Annual Meeting of the German Pharmaceutical Society – DPhG

Pharmaceutical research: From basic research to medical applications

Heidelberg, Germany
September 01 – 04, 2019
at Heidelberg University
Conference Book

Pharmaceutical research: From basic research to medical applications

Annual Meeting of the German Pharmaceutical Society 2019 - DPhG
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Conference Book
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www.2019.dphg.de
Institutional Sponsors

Förderer der DPhG-Jahrestagung 2019
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CONFERENCE COMMITTEES

Scientific committee:
Prof. Dr. Christian Klein
Prof. Dr. Thomas Efferth
Prof. Dr. Irmgard Merfort
Prof. Dr. Ulrike Holzgrabe
Prof. Dr. Michael Lämmerhofer
Prof. Dr. Gerhard Winter
Prof. Dr. Werner Weitschies
Prof. Dr. Dr. Peter Ruth
Prof. Dr. Achim Schmidtko
Prof. Dr. Ulrich Jaehde

Organization committee:
Prof. Dr. Michael Wink
Prof. Dr. Christian Klein
Prof. Dr. Stefan Wölfli
Prof. Dr. Gert Fricker
Dr. Gabriele Reich
WELCOME ADDRESS

Dear colleagues,

the President of the German Pharmaceutical Society (DPhG) and the Congress Chairman would like to welcome you to Heidelberg and Heidelberg University. Heidelberg as the city of sciences with a unique romantic flair is the ideal location for our international annual DPhG meeting 2019. The meeting is cosponsored by one of our most important partners, the Pharmaceutical Society of Japan (PSJ).

The title of this year’s meeting is „Pharmaceutical research: From basic research to medical applications”. A broad range of sessions for oral and poster presentations will cover important aspects of current pharmaceutical research. We are looking forward to exciting talks, presented by many high level speakers – including Nobel Prize winner Prof. Harald zur Hausen - and stimulating discussions. A particularly important part of the meeting is the interaction of younger scientists with other attendees. A dedicated session for poster short-talks will allow selected PhD students to present their work. For excellent young postdocs and group leaders we will have special sessions to give them the opportunity of sharing their research results.

Many thanks to the scientific committee headed by Prof. Ch. Klein and the local organization team, to the scientific chairs as well as to the Pharmaceutical Society of Japan (JPS) for the strong support. This abstract book provides all necessary information on the program as well as on the abstracts of the scientific contributions of plenary lectures, scientific lectures, short poster lectures and posters.

We are glad to have you here for a stimulating meeting in Heidelberg and hope you will enjoy the scientific program as well as the location.

Prof. Dr. Stefan Laufer, DPhG-President

Prof. Dr. Michael Wink, Congress Chairman
GENERAL INFORMATION

The Annual DPhG Meeting 2019 takes place at the Hörsaalzentrum Chemie of the Heidelberg University (Im Neuenheimer Feld (INF) 252).

LANGUAGE

The Conference language is English, no simultaneous translation will be provided.

INSTRUCTIONS FOR USING CONFERENCE WLAN

If your institution is member of the “eduroam” community, you can use the wireless network “eduroam”. The configuration of your device should be the same as instructed by your home institution. Please use your account and the domain of your home institution. If your institution is not member of the “eduroam” community, you can obtain a guest account and a password at the Conference office.

CONFERENCE OFFICE

The Conference office is located at the Conference building Im Neuenheimer Feld 252, 69120 Heidelberg.

Opening hours:
Monday, September 2nd, 2019: 11:00 – 18:00; in the foyer of the main building
Tuesday, September 3rd, 2019: 8:00 – 17:00; in the foyer of the main building
Wednesday, September 4th, 2019: 8:00 – 12:00; in the foyer of the main building

BAGGAGE CHECKROOM

Your baggage can be stored at the conference office during the opening hours (no liability is assumed).

LIABILITY

The Organizers of the Conference cannot be held responsible for any loss, theft, damage or injury to any person or property during the Conference, whatever the cause may be. The liability of persons and enterprises providing means of transportations or other services remains unaffected. Each congress participant and accompanying person takes part in all tours at his/her own risk.
ABSTRACT AND POSTER NUMBERS

Each abstract has a unique identifier, a letter-number combination. Letters refer to the conference topic a contribution was assigned to (i.e. plenary lectures are identified by the letter “P”, scientific lectures by the letters “SL”, and poster presentations by the letters “POS”). Please note that in case of poster presentations the abstract number is identical with the poster number. Please refer to the authors index on page 167 for direct access to specific abstracts.

POSTER SESSIONS

Topics:
Session I: Medicinal chemistry and drug desing and Other topics
Session II: Antiinfectives, Cancer, Inflammation, Analytics, Biotechnology and Biopharmaceutics, Clinical Pharmacy, Natural Compounds, Pharmaceutical technology and biomaterials and Poster Short Talks (mixed topics).
Presenting authors are asked to be present at their poster during the poster sessions.

<table>
<thead>
<tr>
<th>Session</th>
<th>Poster session I</th>
<th>Poster session II</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Monday, September 2\textsuperscript{nd}, 2019, 19:30 – 20:30</td>
<td>Tuesday, September 3\textsuperscript{rd}, 2019 16.00 - 16.30 and 10.00 - 10.30</td>
</tr>
<tr>
<td>Set-up</td>
<td>Wednesday, September 2\textsuperscript{nd}, 2019, before 16:00</td>
<td>Tuesday, September 3\textsuperscript{rd}, 2019 Between 11:00 – 15:00</td>
</tr>
<tr>
<td>Dismantling</td>
<td>Wednesday, September 3\textsuperscript{rd}, 2019, before 10:30</td>
<td>Wednesday, September 4\textsuperscript{th}, 2019, until 12:00</td>
</tr>
</tbody>
</table>

CONFERENCE DINNER

Separate registration necessary (special fee). Please refer to the Conference office for registration and details. The Conference dinner will take place at “Schloßhotel Molkenkur”, Klingenteichstraße 31, 69117 Heidelberg.

BADGES

Badges will be issued to all registered participants and enable access to all scientific sessions.
LOCATIONS

The Congress will take place at Hörsaalzentrum Chemie of the Heidelberg University (Im Neuenheimer Feld (INF) 252).

It’s easy to get to Heidelberg University by public transportation via Tram or bus.

The line 5 takes ten minutes to get from the Main Station (Hauptbahnhof) to Technologiepark Station.
Due to limited parking space, we strongly recommend taking public transport.

For directions please refer to the campus map in the inner cover at the end of the abstract book.
Schloßhotel Molkenkur (Conference Dinner):
The Schloßhotel Molkenkur (Klingenteichstraße 31, 69117 Heidelberg) is located above the city of Heidelberg and invites you to enjoy the wonderful view over the old town and the Neckar and the Rhine valley.

BY PUBLIC TRANSPORTATION
Go from Heidelberg Main Station East to station "Universitätsplatz” by bus 32. Take the bus 30 to station “Molkenkur” (approx. 35 mins).

Alternatively you can use the Heidelberg Bergbahn from station Kornmark to station Molkenkur.
1 CONFERENCE PROGRAM OVERVIEW

Samstag, 31.08.2019

Tag der Offizinpharmazie
Organisationskomitee:
Kathrin Müller, Michael Hannig, Juliane Kresser, Nadine Metzger
(DPhG FG Allgemeinpharmazie und Apothekerkammer Baden-Württemberg)

Veranstaltungsort: Universität Heidelberg, Campus Im Neuenheimer Feld (INF), Hörsaalzentrum Chemie (Gebäude INF-252), 69120 Heidelberg; Großer Hörsaal

Arzneimitteltherapie für Kinder

15:00 – 15:10 Uhr Begrüßung

15:10 – 15:50 Uhr Pädiatrie trifft Pharmakologie: Besonderheiten im Kindes- und Jugendalter
Prof. Dr. Matthias Schwab,
Dr. Margarete Fischer-Bosch-Institut für Klinische Pharmakologie, Stuttgart

15:50 – 16:30 Uhr Kinderarzneimittel in Rezeptur und Defektur
Prof. Dr. Rolf Daniels,
Universität Tübingen

16:30 – 16:45 Uhr Kaffeepause

16:45 – 17:30 Uhr Kinder sind keine kleinen Erwachsenen – Arzneimitteltherapie für Kinder
Margit Schlenk,
Moritz-Apotheke Nürnberg

17:30 – 18:00 Uhr Panel mit den Referenten und Publikumsbeteiligung
Im Anschluß Mitgliederversammlung der DPhG-FG-Allgemeinpharmazie, u. a. mit Vorstandswahl

Wichtig! Nur angemeldete Teilnehmer/innen erhalten Zutritt. Bitte melden Sie sich online unter:
www.lak-bw.de/Fortbildung/Seminarplan an.
### Sunday, September 1st

**Vorsymposium der Fachgruppe „Geschichte der Pharmazie“: Pharmazie in Heidelberg**

Ort: Universität Heidelberg, Campus Im Neuenheimer Feld (INF), Hörsaalzentrum Chemie (Gebäude INF-252), 69120 Heidelberg; Hörsaal West

<table>
<thead>
<tr>
<th>Time</th>
<th>Event</th>
<th>Speaker</th>
</tr>
</thead>
<tbody>
<tr>
<td>14:00</td>
<td>Begrüßung durch den Vorsitzenden der FG Geschichte der Pharmazie</td>
<td>Prof. Dr. Christoph Friedrich, Marburg</td>
</tr>
<tr>
<td>14:15</td>
<td>Entwicklung des Hochschulfaches Pharmazie an der Universität Heidelberg</td>
<td>Dr. Arnt Heilmann, Hirschhorn (Neckar)</td>
</tr>
<tr>
<td>15:00</td>
<td>Aus der Geschichte des Apothekenwesens in Heidelberg</td>
<td>Dr. Albert Borchardt, Heidelberg</td>
</tr>
<tr>
<td>15:45</td>
<td>Kaffeepause</td>
<td></td>
</tr>
<tr>
<td>16:15</td>
<td>Friedrich Bergius (1884-1949) – Chemiker, Unternehmer und Nobelpreisträger</td>
<td>Prof. Dr. Wolf-Dieter Müller-Jahncke, Heidelberg</td>
</tr>
<tr>
<td>17:00</td>
<td>Das Deutsche Apothekenmuseum im Heidelberger Schloss</td>
<td>Dr. Elisabeth Huwer, Heidelberg</td>
</tr>
</tbody>
</table>
### Monday, September 2nd

