SWISS PHARMA SCIENCE DAY 2021

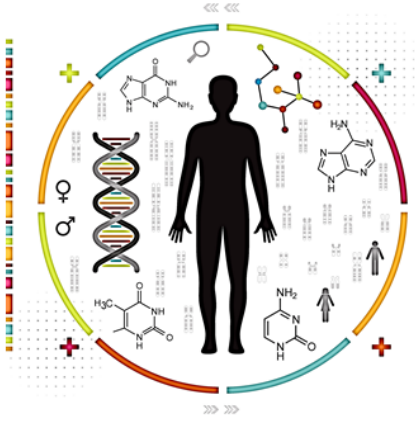
SAPhS Swiss Academy of Pharmaceutical Sciences

14th Swiss Pharma Science Day 2021

Wednesday August 25, 2021

Online Conference

«PRECISION MEDICINE»



Intention

The SWISS PHARMA SCIENCE DAY (SPhSD) is an annual event of the SwissAcademy of Pharmaceutical Sciences (SAPhS, www.saphw.ch). The 1stSPhSD was held on October 9, 2008, at the University of Bern. For congress reports 2008-2019 (2020 canceled due to pandemic) including all lecture and poster abstracts see www.saphw.ch

The SPhSD offers a platform to present, in form of a poster session, the latest research results of Master and PhD students, as well as Post-Docs of the Swiss Academic Institutions for Pharmaceutical Sciences, i.e. ETH Zürich, University of Geneva, University of Basel, University of Bern, and University of Applied Sciences FHNW-School of Life Sciences Muttenz.

The poster session is embedded in a series of lectures given by invited distinguished scientists representing the broad field of pharmaceutical sciences, such as Pharmaceutical Biology, Biotechnology, Technology, Chemistry, Analytics, Engineering, Pharmacology, or Molecular Biology.

One of the primary goals of the SPhSD is to further stimulate professional and social contacts between the students still undergoing training and Alumni, having already a position in industry, hospital, public health administration or public pharmacy. Thus, cooperation and networking between the different institutions in academia and industry and the different fields of pharmaceutical sciences is being promoted.

Last but not least, the SPhSD represents an ideal platform to meet young engineers and scientists, who may be recruited for a position in the academia, hospital, industry, public health administration or public pharmacy.

Organizing Committee:

Prof. Rudolf Brenneisen, PhD, SAPhS, Secretary General, Bern

info@saphw.ch

Prof. Gerrit Borchard, PhD, SAPhS, President

School of Pharmaceutical Sciences, University of Geneva

gerrit.borchard@unige.ch

Program

10:00 – 10:15 **Addresses of Welcome**

- Prof. Gerrit Borchard, PhD,
  University of Geneva, President SAPhS
- Prof. Christian Leumann, PhD,
  Rector University of Bern
- Prof. Rudolf Brenneisen, PhD,
  Secretary General SAPhS

10:15 – 11:30 **Morning Session**

Chair: Prof. Gerrit Borchard

10:15 – 10:45 Keynote Lecture: Dr. Josef-Steiner Cancer Research Awardee 2019
Prof. Serena Nik-Zainal, MD
University of Cambridge, U.K.:

«Accelerating Holistic Cancer Genome Interpretation Towards the Clinic»

10:45 – 11:15 Lecture 2: Women's Health
Prof. Olav Lapaire, MD
University Hospital Basel:

«Personalized Medicine in Women»

11:15 – 11:30 h Discussion Lecture 1 and 2
(Q & A, Chat)

11:45 – 13:00 **Break and e-Poster Session**

Program (cont.)

13:00 – 16:15	**Afternoon Session**
	Chair: Prof. Christoph R. Meier
13:00 – 14:00	**Platform for SAPhS Partner Associations**
13:00 – 13:15	Swiss Society of Industrial Pharmacists (SSIP / GSIA / SSPI)
13:15 – 13:30	Swiss Association of Public Health Administration and Hospital Pharmacists (GSASA)
13:30 – 13:45	Swiss Young Pharmacists Group (swissYPG)
13:45 – 14:00	Discussion Platform Presentations (Q & A, Chat)
14:00 – 14:15	**Award Ceremony**
	SAPhS Fellows 2020 & 2021 Prizes for best posters
14:15 – 15:15	**Short Oral Presentations of Poster Award Winners**
14:15 – 14:30	First Poster Prize
14:30 – 14:45	Prize for best poster in Pharm. Biology/Phytopharmacology
14:45 – 15:00	Prize for best poster in Pharm. Technology

Program (cont.)

15:00 – 16:15	**Afternoon Session (cont.)** **Chair: Prof. Christoph R. Meier**
15:00 – 15:30	**Lecture 3: Pharmaceutical Technology** **Prof. Hans Leuenberger, PhD** **University of Florida, U.S.A.:** **«Spray Freeze-Drying for Precision Medicine and Vaccines»**
15:30 – 16:00	**Lecture 4: Precision Medicine** **Prof. Mark A. Rubin, MD** **Department for BioMedical Research (DBMR)** **Bern Center for Precision Medicine (BCPM)** **University of Bern:** **«Precision Oncology Approaches to Understanding Therapy Resistance»**
16:00 – 16:15	**Discussion Lecture 3 and 4** **(Q & A, Chat)**
16:15 – 16:30	**Closing Remarks** **Prof. Gerrit Borchard, PhD,** **President SAPhS** **Prof. Rudolf Brenneisen, PhD,** **Secretary General SAPhS**

Sponsors

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AKB-Stiftung zur Förderung des Pharmazeutischen Nachwuchses
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Sponsoring 1st poster prize and lecture of Prof. Nik-Zainal

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PHARMAZEUTISCHE
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ZÜRICH

Co-organizer, technical support

Congrex Switzerland, Basel https://congrex.com/

Lectures

L-1

Keynote Lecture: Dr. Josef-Steiner Cancer Research Awardee 2019

Prof. Serena Nik-Zainal, MD, University of Cambridge, UK

Biosketch:

BIOGRAPHICAL SKETCH

Provide the following information for the Senior/key personnel and other significant contributors.
Follow this format for each person. **DO NOT EXCEED FIVE PAGES.**

NAME: Nik-Zainal, Serena, M.A., M.B., B.Chir., Ph.D., M.R.C.P.

eRA COMMONS USER NAME (credential, e.g., agency login): Not applicable

POSITION TITLE: CRUK Advanced Clinician Scientist

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	Completion Date MM/YYYY	FIELD OF STUDY
University of Cambridge	B.A. (hons) - First	1998	Physiology
University of Cambridge	MB, BChir.	2000	Medicine/Surgery
Royal College of Physicians	MRCP	2003	Medicine
Wellcome Trust Sanger Institute	Ph.D.	2012	Cancer genomics
Royal College of Physicians Specialist Register	Certification	2013	Clinical Genetics

A. Personal Statement

Following a first-class degree in preclinical science, I obtained a medical degree from the University of Cambridge in December 2000, sponsored by Petroliam Nasional Berhad Malaysia (PETRONAS) and as a Fellow of the Cambridge Commonwealth Trust. I trained in general internal medicine before specializing in Clinical Genetics. I obtained the Certificate of Specialist Training in Clinical Genetics in January 2013 and have been an Honorary Consultant in Clinical Genetics at Cambridge University Hospitals NHS Foundation Trust since February 2013. I recruited patients with DNA repair defects as part of the Insignia project until December 2018.

I undertook a PhD at Wellcome Sanger Institute (WSI) exploring cancer using next-generation sequencing (NGS) technology in 2009. I was heavily involved in development of the whole genome sequencing (WGS) somatic variation pipeline and the development of an array of analytical principles that revealed the underlying abnormal biology of tumors – including generalized *mutational signatures,* imprints left by mutagenic processes that have occurred through cancer development, a novel phenomenon of localized hypermutation termed "kataegis", and developed the principles of constructing a cancer evolutionary tree from a single tumour sample.

In a post-doctoral role and as an early investigator, I continued bioinformatic exploration of large cancer datasets, leading production and analyses of the largest cohort of WGS cancers of a single tissue-type, of 560 breast cancers. I began pursuing experimental validation of mutational signatures, dissecting mechanisms of mutagenesis using cellular models. Human induced pluripotent stem cells were used to generate CRISPR-Cas9 knockouts of DNA repair genes and were systematically treated with a variety of environmental mutagens. The results from these endeavors serve as a reference resource of validated human mutational signatures.

As a Group Leader at Cambridge, my team continues to advance the whole cancer genomics field through a combination of computational and experimental approaches, to ultimately create clinical applications. With five patent filings made in the last 36 months and a sixth in the pipeline, we are unravelling mutational mechanisms, developing machine-learning based clinical algorithms, and actively connecting with clinical trials to validate our algorithmic tools. We are also deeply embedded with the UK 100,000 Genomes Project and have performed quality control and pan-cancer analyses/interpretation of more than 15,000 whole cancer genomes to date.

B. Positions and Honors

Positions and Employment

1995-2000	Medical student, University of Cambridge
2001-2002	Pre-registration House Officer, East Anglian Deanery
2002-2004	Senior House Officer, Medicine, East Anglian Deanery
2004-2009	Specialist Registrar in Clinical Genetics, East Anglian Deanery
2009-2012	Wellcome Clinical Research Training Fellow, Wellcome Sanger Institute
2012-2013	Specialist Registrar in Clinical Genetics, East Anglian Deanery
2013-2017	Wellcome Intermediate Clinical Research Training Fellow, Wellcome Sanger Institute
2014-2017	Career Development Fellow, Wellcome Sanger Institute
2017-present	CRUK Advanced Clinician Scientist, University of Cambridge

Other Experience and Professional Memberships

2012-2014	Society and Personal Genomes Working Group, Wellcome Sanger Institute
2013-present	British Society of Genetic Medicine member
2014-2016	AURORA-MAB Consortium
2015-present	EACR Ambassador
2017-present	Scottish Genomes Project Scientific Advisory Board
2017-2020	Artios Ltd Scientific Advisory Board
2017-2020	MAP ESMO Scientific Committee
2017-present	CRUK Grant Panel Review committee for DNA repair
2018-2020	Astra Zeneca Expert Scientific Panel Breast Cancer
2019	University of Cambridge Alumnus/Fundraising: Global Cambridge event (11 June, Malaysia)
2019	CRUK Philanthropy Event: Behind the Scenes (14 March, London)
2019-present	Precision Panc Advisory Board
2019-present	Chair of IT Committee, MRC Cancer Unit, University of Cambridge
2019-present	ESMO Congress Scientific Committee
2019-present	Scientific Committee for European Society of Human Genetics (ESHG)
2019-present	German Cancer Aid Grant Panel Review committee for Translational Oncology
2020	BBC Inside Science Radio 4: Human Genome Project 20th Anniversary (aired 25 June)
2020	CRUK Philanthropy Event: Rising to the Challenge (10 June, Virtual)
2020	Co-Lead of the Cambridge Biomedical Research Campus Integrative Genomics Theme

Editorial duties	Advisory Board of *Cancer Cell, Cell Reports in Medicine, Genome Biology*
Clinical duties	Honorary Consultant in Clinical Genetics, Cambridge University Hospital (10-20% time spent in clinical genetics) 2013-present

Honors

2020 Highly-Cited Researcher's List Clarivate 2020
2020 EMBO Elected Fellow
2020 Honorary Fellowship Murray Edwards College, University of Cambridge
2019 Dr Josef Steiner Cancer Research Award
2016 CRUK Pioneer Award
2014 William Bate Hardy Prize
2014 CRUK Future Leaders Prize
2013 Wellcome-Beit Prize
2013 AACR Scholar-in-training Award
2012 EACR Susan G. Komen Prize
2012 Robin Winter Prize

C. Contribution to Science

1. **Exploiting the power and digital nature of whole genome sequencing**: I was instrumental to the development of the whole genome sequencing data-processing-pipeline for somatic variation at the Wellcome Sanger Institute (2009-2012). Subsequently, using the high-quality data generated through that pipeline, I introduced the principles of exploiting the scale of WGS and the digital nature of WGS in order to interpret cancer genomes. Specifically, that the mutations that arose in cancers were not simply random and were due to specific mutagenic processes, generating *mutational signatures* (PMID: 22608084). Also, we uncovered a phenomenon of local hypermutation termed kataegis and the principles underpinning the construction of phylogenetic trees were developed (PMID: 22608083). Subsequently, my research team continued to innovate on these ideas, creating the principles of holistic cancer genome profiling and now have extensive expertise in analysis and interpretation of whole genomes (PMID: 27135926). Beyond this landmark paper in Nature in 2016, the values of early, open data-sharing led to a further fifteen papers since, revealing many new insights and benefiting many careers (PMIDs: 31570896, 30988298, 30692147, 30252041, 30104284, 28977645, 28904067, 28572256, 28288110, 28329761, 28112740, 27498871, 27666519, 27406316, 27136393). These principles of cancer genome profiling have been adopted by various international groups and by the UK 100,000 genomes project (UK 100KGP).
    a. Nik-Zainal S, et al. Mutational processes molding the genomes of 21 breast cancers. **Cell**. 2012 May 25;149(5):979-93. PMID: 22608084
    b. Nik-Zainal S, et al. The life history of 21 breast cancers. **Cell**. 2012 May 25;149(5):994-1007. PMID: 22608083;
    c. Nik-Zainal S, et al. Landscape of somatic mutations in 560 breast cancer whole-genome sequences. **Nature**. 2016 Jun 2;534(7605):47-54. PubMed PMID: 27135926

2. **Innovating on the concept of mutational signatures.** I was part of the team that first introduced the concept of mutational signatures in breast cancers (PMID: 22608084) and later applied this principle to other cancer types (PMID: 23945592). Mutational signatures have now become an accepted part of general analyses of cancer genomics. Latterly, I have been at the forefront of innovating on this principle, deriving rearrangement mutational signatures (PMID: 27135926) and exploring mutagenesis in terms of genome dynamics (PMID: 27136393, 28112740), secondary structures (PMID: 30104284) and delving into the area of rare tumour syndromes to gain insights into the earliest stages preceding carcinogenesis. We have recently reported on tissue-specific behaviours of mutational signatures (PMID: 32118208) and developed a public-facing web-based tool that serves as a reference database for mutational signatures, to make our knowledge accessible instantly and for non-expert users to perform mutational signature analyses (h).
    a. Alexandrov LB, Nik-Zainal S, et al. Signatures of mutational processes in human cancer. **Nature**. 2013 Aug 22;500(7463):415-21. PMID: 23945592.
    b. Morganella S,…. Nik-Zainal S. The topography of mutational processes in breast cancer genomes. **Nat Commun.** 2016 May 2;7:11383. PMID: 27136393.
    c. Glodzik D,… Nik-Zainal S. A somatic-mutational process recurrently duplicates germline susceptibility loci and tissue-specific super-enhancers in breast cancers. **Nat Genet.** 2017 Mar;49(3):341-348. PMID: 28112740
    d. Zou X,… Nik-Zainal S. Short inverted repeats contribute to localized mutability in human somatic cells. **Nucleic Acids Res**. 2017 Nov 2;45(19):11213-11221. PMID: 28977645
    e. Georgokapoulos-Soares I,… Nik-Zainal S. Non-canonical secondary structures arising from non-B DNA motifs are determinants of mutagenesis. **Genome Res.** 2018 Sep;28(9):1264-1271. PMID: 30104284
    f. Davies, H,… Nik-Zainal S,Rajan N. Epigenetic dysregulation underpins tumorigenesis in a rare cutaneous tumour syndrome. **Nat Commun**. 2019 Oct 17;10(1):4717. PMID: 31624251.
    g. Degasperi, A.. Nik-Zainal S. A practical framework and online tool for mutational signature analyses show inter-tissue variation and driver dependencies. **Nature Cancer** 2020 Feb;1(2):249-263. PMID: 32118208.
    h. https://signal.mutationalsignatures.com

3. **Experimental validation of mutational signatures.** My team sought to validate mutational signatures in experimentally-controlled cell-based systems to show that mutational signatures in human cancers are not simply mathematical abstractions - they are true patterns of mutagenesis that arise when normal cellular activity goes awry. We performed a proof-of-principle exercise to show that it is possible to create mutational signatures using CRISPR-cas9 technology to knock out DNA repair genes that sanitize the genome (PMID: 29717121). We published a landmark paper cataloguing the array of mutagens that are present in our environment that can cause DNA damage in normal human cells (PMID: 30982602). We have other pieces of work systematically generating highly-controlled data in order to create a reference set of mutational signatures for the community(c). Given our niche in this area, we have published a "how to" manual for performing such studies, sharing the tips and tricks of the trade (PMID: 32059681).
    a. Zou, X. et al… Nik-Zainal S. Validating the concept of mutational signatures. **Nat Commun.** 2018 May 1;9(1):1744. PMID: 29717121
    b. Kucab, J. et al.. Nik-Zainal S. A compendium of mutational signatures of environmental agents. **Cell** 2019 May 2; 177(4):821-836. PMID: 30982602
    c. Zou, X. et al… Nik-Zainal S. Insights from systematic knockouts of DNA repair/replication genes in a human stem cell system (manuscript in press, **Nature Cancer**)
    d. Koh G, Zou X, Nik-Zainal S. Mutational signatures: experimental design and analytical framework. **Genome Biol**. 2020 Feb 14;21(1):37. PMID: 32059681.

