva.delamo@uef.fi	University of Eastern Finland
Deng Feng	feng.deng@helsinki.fi	University of Helsinki
Depieri Livia	livia.depieri@yahoo.com.br	University of Sao Paulo
Derendorf Hartmut	hartmut@ufl.edu	University of Florida
Dhandhukia Jugal	dhandhuk@usc.edu	University of Southern California
Di Francesco Tiziana	tiziana.difrancesco@unige.ch	University of Geneva
Doty Amy	amydoty@umich.edu	University of Michigan
Engelhardt Konrad	konrad.engelhardt@pharmazie.uni-marburg.de	Philipps-University Marburg
Erdoğar Nazlı	nerdogar@gmail.com	Hacettepe University
Falcao Nivea Maria	niveama@ufl.edu	University of Florida
Filppula Anne	anne.filppula@helsinki.fi	University of Helsinki
Friedman Adam	friedmad@email.unc.edu	University of North Carolina
Fu Jing	jingf@email.unc.edu	University of North Carolina
Fugit Kyle	kdfu222@uky.edu	University of Kentucky
Furini Júnior	jfurini@gmail.com	University of Sao Paulo
Gürsoy Reyhan Neslihan	nesligursoy@gmail.com	Hacettepe University
Garrison Jered	jcgarrison@unmc.edu	University of Nebraska
Ghandehari Hamid	hamid.ghandehari@pharm.utah.edu	University of Utah
Giddam Ashwini Kumar	ashwin.giddam@uqconnect.edu.au	University of Queensland
Gonzalez Daniel	daniel.gonzalez@unc.edu	University of North Carolina
Gordon Sarah	sarah.gordon@uni-saarland.de	Saarland University
Goswami Srijib	srijib.goswami@ucsf.edu	University of California at San Francisco
Gräf Florian	florian.graef@uni-saarland.de	Saarland University
Grobelny Pawel	pawel.grobelny@uconn.edu	University of Connecticut
Groell Floriane	floriane.groell@unige.ch	University of Geneva
Gustafsson Sofia	sofia.gustafsson@farmbio.uu.se	Uppsala University
Gynther Mikko	mikko.gynther@uef.fi	University of Eastern Finland
Hamm-Alvarez Sarah	shalvar@usc.edu	University of Southern California
Hansmann Simone	hansmann@em.uni-frankfurt.de	University of Frankfurt
Hasselt Coen	coenvanhasselt@gmail.com	Leiden University
Heinämäki Jyrki	jyrki.heinamaki@ut.ee	University of Tartu
Helm Nancy	nhelm@ku.edu	University of Kansas
Hens Bart	bart.hens@pharm.kuleuven.be	Katholieke Universiteit Leuven
Hinna Askell	hinna@sdu.dk	Southern Denmark University
Ho Paul	phahocl@nus.edu.sg	National University of Singapore
Huttunen Kristiina	kristiina.huttunen@uef.fi	University of Eastern Finland
Ilina Polina	polina.ilina@helsinki.fi	University of Helsinki
Imai Teruko	iteruko@gpo.kumamoto-u.ac.jp	Kumamoto University
Imanidis Georgios	georgios.imanidis@unibas.ch	University of Basel
Isoherranen Nina	ni2@uw.edu	University of Washington
Janas Christine	c.janas@em.uni-frankfurt.de	University of Frankfurt
Jayagopal Aishwarya	aishwarya.jayagopal@ucsf.edu	University of California at San Francisco

University participants list (cont.)