**Main Symposium (Congress language English)**

<table>
<thead>
<tr>
<th>Time</th>
<th>Event</th>
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</thead>
</table>
| 09:00 – 12:00 | Sitzung des VdPPHI (HS West)  
Chair: B. Clement |
| 13:00 – 13:45 | Opening of the Annual DPhG Meeting 2019 (Großer HS)  
Pharmaceutical research: From basic research to medical applications |
PL.1 |
| 14:30 – 15:00 | Coffee break |

**SHORT TALKS (parallel sessions I)**

<table>
<thead>
<tr>
<th>Time</th>
<th>Session</th>
</tr>
</thead>
</table>
| 15:00 – 16:30 | SL1 (Großer HS)  
New Research, New Researchers I  
Chair: S. Laufer  
15:00  
K. Nakayama: Molecular basis for protein trafficking within cilia and ciliopathies revealed by the visible immunoprecipitation (VIP) assay and CRISPR/Cas9 system |
| 16:45 - 17:30 | Plenary lecture 2, R. Bartenschlager, Curative therapy for chronic hepatitis C: Successes and remaining challenges (Großer HS)  
PL.2 |

<table>
<thead>
<tr>
<th>Time</th>
<th>Session</th>
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</thead>
</table>
| 15:00 | SL1 (Großer HS)  
New Research, New Researchers I  
Chair: S. Laufer  
15:00  
K. Nakayama: Molecular basis for protein trafficking within cilia and ciliopathies revealed by the visible immunoprecipitation (VIP) assay and CRISPR/Cas9 system |
| 15:00 | SL2 (HS West)  
Ion channels as drug targets  
Chairs: P. Ruth, M. Freichel  
15:00  
A. Guse: NAADP: a fundamental modulator of Ca2+ release channels in Lymphocytes |
| 15:00 | SL3 (HS Ost)  
Fachsprecherin  
Dermatopharmakologie: "Predictivity of in vitro tests in pharmacology and toxicology"  
Chairs: G. Weindl, J. Klein  
15:00  
M. Schäfer-Korting: Reconstructed human skin can predict the efficacy and safety of drugs in humans |
| 15:30 | SL1 (Großer HS)  
New Research, New Researchers I  
Chair: S. Laufer  
15:30  
R. Wombacher: Protein Labelling and Manipulation in Living Cells Using Bioorthogonal Chemistry |
| 15:30 | SL2 (HS West)  
Ion channels as drug targets  
Chairs: P. Ruth, M. Freichel  
15:30  
Y-K. Chao: Agonist-Dependent switching of ion selectivity in endolysosomal TPC2 channel |
| 15:30 | SL3 (HS Ost)  
Fachsprecherin  
Dermatopharmakologie: "Predictivity of in vitro tests in pharmacology and toxicology"  
Chairs: G. Weindl, J. Klein  
15:30  
R. Landsiedel: Adverse outcome pathway-based concepts and tools for assessing skin sensitization |
| 16:00 | SL1 (Großer HS)  
New Research, New Researchers I  
Chair: S. Laufer  
16:00  
A. S. Kahnt: Supporting resolution? Biosynthesis of specialized pro-resolving mediators (SPM) in human leukocytes |
| 16:00 | SL2 (HS West)  
Ion channels as drug targets  
Chairs: P. Ruth, M. Freichel  
16:00  
V. Tsvilovskyy: Identification of OCaR1 as a gatekeeper of lysosomal-granular Ca2+ release and regulated exocytosis |
| 16:00 | SL3 (HS Ost)  
Fachsprecherin  
Dermatopharmakologie: "Predictivity of in vitro tests in pharmacology and toxicology"  
Chairs: G. Weindl, J. Klein  
16:00  
G. Weindl: Reconstructed tissue models with integrated immune cells – Do we need advanced in vitro tests for the evaluation of drugs and chemicals? |
| 16:10 | SL1 (Großer HS)  
New Research, New Researchers I  
Chair: S. Laufer  
16:10  
M. Koziolek: The application of salivary caffeine concentrations to study gastric emptying of fluids and formulations in humans |
| 16:15 | SL2 (HS West)  
Ion channels as drug targets  
Chairs: P. Ruth, M. Freichel  
16:15  
J. Camacho-Londono: Determination of fatal ventricular arrhythmias by a new regulator of Ca2+ signaling at the lysosomal-SR junction |
| 16:15 | SL3 (HS Ost)  
Fachsprecherin  
Dermatopharmakologie: "Predictivity of in vitro tests in pharmacology and toxicology"  
Chairs: G. Weindl, J. Klein  
16:15  
C. Zoschke: Human cell-based tumor microenvironment models for improved preclinical drug development |
| 16:45 | SL1 (Großer HS)  
New Research, New Researchers I  
Chair: S. Laufer  
16:45  
M. Koziolek: The application of salivary caffeine concentrations to study gastric emptying of fluids and formulations in humans |
| 16:45 | SL2 (HS West)  
Ion channels as drug targets  
Chairs: P. Ruth, M. Freichel  
16:45  
J. Camacho-Londono: Determination of fatal ventricular arrhythmias by a new regulator of Ca2+ signaling at the lysosomal-SR junction |
| 16:45 | SL3 (HS Ost)  
Fachsprecherin  
Dermatopharmakologie: "Predictivity of in vitro tests in pharmacology and toxicology"  
Chairs: G. Weindl, J. Klein  
16:45  
C. Zoschke: Human cell-based tumor microenvironment models for improved preclinical drug development |

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<table>
<thead>
<tr>
<th>17:30 – 18:45</th>
<th>Meetings der DPhG-Fachgruppen</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fachgruppe Pharm./Med. Chemie</td>
<td>Fachgruppe Pharm. Biologie</td>
</tr>
<tr>
<td>P. Gmeiner (Großer HS)</td>
<td>A. Vollmar (HS Ost)</td>
</tr>
<tr>
<td>Fachgruppe Pharmakologie</td>
<td>Fachgruppe Pharm. Technologie</td>
</tr>
<tr>
<td>J. Klein (HS West)</td>
<td>D. Fischer (Gebäude INF 327, Seminarraum 3, EG)</td>
</tr>
<tr>
<td>Fachgruppe Klinische Pharmazie</td>
<td>Fachgruppe Industriepharmazie</td>
</tr>
<tr>
<td>T. Lehr (HS Med. Klinik, INF 410)</td>
<td>(Gebäude INF 327, Seminarraum 4, EG)</td>
</tr>
<tr>
<td>AG Radiopharmazie, Dr. PD Reischl (Gebäude INF 327, Seminarraum 1, EG)</td>
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<tr>
<td>19:00 – 21:30</td>
<td>Poster Session I (19.30 – 20.30 h) and Welcome Reception</td>
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**Tuesday, September 3rd**

**SHORT TALKS (parallel sessions II)**

<table>
<thead>
<tr>
<th>08:30 – 10:00</th>
<th>SL4 (Großer HS)</th>
<th>SL5 (HS West)</th>
<th>SL6 (HS Ost)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cysteine-reactive inhibitors and probes</td>
<td>Dysfunctional Cellular Signaling in Cardiovascular Diseases</td>
<td>Biopharmacy, Pharmaceutical Technology</td>
<td></td>
</tr>
<tr>
<td>Chair: T. Schirmeister</td>
<td>Chairs: T. Wieland, R. Gilsbach</td>
<td>Chair: G. Fricker</td>
<td></td>
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</tbody>
</table>

<table>
<thead>
<tr>
<th>08:30 SL.13</th>
<th>08:30 SL.16</th>
<th>08:30 SL.19</th>
</tr>
</thead>
</table>

<table>
<thead>
<tr>
<th>09:00 SL.14</th>
<th>09:00 SL.17</th>
<th>09:00 SL.20</th>
</tr>
</thead>
<tbody>
<tr>
<td>M. Gütschow: Novel Cathepsin B Inhibitors with Inversely Oriented Warheads</td>
<td>R. Gilsbach: Identification and modulation of chromatin interactions in cardiac myocytes</td>
<td>Y. Takakura: Development of exosome-based nucleic acid drug delivery system</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>09:30 SL.15</th>
<th>09:30 SL.18</th>
<th>09:30 SL.21</th>
</tr>
</thead>
<tbody>
<tr>
<td>M. Gehringer: Covalent Kinase Inhibition by Reversible and Irreversible Cysteine Targeting</td>
<td>V. Nikolaev: Altered cyclic nucleotide microdomains in cardiac disease</td>
<td>U. Lächelt: Metal-Organic Nanopharmaceuticals</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>09:45 SL.22</th>
<th>10:00 – 10:15</th>
</tr>
</thead>
<tbody>
<tr>
<td>C. Wölk: Development of lipoplex-loaded model surface coatings for the in situ transfection in the field of bone regeneration</td>
<td>Coffee break</td>
</tr>
</tbody>
</table>
# SHORT TALKS (parallel sessions III)

<table>
<thead>
<tr>
<th>Time</th>
<th>SL7 (Großer HS)</th>
<th>SL8 (HS West)</th>
<th>SL9 (HS Ost)</th>
</tr>
</thead>
<tbody>
<tr>
<td>10:15 –</td>
<td><strong>Gold! Hunting treasures in chemistry and pharmacology</strong>&lt;br&gt;Chair: C. Klein</td>
<td><strong>Evidence-based value added to innovative health care by pharmacists</strong>&lt;br&gt;Chairs: H. Seidling, U. Jaehde</td>
<td><strong>Simulation in Pharmaceutical and Formulation Development</strong>&lt;br&gt;Chairs: D. Fischer, P. Langguth</td>
</tr>
<tr>
<td>10:15</td>
<td>S. Hashmi: <strong>Gold Catalysis: Functionalized Gold Carbenes, Unexpected Selectivities</strong></td>
<td>T. Dreischulte: <strong>Evidence-based value added to innovative health care by pharmacists: Ambulatory care</strong></td>
<td>H. Wachtel: Simulations in pharmaceutical technology</td>
</tr>
<tr>
<td>10:45</td>
<td>I. Ott: <strong>Gold Organometallics in Medicinal Chemistry</strong></td>
<td>T. Hoppe-Tichy: <strong>Evidence-based value added to innovative health care by pharmacists: Hospital Setting</strong></td>
<td>M. A. Nguyen: Pharmacokinetic Modelling and Simulation to Support Early Clinical Drug Development</td>
</tr>
<tr>
<td>11:15</td>
<td>A. Miller &amp; N. Gunkel: <strong>Homoleptic gold dithiocarbamate TXNRD1 inhibitors prevent tumor recurrence in a small cell lung cancer mouse model</strong></td>
<td>U. Jaehde: <strong>Evidence-based value added to innovative health care by pharmacists: Long-term care facilities</strong></td>
<td>A. N. Ciciliani: Simulation of pulmonary deposition during inhalation therapy</td>
</tr>
</tbody>
</table>