4. **Translating the concept into clinical applications.** Finally, based on the mechanistic insights gained from *in vitro* systems and from our analysis of *in vivo* signatures from cancers, I am developing algorithms that are intended to assist and improve the interpretation of whole cancer genomes. We already have one of the best tools for investigating *BRCA1*/*BRCA2* deficiency (PMID: 28288110), are developing a suite of tools for an array of other DNA repair/replication defects (PMID: 28904067) – all with the ultimate intention of improving patient stratification for effective therapeutic intervention. We have led the way in how to take an algorithmic technology and validate it in a population setting (PMID: 31501597), applied it into a clinical trial (PMID: 32471999) and am seeking application into various pharmaceutical clinical trials.
    a. Davies H,… Nik-Zainal S. HRDetect is a predictor of BRCA1 and BRCA2 deficiency based on mutational signatures. **Nat Med.** 2017 Apr;23(4):517-525. PMID: 28288110.
    b. Davies H… Nik-Zainal S. Whole genome sequencing reveals breast cancers with mismatch repair deficiency. **Cancer Res.** 2017 Sep 15;77(18):4755-4762. PMID: 28904067
    c. Staaf J, …. Nik-Zainal S. Whole-genome-sequencing of triple negative breast cancers in a population-based clinical study **Nat Med.** 2019 Oct;25(10):1526-1533. PMID: 31570822
    d. Chopra, N, …Nik-Zainal S, Turner N. Homologous recombination DNA repair deficiency and activity of PARP inhibition in primary triple negative breast cancer. **Nat Commun.** 2020 May 29;11(1):2662. PMID: 32471999.

Complete List of Published Work in MyBibliography:

https://www.ncbi.nlm.nih.gov/myncbi/18oRlWoW3TTkj/bibliography/public/

Beyond science, I have been invited to write "Turning points" essays on:
1. Women in Science, Technology and Medicine: Nature Cell Biology in 2018 (PMID: 30696459)
2. How and why I became a physician-scientist: Nature Medicine in 2019 (PMID: 31501597)

D. Additional Information: Research Support

Ongoing Research Support

Basser Gray Foundation Award — Dec 2020-Nov 2024
Dissecting early pathogenesis of BRCA1/2-associated cancer for risk prediction and prevention
The goal of this project is a combination of clinical, genomics and mechanistic exploration of early BRCA1/BRCA2 tumors
Role: Co-PI

BRC Core Grant — Oct 2019-Sept 2022
Foundations of holistic cancer genome interpretation
The goal of this study is to provide foundational support for providing whole genome sequencing services to University of Cambridge
Role: PI

Josef Steiner Award June 2019-May 2023
Accelerating holistic cancer genome interpretation towards the clinic
The goal of this study is consolidating and advancing computational approaches to accelerate the provision of whole genome profiling of the highest standards.
Role: PI

CRUK Early Detection Project Award June 2019-May 2023
Early detection of clinically-relevant mutational signatures
The goal of this study is identifying clinically-relevant mutational signatures and developing assays for detecting them.
Role: PI

CRUK Grand Challenge Award May 2017-April 2022
PRECISION: Prevent DCIS overtreatment now
The goal of this study is to examine multi-faceted consortium (pathology, radiology, physico-chemistry, genomics, mouse models, xenografts) systematically exploring samples from retrospective and prospective DCIS clinical trials in order to understand what are the factors that distinguish indolent from aggressive DCIS
Role: co-PI

CRUK Advanced Clinician Scientist Award Feb 2017-Jan 2022
Advancing the field of mutational signatures: Mechanisms and clinical applications
The goal of this study is to conduct a series of wet-lab experiments aimed at understanding physiological mechanisms of mutagenesis, used to inform and guide the development to the computational algorithms for clinical stratification
Role: PI

Completed Research Support

CRUK Pioneer Award Oct 2016-Nov 2020
Accelerating the translation of mutational signatures into the clinic
The goal of this study is to use algorithmic methods to improve typing of cancers for more meaningful clinical stratification
Role: PI

Wellcome Trust Strategic Award Sept 2013-July 2019
Exploring the biology underpinning mutational signatures
Using isogenic cell-based systems, we are attempting to generate mutational signatures under experimentally-controlled conditions.
Role: co-PI

Wellcome-Beit Prize May 2013-May 2018
Exploring biological processes underlying mutational signatures in human cancer
Prize for outstanding Wellcome Intermediate Clinical Research Fellowship application below
Role: PI

Wellcome Intermediate Clinical Reseach Fellowship Feb 2013-Jan 2017
Exploring biological processes underlying mutational signatures in human cancer
Using computational analyses to explore large cancer datasets; Initiating the Insignia project, recruiting patients with DNA repair defects to study mutational processes in normal cells before carcinogenesis
Role: PI

Lecture Abstract:

«Accelerating Holistic Cancer Genome Interpretation Towards the Clinic»

It took more than 1'000 scientists ten years and ~USD 2.7 billion to sequence the 3-GB genome within the Human Genome Project. Today, it is possible to sequence the whole human genome in a day for under USD 800. This remarkable increase in speed and scale of sequencing permits investigation of diseases defined by mutagenesis, such as cancer, to be explored comprehensively by reading the entire cancer genome of each patient as a matter of course.
For four decades, cancer scientists have sought driver mutations, those recurrent mutations that are causative of carcinogenesis. Whole genome sequencing (WGS) however reveals all substitution, indel and rearrangement drivers, including gene-fusion events and copy number aberrations (amplifications and homozygous deletions) in one experiment. Additionally, the totality of mutagenesis can expose patterns of mutagenesis, or mutational signatures, imprints of DNA damage and repair processes that had occurred through tumorigenesis.
In this lecture, I will describe the concept of mutational signatures derived from WGS cancers. I will explain how we use machine-learning methods to develop clinical algorithms in order to help with cancer genome interpretation. The rate-limiting step in cancer genomics today, is not the ability to perform WGS. It is the substantial expertise required to analyse and clinically interpret the data in a useful way, that remains the hurdle between genomic technology and the clinical context. I will describe how my team are making particular efforts to minimise these hurdles in the pursuit of personalised medicine.

Lecture 2: Women's Health

Prof. Olav Lapaire, MD, University Hospital Basel

Biosketch:

PERSONAL DETAILS

Name:	Olav Lapaire-Mayer M.D.
Date of birth:	April 11, 1970
Place of birth:	Palo Alto, California, USA
Nationality:	swiss

EDUCATION

09/ 2015	**Professorship (title professorship)**
10/2008	**Postdoctoral lecture qualification (Habilitation) in Obstetrics and Gynecology**
11/ 2005	**Certification of the Fetal Medicine Foundation for "Theoretical course for the Certificate of Competence in Ultrasound Examination at 11-14 weeks "**
2005	**Post-doctoral fellowship at Tufts-New England Medical Center, Boston**
10/2004	**Board certification in Obstetrics and Gynecology**
2004	**Certification of Fetal Medicine Foundation for Nuchal translucency measurement in the first trimester**
1998	**Exam of basic surgery, FMH, Bern**
1997	**Doctorate, University of Basel**
1996	**Final examination, University of Basel**

PROFESSIONAL EXPERIENCE

Current position:	**Deputy of the Head of Obstetrics and maternal-fetal medicine**
11/2008 -	**University Women's Hospital Basel** **Head of department: Prof. Dr. med. Viola Heinzelmann**
2006-2008	**University Women's Hospital Basel** **Head of department: Prof. Dr. med. Dr. h.c. mult. W. Holzgreve, MS, FRCOG**
2005	**Tufts-New England Medical Center, Boston** **Head of department: Prof. Dr. med. Diana Bianchi**
2000-2004	**University Women's Hospital Basel** **Head of department: Prof. Dr. med. Dr. h.c. mult. W. Holzgreve, MS, FRCOG**
1999	**Gynecology and Obstetrics, Kantonsspital Bruderholz/Basel** **Head of department: Prof. Dr. med. S. Heinzl**

1998	**Surgery, Gemeindespital Riehen** **Head of department: Dr. med. P. Nussberger**
1996-1997	**Geriatric Medicine, Adullamstiftung Basel** **Head of department: Dr. med. H.-J. Ledermann**

TEACHING (Additional activities)

2012	**Women's University Hospital Zurich, lectures**
2006	**Nursing School, Zurich, lectures**
2006	**University of Basel, Basel, lectures**
2002-2004, 2006	**Nursing School, Basel, lectures**
2003-2004	**International school of Ultrasound, Kosovo**

MEMBERSHIP

2008	**Swiss consortium for perinatal pharmakology (SAPP)**
2007	**Member of the Swiss Society for gynecological and obstetrical ultrasound (Schweizerische Gesellschaft für Ultraschall in der Medizin, Sektion Gynäkologie/Geburtshilfe)**
2006	**Member of the Swiss Academy for Feto-maternal Medicine**
2004	**Swiss Society of Obstetrics and Gynecology**

JOURNAL ACCTIVITIES

2006-08	**Editorial Assistant Journal of Fetal Diagnosis and Therapy**
2009	**Member of the Editorial Board Journal of Fetal Diagnosis and Therapy**
2013	**Member of the Editorial Board, Journal of Disease Markers**
2005	**Reviewer for Placenta, Fetal Diagnosis and Therapy, European Journal of Obstetrics and Gynecology, The Journal of Obstetrics and Gynaecology Research, Prenatal Diagnosis, Archices of Obsterics and Gynecology, Journal of Pregnancy, Trends in Genetics**

Basle (Switzerland), August 9th, 2021

Lecture Abstract:

«Personalized Medicine in Women»

Personalized medicine seeks to identify the right dose of the right drug for the right patient at the right time. The rapidly evolving field of personalized medicine is presenting new and promising opportunities for better patient care, also in obstetrics. The concept of individuali-zation of a medical therapy is based on the pharmacogenomic make-up of the individual and environmental factors that alter drug disposition and response, taking physiological, pregnancy associated changes into account, that can impact the therapeutic efficacy of medications. A genetic makeup-based prescription, a sophisticated design, and diligent implementation of therapy may not only improve the outcome of treatments but also reduce the potential risk of toxicity and other maternal and fetal adverse effects. However, additional future advances and research in the field of diagnosis approaches, data analysis, genomics, and clinical decision-making are needed to speed up the

individualization of therapy based on genetic makeup in the field of obstetrics. Finally, the potential utility of personalized medicine for treating the obstetric patient for hypertension and preterm labor is presented and the impediments of bringing personalized medicine into the obstetrical clinic are discussed.

L-3

Lecture 3: Pharmaceutical Technology

Prof. Hans Leuenberger, Professor emeritus University of Basel, Switzerland & Adjunct Faculty, College of Pharmacy, University of Florida, Orlando Campus, FL 32827, U.S.A.

Biosketch:

Hans Leuenberger studied physics resulting in a PhD in nuclear physics at the University of Basel. He worked for 12 years at Sandoz (today Novartis), and spent a sabbatical at the University of Michigan, Ann Arbor, leading to his habilitation thesis in Pharmaceutics. In 1982 he became full professor of Pharmaceutical Technology at the University of Basel. He served as acting vice president (1993-2001) of the Swiss Academy of Engineering Sciences (SATW), and from1992-1996 as president of its scientific Society of Pharmaceutical Sciences (today Swiss Academy of Pharmaceutical Sciences). Hans Leuenberger received numerous awards: www.ifiip.ch/awards gallery. Dr. Dr. h.c. mult. Hans Leuenberger is Honorary Member of the Swiss Academy of Engineering Sciences, Honorary President of the CISDEM Forum of Latin America, Portugal, Spain & Switzerland. Corresponding Member of the Royal Academy of Pharmacy of Spain; Foreign Member of the Russian Academy of Engineering; Fellow of the Swiss Academy of Pharmaceutical Sciences, and Fellow of the American Association of Pharmaceutical Scientists (AAPS).

Lecture Abstract:

«Spray Freeze-Drying for Precision Medicine &Vaccines»

The majority of the novel highly potent drugs, developed on the basis of modern molecular medicine, taking into account cell surface recognition techniques for precision medicine, are *often thermos-sensitive and have poor water-solubility*. A substantial increase of drug solubility can be obtained by the formulation of nanocomposite pellets using a spray freeze-drying process, which was originally developed at the University of Basel. This process was successfully commercialized by the company Meridion (meridion.de) of Dr. B. Luy. This platform, among others, can be used for manufacturing vaccines, biosimilars, biologics, monoclonal antibodies, antibody-drug conjugates for precision medicine. In this context, it is important to keep in mind that in case of a vaccine formulation the double chamber syringes can be kept at room temperature and do not need to be stored at -77°C. In addition, this platform can be used for medications based on the phage therapy to treat antibiotic resistant (MRSA) bacterial infections. In this context, a specific phage having the property of a virus only infects the bacterial cells. Thus, the infected bacteria cell loses its negative function and starts to serve as a host cell for the replication of more «friendly» viruses. Such an approach is also used for a viral therapy of cancer cells which will lead to cancer therapy without the side effects of traditional chemotherapy and radiation treatment. This type of highly specific targeted medication is the future of precision medicine. At the same time, it can be predicted that this innovative spray freeze-drying technology will be the future of manufacturing lyophilized products.

Keywords: nanoparticles and nano composites, innovation in bulk freeze-drying, precision medicine & vaccine formulation, phage & viral cancer therapy

L-4

Lecture 4: Precision Medicine

Prof. Mark A. Rubin, MD, Department for BioMedical Research (DBMR), Bern Center for Precision Medicine (BCPM), University of Bern

Biosketch:

Mark A. Rubin, MD, Professor, Principal Investigator, and Director of the Department for BioMedical Research (DBMR), University of Bern, Switzerland. Prof. Mark Rubin is a recognized world-renown leader in prostate cancer genomics and pathology, and precision medicine. Dr. Rubin's laboratory led a series of landmark studies defining distinct molecular features of prostate cancer, revealing pathways that are perturbed and drive different types of this cancer. Furthermore, Prof. Rubin has translated many of his genomic discoveries into clinical tests that are currently patented and standardly used in the diagnosis and treatment of prostate cancer. Prof. Rubin founded the Englander Institute for Precision Medicine and, more recently, the Bern Center for Precision Medicine (BCPM).

Lecture Abstract:

«Precision Oncology Approaches to Understanding Therapy Resistance»

Cancer cell resistance to therapy represents a significant gap in biomedical knowledge with major social and economic implications given the downstream effects and costs (repeated expensive imaging and staging, serial genomic testing, and the high costs of targeted secondary and tertiary line therapies including immunotherapy). Research in this field has focused on the resistance encountered by the oncogenic effector that is being targeted by a drug. However, as proposed by Garraway et al. [1], a broader framework to appreciate the complexity of resistance is required. This includes taking into account genomic, transcriptomic, epigenetic, and micro-environmental aspects. There are three key routes to resistance: pathway reactivation, pathway bypass, and pathway indifference. The tumour microenvironment (TME) can modulate each of these mechanisms**.** **Pathway reactivation**: A major focus in understanding resistance has been on new mutations (neo-mutations) or alterations (e.g., amplifications or splice variants) to the index effector or index oncogenic pathway. For lung cancer these would be epidermal growth factor receptor (EGFR) mutations that inactivate sensitivity to primary drugs. For prostate cancer (PCa) this would be mutations in the androgen receptor (AR) that lead to reactivation of the AR signalling pathway. **Pathway Bypass**: Bypass implies the activation of an alternate effector or alternate oncogenic pathway. In PCa, for example, elevated FGF and MAPK activity after AR blockade results in the activation of oncogenic transcriptomic pathways in an AR independent manner. Another example is the activation of the glucocorticoid pathway. **Pathway Indifference**: Indifference refers to adaptation to a new state that is insensitive to the original treatment through lineage plasticity (LP) [2] in PCa after AR blockade. A similar phenomenon has been observed in EGFR-mutant non-small cell lung cancer (NSCLC) following targeted therapy [3]. In both instances, there are no current therapeutic options and clinical demise is rapid.