Name	Email	Affliation
Jensen Sara	saramj@biology.sdu.dk	Southern Denmark University
Jiskoot Wim	w.jiskoot@lacdr.leidenuniv.nl	Leiden University
Jorgensen Lene	lej@farma.ku.dk	University of Copenhagen
Jun Hye-suk	naminra79@nate.com	Chungnam National University
Kärkkäinen Jussi	jussi.karkkainen@uef.fi	University of Eastern Finland
Kalonia Cavan	cavan.kalonia@gmail.com	University of Kansas
Kalonia Devendra	davy.kalonia@gmail.com	University of Connecticut
Kaminska Kamila	kamila.kaminska@nus.edu.sg	National University of Singapore
Kang Bong-Seok	happy.kbs86@gmail.com	Chungnam National University
Kanninen Liisa	liisa.kanninen@helsinki.fi	University of Helsinki
Kari Otto	otto.kari@helsinki.fi	University of Helsinki
Kartal-Hodzic Alma	alma.kartal@helsinki.fi	University of Helsinki
Keusgen Michael	keusgen@staff.uni-marburg.de	Philipps-University Marburg
Khan Saeed	khans@staff.uni-marburg.de	Philipps-University Marburg
Kidron Heidi	heidi.kidron@helsinki.fi	University of Helsinki
Kim JuHeon	moom5611@naver.com	Chungnam National University
Knibbe Catherijne	c.knibbe@antoniusziekenhuis.nl	Leiden University
Knipp Gregory	gknipp@purdue.edu	Purdue University
Kogermann Karin	kkogermann@gmail.com	University of Tartu
Kok Robbert	r.j.kok@uu.nl	Utrecht University
Kokki Emmi	emmi.kokki@uef.fi	University of Eastern Finland
Kokkonen Piia	piia.kokkonen@uef.fi	University of Eastern Finland
Kontturi Leena-Stiina	leena.kontturi@helsinki.fi	University of Helsinki
Kopra Jaakko	jaakko.kopra@helsinki.fi	University of Helsinki
Kraft Jake	kraft@uw.edu	University of Washington
Kramer Katrin	katrin.kramer@otago.ac.nz	University of Otago
Krise Jeffrey	krise@ku.edu	University of Kansas
Kristensen Mie	mie.kristensen@sund.ku.dk	University of Copenhagen
Kroetz Deanna	deanna.kroetz@ucsf.edu	University of California at San Francisco
Kubota Akira	kubota@stu.kanazawa-u.ac.jp	Kanazawa University
Kulczar Christopher	ckulczar@purdue.edu	Purdue University
Kurokawa Keisuke	090p1016@st.kumamoto-u.ac.jp	Kumamoto University
Kuryk Lukasz	lukasz.kuryk@helsinki.fi	University of Helsinki
Kusuhara Hiroyuki	kusuhara@mol.f.u-tokyo.ac.jp	University of Tokyo
Kweon Se ho	sayhokweon@me.com	Seoul National University
Lajunen Tatu	tatu.lajunen@helsinki.fi	University of Helsinki
Lauren Patrick	patrick.lauren@helsinki.fi	University of Helsinki
Laurence Jennifer	laurencj@ku.edu	University of Kansas
Lee Cho-A	wwwdlchdk@naver.com	Chungnam National University
Lee Sang-Eun	nnininn@hanmail.net	Chungnam National University
Lehtinen Julia	julia.lehtinen@helsinki.fi	University of Helsinki
Li Xiao	lixiao19881030@126.com	Shandong University
Liu Garvey	liuxx854@umn.edu	University of Minnesota
Liu Xinyong	wangfanfan@sdu.edu.cn	Shandong University
Lopalco Antonio	lopalco@ku.edu	University of Kansas
Lou Yan-Ru	yan-ru.lou@helsinki.fi	University of Helsinki

University participants list (cont.)