**Poster Short Talks (Großer HS)**<br>Chairs: D. Fischer, P. Ruth, S. Wölfl

<table>
<thead>
<tr>
<th>Time</th>
<th>PST.01&lt;br&gt;K. Mäder: <strong>Intracochlear PLGA based implants for dexamethasone release: challenges and solutions</strong></th>
<th>PST.02&lt;br&gt;F. Mitrach: <strong>Stabilization of Calcium Phosphate nanoparticles as carrier system for siRNA</strong></th>
<th>PST.11&lt;br&gt;M. Felix: <strong>Proteomic Exploration of IDH-Mutant Brain Tumors</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td>11:45</td>
<td>12:14&lt;br&gt;M. Felix: <strong>Proteomic Exploration of IDH-Mutant Brain Tumors</strong></td>
<td>12:17&lt;br&gt;J. Seeger: <strong>Determinants of growth-kill behaviour of fluoroquinolone resistant Escherichia coli under levofloxacin exposure in a dynamic in vitro infection model</strong></td>
<td>12:20&lt;br&gt;A. Müller-Schöll: <strong>Computational treatment simulation to assess the risk for non-efficacy in tamoxifen treated breast cancer patients of different ethnicities</strong></td>
</tr>
<tr>
<td>11:51</td>
<td>12:23&lt;br&gt;X. Cheng: <strong>Triple-color reporter system to follow up directed differentiation of iPSC towards the three germ layers</strong></td>
<td>12:20&lt;br&gt;A. Müller-Schöll: <strong>Computational treatment simulation to assess the risk for non-efficacy in tamoxifen treated breast cancer patients of different ethnicities</strong></td>
<td>12:23&lt;br&gt;X. Cheng: <strong>Triple-color reporter system to follow up directed differentiation of iPSC towards the three germ layers</strong></td>
</tr>
<tr>
<td>11:54</td>
<td>12:20&lt;br&gt;A. Müller-Schöll: <strong>Computational treatment simulation to assess the risk for non-efficacy in tamoxifen treated breast cancer patients of different ethnicities</strong></td>
<td>12:23&lt;br&gt;X. Cheng: <strong>Triple-color reporter system to follow up directed differentiation of iPSC towards the three germ layers</strong></td>
<td>12:23&lt;br&gt;X. Cheng: <strong>Triple-color reporter system to follow up directed differentiation of iPSC towards the three germ layers</strong></td>
</tr>
</tbody>
</table>

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11:57 PST.05
L. Ilia: Identification and quantification of microdialysis variability using a dynamic in vitro microdialysis system and nonlinear mixed-effects modelling

12:00 PST.06
Y-M. Pudritz: Pharmacology meets (clinical) pharmacy – virtual patients for pharmacy students

12:03 PST.07
S. Schwarthoff: Addressing targets relevant in neurodegenerative disorders: synthesis and biological activity of multifunctional heterovalent carboline derivatives

12:06 PST.08
M. Borchers: “Gender Medicine” in the 18th century?! Pharmacotherapy in the hessian hospital Merxhausen

12:09 PST.09
S. Heuter: Platelets induce a chemoresistance of tumor cells by upregulating drug efflux transporters

12:11 PST.10
F. Almouhanna: Glycolytic Flux and p53 status influence Growth Inhibition in response to the G6PD Inhibitor DHEA in Colorectal Cancer Cells

12:26 PST.15
G. Treccani: Ketamine promotes early changes in dendritic morphology in the hippocampus of a genetic rat model displaying depressive-like behaviour

12:29 PST.16
R. Bilancia: Sex dimorphism in rat platelet aggregation

12:32 PST.17
N. Wössner: Thiocyanates as a new class of selective SirT1 inhibitors

12:35 PST.18
B. Praefke: Small molecules as MKK4 inhibitors for the regeneration of hepatocytes

12:38 PST.19
S. Andreev: From JAK to GSK-3β: Synthesis and Biological Evaluation of 7-Chloro-9H-Pyrimido[4,5-b]indoles as Inhibitors of Glycogen Synthase Kinase-3β

12:41 PST.20
J. Hofmann: Bioisosteres of the Natural Product Taxifolin and their Impact on Amyloid-β 42 Aggregation and Intracellular Oxidative Stress

12:45 – 13:45 Lunch break
Workshop PubPharm - Tools für die pharmaziespezifische Recherche, C. Draheim (HS West)

13:45 – 14:30 Plenary lecture 3, W. Haefeli, Achievements, persisting challenges and unmet needs in medication safety (Großer HS)

14:30 – 16:00 SHORT TALKS (parallel sessions IV)

<table>
<thead>
<tr>
<th>14:30</th>
<th>SL10 (Großer HS)</th>
<th>SL11 (HS West)</th>
<th>SL12 (HS Ost)</th>
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</thead>
<tbody>
<tr>
<td>14:30</td>
<td>M. Bünemann: Dynamics of GPCR - G protein interactions as the key to understand coupling selectivity of GPCRs</td>
<td>T. Ross: Visualisation of cardiac fibrosis using [68Ga]MHLL1 – A PET tracer for the fibroblast activation protein</td>
<td>M. Bantscheff: Drug effects on protein homeostasis</td>
</tr>
<tr>
<td>14:30</td>
<td>SL.33</td>
<td>SL.37</td>
<td>SL.41</td>
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<table>
<thead>
<tr>
<th>Time</th>
<th>Session</th>
<th>Title</th>
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<tbody>
<tr>
<td>14:50</td>
<td>SL.34</td>
<td>D. Weikert: Bivalent ligands targeting heterodimers of dopamine receptors</td>
</tr>
<tr>
<td>15:00</td>
<td>SL.38</td>
<td>G. Reischl: Development and validation of 2-nitroimidazole-furanoside based fluorine-18 labeled PET-radiopharmaceuticals for the in-vivo detection of hypoxic tissue in tumors</td>
</tr>
<tr>
<td>15:00</td>
<td>SL.42</td>
<td>K. Marcus: Laser Capture Microdissection/Mass spectrometry- a promising tool in tissue-based clinical proteomics</td>
</tr>
<tr>
<td>15:15</td>
<td>SL.35</td>
<td>M. Decker: Photopharmacology in Alzheimer research: Tools to investigate GPCRs</td>
</tr>
<tr>
<td>15:20</td>
<td>SL.39</td>
<td>M. Keller: Functionalized Nω-carbamoylated arginines give access to labeled peptide receptor ligands</td>
</tr>
<tr>
<td>15:30</td>
<td>SL.43</td>
<td>A. Maurer: GMP Radiolabeling and First-in-Man Imaging of an Antibody for the Diagnosis of Aspergillosis</td>
</tr>
<tr>
<td>15:35</td>
<td>SL.36</td>
<td>G. Wolber: In Silico Pharmacology: A Mechanistic View on GPCR Modulation</td>
</tr>
<tr>
<td>15:40</td>
<td>SL.40</td>
<td>O. Prante: Radioisynthesis, in-vitro and in-vivo studies of 177Lu-labeled neurotensin receptor-1 antagonists as radiotracers for the therapy of pancreatic cancer</td>
</tr>
<tr>
<td>15:45</td>
<td>SL.44</td>
<td>M. Ardelt: Inhibition of Cdk5 – A New Way to Improve the Standard Therapy of Hepatocellular Carcinoma</td>
</tr>
<tr>
<td>16:00 – 16:30</td>
<td></td>
<td>Poster Session II and Coffee Break</td>
</tr>
<tr>
<td>16:30 – 17:15</td>
<td></td>
<td>Plenary lecture 4, S. Knapp, Strategies for selective targeting of protein kinases (Großer HS)</td>
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<tr>
<td>17:30 – 19:00</td>
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<td>DPhG Hauptversammlung (Großer HS)</td>
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<td>Conference Dinner (Restaurant Schlosshotel Molkenkur, Klingenteichstraße 31, 69117 Heidelberg)</td>
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## Wednesday, September 4th

### SHORT TALKS (parallel sessions V)

<table>
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<tr>
<th>Time</th>
<th>SL13 (Großer HS)</th>
<th>SL14 (HS West)</th>
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<tbody>
<tr>
<td>08:30 – 10:00</td>
<td>Microfluidics / Cell-based assays and organoids for drug discovery Chair: S. Wölfl</td>
<td>New Research, New Researchers II Chairs: A. Link, F. Hansen</td>
<td>The promise of cure - recent breakthrough innovation in cell- and gene therapy Chair: H. Apeler</td>
</tr>
<tr>
<td>08:30</td>
<td>C. Merten: Droplet Microfluidics in antibody discovery and personalized cancer therapy</td>
<td>D. Merk: Direct PPARγ activation by L-thyroxin and TETRAC links thyroid hormone and PPAR signaling</td>
<td>K. Cichutek: Cell and gene therapy developments: It is now or never!</td>
</tr>
<tr>
<td>08:30</td>
<td>J. Gerstmeier: Targeting biosynthetic networks of the proinflammatory and proresolving lipid metabolome: a promising pharmacological strategy for intervention with inflammation</td>
<td>J. Stitz: Hybrid retrovirus-transposon vectors</td>
<td></td>
</tr>
<tr>
<td>09:10</td>
<td>S. Wölfl: Analyzing toxicity and metabolic interactions with microfluidic liver-kidney-on-a-chip systems</td>
<td>N. Schützenmeister: Natural Products as Novel Lead Structures: Method Development and Biological Testing</td>
<td></td>
</tr>
<tr>
<td>09:35</td>
<td>X. Cheng: Seeking for chemical OCT4 substitutes for the generation of human iPSCs</td>
<td>D. Kalinin: Small-molecule inhibitors of human coagulation factor XIIa: synthesis and anticoagulant activity</td>
<td>G. Schmiedeknecht: Challenges in manufacturing and Quality Control of ATMPs</td>
</tr>
<tr>
<td>09:45</td>
<td>C. Lamers: Structure-activity study and molecular insights in the mode of action of complement C3 inhibitor Cp40</td>
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<thead>
<tr>
<th>Time</th>
<th>Poster Session II and Coffee Break</th>
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<td>10:00 – 10:30</td>
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## SHORT TALKS (parallel sessions VI)