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e-Posters

I. PHARMACEUTICAL BIOLOGY / PHYTOPHARMACOLOGY

P - I - 1

Antiprotozoal activity of compounds isolated from the root bark of *Ziziphus jujuba*

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Introduction: Human protozoal diseases such as malaria, sleeping sickness, Chagas disease and leishmaniosis remain important health problems, with significant morbidity and mortality, particularly in tropical countries. For centuries, plants have been used by local populations to prevent and cure human diseases in many parts of the world. According to the WHO, approximately 80% of the African population uses traditional medicine for their health care [1].
Aims: As part of our ongoing research on the discovery of new antiprotozoal agents from West African plants, the dichloromethane extract of *Ziziphus jujuba* root bark was investigated.
Methods: Air-dried and powdered *Z. jujuba* root barks (270 g) were extracted at room temperature by maceration using dichloromethane (1:10, m/v, 3 x 24 h). From this extract, sixteen compounds were isolated and their structures were elucidated by 1D, 2D NMR experiments, UV, IR and HRESIMS data. The extract and thirteen of the compounds were evaluated for their antiprotozoal activity towards *Trypanosoma brucei rhodesiense*, *Trypanosoma cruzi*, *Leishmania donovani*, *Plasmodium falciparum,* as well as for their cytotoxicity against L6 rat skeletal myoblast cells.
Results: The dichloromethane extract of *Z. jujuba* root bark exhibited inhibitory activities against *T. cruzi* (IC_{50} = 7.4 µg/mL), *L. donovani* (IC_{50} = 2.7 µg/mL), *P. falciparum* (IC_{50} = 1.7 µg/mL), and *T. b. rhodesiense* (IC_{50} = 10.2 µg/mL). Eight cyclopeptide alkaloids and eight triterpenes were isolated and characterized. Mauritine M is a previously described cyclopeptide alkaloid and is the most active compound with IC_{50} values around 3-4 µM against *T. b. rhodesiense* and *P. falciparum*. Some of the triterpenes showed inhibitory activities against *L. donovani* (IC_{50} < 3 µM) and *P. falciparum* (IC_{50} < 0.2 µM).
Discussion & Conclusions: *Z. jujuba,* commonly known as jujube, is traditionally used against diarrhea, ulcers, vomiting, indigestion, pulmonary ailments, dysentery and fever [2, 3]. To the best of our knowledge, this is the first report on the antileishmanial and antitrypanosomal evaluation of *Z. jujuba*. Furthermore, two compounds have not yet been described in the literature. Active compounds should be further tested to assess their possible use as antiprotozoal drugs.

Keywords: african plant, natural products, neglected tropical diseases, parasitic diseases, *Ziziphus jujuba.*

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P - I - 2

Phytomedicines for mental diseases and the placental barrier: an *ex vivo* study

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Introduction: A recent prevalence estimate in Switzerland reported that 16.7% of perinatal women used mental healthcare. Pharmacotherapy for mental diseases in pregnant women is challenging given that adverse effects on the embryo/foetus have to be considered. Some phytomedicines, such as hops (*Humulus lupulus* L.) and California poppy (*Eschscholzia californica* Cham.*)*, could be used as alternatives to synthetic drugs. However, despite a long track record of use, safety data in pregnancy are largely lacking. Knowledge of foetal exposure to exogenous compounds is crucial for an informed risk assessment in pregnancy. Human *ex vivo* placental perfusion is considered the «gold standard» among translocation models.
Aim: The main aim of this work was to characterise the transplacental transfer of humulone, a characteristic and pharmacologically active compound in hops, and protopine, a major alkaloid in California poppy. The effects of humulone and protopine on the viability of placental tissue and on the production of placental hormones were also investigated.
Methods: Placentas were obtained after informed consent from caesarean sections. In a first step, we established and validated an *ex vivo* placenta perfusion model. Validation was performed with drugs known to cross in this model from the maternal to the foetal circuit, namely antipyrine as a positive control, and citalopram and diazepam as two medications known to cross the placenta barrier. In a second step, we used the same model to characterise the transfer of humulone and protopine. All compounds were quantified by partially validated U(H)PLC-MS/MS bioanalytical methods.
Results: The transfer of citalopram and diazepam was reproducibly observed in our placenta perfusion model, and results were in accord with previously reported data. Only a small portion of humulone initially present in the maternal circuit reached the foetal (1.3% ± 0.6%) and maternal (1.0% ± 0.0%) circuit. As humulone did neither significantly adsorb to the perfusion setup nor bind to the placental tissue, a major amount is likely to have been metabolised in the placenta. This is also in line with the stability data showing that humulone was unstable in contact with placental homogenate. Protopine was transferred from the maternal to the foetal circuit, with a steady-state reached after 90 min. None of the study compounds affected glucose consumption and lactate production during perfusion, and beta-human chorionic gonadotropin and leptin release remained constant.
Discussion and Conclusions: The placental *ex vivo* perfusion model was successfully implemented and used for the first time with phytochemicals. Transplacental transfer occurred with protopine, but only to a negligible extent with humulone. Taken together our results strongly suggest a metabolisation of humulone, but not of protopine, in the placenta. Both compounds had no detrimental effect on placental tissue viability and functionality. For a further assessment of hops and California poppy, additional phytochemicals in these plants have to be investigated, together with testing in additional models (e.g. *in vitro*). The *ex vivo* placental perfusion model will now be used also for transport studies with relevant phytochemicals in other medicinal plants that are candidates for the treatment of mild NMDs in pregnancy.

Keywords: *Humulus lupulus*, *Eschscholzia californica*, placental barrier, *ex vivo* cotyledon perfusion, pregnancy

P - I - 3

Impact of *Petasites hybridus* extract Ze 339 on intestinal handling of histamine

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Introduction: Histamine intolerance (HIT) or food-derived histaminosis is a common diagnosis in Western population with an estimated prevalence of 1% in a population. In patients with HIT exposure to exogenous histamine is linked to various symptoms such as rhinal congestion, dizziness, headache, tachycardia, hypotension, diarrhea, nausea, and flush. The uptake of food-derived histamine is assumed to be modulated by specific mechanisms in the intestine. Here, the diamine oxidase (DAO) and the histamine-N-methyltransferase (HNMT) metabolize histamine, while transport proteins are believed to contribute to the transcellular flux of histamine.
Aims: In previous studies it was shown, that the *Petasites hybridus* CO_2-leaf extract Ze 339 (Tesalin®) decreased histamine levels in nasal laryngeal fluids. The aim of the current study was to investigate the influence of Ze 339 on the intestinal mechanisms involved in the handling of histamine.
Methods: We used the cell model of differentiated Caco-2 cells validated for the presence of DAO, HNMT and organic cation transporter 3 (OCT3; SLC22A3) to investigate the effect of Ze 339 on their mRNA and protein expression by real-time PCR and Western Blot analysis. We further tested the effect of Ze 339 on DAO release from Caco-2 cells polarized on permeable supports (Transwell®) and DAO enzyme activity. We applied Caco-2 Transwell® transport studies to assess the influence of the herbal extract on the transcellular histamine flux. Findings on changes in flux were supplemented with transport studies using MDCKII cells stably overexpression OCT3.
Results: Even though Ze 339 reduced the mRNA levels of HNMT and DAO, there was no influence on their protein levels. Ze 339 neither affected the basal release of DAO from Caco-2 cells, nor changed DAO enzymatic activity. However, testing the interaction of the extract with the transcellular transport of histamine, we observed a significant increase in the basal to apical flux (P_{app} b to a) in presence of high concentrations of Ze 339. This effect was limited to the early phase of the experiment. Since the luminal monoamine transporter OCT3 is one transporter possibly contributing to this finding we examined the influence of Ze 339 on the OCT3-mediated histamine uptake in overexpressing MDCKII cells. We revealed a dose-dependent inhibition with an estimated IC_{50} of 26.9 ug/mL for the extract.
Discussion & Conclusion: In conclusion, we report an effect of Ze 339 on the transcellular transport of histamine resulting in a high efflux ratio, where inhibition of the luminal uptake transporter OCT3 may contribute.

Keywords: histamine intolerance, *Petasites hybridus*, intestinal histamine handling, diamine oxidase, OCT3

P - I - 4

Natural products targeting aberrant ERK/AKT signaling in human melanoma cell lines

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Introduction: The incidence of melanoma, the most fatal dermatologic cancer, has dramatically increased over the last decades. Melanoma show the highest mutation rate of all cancers, with over 10 mutations/Mb. Studies have shown that more than 50% of malignant melanomas exhibit BRAF V600E mutation, leading to constitutive activation of the ERK pathway. Vemurafenib, a specific BRAF V600E inhibitor, has been approved in 2011 for the treatment of metastatic melanomas [1]. Despite spectacular initial results, drug resistance appears within months. Novel inhibitors targeting aberrant proliferation signaling (ERK and AKT pathways) in melanoma are therefore urgently needed. Natural products continuously provide lead compounds for drug discovery. In cancer chemotherapy, they account for over 60% of approved drugs [2].Therefore, we established a high-content screen (HCS) with a bioassay to enable discovery of natural products targeting the complex network of ERK and AKT pathway in living cells.
Aims: Finding new AKT/ERK pathway inhibitors from plant extracts.
Methods: An innovative HCS assay has been developed and combined with our natural product lead discovery platform. The screen was performed on the well-established human melanoma cell line A2058 (harboring a BRAF V600E mutation and a PTEN deletion), as well as on the patient-derived primary cell line MM121224 (harboring BRAF V600 and NRAS Q61R mutations). Both lines therefore exhibit high oncogenic ERK and AKT activity. The cell lines were engineered to express genetically encoded biosensors that report on ERK and AKT activity [3] allowing to screen for compounds that would inhibit ERK/AKT activity.
Results: With this assay we screened a library of 2576 crude extracts and identified 40 hits on the A2058 line and 126 hits on the MM121224 line. 70 extracts were chosen for HPLC micro-fractionation and further testing. The so-called activity-profiles that combine the bioassay results with analytical information (UV-ELSD-ESIMS) enabled the identification of active compounds in complex extracts. Five extracts that were prioritized based on activity profiles were submitted to a targeted isolation of active compounds. A structurally diverse set of molecules active on the ERK/AKT pathway was identified, including flavonoids, brevipolides, piperamides and terpenoidal derivatives. The approach will be explained, and its efficiency highlighted with selected examples.
Discussion and Conclusions: Our approach allows us to explore the natural product chemical space targeting signaling activities in a more comprehensive manner than with classic assays. This may enable the discovery of leads for next-generation drugs for a targeted therapy of melanoma.

Keywords: melanoma, high-content screening, natural products, HPLC, targeted therapy

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II. PHARMACEUTICAL TECHNOLOGY

P - II - 1

Encapsulation of a combination of model microorganisms for fecal microbiota transplantation

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Introduction: Recent research pointed out the relationship between the microbiome and several diseases such as *Clostridioides difficile* infection (CDI), inflammatory bowel diseases, irritable bowel syndrome, obesity, autism spectrum disorder or Alzheimer's disease. The need for methods to modulate the microbiota has then risen: probiotics, prebiotics, post-biotics and fecal microbiota transplantation (FMT). One of the most important challenge of this treatment is the formulation, because of its administration by uncomfortable routes such as naso-duodenal/jejunal tubes, colonoscopy, enema or many voluminous oral capsules, but also its stability during storage. The active component(s) of FMT still remain unclear but the live bacteria constituting the microbiota are considered important. Applying pharmaceutical technologies used for live bacteria formulation will help to produce an efficient formulation for FMT.
Aims: The aim of this project is to produce a dry, stable, compact, gastro-resistant and efficient formulation, which can contain a high amount of live microorganisms and that selectively targets the colon of the patient. In the future, this formulation would ease the administration of FMT and thus, it would reduce costs related to the handling of fecal samples and promote research related to microbiota manipulation therapies.
Methods: Model bacteria strains were grown in their respective culture medium and concentrated prior microencapsulation. The said strains chosen were: *Escherichia coli*, *Enterococcus faecalis* and *Lactobacillus paracasei*. The microencapsulation protocol is based on the extrusion and ionotropic gelation of alginate droplets (1). Concentrated bacteria suspension was mixed with a sodium alginate and trehalose solution. The bacterial suspension was then extruded through a syringe in a calcium chloride solution under magnetic stirring. Next, the obtained particles were harvested by filtration and rinsed twice. Each sample was then frozen at -80°C for at least 1 h. Afterwards, all samples were freeze-dried overnight on automatic cycle. The particles were characterized by optical microscopy and the viability of the entrapped bacteria was assessed by plate-counting and flow cytometry using a LIVE/DEAD™ BacLight™ Bacterial Viability Kit.
Results: Particles of a mean size of ~2 mm were obtained. The appearance of spherical and sturdy gel beads and the mean size is constant between all batches. A high-loading up to 10^{15} and 10^{17} CFU/g of dry product was successfully obtained for single strains and mixed strains, respectively. This corresponded to a viability of 40% and 60%, respectively, for flow cytometry.
Discussion and Conclusions: The extrusion method to formulate alginate particles yielded consistent and robust batches in terms of particles' appearance and size. It was possible to obtain highly-loaded particles with encapsulated live bacteria counts, which is promising as the whole gut microbiota is estimated to contain about 10^{14} bacteria cells. The next steps would be to enhance the formulation so that it will be gastro-resistant and will selectively target the colon. In addition, the application of the technique to clinical stool samples and the evaluation of the effect of the formulation process on the viability, abundance and diversity of the microbiota will be assessed.

Keywords: fecal microbiota transplantation, bacteria formulation, high-loaded particles, alginate particles

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P - II - 2

Drug delivery by amorphous solid dispersions: a randomized pharmacokinetic study in humans

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Introduction: Low oral bioavailability is a recurrent reason for drop-outs of poorly soluble drug candidates in preclinical and clinical stages of drug development, creating a need for reliable drug-delivery systems that can increase bioavailability. A promising candidate platform are amorphous solid dispersions (ASDs). In ASDs, the active pharmaceutical ingredient (API) is delivered in its amorphous state, which is stabilized by a solid polymer matrix. Upon dissolution, a complex system of drug-rich particles can evolve. While ASDs have been extensively investigated *in vitro* and *in vivo* and as well have been marketed in several cases, details on their behavior as a drug delivery platform in humans today are poorly investigated and understood.
Aims: In this clinical study we aimed to investigate the effects of a particle forming amorphous solid dispersions (ASDs) on bioavailability in humans, seeking insights in the complex drug absorption mechanisms from ASDs in a clinical setting.
Methods: We conducted a randomized cross-over design open-label clinical study (NCT03886766) with 16 healthy male volunteers in an ambulatory setting, using micro-dosed efavirenz (EFV) as a model drug. Three interventions (oral ingestion) were performed in block-randomized order: (1) Solid ASD of EFV 50 mg, (2) Dissolved ASD of EFV 50 mg (drug-rich particles), (3) Molecular solution of EFV 3 mg (non-ASD). Endpoints were the pharmacokinetic profiles (EFV plasma concentration *vs.* time curves) and derived pharmacokinetic parameters thereof. Study results were also compared to existing data on a marketed formulation (Stocrin® 50 mg) in a non-cross-over design. Reverse PBPK-modeling was used to simulate the intestinal drug concentration based on measured PK data.
Results: All interventions as well as the marketed formulation showed comparable areas under the curve AUC_{0-t} (scaled to dose). The solution showed the highest maximum concentration C_{max} and the shortest time to reach maximum concentration t_{max}, with a significant difference to both the solid ASD and the marketed formulation ($p < 0.05$). The dissolved ASD showed a slower but comparable absorption compared to the solution. The results of the absorption constant k_a showed statistically significant differences between the liquid (dissolved ASD and solution) and the solid formulations (ASD and marketed formulation). Reverse PBPK-modeling indicated that the dissolved ASD in the human intestine behaved like a solution also at the higher dose.
Discussion and Conclusions: Drug absorption from drug-rich particles, formed upon the dissolution of the ASD, was fast and complete in humans, underlining their potential as drug delivery system. A molecularly dissolved drug concentration beyond aqueous saturation concentration in the intestinal tract is indicated by the reverse PBPK model. The studied drug-rich particles from the ASD might prevent drug crystallization as well as permanent solubilization of drug into micelles
Keywords: bioavailability, drug delivery, amorphous solid dispersion (ASD), clinical study

Reference:
Schittny A et al., Pharmaceutics 2021; 13(3): 401

P - II - 3

Translating thin film rehydration method for cationic liposome manufacture to a microfluidics system for a liposomal pDNA vaccine against SARS-COV2

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Introduction: The severity and the toll on healthcare systems all over the world caused by Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2), led the World Health Organization to declare a pandemic on 11 March 2020. The sudden spread of the disease Since early last year, huge efforts in vaccine research were made. DNA vaccination is considered a potential strategy to meet the challenge. This technique, however, holds two major disadvantages: poor immunogenicity and transfection of the target cell. Both require the development of effective delivery systems and adjuvants. Liposomes are among the more widely investigated vaccine delivery systems. New generation microfluidics devices are a great tool to overcome these problems.
Aims: A plasmid DNA (pDNA) encoding SARS-COV-2 full-length Spike (S) protein, pDNA-S was developed at Chula Vaccine Research Center, Chulalongkorn University, Bangkok, Thailand. An effective DOTAP based liposomal delivery system was prepared, which showed immunogenicity *in vivo*. The thin film protocol was translated to a microfluidics device (NanoAssemblr Ignite, Precision Nanosystems), with the goal of obtaining the same liposomal formulation of similar properties in terms of size, zeta-potential and transfection efficiency.
Methods: DPPC:DOPE:DOTAP (DOTAP4) liposomes where prepared by microfluidics, organic phase was prepared in ethanol, which was later removed by tangential flow filtration (TFF). Dynamic light scattering, zeta-potential measurement, and transmission electron microscopy (TEM) were used to characterize the liposomes. Liposomes and pDNA-S were mixed at different N/P ratios and incubated 30 min at RT to allow complexation. Size, zeta-potential and electrophoretic mobility were measured. HEK293 adherent cells were transfected 24h before the experiment with pDNA-S/liposome complexes and used to detect protein expression. Complexes of Lipofectamine 2000-pDNA S were used as positive control, and pDNA S alone and non-transfected cells were used as negative control. Images were taken using Nikon A1r Spectral.
Results: DOTAP4 blank liposomes prepared by thin film rehydration had a size of 130.9 ± 5.8 nm and a PDI of 0.210 ± 0.028, zeta-potential was +48 ± 12 mV. Liposomal positive charge allowed for the complexation with negatively charged pDNA-S. N/P ratios from 0.25:1 to 100:1 were tested. A size increase with increasing positive charge was observed, which corresponded to a switch from negative charged samples (nucleic acid excess) to positive charged samples (liposome excess), confirming the fact that aggregation is increased when charge is neutral. Gel permeation assay as well as zeta-potential results are equivalent for the two methods. However, some differences were observed for ratios 10:1, 25:1 and 100:1 regarding the size. Transfection was confirmed in cells treated with liposome-pDNA-S complexes, which was observed for both microfluidics manufactured sample and thin film layer rehydration sample at a 1:1 N/P ratio and in pDNA-S/lipofectamine samples.
Discussion and Conclusions: Stable cationic liposomes were formulated by microfluidics and negatively charged pDNA-S was successfully complexed. Characterization showed comparable results between both methods. Further studies need to be performed in order to quantify gene expression. Furthermore, *in vivo* studies will be performed to assess the efficacy of the preparation method compared to thin film rehydration.