Name	Email	Affliation
Lowry Deborah	d.lowry@aston.ac.uk	Aston University
Luciani Paola	paola.luciani@pharma.ethz.ch	ETH-Zürich
Lynch Caitlin	clync005@umaryland.edu	University of Maryland
Mönkäre Juha	j.t.monkare@lacdr.leidenuniv.nl	Leiden University
Mackay J. Andrew	jamackay@usc.edu	University of Southern California
Madsen Cecilie Maria	cecilie.madsen@sund.ku.dk	University of Copenhagen
Magarkar Aniket	aniket.magarkar@helsinki.fi	University of Helsinki
Mahmud K. A. Foyez	foyezmahmud@yahoo.com	Seoul National University
Malinen Melina	melina.malinen@helsinki.fi	University of Helsinki
Malinovskaja Kristina	kristina.malinovskaja@helsinki.fi	University of Helsinki
Marwah Mandeep	marwahm@aston.ac.uk	Aston University
Mastrobattista Enrico	e.mastrobattista@uu.nl	Utrecht University
Maudens Pierre	pierre.maudens@unige.ch	University of Geneva
McNiff Michaela	mmcniff@ku.edu	University of Kansas
Mihajlica Nebojsa	nebojsa.mihajlica@farmbio.uu.se	Uppsala University
Miller Geoffrey	geoffrey.miller@utah.edu	University of Utah
Miller Donald	donald.miller@med.umanitoba.ca	University of Manitoba
Morin Emmanuelle	emmanuelle.morin-picardat@uef.fi	University of Eastern Finland
Morishita Masaki	morishita.masaki.58n@st.kyoto-u.ac.jp	Kyoto University
Munjal Bhushan	bhushanmunjal@gmail.com	NIPER
Munson Eric	eric.munson@uky.edu	University of Kentucky
Nakanishi Takeo	nakanish@p.kanazawa-u.ac.jp	Kanazawa University
Nakayama Takeshi	happy_90_1ucky_09@yahoo.co.jp	University of Tokyo
Naoi Sotaro	tnsphhc191919x@gmail.com	University of Tokyo
Nassta Gemma	gemma.nassta@monash.edu	Monash University
Neumann Silke	silke.neumann@otago.ac.nz	University of Otago
Nicolazzo Joseph	joseph.nicolazzo@monash.edu	Monash University
Nielsen Carsten	carsten.uhdnielsen@sund.ku.dk	University of Copenhagen
Nielsen Line Hagner	line.hagner@sund.ku.dk	University of Copenhagen
Northrup Laura	lnorthrup@ku.edu	University of Kansas
Ohtsuki Shozo	ohtsuki.shozo.57s@st.kyoto-u.ac.jp	Kyoto University
Ohura Kayoko	ohurak@kumamoto-u.ac.jp	Kumamoto University
Ölander Magnus	magnus.olander@farmaci.uu.se	Uppsala University
Öztürk Kıvılcım	kivilcimozturk@gmail.com	Hacettepe University
Pace Samantha	samanthapace@ku.edu	University of Kansas
Pak Yewon	pyewon@umaryland.edu	University of Maryland
Palo Mirja	mipalo@abo.fi	Åbo Akademi University
Pan Yyijun	yijun.pan@monash.edu	Monash University
Papinska Anna	papinska@usc.edu	University of Southern California
Park Jeong-Sook	eicosa@cnu.ac.kr	Chungnam National University
Parrish Karen	karen.e.parrish@gmail.com	University of Minnesota
Paukkonen Heli	heli.paukkonen@helsinki.fi	University of Helsinki
Pelkonen Laura	laura.pelkonen@uef.fi	University of Eastern Finland
Penkina Anna	anna.penkina@ut.ee	University of Tartu
Persson Linda	linda.persson@farmaci.uu.se	Uppsala University
Peters Björn	bjorn.peters@uef.fi	University of Eastern Finland
Phan Stephanie	stephanie.phan@monash.edu	Monash University

University participants list (cont.)