### 10:30 – 12:00

<table>
<thead>
<tr>
<th>SL16 (Großer HS)</th>
<th>SL17 (HS West)</th>
<th>SL18 (HS Ost)</th>
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</thead>
<tbody>
<tr>
<td><strong>Natural Products - From Molecular Pharmacology to Clinical Applications</strong></td>
<td><strong>Bioanalytical Mass Spectrometry</strong></td>
<td><strong>Challenges and opportunities in model based dose adaptation</strong></td>
</tr>
<tr>
<td>Chairs: M. Wink, T. Efferth</td>
<td>Chair: M. Lämmerhofer</td>
<td>Chairs: C. Kloft, T. Lehr</td>
</tr>
<tr>
<td>11:05 M. Wink: <em>Treatment of patients with cardiac AL amyloidosis with epigallocatechin-3-gallate (EGCG) from green tea- results of a clinical study and transcriptomics</em></td>
<td>11:05 C. Hopf: <em>MALDI Mass Spectrometry-Based Applications in Pharmacology</em></td>
<td>11:00 L. Klopp-Schulze: <em>Model-based precision dosing of tamoxifen therapy</em></td>
</tr>
<tr>
<td>11:40 C. Jessen-Trefzer: <em>Targeting the mycobacterial membrane with photosensitizers</em></td>
<td>11:40 S. Pace: <em>Sex bias in colitis yields a protective role of specialized pro-resolving mediators in female mice</em></td>
<td>11:30 S. Wicha: <em>Methodological aspects of model-based dose adaptations</em></td>
</tr>
</tbody>
</table>

### 12:00 – 13:00

**Lunch Break**

### 13:00 – 13:45

**Plenary lecture 5, H. zur Hausen, “Indirect” Mode of Cancer Induction by Infections**

**PL.5**

### 13:45 – 14:30

**Closing ceremony (Großer HS)**
2 PLENARY LECTURES
Critical quality attributes of nanomedicines: What matters and why?

Gerrit Borchard, PharmD, PhD
School of Pharmaceutical Sciences Geneva-Lausanne, University of Geneva, Switzerland
gerrit.borchard@unige.ch

The application of nanotechnology to the development of advanced therapeutics has brought about a number of so-called “nanomedicines”. Such drugs, though showing high variability in terms of size, shape, materials used, etc. share complex structures, which cannot be characterized in their entirety. Being mostly of synthetic origin and their preparation often including a self-assembly step, such drugs are recently referred to as non-biological complex drugs (NBCDs) [1]. Examples include glatiramoids (Copaxone®) [2], liposomal formulations (Doxil®) [3], and nanoparticles such as iron-carbohydrate particles (Venofer®) [4, 5]. Their size and attributes at the molecular scale confer these systems certain properties that impact their interaction with their biological environment, and thus influence PK/PD and safety profiles.

The former FDA commissioner Gottlieb called for an improved review process of generic drug applications (ANDAs) [6]. This must be viewed in relation to nanomedicines, as well, as several follow-on products have entered the market, including a glatiramer generic approved by FDA has now issued a guidance draft on products containing nanomaterials [7] as an answer to a request by the U.S. House of Representatives’ Committee on Energy and Commerce in 2015 to the U.S. Government Accountability Office (GAO).

Even though the draft guidance is suggesting a list of properties of nanomedicines (“Nanomaterial Quality Attributes”) to be tested, it lacks true guidance on individual (groups of) nanomedicines. It presumes levels of knowledge of product behavior that are typically not available, neither for NDA nor for generic, ANDA products. Due to their inherent complex structure, nanomedicines are impossible to be fully characterized by physicochemical methods alone, in vitro as well as in vivo studies, leading up to verification in clinical trials, are required. Therefore, an effort is needed to discover these correlations between specific critical quality attributes (CQA) and their impact on biological activity, ideally to be able to predict in vivo behavior. This can only be realized through a multi-pronged analytical approach and correlation to clinical data.

This presentation will discuss the current state of identification of CQAs using examples from currently marketed nanomedicines.

References:
https://www.fda.gov/Drugs/GuidanceComplianceRegulatoryInformation/Guidances/default.htm
Curative therapy for chronic hepatitis C: Successes and remaining challenges

Prof. Dr. Ralf Bartenschlager
Zentrum für Infektiologie, Molekulare Virologie, Universitätsklinikum Heidelberg, Im Neuenheimer Feld 344, 69120 Heidelberg
Abteilung “Virus-assoziierte Karzinogenese”, Deutsches Krebsforschungszentrum, Im Neuenheimer Feld 242, 69120 Heidelberg
Deutsches Zentrum für Infektionsforschung, Standort Heidelberg

Infections with the hepatitis C virus (HCV) are a global burden and a main cause for acute and especially chronic liver diseases. According to WHO, around 71 million people suffer from chronic hepatitis C worldwide, an estimated 250,000 people in Germany. Although the first molecular cloning of the HCV genome in 1989 marked the starting point to study this medically important virus, for many years it was not possible to propagate HCV in cultured cells. Only with the advent of molecular biology, genetically engineered HCV mini-genomes could be designed that allowed the selection for specific variants capable to replicate with high efficiency in a human hepatoma cell line. This break-through provided the long-sought tool to study the replication of HCV, but also laid the ground for the development of antiviral therapy.

Prior to the availability of selective drugs, patients with chronic hepatitis C were treated by use of an interferon – ribavirin combination therapy. However, this therapy had many contra-indications and numerous side-effects and therefore, the overall success rate of this treatment was rather low. However, with the availability of the HCV cell culture system, highly efficacious drugs could be developed targeting three different HCV proteins: the serine-type protease, the RNA-dependent RNA polymerase and the multi-functional replicase factor NS5A. Drugs targeting the viral protease had been approved for the first time in 2013, but only in combination with interferon. However, following the development of much more potent drugs with higher genetic barrier of resistance and active against all three HCV targets and all HCV genotypes, interferon-free therapy has become possible. Nowadays, all-oral interferon-free therapy of chronic hepatitis C is well established, reaching virus elimination in more than 95% of treated patients with little side effects and only very few contraindications. This result is remarkable, given that HCV is prone to establish persistence, but is due to the peculiar replication cycle of this virus.

This unprecedented efficacy has sparked great hopes to eradicate HCV globally by pure antiviral therapy. However, this aim is questionable for several reasons, including high costs of available drugs, the high number of undiagnosed infections and reinfections after successful virus elimination. For these reasons, a prophylactic vaccine is warranted, but its development is probably one of the biggest challenges towards global eradication of HCV.
Achievements, persisting challenges, and unmet needs in medication safety

Walter E. Haefeli
UniversitätsKlinikum Heidelberg – Medizinische Klinik
Abt. Klinische Pharmakologie & Pharmakoepidemiologie
Im Neuenheimer Feld 410
69120 Heidelberg

There are no drug treatments without risk, but many therapies without benefit, often because essential details of the medication process escape careful attention of either health care professionals or patients and their relatives. In the last decade, in Germany significant progress in medication safety has been made, notably in the area of information about and documentation of drug treatment, transsectoral communication and continuity of care, identification of risks associated with drugs, and the treatment of special patient populations such as children and older patients. However, despite all these efforts, little has been achieved in major areas of concern. As an example, excessive bureaucracy is still present in many essential prescription steps and digitization of the most error-prone steps of the medication process is still not a common standard, thus precluding electronic decision support. Finally, and most notably, the still omnipresent, multifaceted challenge of non-adherence and non-persistence is largely unchanged, limiting the success of many potentially effective therapies much too often.
Strategies for selective targeting of protein kinases

Stefan Knapp
Johann Wolfgang Goethe-University, Institute for Pharmaceutical Chemistry, Max-von-Laue-Str. 9, D-60438 Frankfurt am Main, Germany.
Johann Wolfgang Goethe-University, Structural Genomics Consortium, Buchmann Institute for Life Sciences; Max-von-Laue-Str. 15, D-60438 Frankfurt am Main, Germany.

Protein kinases are enzymes with remarkable domain plasticity, a property that plays a key role not only in their regulation but also in the mode of action of inhibitors and drugs targeting diverse activation states. In this presentation I will outline some of the challenges targeting conformational states of kinases as well as new opportunities for drug discovery. I will present structure based design strategies for the development of highly selective inhibitors including inhibitors binding to allosteric sites and I will discuss consequences targeting distinct activation states of kinases. Modulators of kinase scaffolding function and the potential targeting pseudokinases will also be discussed.

References:
“Indirect” Mode of Cancer Induction by Infections

Harald zur Hausen, Timo Bund, and Ethel-Michele de Villiers,
Deutsches Krebsforschungszentrum 69120 Heidelberg, Germany

Chronic inflammation induced by infections may lead to malignant proliferations, as evidenced by gastric cancers linked to Helicobacter pylori infections, by bladder cancer as the consequence of persisting schistosoma infections, and more recently by chronic hepatitis C virus infections resulting in liver cancer. In none of these cancers, persistence has been noted of the respective agents within the cancer cells.

Global epidemiological studies suggested a joint risk for colon- and breast cancers after consumption of red met or milk products of Eurasian dairy cattle (zur Hausen, 2009, 2012, zur Hausen and de Villiers, 2014, 2015, zur Hausen et al., 2017, 2019). Our group cloned and sequenced a large number of small single-stranded circular DNAs (1400-200 Nucleotides) initially from bovine sera and dairy products of Eurasian cattle. They revealed structural characteristics of specific bacterial plasmids, but also DNA elements characteristic for viruses (de Villiers et al., in print). Analyzed types revealed transcription, translation and replication in specific human cells. Monoclonal antibodies permitted the demonstration of their major replication protein in peri-tumoral tissue of colon cancer cells, regularly surrounding the Lieberkühn’s crypts, from which cancer arises. By micro-dissection the genomes of specific BMMFs were recovered and analyzed. Since sugars in human milk protect against these infections, the prime risk phase for acquiring them is the weaning and post-weaning period. Cow milk does not contain the same protective sugars.