Keywords: DNA vaccines, liposomes, microfluidics

P - II - 4

Efficient colonic drug delivery – novel tablet formulation with double control concept

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Introduction: Efficient and selective drug delivery to the colon has not yet been achieved. This is especially relevant for local treatment of inflammatory bowel diseases. Available formulations mainly focus on only one release-controlling mechanism, predominantly by using pH-sensitive coatings. Due to inherent variability of pH values through the gastrointestinal tract, both premature and delayed active pharmaceutical ingredient (API) release were shown, causing systemic side effects and therapy failure, respectively.
Aims: We developed a new oral formulation for targeted colonic delivery by implementing two control mechanisms: A pH-sensitive coating preventing drug release in the upper gastrointestinal tract and a xyloglucan-based matrix inhibiting drug release before entry into the large intestine. Bacterial enzymes present in the colonic microbiome cleave this matrix polysaccharide, ensuring drug release at the targeted site.
Methods: Coated matrix tablets were tested in vitro for their drug release by simulating complete mouth-to-colon transit. The passage through stomach, upper and lower small intestine was mimicked by step-wise pH changes. Xyloglucanase was added in the final stage of dissolution testing, representing the enzymatic activity of the microbiome. The effect of different enzyme concentrations on drug release was investigated. Drug release was quantified spectrophotometrically while matrix degradation was measured in the form of reducing sugars being created during the enzymatic polysaccharide cleavage.
Results: The applied Eudragit® FS coating layer successfully prevented drug release in the upper gastrointestinal tract. Upon contact with fluids, the xyloglucan matrix formed a highly viscous mass and thereby prevented major drug release in the simulated lower small intestine. In the absence of xyloglucanase, drug release was slow and required up to 46 h for complete dose dissolution. Drug release from the matrix tablet was accelerated with increasing enzyme concentration. Reducing sugar quantification showed parallel profiles for matrix degradation and API dissolution, indicating erosion-controlled drug release.
Discussion and Conclusions: Efficient and selective drug delivery to the colon was demonstrated *in vitro*. Our new oral formulation with double control mechanism prevented premature drug release by the use of two control concepts; an enteric coating layer and a biodegradable xyloglucan matrix. Hydrolysis of the matrix polysaccharide by enzymes present in the human colonic microbiome accelerated drug release at the desired site of action. Under physiologically relevant enzyme concentrations, complete dose release was achieved within the expected human colonic transit time.

Keywords: colonic drug delivery, matrix tablets, xyloglucan, *in vitro* dissolution, microbiome

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Polyethylenimine/cGAMP complexes for STING-mediated cancer immunotherapy: formulation and characterization with orthogonal techniques

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Introduction and Aim: Cyclic GMP-AMP (cGAMP) is lately extensively investigated in cancer immunotherapy, due to its activation of innate immunity pathway – STING within the antigen presenting cells (APC) and later increase in tumor specific CD8+ T cells. As a negatively charged dinucleotide prone to degradation by enzymes before being taken up by APC, there is a need for appropriate carrier. Therefore, polyethylenimine (PEI), as a golden standard for oligonucleotide delivery has been chosen.
Material and Methods: Molecular weight (1.2, 4, 10 and 25 kDa), type of PEI (linear and branched), and N/P ratio between PEI/cGAMP (1/1 and 2/1) are affecting toxicity, efficacy and physicochemical properties of complexes such as size, zeta potential and shape. In order to examine those parameters several complementary methods were employed, such as dynamic light scattering (DLS), nanoparticle tracking analysis (NTA) and online Asymmetric Flow Field Flow Fractionation (AF4) connected to DLS.
Results and Discussion: 2/1 ratio of linear PEI 25 kDa/cGAMP as a bigger, positively charged particles with elongated bacteria look alike shape were shown to have the best toxicity/efficacy ratio among different PEIs and ratios tested.
Conclusion: linear PEI 25/cGAMP complexes were chosen to continue with the further *in vivo* studies.

Keywords: cGAMP, polyethyleneimine, dynamic light scattering DLS, asymmetric flow field flow fractionation AF4, nanoparticle tracking analysis NTA

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Zebrafish (*Danio rerio*) embryo as an *in vivo* vertebrate model to study renal function

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Introduction: The study of renal function remains a challenge. *In vitro* cell based assays are approved to study e.g. ABC/SLC mediated drug transport but do not cover other renal functions as glomerular filtration and tubular reabsorption. For this purpose, *in vivo* studies are needed, which are time-consuming, expensive, and often rely on *in vivo* experimentation with higher vertebrates. However, they often need to be combined with *in vitro* or *ex vivo* transporter assays to provide mechanistic insights at a cellular level. In view of these limitations, there is a high unmet need for cost-effective and animal reducing *in vivo* test systems that include mechanistic studies at a cellular level and allow a translation to humans.
Aims: Since the zebrafish embryo (ZFE) pronephric kidney shares high similarity with the anatomy of nephrons in higher vertebrates, including mammals and does not count as an animal, we explored in the present study whether 3 to 4 days old ZFE have a fully functional pronephron. The aim is to use ZFE as an *in vivo* vertebrate model to study glomerular filtration, ABC/SLC mediated drug transport and folate receptor 1 mediated tubular reabsorption.
Methods: Polymers of interest were fluorescently labelled using click-chemistry (e.g. FITC-PEG) and characterized using Fluorescence Correlation Spectroscopy to determine purity and the hydrodynamic diameter. These polymers and fluorescent model substrates of specific drug transporters combined with their corresponding inhibitors were intravenously injected into 3-4 days old anaesthetized ZFE. Fluorescent test compounds were localized within the tubular volume or the central blood compartment using confocal fluorescence microscopy and recombinant marker zebrafish lines.
Results: Intravenous injection of fluorescent PEG and dextran derivatives of different molecular weights revealed a cut-off of 4.4 to 7.6 nm in hydrodynamic diameter for passive glomerular filtration, which agrees with corresponding values in rodents and humans. Distal tubular reabsorption of a FITC-folate conjugate, covalently modified with PEG_{2000}, was mediated by the folate receptor 1 (folr1). Transport experiments in the presence and absence of specific inhibitors confirmed functional expression in the proximal tubule of oat/slc22, mrp1/abcc1, mrp2/abcc2, mrp4/abcc4 and the ZFE p-glycoprotein analogue abcb4. These results were confirmed by corresponding *ex vivo* experiments in killifish (*Fundulus heteroclitus*) proximal kidney tubules.
Discussion and Conclusions: We conclude that ZFE has a fully functional pronephron at 4 days post fertilization and is, therefore, an attractive translational vertebrate screening model to bridge the gap between cell culture-based test systems and pharmacokinetic experiments in higher vertebrates.

Keywords: zebrafish, renal function, drug transporter, glomerular filtration, kidney tubule

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Artificial intelligence in the analysis of time-resolved micro-computed tomography data

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Introduction: Understanding the behavior of pharmaceutical tablets in physiological liquids, i.e. dissolution and disintegration, is of great importance to controlling a formulation's performance *in vivo*. Due to the complexity of the mechanisms involved and the impossibility of directly observing the process, the mechanistic understanding is still far from complete.
Aims: We aim to develop tools to study disintegration mechanisms by providing direct and dynamic observations of the process. We applied 4-dimensional micro-computed tomography imaging combined with deep learning based computer vision.
Methods: A set of 64 mini-tablets with different compositions was imaged at the Swiss Light Source synchrotron at the Paul Scherrer Institute. Their highly specialized facility provides the means necessary to generate time-resolved micro-computed tomography (µCT) images of the tablets as they disintegrate in water.
Results: A custom µCT data reconstruction pipeline was implemented in Python and CUDA C++. To enable mathematical analysis, the reconstructed images were segmented using a deep learning approach based on a custom 3D convolutional neural network.
Discussion and Conclusions: Our approach demonstrates the power of deep learning when it is applied in a suitable setting. Due to the non-linearly separable nature of the data, the convolutio-nal neural network outperforms traditional image segmentation approaches and is able to process massive amounts of data without user intervention.

Keywords: artificial intelligence, time-resolved micro-computed tomography, tablet disintegration

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Thermoresponsive hyaluronan-based hydrogels for the formulation of standardized transplants in tendon regenerative medicine

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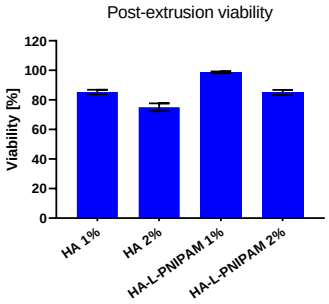
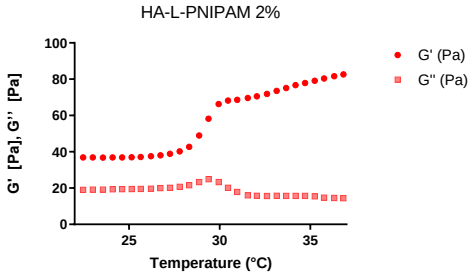
Introduction: Thermoresponsive hyaluronan-based hydrogels enable facilitated injection of products developing high viscosity values *in situ* and thus present potential for delivery of therapeutic cellular materials in specific regenerative medicine applications (*e.g.*, repair stimulation of structurally and functionally impaired hand tendons). Therein, cultured human fetal progenitor tenocytes (hFPT) have been identified as potentially optimal therapeutic cell sources for manufacture of homologous therapeutic standardized transplants.
Aims: The aim of the present study was to validate the combination of product prototypes, composed of viable hFPTs and thermoresponsive hyaluronan-based hydrogels named HA-L-PNIPAM.
Methods: Hyaluronic acid (HA) was grafted with poly(N-isopropylacrylamide) (PNIPAM) using a spacer. Structures of HA-L-PNIPAM were determined from their ^{1}H NMR spectra. The final cell content in the respective combination product formulations was of 5 x 10^{6} cells/mL of gel. Using HA as a baseline, the different preparations were extruded through 26G needles to evaluate cellular integrity/viability maintenance after application. Rheological behaviours of the products were determined as a function of the temperature.
Results:





Discussion and Conclusions: HA-L-PNIPAM formulations outperformed HA formulations in terms of maintenance of cellular integrity/viability after extrusion. Specific characterization workflows allowed to experimentally confirm the spontaneous and reversible microgel-structure thermoformation above 27°C, while maintaining adequate viability of therapeutic cellular payloads. The thermosensitive viscous behaviour of the hydrogel during the injection may play a major role in the preservation of cell viability. This study provides the technical basis for further formulation optimization in translational musculoskeletal regenerative medicine, with specific focus set on hand tendon disorder management.

Keywords: cell therapy, regenerative medicine, thermoresponsive hydrogels, hyaluronic acid

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Mesoporous silica particles as drug delivery system offering prolonged drug release

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Introduction: Mesoporous silica particles (MSP) are inorganic nanoparticles and have gained great popularity in pharmaceutics over recent years. Their applications range from diagnostic to therapeutic purposes. Due to their controllable and uniform pore sizes, flexible surface functionalization, significant biocompatibility, biodegradability and lack of toxicity, they are of high interest as carriers for a targeted drug delivery. A variety of differently arranged MSPs have already been synthesized and previously been characterized as drug carrier systems. In this study Mobil Crystalline Materials (MCM-41) MSPs were investigated with regard to their potential to be used as drug carriers allowing a controlled release of drugs.
Aims: Specifically, the aim of this project was to establish a strategy, to chemically modify MCM-41 MSPs in order to achieve a sustained release of drugs, loaded in these modified MSPs, over prolonged time periods of days to weeks. We chose two approaches to do this: (i) MSPs were functionalized with 3-aminopropyltriethoxysilanes (APTES), which has previously been suggested to prolong the release of drugs from MSPs in several studies [1, 2] and (ii) drug loaded MSPs were coated with a synthetic polymer to prolong the drug release.
Method: In a first step, the drug loading efficiencies of the MSPs were analyzed. For initial proof of concept, MSPs were functionalized with different concentrations of APTES according to established procedures [3] and then equilibrated in a solution with equal concentrations of fluorescein free acid (FFA) as model drug. The release of FFA from MSPs was analyzed by UHPLC. In subsequent experiments, APTES functionalization protocols were varied by performing the APTES functionalization of MSPs in the presence or absence of humidity, before or after drug loading to test the feasibility of further prolonging drug release. For this second round of experiments, ibuprofen (IBU) was used as a model drug. The release of IBU from MSPs, as powder or tableted to disks, was measured in phosphate buffered saline (pH 7.4). To investigate if a polymer coating could be used to achieve a sustained drug release, the ibuprofen loaded MSPs were coated with the model polymer Poly (2-hydroxyethyl methacrylate) (HEMA). Drug release was, due to the lack of water solubility of HEMA, tested in organic solvents such as ethanol.
Results: By UHPLC analysis, a linear dependence of FFA loading on APTES concentration was observed (R^2 0.992). Independent of the specific APTES functionalization protocol tested, drug release of the MSP powder could not be prolonged for more than 3 h using this approach. However, in a further experiment, IBU-loaded MSPs were tableted into discs before *in vitro* drug release. In agreement with the publication of Manzano et al. [1], a sustained release of IBU over 50 h was observed. In addition, the performed proof of concept study showed, that the IBU- loaded MSPs, which were coated with HEMA displayed a prolonged release of IBU over several days in organic solvents.
Discussion and Conclusion: In this study it was possible to manufacture mesoporous silica particles with different drug loadings. A sustained drug release could be achieved by either tableting the functionalized MSP, or by coating the IBU-loaded MSPs with HEMA. Polymer coating is hence an option to achieve sustained release of drugs from MSPs. For further experiments, a biodegradable polymer such as Poly (lactic-co-glycolic acid) should be chosen, where the drug release kinetics are controlled through the degradation time of the polymer.