Name	Email	Affliation
Pikal Michael	michael.pikal@uconn.edu	University of Connecticut
Pindrus Mariya	mariya.pindrus@uconn.edu	University of Connecticut
Pinnapireddy Shashank Reddy	psr_oxyozone@yahoo.com	Philipps-University Marburg
Pizzo Michelle	kropf@wisc.edu	University of Wisconsin
Podracka Lucia	lucia.podracka@uef.fi	University of Eastern Finland
Rahimian Sima	s.rahimian@uu.nl	Utrecht University
Ramsay Eva	eva.ramsay@helsinki.fi	University of Helsinki
Rautio Jarkko	jarkko.rautio@uef.fi	University of Eastern Finland
Reinisalo Mika	mika.reinisalo@uef.fi	University of Eastern Finland
Richardson Dominique	dominique.richardson@helsinki.fi	University of Helsinki
Rimpelä Anna-Kaisa	arto.urtti@uef.fi	University of Helsinki
Rubinstein Abraham	avrir@ekmd..huji.ac.il	Hebrew University of Jerusalem
Ruponen Marika	marika.ruponen@uef.fi	University of Eastern Finland
Saarinen Jukka	jukka.saarinen@helsinki.fi	University of Helsinki
Salim Mohsin	mohsin1993@aol.com	University of Eastern Finland
Samadi Sahar	samadis@staff.uni-marburg.de	Philipps-University Marburg
Sandler Niklas	niklas.o.sandler@gmail.com	Åbo Akademi University
Sasko Eric	ericsas@t-online.de	Philipps-University Marburg
Schäfer Jens	j.schaefer@staff.uni-marburg.de	Philipps-University Marburg
Schipper Pim	p.schipper@lacdr.leidenuniv.nl	Leiden University
Schoneich Christian	schoneic@ku.edu	University of Kansas
Schwendeman Steven	thardy@med.umich.edu	University of Michigan
Shekh Ahmad Tawfeeq	tawzedahmad@yahoo.com	Hebrew University of Jerusalem
Shi Wen	wen.shi@unmc.edu	University of Nebraska
Shi Yang	y.shi@uu.nl	Utrecht University
Siahaan Teruna	siahaan@ku.edu	University of Kansas
Sisco Jay	jaysiscollc@aol.com	University of Kansas
Sjöstedt Noora	noora.sjostedt@helsinki.fi	University of Helsinki
Sohn Myriam	myriam.sohn@unibas.ch	University of Basel
Soininen Suvi	karoliina.soininen@uef.fi	University of Eastern Finland
Soundararajan Ramesh	ucnvso1@live.ucl.ac.uk	UCL College of Pharmacy
Sousa Sofia	sofia.sousa@uef.fi	University of Eastern Finland
Stappaerts Jef	jef.stappaerts@pharm.kuleuven.be	Katholieke Universiteit Leuven
Steffansen Bente	bente.steffansen@sund.ku.dk	University of Copenhagen
Stephenson rachel	r.stephenson@uq.edu.au	University of Queensland
Stobaugh John	stobaugh@ku.edu	University of Kansas
Stocke Nathanael	nas587@gmail.com	University of Kentucky
Strachan Clare	clare.strachan@helsinki.fi	University of Helsinki
Subrizi Astrid	astrid.subrizi@helsinki.fi	University of Helsinki
Sun Zhizhi	sam.zhizhisun@gmail.com	University of Manitoba
Swaan Peter	pswaan@rx.umaryland.edu	University of Maryland
Takakura Yoshinobu	takakura@pharm.kyoto-u.ac.jp	Kyoto University
Takeuchi Ryota	r.takeuchi@stu.kanazawa-u.ac.jp	Kanazawa University
Tallapaka Shailendra	shailendrabhara.tvs@unmc.edu	University of Nebraska
Thakker Dhiren	dhiren_thakker@unc.edu	University of North Carolina
Thati Sharadvi	sthati@ku.edu	University of Kansas

University participants list (cont.)

Name	Email	Affliation
Thormann Ursula	ursula.thormann@unibas.ch	University of Basel
Torge Afra	torge@staff.uni-marburg.de	Philipps-University Marburg
Toth Istvan	i.toth@uq.edu.au	University of Queensland
Tscheka Clemens	clemens.tscheka@pharmazie.uni-marburg.de	Philipps-University Marburg
Tuominen Raimo	raimo.tuominen@helsinki.fi	University of Helsinki
Urtti Arto	arto.urtti@helsinki.fi	University of Helsinki
Välitalo Pyry	pyry.valitalo@gmail.com	Leiden University
Vakili Hossein	hvakili@abo.fi	Åbo Akademi University
Vellonen Kati-Sisko	kati-sisko.vellonen@uef.fi	University of Eastern Finland
Viitala Tapa1ni	tapani.viitala@helsinki.fi	University of Helsinki
Voelkner Alexander	alex.voelkner@ufl.edu	University of Florida
Vornicescu Doru	doru.vornicescu@staff.uni-marburg.de	Philipps-University Marburg
Wacker Matthias	wacker@em.uni-frankfurt.de	University of Frankfurt
Wagner Zoe	folchman@usc.edu	University of Southern California
Walker Greg	greg.walker@otago.ac.nz	University of Otago
Wickström Henrika	hwickstr@abo.fi	Åbo Akademi University
Williams Desmond	des.williams@unisa.edu.au	Adelaide University
Wilson Venecia	vwilson@purdue.edu	Purdue University
Wiltshire Tim	timw@unc.edu	University of North Carolina
Wissel Gloria	gloria.wissel@helsinki.fi	University of Helsinki
Woon Esther	esther.woon@nus.edu.sg	National University of Singapore
Xiong May	mpxiong@pharmacy.wisc.edu	University of Wisconsin
Yildir Emrah	eyildir@abo.fi	Åbo Akademi University
Ylikangas Henna	henna.ylikangas@uef.fi	University of Eastern Finland
Yliperttula Marjo	marjo.yliperttula@helsinki.fi	University of Helsinki
Yonemitsu Takahiro	141y1006@st.kumamoto-u.ac.jp	Kumamoto University
Yu Victor	phayuv@nus.edu.sg	National University of Singapore
Zane Nicole	nrzane@unc.edu	University of North Carolina
Zerkoune Leïla	leila.zerkoune@u-psud.fr	Université Paris-Sud
Zhai Guangxi	zkyjd@sdu.edu.cn	Shandong University
Zhang Peng	zhangpeng1202@gmail.com	National University of Singapore
Zhao Ma	sdmazhao@163.com	Shandong University
Zlatev Hristo	hristo.zlatev@uef.fi	University of Eastern Finland