Available data point to decade-long chronic inflammations by infections as specific triggers for random mutations (characteristic for indirect carcinogenesis) via oxygen radical production, resulting in colon cancer in average 40-70 years later. Besides colon cancers, breast and prostate cancers emerge as additional “hot” candidates. Due to the obvious plasmid origin of our isolates, we proposed to label these diseases as plasmidoses.
3 SCIENTIFIC LECTURES
3.1 New Research, New Researchers I

Chair: S. Laufer

**Molecular basis for protein trafficking within cilia and ciliopathies revealed by the visible immunoprecipitation (VIP) assay and CRISPR/Cas9 system**

*Kazuhisa Nakayama¹, Yohei Katoh¹*

¹Graduate School of Pharmaceutical Sciences, Kyoto University, Sakyo-ku, Kyoto 606-8501, Japan

Primary cilia are microtubule-based projections from the surface of various eukaryotic cells, and function as cellular antennae by sensing extracellular stimuli, such as fluid flow, and by receiving and transducing developmental signals, such as Hedgehog signalling. Owing to their crucial roles, defects in ciliary assembly and ciliary protein trafficking result in a wide range of hereditary diseases, collectively called the ciliopathies. The biogenesis and functions of cilia rely on bidirectional trafficking of proteins along the axonemal microtubules, which is mediated by the intraflagellar transport (IFT) machinery. The IFT machinery is composed of large multiprotein complexes, IFT-A (composed of 6 subunits), IFT-B (16 subunits), and BBSome (8 subunits), with the aid of two motor protein complexes, kinesin-2 (3 subunits) and dynein-2 (11 subunits) [1].

To reveal the molecular basis for the ciliary protein trafficking, we first tried to delineate the overall architectures of the large protein complexes. To this end, we developed a novel strategy for detection of protein–protein interactions, named the visible immunoprecipitation (VIP) assay, in which not only binary but also one-to-many and many-to-many protein interactions can be visually detected, without the need of Western blotting [2]. By utilizing the VIP assay, we revealed the overall architectures of the IFT-A [3,4], IFT-B [5,6], BBSome [2,7], kinesin-2 [8], and dynein-2 [9,10] complexes. Furthermore, we determined the interaction modes among these multiprotein complexes.

On the other hand, to determine the roles of individual subunits, we developed a practical strategy to disrupt specific genes using the CRISPR/Cas9 system [11]. By using this knockout strategy, we revealed the roles of individual subunits of the complexes [3,4,6–11] and the molecular basis for the ciliopathies [4,10].

**Acknowledgements:** Our study was supported in part by grants from JSPS, MEXT, Astellas Foundation, and Uehara Memorial Foundation.

**References:**

Protein Labelling and Manipulation in Living Cells Using Bioorthogonal Chemistry

Richard Wombacher*, Philipp Werther, Mathis Baalmann, Michael Ziegler
1Department of Pharmaceutical Chemistry, Institute of Pharmacy and Molecular Biotechnology, Heidelberg University, INF 364, 69120 Heidelberg

The ability to visualize or rapidly perturb specific molecular interactions within cellular protein networks is of major importance to investigate the role of individual biomolecules in the temporal and spatial context of a living cell or organism. Our research is focused on the use of small molecules as probes to either manipulate the cellular localization of proteins [1-3] or to specifically label proteins in living cells [4]. Particularly small chemical fluorophore probes with tailor-made photo-physical properties have gained prime importance for modern microscopy technologies. Ideally a fluorescent probe should have excellent specificity (low background) for its target, high photo-stability and photon output as well as optimal spectral properties suited for the microscopic technology and biological question to study. Fluorogenic probes for bioorthogonal labelling chemistry are highly beneficial to improve target specificity and to reduce background signal in fluorescence microscopy imaging [5]. We recently reported a palette of fluorogenic dyes for intracellular live cell protein labelling [6]. We demonstrate the power of this fluorogenic dyes in multi-colour live cell imaging under no-wash conditions. Moreover, we adapted our concept of fluorogenicity to fluorophores that possess spontaneous-blinking based on intramolecular spirocyclization at physiological pH [7]. We successfully applied these fluorogenic spontaneously blinking fluorophores (fSBF) in live cell single-molecule localization microscopy (SMLM). With our probes we are able to visualize cellular structures with a localization precision of ~20 nm, far below the diffraction limit. Further, we utilize the probes to visualize dynamic cellular events with high temporal and spatial resolution in living cells [8, 9].

Acknowledgments: We gratefully acknowledge financial support by the DFG-SPP1623 and thank our collaboration partners Dirk Ollech and Dr. Dr. Ada Cavalcanti-Adam (MPI for Medical Research) and Klaus Yserentant, Felix Braun and Prof. Dr. Dirk-Peter Herten (Heidelberg University, Institute of Physical Chemistry).

References:
Supporting resolution? Biosynthesis of specialized pro-resolving mediators (SPM) in human leukocytes

Ebert R1; Cumbana R1; Spohner, K1; Kutzner L1; Ferreirós N2; Schebb NH3; Steinhilber D1; Kahnt AS1
1 Goethe-University, Institute of Pharmaceutical Chemistry, ZAFES, Max-von-Laue-Str. 9, D-60438 Frankfurt/Main, Germany
2 Goethe University, Institute of Clinical Pharmacology, Pharmazentrum Frankfurt, ZAFES, Theodor Stem Kai 7, D-60590 Frankfurt/Main, Germany
3 Chair of Food Chemistry, Faculty of Mathematics and Natural Sciences, University of Wuppertal, Germany

The resolution of inflammation is an active process controlled by endogenous specialized pro-resolving mediators (SPM). Derived from polyunsaturated fatty acids such as arachidonic and docosahexaenoic acid these lipid mediators are formed by various immune cells during the course of an acute inflammatory response by the concerted action of different lipoxygenases and cytochrome P450 enzymes. However, the detailed molecular mechanisms underlying SPM biosynthesis are not completely understood. Yet, this knowledge is of great importance for the development of pro-resolving pharmacotherapies as well as a better comprehension of potential resolution-toxic effects induced by already existing anti-inflammatory therapies. Therefore, we investigated lipoxygenase expression and SPM formation in primary human immune cells such as neutrophils, polarized macrophages and various cell lines. We found that enzymes known to be involved in the biosynthesis of pro-inflammatory leukotrienes such as FLAP, cPLA_2alpha, LTA_4 hydrolase and LTC_4 synthase influence transcellular lipoxin and resolvin formation. Furthermore, macrophage polarisation and activation considerably rearranged the enzymatic layout of the cells leading to substantial changes in enzyme, lipid mediator and cytokine patterns. In addition, an outlook on possible new players involved in SPM biosynthesis will be given.

References:
The application of salivary caffeine concentrations to study gastric emptying of fluids and formulations in humans

Maximilian Sager1, Philipp Jedamzik1, Michael Grimm1, Werner Weitschies1, Mirko Koziolek1
1 Department of Biopharmaceutics and Pharmaceutical Technology, Institute of Pharmacy, University of Greifswald, Felix-Hausdorff-Straße 3, 17489 Greifswald, Germany

For many orally administered drugs, the kinetics of drug plasma concentrations are closely correlated with the kinetics of gastric emptying. Hence, it is essential to understand the process of gastric emptying in healthy subjects but also in different groups of patients. Typically, in vivo methods such as magnetic resonance imaging (MRI), scintigraphy or the paracetamol absorption test are used to study gastric emptying of drinks, food or oral formulations in humans. However, these methods are either not applicable on a routine basis, costly and time-consuming or require the presence of medically trained personnel. It was therefore our aim to find an in vivo method that can be used to determine gastric emptying of fluids and formulations in humans in a simple, inexpensive and non-invasive manner.

Since saliva is an easily accessible body fluid that can be sampled at a high frequency, we aimed to find a marker for gastric emptying that shows a high correlation between plasma and saliva concentrations. In this regard, caffeine seemed to be well suited in humans. In a proof-of-concept study with 6 healthy subjects, a low dose of caffeine (35 mg) was administered in fasted and fed state together with 240 mL of water. To prevent the contamination of the oral cavity but still to allow the labelling of the liquid phase, caffeine was administered in form of an ice capsule. Subsequently, we compared caffeine concentrations in saliva determined by LC-MS/MS with gastric emptying kinetics determined by MRI. The results of this study confirmed the hypothesis that the caffeine concentrations in saliva are able to describe the gastric emptying kinetics of water in humans.1 In another study, hard gelatin capsules containing 50 mg of caffeine were administered in fasted state with 240 mL of water. In order to directly compare the disintegration times of the capsules, saliva sampling and MRI scans were performed simultaneously.2 The comparison of the disintegration times revealed a difference of around 4 min between the two methods. Since capsule disintegration occurred mostly in the stomach, salivary caffeine concentrations only increased after disintegration was observed in MR images. This delay was caused by gastric mixing and emptying as well as by intestinal absorption of caffeine. Nonetheless, the application of caffeine was still useful for characterizing the in vivo behavior of oral formulations and thus, was used in follow-up studies.

In the future, this simple and reliable method shall be applied in different groups of patients (e.g. geriatric patients, patients with diabetes) to study gastric emptying of various fluids as well as to describe the in vivo transit and disintegration behavior of different oral formulations. These data will be directly implemented into physiologically relevant in vitro test methods and physiologically based pharmacokinetic (PBPK) models in order to predict the extent of changes associated, for instance, with age or certain diseases. This technique will therefore be very useful in optimizing oral pharmacotherapy by better understanding the behavior of oral drug products in the human gastrointestinal tract.

This work was partly performed within the OrBiTo project (http://www.orbitoproject.eu), which is funded by the Innovative Medicines Initiative Joint Undertaking (http://www.imi.europa.eu) under Grant Agreement no. 115369, resources of which are composed of financial contribution from the European Union’s Seventh Framework Program and EFPIA companies in kind contribution.

References:
Nicotinamide adenine dinucleotide (NAD) has long been known as cofactor of oxidoreductases. For a long period of time this task in metabolism has been recognized as major, if not the only function of NAD. However, over the past couple of years it became clear that NAD also serves as (i) precursor for Ca\(^{2+}\) mobilizing second messengers, (ii) substrate for posttranslational protein modifications (mono- or poly-ADP-ribosylation), and (iii) as acceptor for acetyl groups during de-acetylation reactions.

Ca\(^{2+}\) mobilizing second messengers play an important role in almost all cell types and in many signaling processes. In particular, Ca\(^{2+}\) signaling during T-lymphocyte activation is essential for the adaptive immune response. In recent years, we have analyzed the role of NAD-derived Ca\(^{2+}\) mobilizing second messengers during this process.

Biochemical time course analysis in T cells stimulated via the T cell receptor/CD3 complex revealed nicotinic acid adenine dinucleotide phosphate (NAADP) as earliest 2\(^{nd}\) messenger, followed by D-myo-inositol 1,4,5-trisphosphate (IP\(_3\)) and cyclic ADP-ribose. On the sub-second and second timescale of T cell activation we recently characterized Ca\(^{2+}\) microdomains involving type 1 ryanodine receptors activated by NAADP (1,2). In addition, Ca\(^{2+}\) entry via ORAI1, controlled by STIM1 and STIM2, constitutes a further essential mechanism underlying Ca\(^{2+}\) microdomains (2).