Keywords: mesoporous silica particles (MSP), drug delivery, prolonged drug release, functionalization

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Electrospun poly(L-lactide-co-ε-caprolactone) (PLCL) fibres loaded with mesoporous silica particles to prevent bacterial biofilms on medical implants

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Introduction: Implant innovation represents an important field of medical research. One of the complications associated with medical implants are bacterial infections and subsequent formation of bacterial biofilms. Directly after implant surgery, a so-called «race to the surface» pro cess takes place, which involves the competitive attachment of host cells such as fibroblasts and bacteria on the implant surface. In case bacterial colonisation outcompetes growth of mammalian cells, the adhesion of bacteria leads to a successive formation of several bacterial layers [1]. A protective matrix forms around the bacterial layers in a biofilm, which prevents the penetration of antibiotics and can give rise to systemic bacterial infections, potentially life-threatening to patients. One approach to avoid implant associated bacterial biofilms is to embed medical implants in a biodegradable, protective wrap, pharmacologically preventing the adhesion of bacteria.
Aims: The current study focuses on the development of a protective shell of polymer fibres, which could prevent the bacterial adhesion through its nanostructured surface obtained by electro-spinning. Furthermore, the shell contains drug carriers such as mesoporous silica particles enabling sustained release of anti-inflammatory and anti-bacterial drugs.
Methods: For the manufacturing of the fibres, the poly(L-lactide-co-ε-caprolactone) (70:30) (PLCL) was solved in a mixture of dichlormethane and dimethylformamide (7/3). After adding MSPs to the solution, nanofibers were spun out of the suspension via electrospinning. The uniformity and morphology of the obtained fibres were observed by scanning electron microscopy (SEM) and fluorescence microscopy. For *in vitro* experiments NIH 3T3 fibroblasts were cultivated on the obtained PLCL fibres. The integration and adhesion of the cells in the fibres were observed using fluorescence microscopy. Additonally a MTT ((3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) assay was performed to examine the viability of the fibroblasts.
Results: The development and optimisation of the key parameters (polymer concentration, flow rate, voltage) during the electrospinning process ensured a suitable quality of the polymer fibres with an average fibre diameter between 0.5 - 1.2 μm. According to the obtained SEM images, MSPs were succesfully incorporated in the spun fibres, however MSP agglomerates rather than single particles were observed. In cell experiments fibroblasts showed an efficient immigration into the different layers of the scaffold. Furthermore the polymer fibres had only a minor effect on the cell viability.
Discussion and Conclusions: The polymer PLCL is suitable to produce nanofibre-meshes using electrospinning. Moreover, the herein produced polymer scaffolds showed a good biocompatibility as demonstrated by the cultivation of fibroblast within the scaffolds. To further promote the migration of the fibroblasts and improve the tissue-anchoraing of the implant, an improvement of the 3D geometric scaffold-structure would be necessary. The integration of MSPs into the fibres during electrospinning is possible, but requires further optimization. In addition, the tendency, of the drug carrying MSPs, to agglomerate must be overcome and a suitable method to achieve the release of the MSPs and the drugs loaded onto them out of the fibres during biodegradation must be established in further experiments.
Keywords: implant infection, electrospinning, mesoporous silica particles (MSP), fibroblasts (NIH 3T3)

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P - II - 11

Influence of manufacturing parameters during high-pressure homogenization on oxidation products in parenteral lipid emulsions

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Introduction: Lipid emulsions containing polyunsaturated fatty acids are susceptible to oxidation. Diene radicals can react with oxygen and form lipid peroxides. In the body, they can modulate and disturb the basal metabolism and functional state of cells and organs [1]. To finely disperse the immiscible oil droplets in the outer aqueous phase and form emulsions, high-pressure homogenization is employed. Exposure to high process pressures as well as atmospheric oxygen during the manufacturing can deteriorate the quality of the resulting product. The use of a backpressure module during homogenization alleviates the pressure drop from process pressures of up to 2000 bar back to atmospheric pressure at the outlet.
Aims: The goal of the present study was to examine the influence of the installation of a back-pressure module on the formation of oxidation products in lipid emulsions.
Methods: Lipid emulsions consisting either of soybean, fish oil or a mixture of the two with medium-chain triglycerides were prepared using the Dyhydromatics HL60 high-pressure homogenizer. The effect of a backpressure module on emulsions prepared at different process pressures and homogenization cycles was evaluated by measuring the primary and secondary oxidation products. Established assays for the quantification of oxidation products, namely modified ferrous oxidation xylenol orange assay [2] and thiobarbituric acid reactive substances assay [3], were adapted to work with minimal sample amount and optimized throughput in a microplate reader setup. These assays were subsequently used for the quantification of lipid degradation products in the manufactured emulsions. The results were compared with values obtained with the same assays for three commercially available reference emulsions.
Results: The installation of a backpressure module led to less efficient homogenization and increased size of the lipid droplets, in turn more cycles or a higher process pressure was necessary. Altogether this led to an increase in primary and secondary oxidation products of up to 1.7-fold when compared to the respective formulations produced without a backpressure module. Compared to commercial references, the values of emulsions prepared in-house were within 75-140% of the references for primary and 33-83% for secondary oxidation products.
Discussion and Conclusions: Assays were optimized to quantify primary and secondary oxidation products in lipid emulsions for parenteral nutrition. Levels of primary and secondary oxidation products of emulsions prepared by high-pressure homogenization were in the range of commercial reference emulsions. Lipid oxidation was not supressed by installation of a backpressure module.

Keywords: lipid emulsion, high-pressure homogenization, backpressure module, lipid oxidation

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P - II - 12

Colonic capsules for bacterial suspension

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Introduction: Recent studies have revealed the important contribution of the human microbiome in modulating many biological pathways ranging from metabolic functions to preservation of the health. Despite all the efforts invested in understanding the complex relationship that governs the interaction between the microbiome and host cells, many functions remain unknown, and efficient procedures to harvest the benefits of microbiome modulation are far from reach. In particular, certain steps (i.e. drying or compression) involved in the manufacturing of classical dosage forms (e.g. enteric-coated tablets and capsules) lead to a significant loss of bacterial survival. This often results in the administration of high doses to compensate the bacterial loss or in using invasive practices to deliver the microorganisms.
Aim: The objective of this work is to engineer a capsule capable of delivering aqueous suspensions of live bacteria to the ileum/colon.
Methods: The approach is based on a hydrophobic double-layered coating whose resistance to pressure decreases as the capsule moves along the gastrointestinal (GI) tract. Commercial capsules were modified with hydrophobic cellulose derivatives and methacrylic acid copolymers using the dip-coating method.
Results: These capsules withstood aqueous media, while breaking at the intestinal pH when a certain pressure was reached. In simulated gastric fluids, they prevented the acidification of the encapsulated aqueous fluid. Disintegration tests confirmed that the capsules maintained their integrity under simulated GI conditions, while breaking at pressures encountered in the ileum/colon.
Discussion and Conclusion: This simple delivery system may allow in the future to study the effect of microbiota supplementation such as fecal matter transplantation with minimum handling while providing higher amounts of live bacteria to the distal part of the GI tract.

Keywords: microbiome, capsules, aqueous suspensions, colonic colonisation

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III. PHARMACOEPIDEMIOLOGY

P - III - 1

Incidence rates (IR) and IR ratios of alzheimer's disease in patients with cataract compared to the general population: analyses using The Clinical Practice Research Datalink (CPRD)

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Introduction: Previous research suggests a link between cataract and Alzheimer's disease (AD), as postulated by one observational study (1).
Aims: To analyse the incidence of AD in patients with or without cataract.
Methods: In the UK-based CPRD database, we identified incident AD patients in a cohort of patients with cataract (i.e., the exposure of interest), aged over 39 years, between 1995 and 2019, and in a random sample of the general population without cataract, matched for age, sex, GP and calendar time.
We assessed AD incidence rates (IRs) and incidence rate ratios (IRRs). We defined AD by diagnosis code plus one or more prescriptions (Rx) for anti-AD medication. We excluded individuals with a diagnosis of vascular or other dementias at any time in their patient record as well as patients with a recorded Rx for an anti-AD medication before cohort entry.
We assessed AD cases by age, sex, exposure, and year. We calculated person-time of exposure by adding up individual exposure times.
Results: In the main analysis, we included 403'564 patients with cataract and an equal number of cataract free individuals. During follow-up, we identified 5'932 cataract patients who developed AD and 5'944 AD cases in the comparison group. The crude IR of AD was 2.55 (95% CI 2.48-2.61) per 1'000 person-years (py) in patients with cataract, and 2.97 (2.89-3.04) per 1'000 py in the comparison group. The IRR was 0.86 (0.83-0.89). The IRR was similar in men and women as well as in individuals aged 40 to 79 years compared to those ≥80 years.
Discussion and Conclusion: In contrast to a previous study, we did not observe an increased risk of AD in patients with cataract compared to the general population.

Keywords: observational study, incidence rates, alzheimer disease, cataract

Reference:

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P - III - 2

Changes in serum creatinine values during and after pregnancy in a cohort of women with or without pre-existing chronic kidney disease

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Introduction: Chronic kidney disease (CKD) occurs in up to 3% of women of childbearing age. Impaired kidneys may not sufficiently adapt to physiological changes during pregnancy that may result in accelerated CKD progression.
Aims: To describe changes in renal filtration during and after pregnancy in women with or without CKD.
Methods: We performed a longitudinal descriptive study using data from the UK-based Clinical Practice Research Datalink (2000-2018). We included pregnancies ending in live birth if they had ≥ 1 serum creatinine (SCr) value during a 1-year baseline period before the estimated last menstrual period or during trimester 1. Pregnancies were categorized into 3 groups based on the mean estimated glomerular filtration rate (eGFR [mL/min/1.73 m^2], CKD-EPI formula) during baseline/trimester 1: no CKD (eGFR ≥ 90), mild CKD (eGFR = 60-90), moderate-severe CKD (GFR < 60). We captured demographics and risk factors for CKD and calculated percentage changes of mean SCr levels (group means) as a proxy for renal filtration in 3-months intervals during and until 1 year after pregnancy. We quantified the difference between the mean baseline eGFR and the mean eGFR 6-12 months after delivery.
Results: Of 169'855 included pregnancies, maternal moderate-severe CKD was present in 981 (0.6%), mild CKD in 29'433 (17.3%), and no CKD in 139'441 (82.1%) pregnancies. Pre-existing hypertension, diabetes, and/or obesity was recorded in 37% in moderate-severe CKD, and in 30% in the other 2 groups. Autoimmune diseases pre-existed in 3-4% in all groups. Mean reduction in SCr levels were larger between baseline and the end of trimester 2 in women without CKD (-22%) and with mild CKD (-24%) than in women with moderate-severe CKD (-14%). Thereafter, mean SCr levels increased, to level off around 6 months after delivery. In women without CKD, mean eGFR levels 6-12 month after delivery (118 mL/min, SD 15) were comparable to baseline SCr levels (119 mL/min, SD 13). Women with mild CKD revealed an increased eGFR (97 mL/min, SD 18) after delivery (baseline eGFR 88 mL/min, SD 9). In women with moderate-severe CKD, the post-pregnancy eGFR (55 mL/min, SD 23) was similar as in baseline (50 mL/min, SD 14), but sample size was small due to incomplete lab data.

Discussion and Conclusions: Our results suggest that kidneys of women with moderate-severe CKD adapted to a lesser degree to physiological changes during pregnancy, but sample size was small. Renal filtration was not decreased within 1 year after delivery in all groups. The increased post-pregnancy eGFR in women with mild CKD may reflect continued hyperfiltration or random variability.

Keywords: chronic kidney disease, serum creatinine, pregnancy, disease epidemiology, database research

P - III - 3

Patient groups in rheumatoid arthritis identified by deep learning respond differently to biologic or targeted synthetic disease modifying antirheumatic drugs

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Introduction: Cycling of biologic or targeted synthetic disease modifying antirheumatic drugs (b/tsDMARDs) in rheumatoid arthritis (RA) patients due to non-response is a problem preventing and delaying disease control.
Aims: To assess and validate treatment response of b/tsDMARDs among clusters of RA patients identified by deep learning.
Methods: In the Swiss Clinical Quality Management of Rheumatic Diseases registry (SCQM), we clustered RA patients at first-time b/tsDMARD (i.e. cohort entry) through deep embedded clustering. Within 15 months, we assessed b/tsDMARD stop due to non-response, and separately a ≥20% reduction in DAS28-esr (RA disease activity measured in 28 joints and using erythrocyte sedimentation rate measures) as a proxy for treatment response. We assessed comparative effectiveness of b/tsDMARDs (ref. adalimumab) using Cox proportional hazard regression in each cluster estimating hazard ratios (HR) with 95% confidence intervals (CI). We validated results through stratified analyses according to most distinctive patient characteristics of clusters.
Results: Clusters comprised between 362 and 1481 patients (among 3516 unique patients). Comparative effectiveness results among validation strata confirmed comparative effectiveness results among clusters: Patients with at least two conventional synthetic DMARDs and prednisone at b/tsDMARD initiation, male patients, as well as patients with a lower disease burden responded better to tocilizumab than to adalimumab (HR 5.46, 95% CI [1.76-16.94], and HR 8.44 [3.43-20.74], and HR 3.64 [2.04-6.49], respectively). Furthermore, seronegative women without use of prednisone at b/tsDMARD initiation as well as seropositive women with a higher disease burden and longer disease duration had a higher risk of non-response with golimumab (HR 2.36 [1.03-5.40] and HR 5.27 [2.10-13.21], respectively) than with adalimumab.
Discussion and Conclusions: Our results suggest that RA patient clusters identified by deep learning may have different responses to first-line b/tsDMARD. Thus, our results may suggest optimal first-line b/tsDMARD for certain RA patients, which is a step forward towards personalizing treatment.

Keywords: rheumatoid arthritis, biologics, treatment response, personalized medicine

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P - III - 4

Attitudes and expectations of patients on home parenteral nutrition towards e-Health

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Introduction: eHealth denotes the use of electronic tools in healthcare to improve processes and connect patients and health care personnel. We are developing an eHealth platform for home parenteral nutrition (HPN) patients, including video consultations, instructions (e.g. for patient education), and interaction with patient support groups. In addition, this platform will serve as a central repository for treatment- and care-related data for patients and medical staff.
Aims: Our aim was to identify the attitudes and expectations of HPN patients towards eHealth as a basis for the creation and implementation of an eHealth platform.
Methods: We conducted an anonymous survey on the attitudes and expectations of HPN patients towards eHealth. We interacted with patients in person or by phone. The questionnaire consisted of 18 questions on HPN care, familiarity and experience with digital devices, attitudes and expectations towards video consultations and other components of the intended platform.
Results: We included 25 HPN patients (60% females) looked after by two different HPN centers. Mean (SD) age was 55 (14) years and median (range) duration of HPN was 305 (29-4528) days. A majority of participants (n = 21, 84%) reported using a smartphone, tablet or computer and 16 (64%) rated their digital skills as proficient. Almost half of the participants (n = 11, 44%) found it cumbersome to go to the hospital for follow-up visits and 19 (76%) were open to video follow-up visits. Easy operation of the platform was important to 16 participants (64%). The following proposed components of the platform were frequently rated as important: Videoconferencing with physicians (n = 20, 80%) and dieticians (n = 19, 76%), checklists for PN, catheter and pump handling, data collection and storage, data protection (n = 20, 80%), video instructions (n = 18, 72%), automatic ordering of PN material (n = 17, 68%), and automatic dietary records (n = 16, 64%). A majority of participants rated the collection and storage of the following data as important: Weight (n = 24, 96%), infusion plan and administration details (n = 23, 92%), medication plan and intake (n = 22, 88%), laboratory parameters (n = 21, 84%), blood pressure, catheter photos, pain, nausea, other test results (n = 20, 80%), stool frequency and consistency, reports from different hospitals/practices (n = 19, 76%), body temperature, blood glucose (n = 18, 72%), and dietary records (n = 16, 64%).
Discussion & Conclusions: HPN patients are open towards an eHealth platform for care support, including video follow-up visits. This is especially useful in a pandemic. Important criteria for the design of the eHealth platform were identified and confirmed by HPN patients. We plan a validation study to evaluate the benefits of follow-up visits via videoconferencing versus in person usual care in those patients.

Keywords: eHealth, home parenteral nutrition, telemedicine

IV. CLINICAL PHARMACY / CLINICAL PHARMACOLOGY

P - IV - 1

Markers of complement and coagulation in diabetes patients treated with dapagliflozin

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Introduction: Diabetes mellitus is a major cause of morbidity and mortality nowadays. It is characterised by an increase in blood glucose levels due to absolute (type 1 diabetes) or relative (type 2 diabetes) insulin deficiency and associated with multiple complications. Subjects with diabetes have an increased thrombotic tendency which can promote the development of cardio-vascular diseases. This tendency is partially due to alterations in plasma levels of coagulation proteins. Moreover, diabetes is associated with a proinflammatory state partially due to alterations in complement proteins.
Aim: Dapagliflozin, a sodium-glucose cotransporter-2 (SGLT-2) inhibitor, is an oral antidiabetic drug used to treat type 2 diabetes. It provides an insulin-independent way of lowering blood glucose levels and could be considered as a treatment option for type 1 diabetes. A recent pilot study suggested that short-term dapagliflozin treatment in type 1 diabetes patients leads to a higher increase in the incretin hormone glucagon-like peptide-1 (GLP-1) following oral glucose intake than placebo treatment (1). Since GLP-1 receptor agonists have been shown to inhibit thrombus formation, a treatment with dapagliflozin might have a beneficial impact on the thrombotic risk profile in type 1 diabetes patients. Therefore, the effects of a short-term dapagliflozin treatment for type 1 diabetes patients on the plasma levels of coagulation markers were investigated. As there are many interactions between the plasmatic coagulation system and the complement system, the effects of the treatment on the plasma levels of complement markers were investigated as well. For both systems, it was focused on the markers which are known to be elevated in diabetes.
Methods: The participants of two cross-over intervention studies received 10 mg dapagliflozin or 10 mg placebo per day for 7 days (Dapa01; n = 13) and 17 days (Dapa02; n = 7). Plasma samples were taken before treatment (baseline) and after 7 days (Dapa01) and 14 days (Dapa02) of dapagliflozin or placebo treatment. Plasma levels of Factor VII, Factor XIII, tissue factor, plasminogen activator inhibitor-1, tissue plasminogen activator, DDimer, mannose-binding lectin, C3, terminal complement complex and high-sensitive C-reactive protein (hsCRP) were determined using sandwich ELISAs. The participants' coagulation and fibrinolytic capacities were measured using a turbidimetric clot formation and lysis assay.
Results: After 7 days of dapagliflozin intake, Factor XIII and hsCRP levels were significantly higher than after placebo intake. There was no significant change in any of the other markers. The coagulation and fibrinolytic capacities after dapagliflozin treatment did not differ significantly from the capacities at baseline and after placebo intake. After 14 days of dapagliflozin intake, there were no significant differences in any of the measured markers.
Discussion and Conclusion: The results indicate, that the treatment with dapagliflozin has no beneficial impact on the thrombotic risk profile in type 1 diabetes. Nevertheless, dapagliflozin can still be considered as an option for the treatment of type 1 diabetes since it does not worsen the prothrombotic and proinflammatory state present in diabetes by further increasing the already elevated complement and coagulation markers. However, the data from these small pilot studies are limited and further studies are needed.