Industrial participants list

Name	Email	Affliation
Andersson Tommy	tommy.b.andersson@astrazeneca.com	AstraZeneca
Berndl Gunther	gunther.berndl@abbvie.com	AbbVie
Borchardt Thomas	thomas.borchardt@abbvie.com	AbbVie
Brewster Marcus	mbrewste@its.jnj.com	Janssen Research & Development, A Division of Johnson & Johnson
Chakravarty Paroma	chakravarty.paroma@gene.com	Genentech, Inc.
Charlton Stuart	stuart.charlton@bms.com	Bristol-Myers Squibb Co.
Davies Nigel	nigel.davies@astrazeneca.com	AstraZeneca
Ditter Dominique	dominique.ditter@roche.com	F. Hoffmann-La Roche AG
Liederer Bianca	biancal@gene.com	Genentech, Inc.
Oliyai Reza	roliyai@gilead.com	Gilead Sciences, Inc.
Patel Sulabh	sulabh.patel@roche.com	F. Hoffmann-La Roche AG
Koskinen Mikko	mikko.koskinen@orionpharma.com	Orion Corporation
Rowland Martin	Martin.Rowland@pfizer.com	Pfizer Pharmaceutical Sciences
Weber Benjamin	benjaminweber@ufl.edu	Boehringer-Ingelheim Pharmaceuticals Inc.