Though TRPM2 does not appear to be involved in Ca\(^{2+}\) microdomains, we recently identified 2'-deoxy-ADPR (2dADPR) as a significantly better TRPM2 agonist than ADPR (3). Using HPLC and mass spectrometry, endogenous 2dADPR was detected in Jurkat T-lymphocytes. Consistently, cytosolic nicotinamide mononucleotide adenylyltransferase 2 (NMNAT-2) and NAD-glycohydrolase CD38 are able to sequentially catalyze synthesis of 2dADPR from nicotinamide mononucleotide and 2'-deoxy-ATP \textit{in vitro}.

Taken together, adenine nucleotide 2nd messengers NAADP and 2dADPR play significant roles in shaping T cell Ca\(^{2+}\) signaling.

Agonist-Dependent switching of Ion selectivity in endolysosomal TPC2 channel


1Walther Straub Institute of Pharmacology and Toxicology, Faculty of Medicine, Ludwig-Maximilians-Universität, Munich, Germany
2Department of Pharmacy – Center for Drug Research, Ludwig-Maximilians-Universitat, Munich, Germany
3Department of Cell and Developmental Biology, University College London, London, UK.

Ion selectivity is a defining feature of a given ion channel and is considered immutable. Here we probed the ion selectivity of the lysosomal ion channel TPC2 that is hotly debated. Both the ion selectivity and activating ligand(s) of TPCs are equivocal. Initial studies characterized TPCs as non-selective Ca2+-permeable channels activated by NAADP. But other studies indicate that TPCs are highly-selective Na+ channels and activated directly by PI(3,5)P2. We identify two structurally distinct, cell-permeable TPC2 agonists through a high throughput screen. One of these evoked robust Ca2+ signals in live cells and non-selective cation currents in direct endo-lysosomal patch clamp recordings. In contrast, the other evoked weaker Ca2+ signals and Na+-selective currents. Physiologically, these properties were mirrored by the Ca2+ mobilizing messenger, NAADP and the phosphoinositide, PI(3,5)P2, respectively. Novel small molecules thus mimic disparate intracellular signalling cues in switching the ion selectivity of TPC2. A new paradigm whereby a single ion channel mediates distinct ionic signatures on demand emerges.
Identification of OCaR1 as a gatekeeper of lysosomal-granular Ca\textsuperscript{2+} release and regulated exocytosis

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Exocytosis triggered by agonists depends on an increase of the Ca\textsuperscript{2+} concentration in close vicinity of secretory granules and is strictly limited in resting cells. We identified a novel organellar calcium regulator we termed OCaR1, which tightly controls Ca\textsuperscript{2+} release from lysosomal and secretory granules. OCaR1 is a putative transmembrane protein that co-localises with markers of endo-lysosomal organelles. In secretory cells such as pancreatic acinar cells OCaR1 also locates to secretory granules as demonstrated using cells from OCaR1-YFP knock add on mice. Acinar cells of OCaR1-deficient mice exhibit an increase in both spontaneous and regulated exocytosis which goes along with an enlargement and globalisation of intracellular Ca\textsuperscript{2+} oscillations. Notably, exaggerated Ca\textsuperscript{2+} oscillations in OCaR1-deficient acini can be suppressed by a NAADP antagonist or deletion of two pore channel 2 (TPC2). OCaR1 controls spontaneous and agonist-evoked Ca\textsuperscript{2+}-dependent exocytosis also in mast cells, another secretory cell type, suggesting a broader prevalence of this regulatory mechanism. Together, our results provide the first demonstration that OCaR1 in lysosomes and secretory granules operates as a gatekeeper of exocytosis, a process, known to contribute in the development of pancreatitis and, possibly, to pancreatic malignancies.
Determination of fatal ventricular arrhythmias by a new regulator of Ca\textsuperscript{2+} signaling at the lysosomal-SR junction

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Altered Ca\textsuperscript{2+} release from intracellular stores in cardiomyocytes is a trigger of ventricular arrhythmias. Recently, it was shown that Two-pore channels (TPC) are activated by nicotinic acid adenine dinucleotide phosphate (NAADP) after catecholamine stimulation in cardiomyocytes, and that TPC2 at lysosomal/sarcoplasmic reticular junctions contribute to the development of cardiac hypertrophy and arrhythmia through β-adrenergic signaling. We identified novel Organellar Ca\textsuperscript{2+} Regulator proteins (termed OCaR1 and OCaR2) contributing to Ca\textsuperscript{2+} release from acidic organelles and we aimed to determine their implication in pathological cardiac remodeling.

Methods: We generated global and cardiomyocyte-specific OCaR1- and OCaR2-deficient mice (KO) by gene targeting in ES cells. Ca\textsuperscript{2+} signaling was analyzed in isolated adult cardiomyocytes under resting and pacing conditions, and using several agonists/antagonists to address NAADP-sensitive Ca\textsuperscript{2+} stores including NAADP, Isoproterenol and Ned-19 among others. We induced cardiac remodeling by chronic Isoproterenol (ISO) infusion. Cardiac contractility was determined \textit{in vivo} by pressure-volume (P/V) loop measurements under Isoflurane anesthesia. Telemetric ECGs were recorded in conscious freely moving mice. Whole transcriptome sequencing was done by NGS and we analyzed changes in gene expression during cardiac remodeling.

Results: OCaR1 and OCaR2 transcripts are detected in isolated adult mouse cardiomyocytes. In resting cardiomyocytes from global (KO) or cardiomyocyte-specific (OCaR2\textsuperscript{fx/fx}/αMHC-Cre\textsuperscript{pos}) OCaR2-deficient mice, ISO and NAADP evoked spontaneous intracellular Ca\textsuperscript{2+} oscillations that were not observed in cells from corresponding control mice or from OCaR1-KO mice. Exaggerated ISO-evoked Ca\textsuperscript{2+} transients in OCaR2-deficient cardiomyocytes were blocked by the NAADPantagonist Ned-19. In beating (1Hz pacing) OCaR2-deficient myocytes, the occurrence of spontaneous diastolic Ca\textsuperscript{2+} transients was increased by more than 50% under ISO (100nM) stimulation. After chronic (7d) ISO treatment, OCaR2 cardiomyocyte-specific mice developed increased cardiac hypertrophy accompanied by significantly different gene regulation. In contrast, under basal conditions and ISO stimulation, P/V loop analysis revealed no differences in cardiac contractility parameters in cardiomyocyte-specific OCaR2-deficient mice. However, ECG telemetry revealed that the overstimulation of the β-adrenergic axis in OCaR2\textsuperscript{fx/fx}/αMHC-Cre\textsuperscript{pos} mice evoked polymorphic ventricular tachycardia that resulted in sudden cardiac death within 20-40 min that was not observed in any of the control animals. Additionally, after a cardiac model of ischemia/reperfusion OCaR2 cardiomyocyte-specific mice presented and increased occurrence of ISO-triggered salvos accompanied by decreased cardiac function.

Conclusion: We identified OCaR2 proteins in cardiomyocytes as key regulators of Ca\textsuperscript{2+} oscillations arising from NAADP-sensitive Ca\textsuperscript{2+} stores that are triggered by β-adrenorecepto stimulation. Inactivation of OCaR2 proteins in murine cardiomyocytes had no impact on basal cardiac contractility or on the inotropic response to catecholamines. In contrast, OCaR2 proteins are critical determinants of neurohumoral-induced cardiac hypertrophy as well as of the development of fatal catecholaminergic ventricular tachycardia and cardiac dysfunction.
Reconstructed human skin can predict the efficacy and safety of drugs in humans

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The poor success rate of drugs in clinical studies which have been successfully tested preclinically (3.4-33%; Wong et al., 2019) indicates the need for improved preclinical approaches. Reconstructed human tissues overcome the species gap between animal and man. Respective models of diseased tissues and introducing diversity should also restrict other limitations of animal experiments. Testing in rodents often makes use of a single strain and animals are of single sex and close in age – in order to keep data variability and thus sample size low. Yet, thereby diversity is eliminated.

At Freie University Berlin we have established the technique of reconstructing full-thickness human skin (RHS) by standardized coculture of juvenile human keratinocytes and fibroblasts. Atopic dermatitis can be modeled by the knock-down of the filaggrin gene and co-culture with the proinflammatory cytokines IL-4 and interleukin-13 resulting in a less developed stratum corneum barrier and a shift of the normal skin surface pH to pH 6.4 (Hönzke et al., 2016). The efficacy of topical applied glucocorticoids can be derived from the nuclear translocation of the glucocorticoid receptor resulting in a decline in inflammatory proteins. Drug penetration into the tissue can be quantified by chromatographic approaches as well as by stimulated X-ray microscopy, the latter allows for high-end local resolution (Yamamoto et al., 2016). Dexamethasone penetration into RHS and human skin ex vivo is close (Wanjiku et al., in press). The model of atopic skin even allows to study drugs delivered by nanoparticulate carrier systems (Giulbudagian et al., 2017). Skin thinning, the dominant glucocorticoid induced adverse drug reaction following the topical use becomes obvious by impaired collagen synthesis (Weindl et al., 2011).

Glycation induced clumpy collagen characterizes the skin in old age and in diabetes mellitus. This damage can be included into RHS by collagen pre-incubation with ribose (Balansin Rigon et al., 2018). An alternative approach to aged RHS is the use of fibroblast from aged donors (Hausmann et al., 2019a). Those constructs depict the typical morphological changes and the poorer access of drugs to viable skin which is in accordance with studies on fentanyl penetration of skin obtained from aged donors (Holmgaard et al., 2013).

Acceptance of the proposed preclinical approach will increase with proven predictivity. The qualification of the human-based testing can be documented by studying drug effects in the model and in human – preferably first in the model and next in human. The protocols need to confirm with the high standards of clinical studies. Using reconstructed human epidermis for the in vitro approach, we observed a major increase in filaggrin formation and less caffeine permeation following Lactococcus lysate. The improved barrier in the construct is paralleled by a decrease in transepidermal water loss in 30 female volunteers. Skin irritation was neither detected in RHE nor reported by the volunteers. Because of an enhanced beta-defensin-2 formation in the construct, the antimicrobial barrier may improve, too (Hausmann et al., 2019b).

Taken together the results show the possibility to introduce disease and diversity into a reconstructed human tissue as well as a first approach into the essential qualification of the human-based testing in preclinical research.