Keywords: type 1 diabetes mellitus, complement, coagulation, dapagliflozin

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V. MOLECULAR PHARMACOLOGY / MOLECULAR MEDICINE

P - V - 1

Establishing a screening platform for the biological evaluation and modulation of complement-related integrin receptors

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Introduction: The complement system serves as a first line of defense against pathogens and endogenous threats. However, due to its integral roles in the maintenance of immune and inflammatory reactions, inappropriate complement activation is involved in several clinical conditions. The modulation of cell adhesion and inflammatory signaling via complement receptors of the β_2 integrin family is therefore considered an attractive yet, due to the poor druggability of the targets, scarcely explored option for drug development.
Aims: This project aims to characterize β_2 integrin receptors, which are involved in the migration/adhesion of leukocytes to sites of inflammation and phagocytic removal of complement-opsonized particles, as potential therapeutic targets. For this purpose, a library of the major ligand-binding domains (αI) and physiological or therapeutic ligands is compiled to allow for direct binding and functional assays, with an initial emphasis on the complement receptors CR3 and CR4.
Methods: Recombinant forms of the αI domain of all four β_2 integrin family members (i.e., CR3, CR4, LFA-1, and CD11d/CD18) were expressed in *E. coli* and purified by affinity chromato-graphy. Whereas surface plasmon resonance (SPR) was used to determine affinity and kinetic profiles, adhesion assays are currently established to determine the functional and selectivity spectra of the proteins.
Results: By optimizing the expression parameters and selecting appropriate purification tags, the αI domains of all four β_2 integrins could be expressed in baseline and high-affinity states with high yield and purity. SPR studies confirmed activity and selectivity of the recombinant CR3 and CR4 αI domains for complement opsonins C3b, iC3b and C3d with distinct kinetic profiles. Moreover, the effect of cofactors and small molecule modulators could already be investigated with this platform.
Discussion and Conclusion: In this study, we established the recombinant expression of the major ligand-binding domain of CR3, CR4, LFA-1, and CD11d/CD18 and validated the activity/ selectivity profile of CR3 and CR4 by SPR. It is anticipated to use the recombinant αI domains of β_2 integrins in functional studies and for CR3/CR4-targeted drug development, with a potential expansion to the other β_2 family members at a later stage.

Keywords: innate host defense, integrins, complement

P - V - 2

Targeting protein-protein interactions of DNA polymerase ζ to circumvent chemotherapy resistance

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Introduction: Cisplatin and oxaliplatin are platinum-based chemotherapeutic agents used to treat a diverse range of cancer types. After activation in the body, these drugs covalently bind to DNA to form DNA adducts that evoke cell death in cancerous cells. The most prevalent DNA adduct is an intrastrand cross-link between two adjacent guanines (Pt-GG). An adverse outcome of platinum-based therapy is the development of drug resistance. One strategy to combat this resistance is inhibiting enzymes involved in the tolerance of these DNA adducts during DNA replication. Human DNA polymerases (hPol), such as human Pol ζ, participate in Translesion DNA synthesis (TLS) to replicate past cisplatin and oxaliplatin DNA adducts. Human Pol ζ is a tetrameric protein composed of a catalytic subunit (Rev3), a non-catalytic subunit (Rev7) and two accessory subunits. The binding of Rev3 to the “safety belt” region of Rev7 is essential for the activity of the enzyme and induces a conformational change in Rev7, from an «open» to «close» state. Human Pol ζ expression is found to increase upon platinum drug therapy and, therefore, can contribute to drug resistance by increasing cellular tolerance to Pt-GG DNA adducts.
Aims: (i) Design and synthesize small molecule inhibitors targeting the Rev7-Rev3 interface, (ii) develop biochemical assays to evaluate the effectiveness of the small molecule inhibitors, (iii) characterize the mechanism of Rev3 displacement from Rev7.
Methods: (i) Computational modeling and chemical synthesis to generate inhibitors of the Rev7-Rev3 interaction, (ii) protein expression and purification of Rev7-Rev3 complexes, (iii) development of microscale thermophoresis and Ni^{2+} pull-down assays to study the structure-activity relationship of the small molecules.
Results: *In silico* modeling identified a potential binding pocket for the small molecule inhibitors that would result in the disruption of the Rev7-Rev3 protein-protein interaction. After the chemical synthesis of a series of potential inhibitors, biochemical assays have been developed to assess their effectiveness in disrupting the Rev7-Rev3 interaction within a purified protein complex. The results of these biochemical assays are expected to provide mechanistic insights into the function of hPol ζ.
Discussion and Conclusions: The development of new inhibitors that target the Rev7-Rev3 protein-protein interaction of hPol ζ will contribute to the development of more effective chemotherapeutic strategies aimed at combating drug resistance.

Keywords: DNA polymerase, translesion DNA synthesis, platinum-based drugs, drug resistance, drug discovery

P - V - 3

Structure-activity assessment of the leech-derived complement inhibitor BD001

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Introduction: The complement system is a vital first-line-of-defense barrier against microbial intruders, yet can turn against host cells/tissues to cause clinical conditions such as hemolytic anemia or reperfusion injury (e.g., following transplantation or stroke). The interest in thera-peutics that can modulate complement activation is therefore growing. Owing to the tight regulation of complement by our body and pathogens alike, nature can provide intriguing lead structures for complement inhibitors, which can be optimized towards drug like compounds. Parasites are of particular interest, as they have developed fascinating strategies to overcome host defense responses. The giant Amazon leech produces a protein, called BD001, which impairs two serine proteases (C1s, MASP2) that are vital for complement activation via the classical (CP) and lectin pathway (LP), respectively [1, 2]. Our group previously succeeded in expression of BD001 in prokaryotic systems with high yield, purity and activity, which greatly facilitates an evaluation of the lead in structure-activity relationship (SAR) studies.
Aim: This study aimed at developing BD001 derivatives with enhanced target activity and distinct pathway selectivity profiles, based on *in silico* predictions and protein engineering.
Methods: Commercial/public software tools were initially used to predict changes in C1s affinity by performing a mutational scan of BD001. Based on these *in silico* predictions, 16 mutants with either positive or negative effect on C1s affinity were selected for experimental testing. The selected mutants were expressed in *E. coli* (Rosetta-gami), purified by affinity chromatography, and characterized by SDS-PAGE and nanoDSF. The activity of the inhibitors was tested in different biochemical assays, including chromogenic substrate assays, classical and lectin pathway ELISAs, and haemolytic assays.
Results: The three software tools used in the study resulted in some common and some distinct mutagenesis predictions. All 16 mutants that were selected after visual inspection of the binding mode could be expressed and showed comparable nanoDSF profiles to the wild type BD001, suggesting that the protein folding was correct. The mutants showed distinct activity profiles in the functional assays, with both drops and notable enhancements in activity being observed. However, the experimentally determined activity changes did not always correlate with the *in silico* predictions. Intriguingly, some mutants featured a stronger activity in LP inhibition while CP activity was unaffected or even reduced.
Discussion and Conclusion: Our initial SAR study already provided important insight into the molecular determinant of target activity and selectivity of BD001, thereby paving the way for more precise protein engineering projects for tailoring the 122 amino acids-long protein to a drug-like compound. It also showed that the use of off-the-shelf tools for mutant screening are of limited predictive value. We are therefore currently establishing enhanced computational methods for the next optimization steps. Overall, our findings establish the BD001 derivatives as interesting leads for further development towards treatment options for diseases such as autoimmune haemolytic aneamia or ischemic-reperfusion injuries.

Keywords: complement system, immunology, immunotherapy, protein therapeutics

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P - V - 4

Insight into mode-of-action and structural determinants of the compstatin family of clinical complement inhibitors

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Introduction: The complement system serves as «first line of defense» against injurious stimuli and invading pathogens in a series of cascading enzymatic reactions which lead to pathogen clearance and opsonic cell killing. Yet complement has gained increasing interest as a potential drug target, since it may be inadvertently triggered, thereby contributing to clinical complications in the pathogenesis of various autoimmune, inflammatory and age-related diseases [1]. However, the arsenal of available complement therapeutics has long been limited both in numbers and target selectivity. It was only May 2021, that with pegcetacoplan (Empaveli, Apellis), a second class of complement-specific drugs has been approved by the FDA. Pegcetacoplan (i.e., Cp05-PEG-Cp05) is based on a second-generation analog of the peptidic C3 inhibitor compstatin (Cp05), which has been originally identified by phage display [2]. Several derivatives of the compstatin family have reached clinical development for the treatment of paroxysmal nocturnal hemoglobinuria (PNH), age-related macular degeneration (AMD), periodontal disease, and COVID-19-induced acute respiratory distress syndrome. Among those is Cp40 (AMY-101, Amyndas), a third-generation analog. A detailed molecular understanding of its target binding, species specificity, and mode of action is critical for design of future compstatin analogs.
Aim: We aim to provide a detailed understanding of target binding, species specificity and mode of action of the 3rd generation compstatin derivatives.
Methods: In this study, we combined a newly resolved co-crystal structure of Cp40 in complex with C3b with molecular dynamics simulations and direct binding studies using a panel of compstatin derivatives to arrive at a detailed structure-activity-relationship profile. We compared the binding modes of mono- and bivalent compstatin derivatives concerning target binding in solution (ITC) and on surfaces (SPR) by using a surrogate of pegcetacoplan. Finally, by employing surface plasmon resonance studies we investigated the molecular mechanism of C3 inhibition by Cp40.
Results: We identified dTyr-1, (1Me)W-5, Gln-6, Trp-8, Sar-9, Ala-10, His-11, mIle-14 as key contact residues. Interestingly, our analysis also revealed major contributions of intramolecular interactions and structural water and suggested an influence of amino acids that are not in direct contact with the target. For the molecular mechanism we could show that C3 is recruited to surface-bound C3 convertases and that Cp40 prevents recruitment and activation of C3 by blocking the dimerization interface between the C3 substrate and the convertase component C3b.
Discussion and Conclusion: The results of our study may not only guide the future development of a promising class of complement inhibitors but also offers new details about the molecular mechanisms of complement activation and regulation.

Keywords: complement system, peptides, solid-phase-peptide-synthesis, co-crystal structure, structure-activity-relationship

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VI. RADIOPHARMACEUTICS

P - VI - 1

ICP-MS as tool for *in vitro* and *in vivo* characterization of imaging probes and radioligand therapy candidates

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Introduction: In radiopharmaceutical sciences, target-specific small molecules, peptides and biologics vectors are labeled with radionuclides either for diagnostic (tracer) or therapeutic (radioligand therapy RLT, radioimmunotherapy) applications. Very often such radionuclides are transition metals or lanthanides (e.g. gallium-68, lutetium-177 etc.). In-depth *in vitro* and *in vivo* characterization of these ligands is key for the success of the radiopharmaceutical development. The characterization of these radioconjugates can be performed only at centers that are specialized in radiopharmaceutical sciences to handle radioactive substances. To perform similar experiments with the same sensitivity and accuracy in a conventional laboratory would be of great interest. The extremely high sensitivity of inductively coupled plasma mass spectrometry (ICP-MS) and its wide dynamic range allow the detection of various non-radioactive metals in low quantities. Thus, ICP-MS might have the potential to support early step evaluation of new targeting agents without the need of the radioactive isotope of a given element.
Aims: To test if ICP-MS can be implemented for radioligand candidates discovery efforts and would deliver comparable results to the state-of-the-art assays that use radioconjugates for the characterization of imaging probes and RLT candidates.
Methods: Reversed phase chromatography (RPC) and size exclusion chromatography (SEC) were coupled to the ICP-MS to do quality control (QC) of peptide and biologics conjugates after complexation with stable isotopes of the most commonly used radionuclides. Constant infusion methods of ICP-MS were used to analyze cell samples of *in vitro* cellular binding and internalization assays to characterize the binding behavior of the metal conjugates.
Results: A linear calibration range for [^{175}Lu]Lu-DOTA-peptide was set up between 2 nM and 2.5 µM to do QC of peptidic conjugates. For [^{175}Lu]Lu-DOTA-single domain antibodies (sdAB), a linear calibration range was set up between 2.5 nM to 123 nM for QC of biologics conjugates. To prevent the formation of black carbon deposit on the cones when coupling the RPC to the ICP-MS, 5% oxygen was added to the nebulizer argon flow. For SEC-ICP-MS, 20 mM ammonium acetate buffer was selected as mobile phase to obtain good chromatographic separation of the free lutetium-175 and [^{175}Lu]Lu-DOTA-sdAB without blocking the sample introduction system of the ICP-MS by the formation of salt crystals. Apomyoglobin was added to the biologics samples and rat blood extract to the peptide samples, plus DTPA to complex free lutetium-175 to hinder adhesion of the sdAB/peptide and the free lutetium-175 to the tubing of the system and the column. To have a robust and accurate method for quantification of lutetium-175 in *in vitro* cellular binding assays, nitric acid was added to the cell samples and samples were digested in a microwave at 150 °C for 10 min. For the direct infusion method, a LOQ of 0.12 nM for lutetium-175 in cells was reached.
Discussion and Conclusions: Various methods were successfully established to use the ICP-MS in combination with either RPC, SEC or in constant infusion mode to characterize molecular imaging probes and RLT candidates. These characterization methods include QC of the ligands after complexation with stable isotopes before they go *in vitro* or *in vivo*, and defining the ligands' binding behavior with *in vitro* cellular uptake experiments. Further experiments are planned to test

the applicability of RPC/SEC-ICP-MS for *in vitro* serum stability experiments but also the constant infusion methods for *in vivo* biodistribution experiments in tumor bearing mouse models.

Keywords: inductively coupled plasma mass spectrometry (ICP-MS), radioligand therapy

VII. ANALYTICS

P - VII - 1

Authenticity control of pine essential oils by chemometric analysis and chiral gas chromatography

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Introduction: Numerous terpenes present in essential oils (EOs) display one or more chiral centers [1]. Within the same genus the enantiomeric ratio of these compounds can be different [2]. Thus, the determination of enantiomers is a valuable tool to evaluate authenticity and quality of Eos [3]. In here, the terpene profile of primary and commercial pine EOs was analysed by conventional and chiral gas chromatography coupled to a flame ionization detector.
Aims: The present work evaluates the chiral gas chromatographic-flame ionization detector (GC-FID) profile of primary and commercially available pine EOs as additional tool for authenticity control.
Methods: Primary pine EOs were obtained by steam distillation from needles and twigs collected in Europe, Sibiria and Canada. Commercially available EOs of *Pinus sylvestris* L. were purchased from different suppliers. EOs were analysed by chiral GC-FID.
Results: *Pinus sylvestris* L. and *Pinus nigra* J. F. ARNOLD are morphologically similar and share the same habitat. However, primary *Pinus sylvestris* L. EOs of different origin showed an enantiomeric excess (ee) of (+)-α-pinene whereas the predominant enantiomer of *Pinus nigra* J. F. ARNOLD was (-)-α-pinene. Furthermore, commercial EOs showed an enantiomeric excess of (-)-α-pinene. Lack of botanical knowledge could have led to misidentification and confusion in the raw material. In our study, a group of primary EOs, initially labeled as *Pinus sylvestris* L., still remained unclassified by applying our previously published PLS-DA model [2]. Detailed analysis of plant material and consideration of their Sibirian origin revealed that these EOs were obtained from *Pinus sibirica* DU TOUR, a known subspecies of *Pinus cembra* L. [3]. Both EOs were not significantly different in terms of their ee of (+)-α-pinene ($p > 0.05$, ns). However, the difference of the ee to *Pinus sylvestris* L. was highly significant ($p < 0.0001$: ****).
Discussion and Conclusion: For the evaluation of the correct herbal substance and preparation in the field of pine EOs – next to a traceable supply chain – the unique chromatographic quality including analytical markers is crucial. Chromatographic profile obtained by GC-FID is able to distinguish between primary and commercial pine EOs. Chiral analysis provides additional significant information on the authenticity of pine EOs and allows to uncover possible mislabelling, the use of wrong herbal substances and/or adulteration within the supply chain.