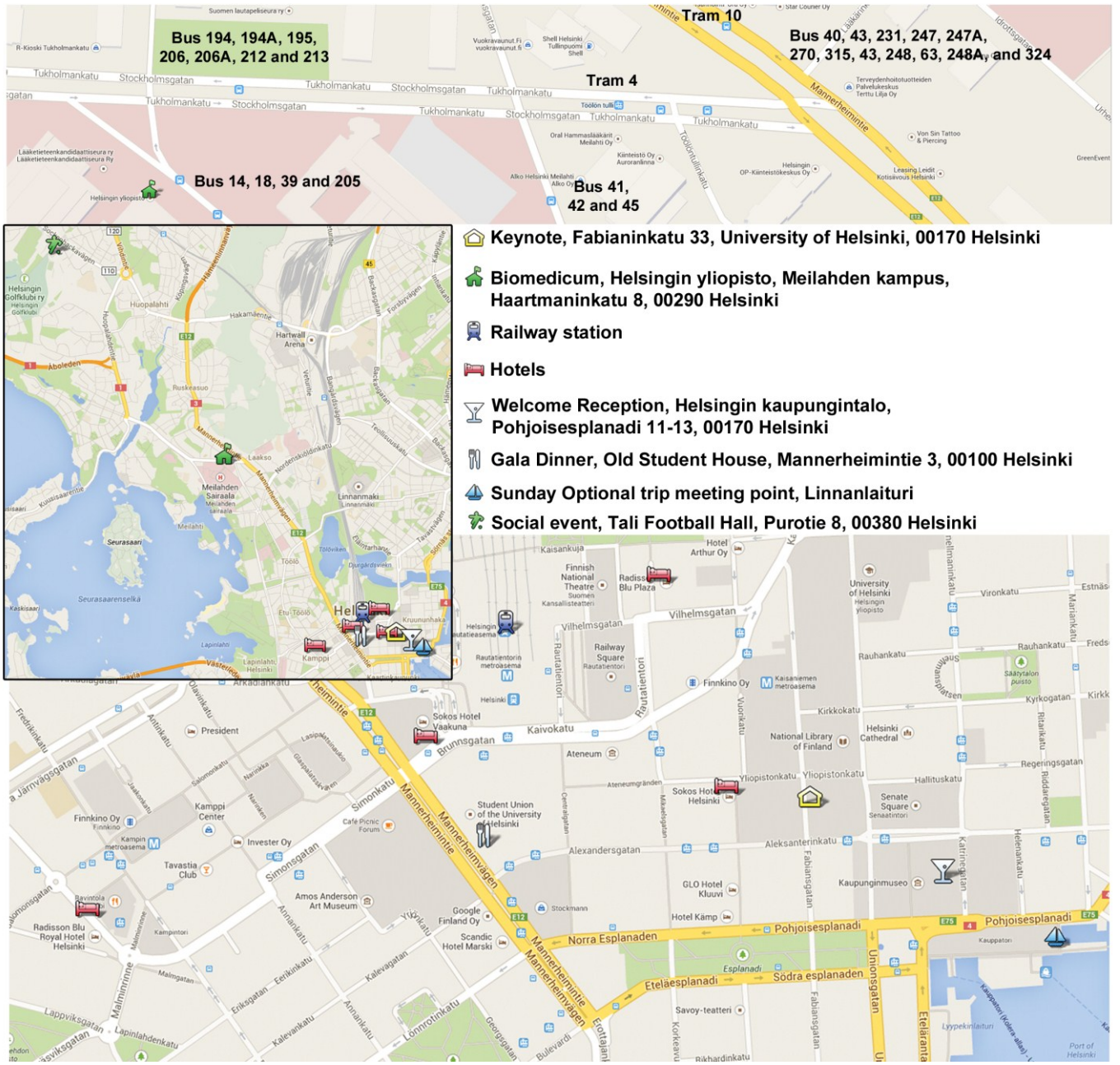
TRANSPORTATION IN HELSINKI

The travel card in your conference package can be used on the bus, tram, train within Helsinki, Espoo, Vantaa, Kaunianen and the Suomenlinna ferry from the 27th to the 30th of August.

Otherwise, single tickets cost 3 euro (valid for 1 hour) or 8 euro (valid for 1 day).

For more information on timetables and routes, please see the Helsinki Region Transportation website: http://www.reittiopas.fi/en/index.php

For a taxi (cab) please call +350 0100 0700 or ask the reception of your hotel to order one.



EMERGENCY INFORMATION

The emergency number in Finland is 112

BIOMEDICUM

Biomedicum Helsinki 1

Haartmaninkatu 8, FI-00290 Helsinki, Finland

P.O.Box 63, FI-00014 University of Helsinki, Finland

P.O.Box 700, FI-00029 HUS, Finland

Tel: +358 294 1911 / +358 9 4711

1. Lecture Hall 1
2. Lecture Hall 2
3. Lecture Hall 3
4. Seminar room 1-2
5. Seminar room 3
6. Meeting room 1
7. Meeting room 4
8. Meeting room 8-9
9. Meeting room 10
10. Meeting room 11
11. Meeting room 12
12. Courtyard B1
13. Courtyard B2
14. Courtyard C1
15. Courtyard C2

GPEN 2014 CONFERENCE PERSONNEL CONTACT INFORMATION

Noora Sjöstedt (Co-Chair Student Organizing Committee)
Tel: +358 50-4480817
Email: noora.sjostedt@helsinki.fi

Arto Urtti (Co-Chair Organizing Committee)
Tel: +358 40-5402279
Email: arto.urtti@helsinki.fi

GPEN 2014 HOTELS

Radisson Blu Royal Hotel
Runeberginkatu 2, 00100 Helsinki
Tel: +358 20 1234 701
Fax: +358 20 1234 702

Radisson Blu Plaza Hotel
Mikonkatu 23, 00100 Helsinki
Tel: +358 20 1234 703
Fax: +358 20 1234 704