Federal Ministry of Education and Research (BB3R), German Research foundation (CRC 1122), Sebapharma GmbH & Co. KG

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Adverse outcome pathway-based concepts and tools for assessing skin sensitization

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In general, no single non-animal method can fully cover the complexity of any given animal test; rather, data from several approaches are needed to address an in vivo toxicity endpoint. Chemicals often induce specific molecular key events that can initiate a sequence of early cellular key events (KE), which may result in an observable toxic effect in vivo. This sequence is termed an adverse outcome pathway (AOP). Integrating information from in vitro, in silico, and in chemico methods into toxicity testing strategies has been widely considered the way of providing adequate and relevant information about chemicals’ hazardous properties.

Skin sensitization test data are required or considered by chemical regulation authorities around the world. These data are used to develop product hazard labeling for the protection of consumers or workers and to assess risks from exposure to skin sensitizing chemicals [1]. There has been significant progress in recent years in the development and application of new methods for assessing the skin sensitization potential of chemicals. The KE of in skin sensitization have been described in an AOP [2]. To date, OECD test guidelines are available for non-animal test method addressing the first three KE: protein binding (direct peptide reactivity assay, DPRA, OECD 442C), keratinocyte- (LuSens and KeratinoSens, OECD 442D) and dendritic cell activation (e.g. h-CLAT, OECD 442E). Numerous prediction models have been proposed to combine the results of three assays (and other information sources); 12 of them have been listed as case studies by the OECD [3]. Among them, the “2 out of 3” integrated testing strategy (ITS) is a simple defined approach (DA) that has demonstrated very good sensitivity, specificity and overall accuracy numbers for predicting the skin sensitization potential. Chemicals with at least two positive results in tests addressing KE 1–3 are predicted sensitizers, while chemicals with none or only one positive outcome are predicted non-sensitizers. The ‘2 out of 3’ prediction model achieved accuracies of 90% or 79% when compared to human or LLNA data, respectively [4] and thereby even exceeded the predictivity of the LLNA. The methods used by the “2 out of 3” ITS prove to also recognise pre- and prohaptens [5]. A recent analysis of discordant results (1 out of 3 positive) found that most of these cases are due to borderline results of an assay [6]. The problem of borderline results (i.e. assay read-outs close to the classification thresholds) has been recognised and described for skin sensitization methods in vitro and in vivo [7].

While the skin sensitization hazard of substances can already be identified by non-animal methods, the classification of potency is still challenging. A kinetic DPRA (kDPRA) method was developed to determine the reaction rate constant of haptens with model peptides [8]. In a recent ring-trial, the method proved to be robust and reproducible; chemicals’ reaction rate constants correlated well with their skin sensitization potencies.

Testing for skin sensitization is regarded a vanguard of modern toxicological testing, addressing complex toxic effects by combining different assays which address KEs of an AOP. For skin sensitisation, we have sufficient methods and prediction models at hand, and we know their applicabilities and limitations. This should allow for a full replacement of animal studies, which is currently not quite completed [9].

References:


Reconstructed tissue models with integrated immune cells – Do we need advanced in vitro tests for the evaluation of drugs and chemicals?

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To ensure human safety, the benefit-risk assessment of drugs and chemicals requires relevant and reliable test methods. Animal models have historically been widely used for pharmacological and toxicological studies, however, the limited biological relevance of animal models to human as well as ethical and regulatory pressure underlines the urgent need for alternative in vitro test systems. Three-dimensional multilayer skin constructs are one of the most developed and advanced in vitro engineered tissue models that eliminate species differences while at the same time saving animal experiments. Besides testing for skin irritation, corrosion and phototoxicity, reconstructed skin is increasingly playing a role in the evaluation of complex endpoints such as genotoxicity or sensitization. Furthermore, engineered in vitro skin disease models have been established in basic and preclinical research. The immune system is critically involved in most human skin diseases. Skin-resident dendritic cells, including Langerhans cells localized in the epidermis, are the most relevant subset of immune cells during the sensitization phase of allergic contact dermatitis and the initial mechanisms of skin inflammation in atopic dermatitis. Yet, commercially available models including reconstructed human epidermis and reconstructed human full-thickness skin, which is comprised of an additional dermis equivalent, largely lack immunocompetent cells. To overcome this limitation, in-house skin models with integrated immune cells have been successfully developed although the stable integration into reconstructed skin poses a particular challenge. The phenotype and functionality of the integrated immune cells in the in vitro environment must be preserved to study major processes in dendritic cells such as differentiation, antigen uptake, maturation and migration. The technically challenging and labour-intensive reconstruction of complex tissue models may be less relevant for regulatory testing which requires robust, transferable and standardised test methods. In preclinical research, advanced tissue models reflect the physiological and pathophysiological processes more reliably than cell-based assays in monoculture or simpler tissue models and allow the analysis of immune-molecular mechanisms under controlled experimental conditions.

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Human cell-based tumor microenvironment models for improved preclinical drug development

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Epithelial carcinomas like head and neck and non-melanoma skin cancer are among the most frequent cancers in man. Thereby, squamous cell carcinomas of the head and neck metastasize more frequently than their cutaneous counterparts, contributing to the high mortality. Recent studies in various cancer entities suggest the determining role of cancer microenvironment for the tumor's response to chemotherapy together with the challenges of intra-tumor heterogenicity (Stanta et al. 2018 Front Med, Ren et al. 2018 Mol Cancer). Herein, we emulate oral and cutaneous squamous cell carcinoma in stratified tumor microenvironment models and test drug efficacies. Moreover, we investigate the effects of confounders like body-site or the glycation of the extracellular matrix on epithelial differentiation.

Cancer cells, either from patient derived tumor tissue or cell lines, were grown together with normal primary keratinocytes and fibroblasts in a 3D in vitro model.

Oral mucosa models with patient-derived cancer cells reliably preserved the tumor grading, while the cell lines reproducibly emulated one tumor grading. While three applications of 0.7 µg/mL docetaxel induced cell death and reduced proliferation, cetuximab (10 µg/mL or 100 µg/mL) failed to alter these parameters. Ingenol mebutate (150 µg/g) almost completely eradicated cancer cells from cutaneous cancer models following three applications of the commercial gel (Zoschke et al. 2016 J Control Rel).

The body site of which fibroblasts were isolated determined the epithelial differentiation in the respective skin constructs as shown for altered expression of e.g. granulocyte-macrophage colony-stimulating factor, e-cadherin, and filaggrin (Hausmann et al. 2019 Sci Rep). Next, we replaced included a glycated extracellular matrix, being typical for aged or diabetic persons. These glycated constructs showed increased proliferation and differentiation in the epithelial layers (Rigon & Kaessmeyer et al., 2018 Int J Mol Sci).

In conclusion, stratified tumor microenvironment models closely mimic epithelial cancers in vitro and capture certain drug effects. However, fibroblasts and the extracellular matrix extensively modulate epithelial differentiation in these models. Emulating heterogeneous cancers in heterogeneous tumor microenvironments might improve the currently low predictive capacity of in vitro models for anti-cancer effects in patients.

Federal Ministry of Education and Research (BB3R), German Research foundation (CRC 1122)

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3.4 Cysteine-reactive inhibitors and probes

Chair: T. Schirmeister

Cysteine, pKa, covalent attack: any correlation?

Czodrowski, P.

Abstract not available.
Novel Cathepsin B Inhibitors with Inversely Oriented Warheads

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Cathepsin B, a papain-like cysteine protease, is involved in various physiological processes. In tumor cells, it adopts a peripheral distribution and affects the extracellular matrix either directly by extracellular proteolytic degradation of its components or indirectly via activation of other proteases. The breakdown of the extracellular matrix remodels the tumor environment, promotes tumor invasion, and enables angiogenesis and metastasis. The crucial roles of cathepsin B at multiple points of the tumor development have been established in several in vitro and in vivo models and cathepsin B was proposed to be a prognostic marker in patients with various types of cancer [1,2]. The typical feature of peptidic inhibitors of cysteine proteases includes an N-capped peptide structure bearing an electrophilic warhead (e.g. a nitrile [3-7], aldehyde, halomethyl or acyloxymethyl ketone or Michael acceptor) in place of the scissile peptide bond. A linker can direct a carboxylic group to the S' region to allow for an advantageous salt bridging with the histidine residues of the occluding loop of cathepsin B, thus enhancing cathepsin B selectivity [8]. In E-64-derived epoxysuccinyl derivatives, a peptide part can bind along the S subsites of cathepsin B, while a dipeptide moiety is oriented towards the S’ sites [9,10]. We have designed cathepsin B inhibitors with dipeptide portions directed towards the occluding loop and equipped with fine-tuned, inversely oriented warhead structures which are cleaved upon the action of the active site cysteine leading to irreversible inhibition.

![Formation of an S-carbamoyl enzyme through the reaction of cathepsin B with a representative inhibitor.](image)

Kinetic data at four human cathepsins obtained for an extended series of around 200 unpublished representatives of this chemotype demonstrated their selectivity for cathepsin B, supported the exciting mode of action and allowed to draw detailed structure-activity relationships. The synthesis and the biochemical characterization of these novel cysteine protease inhibitors will be presented. The tailored structure of our new inhibitors allows the design of PET ligands and (in continuation of our previous studies [11-13]) linker connection for the assembly of activity-based probes.

References:
Covalent Kinase Inhibition by Reversible and Irreversible Cysteine Targeting

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Covalent inhibitors have a long history in medicinal chemistry and over 40 drugs relying on a covalent mechanism are currently approved. Typically, such compounds address catalytic nucleophiles (e.g. cysteine, serine or threonine residues) in the active sites of enzymes. The mode of action of most approved covalent modifiers has been discovered serendipitously while the use of reactive groups in rational drug design has long been regarded with skepticism due to potential issues arising from irreversible off-target modification, haptenization and idiosyncratic toxicity.[1] Within the last decades, however, covalent targeting resurged as a promising strategy in drug discovery and the concept of "targeted covalent inhibitors" (TCIs) evolved.[2] TCIs address non-catalytic amino acid side chains by means of covalent reactive groups (CRGs) frequently termed "warheads". The latter are supposed to react with the target amino acid upon ligand binding in a proximity-driven manner and inherent warhead reactivity should be limited to a necessary minimum. If properly designed, TCIs can have various advantages such as increased potency and specificity, limited competitiveness with natural ligands or substrates (e.g. ATP in the case of kinases) and, depending on the target's turnover rate, a decoupling of pharmacodynamics from pharmacokinetics enabling prolonged therapeutic effects while minimizing exposure.[1,2] The TCI concept has been most successfully applied to kinases[3] with currently six drugs approved by the FDA. Due to its high intrinsic nucleophilicity, cysteine is the residue most amenable to TCI design. All of the approved covalent kinase inhibitors, as well as the vast majority of experimental TCIs address poorly conserved cysteines by using carboxamide-derived Michael acceptors as the electrophiles. However, with the employment of more diverse warhead types, other residues such as tyrosine or lysine are becoming increasingly ligandable.[4] Moreover, covalent-reversible targeting approaches, which might resolve some of the aforementioned liabilities associated with irreversible TCIs, have recently gained attention.[4]

Our current research is focused on the identification of highly specific covalent kinases inhibitors. We employ structure-based design approaches to address particular cysteines with classical electrophiles (i.e. α,β-unsaturated amides), but also with less common warhead chemistries (e.g. S₅Ar-reactions). By applying covalent trapping strategies, we were able to generate irreversible and covalent-reversible inhibitors with exquisite selectivity. For example, we designed highly isoform- and kinome-selective Janus kinase (JAK) 3 inhibitors by engaging a poorly conserved cysteine absent in the other JAK family members.[5,6] These efforts, as well as recent work on covalent probes for kinases featuring a cysteine residue in the so-called "hinge region", will be presented.