Keywords: authenticity control, chiral GC analysis, primary essential oil, chemometric analysis, α-pinene

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P - VII - 2

Determination of ammonia in different matrices using LC-MS

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Introduction: Ammonia plays a key role in the nitrogen cycle among aquatic life, and humans. The human microbiome contributes to over 50% of ammonia production in mammals. Dysfunction of ammonia metabolism results in many diseases, for example, hepatic encephalopathy, cancer, urea cycle disorders and cerebral dysfunction [1,2]. The quantitative determination of ammonia attracts a wide range of research topics. Currently, colorimetric, enzyme-based assays or ion-selective electrodes can be used for quantification. However, these methods show some limitations like sensitivity, selectivity, cross reactivity and the use of toxic solvents. Therefore, a sensitive and selective LC-MS method is needed for the determination of ammonia in complex matrices, like urine and surface waters [3].
Aims: The aim of our study is the development of a new and highly selective LC-MS method to detect ammonia in aqueous solutions.
Materials and Methods: Ammonia was derivatized with phenethyl isothiocyanate (PEITC) to phenethylthiourea (PETU) in PBS/MeCN. Benzylthiourea (BTU) was used as an internal standard. PETU was quantified by LC-MS. Analysis was performed using a LTQ-XL linear ion trap (Thermo Scientific, San Jose, USA) mass spectrometer coupled to a Waters Acquity™ UPLC system (Milford, USA). Detection of PETU and BTU was done in ESI positive mode. Chromatographic separation was done on an ACE Excel 2 C-8 column (30 x 2.1 mm, 2 µm,100A).
Results: Chromatographic analysis was achieved within 10 min. The developed method was validated with respect to the accuracy, precision, stability, matrix effect, and derivatization efficiency. The physiological range of ammonia in urine is between 4 mM and 107 mM and samples were analyzed after a 500-fold pre-dilution with distilled water. The selected workup of urine provided recoveries of more than 87% for PETU. At QC_{Low}-level, matrix effects for urine were 1.0% and 2.1% at QC_{High}, respectively. In the autosampler (@ 10°C), the samples of QC_{Low} and QC_{High} were stable for at least 12 weeks, as no significant changes in the peak areas of the analyte were detected. Derivatization was quantitative at 10 µM ammonia, when it took place for 24 h, at pH 7.4 and 60°C. The calibration range for ammonia was between 200 nM and 30 µM.
Discussion and Conclusion: After derivatization, ammonia was sensitively detected as PETU by LC-MS. Compared to previously published methods, our method is faster, less expensive and more ecologically friendly. Moreover, quantification limits were lowered to 200 nM. The described method represents a valuable starting point for biomarker detection in clinical screenings but also for upcoming analysis of surface waters.

Keywords: ammonia, LC-MS, quantification, derivatization, isothiocyanate

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P - VII - 3

Fluorescence-based detection method for the separation and quantification of hydrolysis products in oil-in-water emulsions

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Introduction: Provision of essential nutrients via parenteral nutrition (PN) is a crucial way to ensure survival of preterm infants. Triglycerides present in lipid emulsions as part of PN can be hydrolyzed to free fatty acids (FFA). Released FFA in turn can bind to albumin and compete with bilirubin [1]. Especially in preterm infants, there is the risk of developing hyperbilirubinemia, leading to jaundice and in severe cases to neurotoxicity if excess FFA are provided through degraded lipid emulsions. An improved HPLC method was thus developed to accurately quantify the amount of FFA in oil-in-water emulsions for PN prior to administration.
Aims: The objective was to develop an improved method for the separation and quantification of individual fatty acid (FA) species by HPLC with fluorescence detection. Insights into different ratios between individual FFA species in contrast to simply assessing the level of total FFA can further enhance our understanding of underlying degradation mechanisms.
Methods: The method is based on activation of extracted FFAs with the triazine derivative DMT-MM as a coupling agent and subsequent conjugation with the fluorescent dye DBD-PZ [2]. After 2 h of incubation, aliquots were quenched with diluted acid to terminate the coupling reaction and analysed by HPLC. The method utilizes minimal sample volumes of 7.5 µL emulsion. Quantification takes place via standardization to two non-naturally occurring FAs as internal standards and comparison with calibration curves prepared for ten FAs from commercially available standards.
Results: Over a 25-min run time, we were able to separate ten natural FAs commonly present in the studied oil sources as well as two non-natural FAs as internal standards. Comparison of the retention times with commercially available standards allowed to confirm the identity of all the peaks. The limit of quantification was in the one-digit pmol range for all studied FFAs. The method was validated with commercially available lipid emulsions as well as in-house manufactured mimics. Based on the oil source – soybean, fish oil or a mixture of the two with medium-chain triglycerides – distinct FA profiles were observed. The concentration of the coupling agent influenced the reaction velocity of the labelling reaction. The influence of the fluorophore concentration was minor and the consumption of the costly dye was successfully reduced compared to previous reports [2]. While common excipients such as phospholipids or antioxidants did not interfere with the analysis, sucrose esters of inferior quality used as co-surfactants contributed to the signal.
Discussion and Conclusions: Experiments showed that the identification and quantification of hydrolysis products in lipid emulsions intended for clinical application in total parenteral nutrition is possible with minimal sample consumption and satisfactory sensitivity. Optimal concentrations of the reactants were determined to allow a rapid and accurate quantification. This method may provide a critical contribution to quality control of a life-saving therapy, especially when intended for preterm infants, and prevent severe complications.

Keywords: free fatty acids, hydrolysis of triglycerides, fluorescence detection, HPLC, neonatal hyperbilirubinemia

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VIII. PHARMACOLOGY

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Pharmacogenetic analysis of voriconazole in children

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Introduction: Invasive fungal infections (IFIs) are a serious complication and a major cause of morbidity and mortality in immunocompromised children. The first-line antifungal drug to treat IFIs in children is voriconazole. This drug is known for its narrow therapeutic range and its non-linear pharmacokinetic profile with pronounced inter- and intra-individual variability. Due to the high risk for therapy failure if the therapeutic range is not reached, therapeutic drug monitoring is strongly recommended. Drug-drug interactions and patient specific characteristics as age, weight, liver function, and also the phenotype of the metabolizing enzyme cytochrome P450 2C19 are known factors to influence the *in vivo* behaviour of voriconazole. In addition to genetic variants of CYP2C19, single nucleotide polymorphisms (SNPs) in other genes involved in the metabolism and transport of voriconazole assumed to influence serum concentrations and thus the therapeutic outcome. Accordingly, understanding how genetics affect dosage, efficacy and safety of voriconazole in children is essential to optimally dose and reduce the risk of treatment failure.
Aims: Our aim was to investigate the impact of genetic variants on voriconazole serum levels in order to allow future optimization of voriconazole dosage and improve the therapeutic outcome in IFIs.
Methods: We performed a retrospective data analysis, where we used data on medication including trough levels, health-related personal data, co-medication and biological material from 36 children suffering from IFI and treated with voriconazole between 2014 and 2019 in two different children's hospitals. Data were extracted from the clinical and laboratory information systems of the hospitals with the recently built Swiss Personalized Health Network infrastructure SwissPK^{cdw}. We collected a total of 395 voriconazole trough levels. Among 23 individuals with samples available for DNA extraction, 12 selected SNPs within the voriconazole metabolizing enzymes CYP2C19, CYP3A4, and CYP3A5 and transporters ABCC2, ABCG2, and SLCO1B3 were genotyped using commercially available TaqMan® real-time PCR assays.
Results: 195 (49%) of the voriconazole trough levels measured were outside the recommended therapeutic range of 1-5.5 mg/L, of which 169 (87%) were at sub-therapeutic level. Analysis of trough levels with genotyping revealed statistically significant differences in mean voriconazole serum concentrations associated with 5 of 12 genetic variants tested. SNPs within CYP2C19 (rs4244285, $P < 0.001$), ABCG2 (rs2231142, $P = 0.001$), ABCC2 (rs2273697, $P = 0.005$ and rs717620, $P < 0.001$), and SLCO1B3 (rs4149117, $P = 0.001$) influenced dose-corrected trough levels. No association was observed for the CYP2C19 (rs12248560 and rs4986893), ABCG2 (rs13120400), CYP3A4 (rs35599367), and CYP3A5 (rs776746) genotype. None of the patients harboured the variants CYP2C19 (rs28399504) and CYP3A5 (rs10264272).
Discussion and Conclusions: Our results suggest that voriconazole serum concentrations in children are affected by several genetic polymorphisms. In order to prescribe an optimal drug dosage, pre-emptive PGx testing in addition to therapeutic drug monitoring might be helpful for patients treated with voriconazole. The results of this retrospective study need to be confirmed prospectively in order to guide voriconazole dosing in critically ill children.

Keywords: children, paediatric pharmacology, therapeutic drug monitoring, pharmacogenetics

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Inflammasome activation increases with age and positively correlates with the high risk of CMML

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Introduction: As the human life span increases, chronic, as well as age related health problems have become a major issue in health care. Aging related changes in one's body result in the dysfunction of the immune system. Elderly people show diminished protection against infections and they do not respond as effectively to vaccines as younger individuals would. Age related chronic low-grade inflammation is known as inflammaging and is a risk factor for several non-communicable chronic disorders such as arthritis, diabetes, and cardiovascular diseases including myeloid leukemias, such as Chronic Myelomonocytic Leukemia (CMML). Recent studies suggest that NLRP3 inflammasome activation plays a critical role in the development of inflammaging [1]. NLRP3 inflammasome is a multiprotein signalling complex formed in the cytosol of stimulated immune cells such as monocytes. Inflammasomes control the caspase-1 mediated release of the proinflammatory cytokine IL-1β. The canonical NLRP3 inflammasome activation is a two-step mechanism. The initial priming step involves an NF-κB-activating stimulus, such as LPS binding to TLR4, that induces transcription and translation of the IL-1β precursor as well as increased expression of NLRP3 protein. The second step results in an activation of caspase-1. This step can be induced by various triggers such as Nigericin, MSU etc. However, an alternative inflammasome pathway had previously been discovered specifically arising in human monocytes. Herein LPS directly activates this inflammasome via TLR4 and caspase-8 without requiring a second signal [2].
Aims: It is known that aging causes chronic low-grade inflammation. However, molecular mechanisms that contribute to low grade inflammation are not entirely understood. Furthermore, it is not yet known if any cell intrinsic changes in innate immune cells contribute to chronic inflammation. Since NLRP3 inflammasome has been involved in chronic inflammation in the elderly, we investigated NLRP3 activation in monocytes derived from younger (< 40 years) and older (> 60 years) healthy human donors. Additionally, we investigated the role of NLRP3 inflammasome activation in patients suffering from CMML. It is currently unclear how changes during the physiological aging process can affect the susceptibility for chronic diseases.
Methods: CD14+ monocytes were isolated from peripheral blood. Monocytes were stimulated with NLRP3 agonists. ELISA and Western blot analysis were performed to check IL-1β levels and caspase-1 activation in cell lysates and supernatants. In addition, a diverse set of pharmacological inhibitors were used to gain a greater insight into the molecular mechanism of inflammasome signaling during the aging process. The clinical data of CMML patients was prospectively collected and analyzed.
Results: We found that CD14+ monocytes isolated from older healthy donors produced significantly elevated IL-1β levels compared to younger donors after canonical and alternative NLRP3 inflammasome activation. Priming and activation signals were both increased in older healthy donors. Interestingly, we found that TLR1/2 agonist Pam3CSK4 directly activates NLRP3 inflammasome independent of canonical inflammasome stimuli in monocytes from older donors. At the molecular level, Pam3CSK4 induced NLRP3 inflammasome activation pathway is dependent on caspase-8. Surprisingly, we observed a dichotomy in NLRP3 inflammasome activation in elderly CMML patient monocytes. Intriguingly, increased inflammasome activation in patients positively correlates to the disease severity. In general, high NLRP3 reactivity is associated with splenomegaly, a more advance disease stage, a shorter time to treatment and a shorter time to death.

Discussion and Conclusions: Collectively, our results suggest that aging causes dysregulation of and sensitivity to NLRP3 inflammasome activation at the cellular level, which may explain increased inflammation and immunopathology in the elderly. These results are of high clinical relevance as they allow for a more detailed insight into the physiological mechanism of aging regarding NLRP3 inflammasome activation pathway at a cellular level. The proinflammatory cytokine IL-1β could possibly be used as a prognostic factor or as a basis for a future drug target for CMML patients. However, further studies are needed to explore the role of IL-1β in the human aging process and in the pathogenesis of CMML.

Keywords: NLRP3 inflammasome, aging, chronic inflammation, inflammaging, chronic myelomonocytic leukemia (CMML)

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Extracellular vesicles from human hepatic stellate cells provide insights into liver fibrosis treatments using proteomic data mining

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Introduction: The evolution of liver fibrosis into liver cirrhosis leads to over a million deaths every year worldwide. The transdifferentiation of hepatic stellate cells (HSCs) from a quiescent into an activated, pro-fibrotic status plays a key role in liver fibrogenesis [1].
Aims: Given the function of extracellular vesicles (EVs) in intercellular communication, we aimed to thoroughly analyze EVs shed by HSCs in their different phenotypical states, potentially paving the way to non-invasive and less painful methods for the evaluation of liver fibrosis therapy [2].
Methods: We isolated and characterized EVs from differently treated LX-2 (human HSCs cell line) in vitro, and we investigated the biological effect they exert onto naïve cells by fluorescence microscopy. LX-2 cells were untreated, treated with retinol and palmitic acid (quiescent), or with profibrotic TGF-β1 (perpetuated activated status). Additional treatments including polyenyl-phosphatidylcholines (PPC)-rich lipid S80 were also evaluated. EVs were isolated from conditioned cell culture medium by ultracentrifugation, and they were purified by size exclusion chromatography (SEC) or asymmetrical flow field-flow fractionation (AF4) [3]. The concentration and size distribution profiles of EVs in the purified fractions were assessed by nanoparticle tracking analysis. EV-morphology was observed by scanning and cryogenic transmission electron microscopy. Peptides were analyzed by liquid chromatography tandem mass spectrometry (nano-LC-MS/MS).
Results and Discussion: EVs were found to play an active role in affecting neighboring HSCs' status. Differently treated LX-2 produced EVs in similar yields (10^{10} particles/mL), sizes (180 ± 90 nm) and zeta potential values (-36 ± 3 mV), generated by ca. 10^7 cells (>96% cell viability). Successful SEC and AF4 purifications revealed EV subpopulations with different physico-chemical behaviors. Electron microscopy imaging corroborated the size polydispersity and EV-morphology. Peptide analysis by nano-LC-MS/MS revealed EV-associated proteins whose expression correlated with HSCs treatment. Complex proteomic datasets were mined to develop a screening panel of 44 proteins that can be used to effectively distinguish between treatments and cell status. For the first time, we could measurably correlate the cellular response to PPC-treatment to the relative presence of candidate protein markers on the released EVs.
Conclusions: Our results confirmed differences in EV populations originating from the same cells in either healthy or diseased state, paving the way for more precise and less invasive *ex vivo* analyses.

Keywords: extracellular vesicles, liver, fibrosis, proteomics

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HDAC-based multi targeted therapies against multiple myeloma

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Introduction: Multiple myeloma (MM) still remains a fatal disease despite relevant progresses in the standard care with the introduction of novel classes of drugs. Therefore, new therapeutic strategies are urgently needed [1]. In the past few decades, proteasome inhibitors, such as bortezomib, have shown satisfactory clinical outcomes and became one of the backbone regimens against MM [2]. Unfortunately, drug resistance and relapse are unavoidable, and life expectancy is less than 10 years. MM cells bypass the proteasome pathway by using the alternative aggresome-autophagy pathway for protein degradation in which histone deacetylase 6 (HDAC6) is a key player [3]. Recent findings suggested that HDAC inhibition could reestablish responsiveness to bortezomib-resistant MM. Thus, the combination of proteasome and HDAC inhibitors has been emerging as a well-recognized approach in the field.
Aims: Evaluation of dual HDAC and proteasome inhibitors to overcome drug resistance in MM.
Methods: Seven hybrid compounds, bearing both HDAC and proteasome inhibitor pharmacophores were synthesized. All hybrids were composed of a portion of entinostat (pan-HDAC inhibitor) and part of bortezomib, and their cytotoxic activity was evaluated in MM cells. For this, RPMI 8226 cells were treated with the compounds at different concentrations. After 72-h incubation, the antiproliferative activity of the compounds was measured using the XTT assay and those that showed an IC_{50} value < 500 nM were tested in RPMI 8226 cells resistant to proteasome inhibitors established in our lab. Furthermore, HDAC and proteasome inhibitory activities were evaluated.
Results: The strongest antiproliferative activity was obtained by the hybrid displaying the boronic acid of bortezomib as pinanediol ester and bearing the arylamide zinc binding group of entinostat. It showed an IC_{50} value of 9.5 nM in RPMI 8226 cells and of 18.9 nM in the cells resistant to proteasome inhibitors. In comparison, values obtained with bortezomib and entinostat were of 2.1 and 328.9 nM, respectively in RPMI 8226 cells and of 6.0 nM and 524.1 nM, respectively in cells resistant to proteasome inhibitors. Finally, the compound inhibited 28% of HDAC enzymatic activity at 10 µM in RPMI 8226 cells.
Discussion and Conclusions: The most active compound showed antiproliferative activity in the low nM range in both cell lines. Various clinical studies reported that dual-inhibitors were as efficient as drug combinations with the advantage of having both pharmacophores in a single molecule. In MM, HDAC6 plays a significant role in the progression of the malignancy. Therefore, the substitution of entinostat with a selective HDAC6 inhibitor is currently being pursued with the expected advantage of increasing specificity and decreasing side effects.