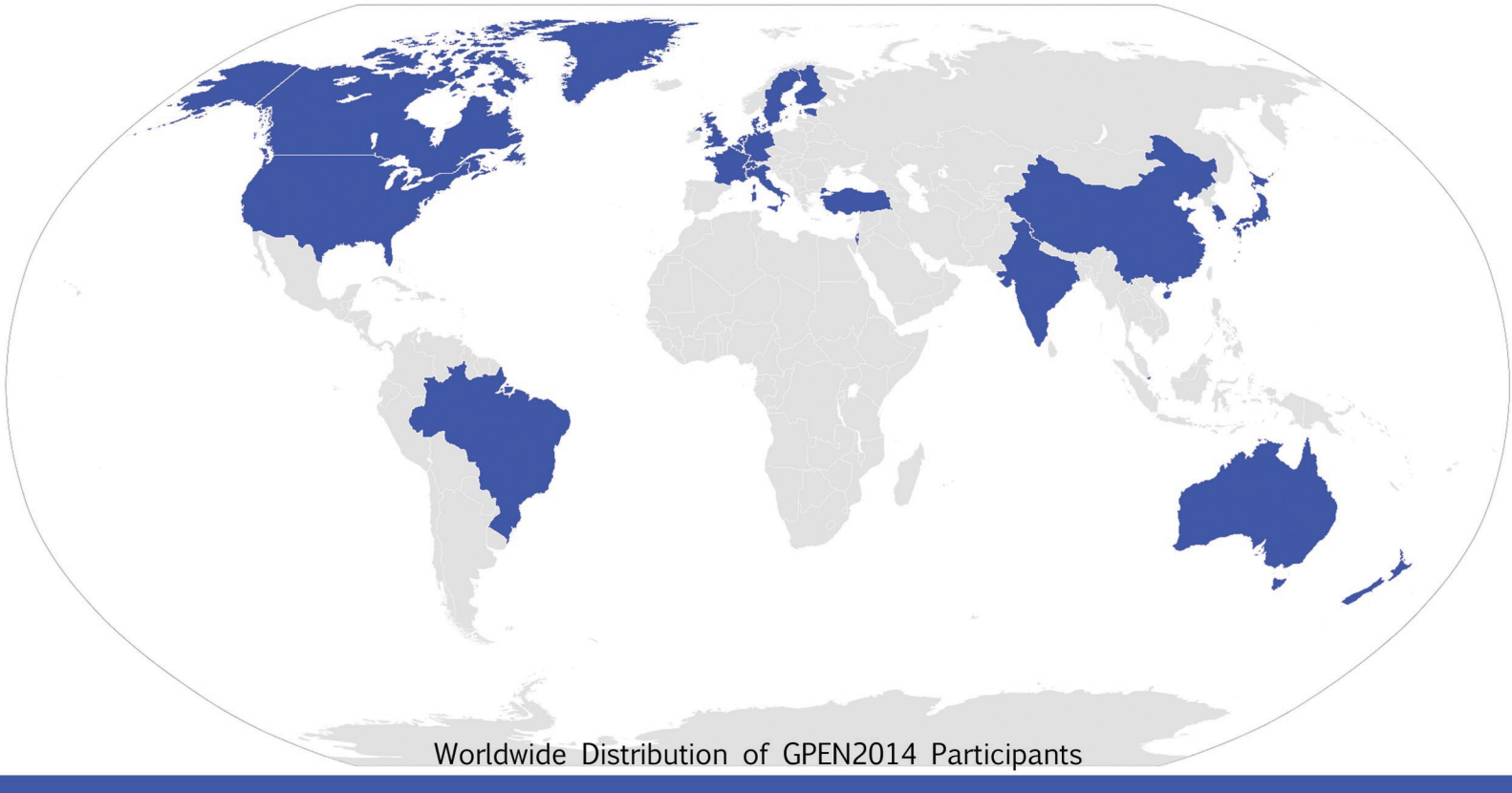
Original Sokos Hotel Helsinki
Kluuvikatu 8, 00100 Helsinki
Tel: +358 20 1234 601
Fax: +358 9 176 014

Original Sokos Hotel Vaakuna
Kaivokatu 3, 00100 Helsinki
Tel: +358 20 1234 601
Fax: +358 9 176 014

SEE YOU AT

KU
THE UNIVERSITY OF
KANSAS

GPEN 2016!



Worldwide Distribution of GPEN2014 Participants