Keywords: multiple myeloma, proteasome inhibitors, HDAC inhibitors, resistance, dual-inhibitors

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A novel and flexible strategy to functionalize extracellular vesicles for targeted tumor therapy

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Introduction: Extracellular vesicles (EVs) are lipid bilayer enclosed vesicles, released by almost all eukaryotic cells. The ability of EVs to deliver functional cargoes and to modify recipient cells have instigated their use as endogenous nanocarrier system for targeted drug delivery. In comparison to synthetic nanocarriers such as liposomes, EVs offer several advantages: they are non-toxic and non-immunogenic, show great biocompatibility and stability and can efficiently cross biological barriers such as the blood brain barrier. However, EVs exhibit low targeting specificity and an unfavorable pharmacokinetic profile. To overcome these limitations, surface functionalization with specific ligands directing EVs to specific tissues is essential. In this project, a novel and flexible strategy for surface functionalization of EVs with small molecule ligands to target them to tumor cells was implemented. The surface functionalization strategy established is based on a simple «building block» approach involving the avidin-biotin. For proof-of-principle, carbonic anhydrase IX (CAIX), a hypoxia inducible transmembrane protein overexpressed in most solid tumors, is used as target protein. C51 cells, genetically engineered with an estab-lished gycosylphosphatidyl-anchored avidin (GPI-avidin) DNA construct, driving hypoxia induced expression of avidin on the cell surface, are used as EV donor cells. EVs isolated from these cells, presenting avidin on the outer membrane side, are implemented for further functionali-zation with biotinylated ligands.
Aims: The specific aims of this project are to (i) establish a protocol for efficient EV isolation, (ii) confirm the presence of GPI-avidin on the surface of donor cells and EVs, (iii) assess the expression of CAIX on the different cancer cell lines used in these experiments, (iv) characterize the binding affinity of *de novo* synthesized biotinylated CAIX ligands, (v) investigate the binding and internalization of functionalized EVs by CAIX positive cells.
Methods: EV isolation is performed using size exclusion chromatography and ultracentrifu-gation. Isolated EVs are characterized by scanning electron microscopy (SEM) and nanoparticle tracking analysis (NTA). The presence of GPI-Avidin on C51 cells and EVs is assessed by immunofluorescence staining and confocal microscopy or NTA. CAIX expression on cancer cell lines is verified using immunofluorescence staining and flow cytometry. Fluorescence cell-based staining is implemented to evaluate the binding affinity of *de novo* synthesized biotinylated ligands against CAIX. The specific targeting efficacy of functionalized EVs loaded with a fluorescent dye to CAIX positive cells is characterized by confocal microscopy and flow cytometry.
Results: EVs are successfully isolated from C51 cells and display a size range between 30- 300 nm. Moreover, high levels of GPI-Avidin on C51 cells are found after 48 h of incubation at hypoxic condition and the presence of GPI-Avidin on isolated EVs is demonstrated by NTA. The expression of CAIX on cancer cells is significantly induced by hypoxia in all cell lines used. GPI-Avidin EVs are successfully functionalized with biotinylated ligands and shown to specifically bind CAIX expressing tumor cells.
Discussion and Conclusion: Overall, this project provides first evidence that EVs can be flexibly functionalized using the GPI-Avidin system. GPI-Avidin EVs functionalized with ligands targeting CAIX specifically bound to and were internalized by CAIX expressing cancer cells within 6 h, revealing the appropriateness of EVs as drug delivery system. However, for the translation into the clinic and for the application of precision and personalized medicine, EVs will have to be characterized with regard to their biodistribution and targeting capacity in preclinical animal models. Furthermore, EVs from human mesenchymal stem cells, associated with minimal immunogenicity according to first clinical studies or autologous patient cells will have to be stably transfected to express GPI-Avidin and used for further experiments.

Keywords: extracellular vesicles, drug delivery system, target therapy, functionalization of EVs, tumor therapy

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FOLFOXIRI-resistance induction in colorectal carcinoma *in vitro* models

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Introduction: FOLFOXIRI **(**folinic acid, 5-fluorouracil, oxaliplatin and/or irinotecan) is a standard of care for colorectal cancer (CRC), yet an aggressive and non-personalized treatment option. The treatment efficacy remains low du to patient-intolerance and induced resistance.
Aim: We aimed to generate CRC cells that would reflect the clinical situation of stage IV CRC patients long term treated with FOLFOXIRI. This would serve as a platform to study how an induced drug resistance to this chemotherapeutic mixture could alter the response to targeted treatment.
Methods: Four human CRC cell lines (HCT116, LS174T, DLD1 and SW620) were chronically treated with their corresponding optimized FOLFOXIRI mixture, once weekly, for 30-62 weeks. Decrease in cell sensitivity to FOLFOXIRI was evaluated every two weeks using cell metabolic activity assay and compared to the parental, treatment-naïve cells.
Results: Significant loss in sensitivity to FOLFOXIRI was observed in all four chronically treated CRC cell lines, with up to 40% in LS174T cell line, compared to their parental treatment-naïve counterpart, in both 2D and 3D co-cultures of the pre-treated cell lines together with endothelial cells and fibroblasts. Induced morphometric changes are also observed in the pre-treated cells. Moreover, our results show that optimized synergistic drug combinations (ODC) overcome resistance to FOLFOXIRI.
Discussion and Conclusion: By chronically exposing human CRC cell lines to a cell line specific FOLFOXIRI mixture, resistance is obtained over time. The latter can be overcome using ODC. RNA sequencing analysis is being conducted to highlight the different signaling pathways implicated in the resistance mechanisms.

Keywords: colorectal cancer, FOLFOXIRI, drug-resistance, drug combinations

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Development of a complex organoid platform to evaluate the impact of synergistic drug combinations for colorectal cancer treatment

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Introduction: Colorectal cancer (CRC) is one of the three most commonly occurring cancer types with 1.8 million new cases in 2018 while its incidence is expected to rise by 60% until 2030. Current standard therapies for CRC consisting of combined chemotherapeutics are facing some limitation, especially in the late stage of the disease, and, therefore, novel drug-based therapies are urgently required. In our previous studies we have identified promising synergistic low-dose four drug mixtures for treatment of human CRC. The optimization was performed using our validated phenotypic Therapeutically Guided Multidrug Optimization (TGMO) platform, a technology that allows rapid identification of synergistic multidrug combination at low doses. Validation of the optimized drug combinations (ODCs) efficacy is expected to give important insights into their potency in a representative model of CRC.
Aim: We aimed to develop a complex organoid platform to validate the efficacy of promising ODCs.
Methods: We established reproducible short-term 3D cultures of single intestinal organoids, generated from a genetically modified C57 Black 6 Mouse (Apcfl/fl;Krasfl-LSL-G12D-fl/+;p53fl/fl; villin-CreERT2). The efficacy of the ODCs validated in previous *in vitro* and *in vivo* models was assessed within the newly formed platform.
Results: The organoid platform generated single organoids of a standardized size of 300 to 500 μm 48 h after the seeding, with a success rate of 95%. The ODCs successfully demonstrated their cytotoxic effect in intestinal murine organoids. The two tested ODC inhibited organoid cell viability by 91% and 89%, respectively, and growth inhibition over time by approximately 60% in comparison to the control, 48 h after the treatment.
Discussion and Conclusion: The organoid platform was successfully established with high reproducibility and a 95% success rate of single organoid formation. Moreover, the previously identified ODCs maintained their cytotoxic effect in the newly created organoid model. We are currently enriching the platform with immune cells to evaluate the effect of ODCs on the immune system.

Keywords: organoids, colorectal cancer, drug combination

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Squalene-NIR dye nanoassemblies for treatment of cancer by phototherapy

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Introduction: Squalene (Sq) is a natural precursor of cholesterol [1]. The conjugation of Sq to a drug produces bioconjugates that self-assemble in water to give nanoassemblies (NAs) [2]. Near-infrared (NIR) fluorescence has great potential for *in vivo* tumour imaging. NIR dyes such as the recently synthesized IR-774 [3] are lipophilic cations with preferential accumulation in the mitochondria of cancer cells. They also have photosensitizing properties inducing tumour cell death after NIR light irradiation.
Aims: This study aims to develop Sq-IR-774 NAs targeting mitochondria, with imaging and photosensitizing properties and to assess their capacity to induce tumour cell death by phototherapy after NIR light irradiation. Here, we report the *in vitro* fluorescence imaging, specific mitochondrial localization and antitumour effect of Sq-IR-774 NAs after NIR light irradiation.
Material and methods: For this study, we used MCF7 human breast cancer cells and MCF-10 non-tumorigenic human breast cells to assess the specific localization of Sq-IR-774 NAs in the mitochondria of cancer cells. MCF7 and MCF10 were incubated with different concentrations of IR-774 or the Sq-IR-774 NAs for 1 h. Mitochondria were stained with MitoTracker® Orange and nuclei with Hoechst®. Cells were observed by live-cell imaging using the automated IXM-XL microscope (Molecular devices, 1 x 40). To study the photosensitizing properties of our product, we incubated MCF7 cells with 20 µM of IR-774 and Sq-IR-774 for 1 h. The cells were washed and irradiated with a NIR LED lamp at 660 nm, at 100mW/cm^2 for 10 min. We performed the cytotoxicity assay after 24h using WST-1 cell proliferation assay.
Results: Fluorescence imaging of MCF-7 cells demonstrated effective localization of both IR-774 and Sq-IR-774 NAs in mitochondria of cancer cells by the co-staining with MitoTracker® Orange. The comparison with MCF10 non-tumorigenic breast cells demonstrated the preferen-tial accumulation of our product in cancer cells. The WST-1 cell proliferation assay indicated that both IR-774 and Sq-IR-774 induced tumour cell death after light irradiation compared to the control (dark condition).
Discussion and Conclusion: This study demonstrated the preferential accumulation of Sq-IR-774 in the mitochondria of cancer cells by comparison to healthy cells. Sq-IR-774 induced cancer cell death after NIR light irradiation. The fluorescence and photosensitizing properties of the Sq-IR-774 NAs suggest their potential use as a nanotheranostic agent for imaging and treatment of cancer by phototherapy.

Keywords: squalene, NIR-dye, nanoassemblies, cancer, phototherapy

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Investigating the effects of targeted drug combinations on colorectal cancer

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Introduction: Current therapy used in the clinic for the treatment of colorectal carcinoma (CRC) are composed mostly of chemotherapeutic drugs, which have a less-than-optimal therapeutic window and induce side effects. An interesting alternative is the use of targeted drugs instead. Used as monotherapies, those targeted drugs are however often rapidly followed by the apparition of resistance in the tumors. To challenge these issues, we identified from the large spectrum of possibilities a low-dose synergistic targeted drug combinations (ODC), which are both selective and effective in *in vitro* and *in vivo* models of human CRC.
Aim: We aimed to characterize the effects of two cell subtype specific ODCs, composed of Regorafenib, Selumetinib, Vemurafenib and either Erlotinib or GDC-0994, on human CRC grown in mice.
Methods: We mounted 0.5-µm slices of ODC-treated tumors from our *in vivo* tests on glass slides and analyzed them using different histological staining: H&E for comprehensive micro-anatomy picture, Ki67 + DAPI immunofluorescent staining for proliferation characterization, CD31 staining for blood vessels density and their morphology, and ER-TR7 staining for fibroblasts characterization.
Results: Tumors treated with the respective ODC showed significantly reduced cell and vessel density (minus 17.6% and 26% for cell density and minus 69.7% and 61.7% for vessel density), as well as reduced cell proliferation (minus 42.8% and 20.7%) when compared to control and respective monotherapy treated tumors. Qualitative test for other characteristics could also hint for a fibroblast density reduction effect in SW620 ODC-treated tumors and an apoptosis induction in DLD1 ODC-treated tumors.
Discussion and Conclusion: The treatment with ODC improved antitumor activity showing encouraging results toward the use of personalized drug combinations in the clinic.

Keywords: colorectal cancer, drug combination, immunohistochemistry

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Low-dose multidrug combination preventing spindle pole clustering in dividing cancer cells: mechanism of action and activity in colorectal carcinoma *ex vivo* models

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Introduction: Combinatorial treatment approaches are a promising anti-cancer strategy to achieve high efficacy, decrease dose-related toxicities, as well as acquired drug resistance. Our validated phenotypic Therapeutically Guided Multidrug Optimization platform allowed for the identification of a synergistic low-dose four-drug combination highly effective in renal cell carcinoma, colorectal carcinoma (CRC) and melanoma, while preserving non-malignant epithelial cells [1]. This optimized drug combination (ODC) consists of two tyrosine kinase inhibitors (dasatinib, erlotinib) and two histone deacetylase inhibitors (tacedinaline, tubacin). ODC efficacy strongly correlated with the prevention of spindle pole clustering, a survival mechanism allowing cancer cells with centrosome abnormalities or mitotic spindle defects to cluster their multiple spindle poles into a bipolar configuration, thus avoiding death via multipolar divisions [1,2].
Aims: We aim to identify the full mechanism of action of ODC and causally link its efficacy to spindle pole clustering inhibition. We also aim to evaluate ODC efficacy in CRC *ex vivo* models, highly relevant to human physiopathology, and ultimately facilitate *in vivo* translation.
Methods: We performed a cell metabolic activity assay on six CRC cells lines prone to form multipolar spindles to evaluate ODC efficacy, as well as on CRC patient-derived organoids. Immunofluorescence microscopy and live cell imaging were used to quantify ODC impact on centrosome amplification, multipolar spindle formation, and mitotic progression.
Results and Discussion: ODC showed highest efficacy in COLO205 (78% cell viability inhibition vs. control), the cell line with the highest centriole amplification rate in our panel, while showing negligible toxicity towards non-malignant epithelial colon cell line CCD841 (18% cell viability inhibition vs. control). Preliminary results showed ODC activity in CRC patient-derived organoids. After ODC treatment, COLO205 displayed an increased number of multipolar cells (12.5% vs. 7.6% in control) and a decreased proportion of cells displaying centriole amplification (23.8% vs 35.9% in control). This supports the hypothesis ODC targets cells prone to form multipolar spindles. Live cell imaging revealed an increase in mitotic timing and a 2-fold reduction of mitotic index, suggesting additional mitosis-independent mechanisms of actions need to be investigated.
Conclusion: ODC selectively target dividing cancer cells prone to form multipolar spindles and its activity in CRC patient-derived organoids reveals potential for clinical translation.

Keywords: drug combination, colorectal cancer, mitosis, spindle pole clustering

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Oatp2b1 influences coproporphyrin serum levels as determined in a novel *Slco2b1*$^{-/-}$ rat model

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Introduction: Coproporphyrin (CP) I and III are isomeric byproducts of the heme metabolism which are handled as potential biomarkers to indicate drug-drug interactions involving hepatic Organic Anion Transporting Polypeptide (OATP) 1B. OATP2B1 is another hepatically expressed member which is known to specifically transport CP-III.
Aims: We wanted to test whether CP-III is a potential biomarker for OATP2B1 transporter function by investigating CP levels in serum of wildtype and *Slco2b1*$^{-/-}$ rats.
Methods: In *in vitro* experiments we used transfected HeLa cells to determine CP-III transport kinetics by human an rat orthologues. A novel *Slco2b1*$^{-/-}$ rat model was generated and characterized for physiological parameters including LC-MSMS based quantification of CP-I and CP-III serum levels. Other major drug transporters, which are assumed to be involved in the CP-handling, were analyzed for their hepatic and renal expression using Real-Time PCR, Western blot analysis and immunohistochemistry.
Results: *In vitro* transport experiments revealed differences in transport kinetics comparing human and rat OATP2B1/rOatp2b1, while a consistent interplay with MRP3/rMrp3 was observed. Offspring of homozygous *Slco2b1*$^{-/-}$ rats were viable and fertile, with gender-dependent differences in phenotypic parameters and CP serum levels: serum CP-III levels were indifferent while in male and female *Slco2b1*$^{-/-}$ rats lower CP-I levels were observed compared to wildtype animals. Western blotting analysis revealed changes in Mrp2 protein levels which showed to be higher in the liver of male and in the kidney of female knockout rats.
Discussion and Conclusion: Lower CP-I levels are caused by an Oatp2b1 deficiency although it is not a substrate. This suggests the influence on the hepatic handling of CPs by other factors beside the OATP1B-activity.

Keywords: drug transport, OATP2B1, coproporphyrin, biomarker, *in vivo*

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Replaced PharmaLunch, 3-4 times per year, usually in Basel, currently as online webinar.

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The SAPhS

- fosters nationally the scientific exchange and the cooperation of Swiss pharmacy, especially its scientific interests
- fosters and supports the pharmaceutical research, thereby complying with the principles of the pharmaceutical scientific ethics
- fosters the young pharmaceutical scientific academics
- fosters the communication between the pharmaceutical sciences in Switzerland and other scientific organisations, as well as between pharmaceutical societies, their institutions and members
- represents the Swiss pharmaceutical sciences on a national and international level.

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The SAPhS

- fosters the scientific exchange in adequate platforms, such as the Swiss Pharma Science Day (SPhSD), the PharmaTalk and the PharmApéro
- fosters the pharmaceutical education and post-graduate education, and coordinates the continuing education by combining education and research at a high scientific level
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- develops recommendations and guidelines for the university education in pharmaceutical sciences
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- represents the pharmaceutical sciences in the public
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- assists in the preparation of Swiss science politics and represents the interests of all pharmaceutical disciplines in the Swiss universities' politics

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Tasks and Activities

- releases scientific publications and publishes articles, e.g. in the journals «Swiss Pharma» and «PharmaJournal»
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