References:

3.5 Dysfunctional Cellular Signaling in Cardiovascular Diseases

Chairs: T. Wieland, R. Gilsbach

Selective inhibition of nuclear ERK1/2 functions – two quite distinct implications: cardiac hypertrophy and cancer?

Lorenz, K.

Abstract not available.
Identification and modulation of chromatin interactions in cardiac myocytes

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Background: Epigenome studies in cardiac myocytes revealed dynamic establishment of active regulatory sites during development and disease (Gilsbach et al., Nat. commun. 2018). The aim of this project was to identify target promoters of distal regulatory sites and to prove the functional relevance of chromatin interactions in cardiac myocytes.

Methods: We performed Hi-C and Promoter Capture Hi-C experiments to analyze higher order chromatin organization and promoter interactions in cardiac myocytes. We perturbed active regulatory sites using the CRISPRi system. CRISPRi utilizes a programmable catalytic-deficient Cas9 fused to the KRAB repressor domain. ChIP-seq, RNA-seq and Western Blot analysis were carried out to access the functional consequences of promoter and enhancer perturbation.

Results: Our Hi-C experiments revealed that the higher order chromatin organization, including TADs and A/B compartments, remains stable in cardiac myocyte postnatal development and disease. We furthermore generated a promoter interaction map for newborn, adult and diseased cardiac myocytes. In total we detected more than 30,000 genomic elements contacting 2432 genes. These interactions were strongly enriched for enhancer-promoter and promoter-promoter interactions. Among those were for example a spatial interaction between the NPPA and NPPB promoter and between a putative enhancer and Gata6. CRISPRi-mediated silencing of the NPPA promoter induced heterochromatin formation at the NPPA promoter and silencing of NPPA and NPPB gene expression, indicating that the NPPA promoter has enhancer function and controls NPPB gene expression. Since Gata6 has been shown to be implicated in pathological cardiac hypertrophy, we further used CRISPRi to silence a putative enhancer 320kb downstream of Gata6. This resulted in reduced gene expression, protein levels and genome-wide binding of Gata6. RNA-seq experiments revealed that enhancer-mediated silencing of Gata6 enhancer affected more than 600 genes. These genes were significantly associated with cardiovascular developmental and stress processes.

Conclusion: This study unraveled promoter interactions in cardiac myocytes and showed that functional epigenetic-modulation of distal regulatory elements allows steering of gene expression programs. Furthermore, will this project show the dynamics of promoter interactions in cardiac myocyte development and disease.
Altered cyclic nucleotide microdomains in cardiac disease

Nikolaev, V.

Abstract not available.
3.6 Biopharmacy, Pharmaceutical Technology

Chair: G. Fricker

Molecular Therapeutics from DNA to Cas9/sgRNA: Chemical Evolution of Nanocarriers

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It took five decades from first gene transfections to approval of gene therapies as medical drugs. Up to date, >2930 clinical gene therapy trials have been initiated, 9 gene therapy products, 1 siRNA drug, and 8 oligonucleotide drugs received market authorization. Intracellular delivery has been critical for the success of these therapeutic macromolecules. A further refinement of delivery carriers will have a tremendous impact for efficacy of future nanomedicines. Different chemical evolution approaches are pursued toward synthetic nucleic acid or protein nanocarriers. Our strategy focuses on a bioinspired sequence-defined process including (i) artificial amino acids active in specific delivery steps, (ii) precise assembly into defined sequences by solid phase-assisted synthesis, (iii) screening for a pre-defined delivery task and selection of top candidates, followed by random or educated variation for a next round of selection. The selected type of cargo (natural product, proteins, nucleic acids including pDNA, siRNA, miRNA, PMOs, mRNA, or Cas9/sgRNA) directs the optimal motifs and sequences of nanocarriers.

References
Development of exosome-based nucleic acid drug delivery system

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A variety of nucleic acid drugs have been studied as promising drug candidates for the treatment of a wide range of diseases, including cancer, viral diseases and other incurable diseases. For a successful therapy with these nucleic acid drugs, it is important to establish a rational strategy for the delivery of the nucleic acid drugs. Exosomes are extracellular vesicles secreted from most types of cells. As exosomes are endogenous delivery vehicles responsible for intercellular transport of biomolecules including nucleic acids, natural and genetically engineered exosomes are expected to become potent delivery systems for nucleic acid therapeutics. We have developed novel methods of labelling exosomes with luciferase and $^{125}$I using fusion proteins to study pharmacokinetics of exogenously administered exosomes, one of the most important issues for the development of exosome-based delivery systems. Based on the findings, we have developed a novel exosome-based antigen-adjuvant co-delivery system using genetically engineered tumour cell-derived exosomes containing endogenous tumour antigens and CpG DNA, a potent adjuvant. Vaccination with CpG DNA-modified exosomes exhibited a strong antitumor immunity, indicating that our novel exosome-based antigen-adjuvant co-delivery system can be a useful approach in cancer immunotherapy.

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Metal-Organic Nanopharmaceuticals

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The combination of organic building units with Lewis base function and inorganic metal ions or metal oxide clusters creates a diverse chemical space of hybrid materials. Crystalline metal-organic frameworks (MOFs) with tuneable size and porosity are an interesting compound class for the utilization in biomedical applications such as imaging, photodynamic therapy or drug delivery. Analog to more established drug delivery systems, MOFs require suitable functionalization approaches to enable change and control of the behaviour in a biological environment. On the other hand, selected drug molecules can serve as organic linkers in coordination polymers themselves and enable the formation of drug-metal nanopharmaceuticals with very high drug loading capacity. Here, different concepts for the functionalization of MOF nanoparticles (Figure 1) [1-3] and the assembly of direct drug-metal nanoparticles (Figure 2) with the aim of potential utilization in biomedical applications are presented.
Development of lipoplex-loaded model surface coatings for the \textit{in situ} transfection in the field of bone regeneration

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The German legislation modernised and expanded the definition of medicines for new therapies by including the advanced therapy medicinal products (ATMPs) in the § 4b of the German Medicines Act (AMG). The term ATMPs covers gene therapy medicinal products, somatic cell therapy medicinal products and tissue engineered products, which are defined in the EU guideline 2009/120/EG.

An interesting field of research and one application of ATMPs is the DNA-induced bone regeneration in the field of regenerative medicine. In the Western World 5 to 10\% of bone fractures show insufficient healing which results in a longer leave of absence and higher costs for the health care system. Hence, numerous strategies in the regenerative medicine focus on initiating fast osteogenesis on artificial scaffolds to stimulate the healing of bone fractures. However, these strategies primary use recombinant proteins to induce osteogenesis, which may result in complex biotechnological manufacturing and therefore high therapy costs.

In our project we produce functionalized material films loaded with DNA. Functionalised material coatings are developed based on polyelectrolyte multi layers (PEMs, see Figure 1). These PEMs are composed of biocompatible polymers and were loaded with layers of DNA. The incorporated DNA is complexed by highly effective cationic lipid composites [1]. This lipid encapsulation has protective and transfection enhancing effects. The project focuses on the development of methods to achieve effective loading of the PEMs with lipid/DNA complexes (lipoplexes) and on the intensive characterization of DNA-loaded LbL-films using surface sensitive biophysical methods, namely, atomic force microscopy, raster electron microscopy, zeta potential measurements, and fluorescence microscopy. Furthermore, the interactions of cells with the functionalized PEMs were investigated using molecular biological methods to determine cell attachment, viability parameters, and effectivity of DNA transfer. Final experiments using the chorio-allantoic membrane of the chicken egg as \textit{in vivo} system demonstrate the high efficacy of the DNA-loaded surface coatings in complex tissues.

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References:

Gold Catalysis: Functionalized Gold Carbenes, Unexpected Selectivities

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Only after two papers from 2000 had demonstrated the full potential of gold catalysis for organic transformations by a high increase of molecular complexity,1,2 homogeneous gold catalysis was developed to a versatile tool for organic synthesis.3,4 For a long time the field was exclusively focusing on electrophilic and nucleophilic species, radical intermediates were not involved, but this changed in 2013.5

Apart from the synthesis of different heterocycles (Scheme 1), the use of these principles also allows a number of C-C coupling reactions, which in a formal sense can also address C,H bonds.6 Principles like dual activation, selectivity control by remote steric repulsion and the use of di- and even mononuclear gold(I) complexes for photochemical reactions will be discussed. All these results will include results from computational chemistry.

![Scheme 1][1]

**Scheme 1.** Intermolecular reaction of an anthranil derivative with an ynamide to provide a multi-annulated heterocyclic system in only one step.

References
Gold Organometallics in Medicinal Chemistry

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Organometallic gold complexes have recently attracted major attention in inorganic medicinal chemistry based on their strong cytotoxic activities and their multiple pharmacological effects against tumor cell proliferation.[1,2] The enhanced stability of their metal-carbon bonds has also been a driving argument regarding the design of potent gold metallodrugs with an improved stability under physiological conditions.

We and other groups have recently reported on gold(I) complexes with N-heterocyclic carbene (NHC) or alkynyl ligands and their potential as anticancer agents (see the figure for some examples of the [Au(I)(NHC)₂⁺] and alkynylAu(I)(NHC) types).[3-6] The complexes generally triggered notable cytotoxic activities, displayed an efficient cellular uptake, and were good inhibitors of the selenoenzyme thioredoxin reductase (TrxR). Other relevant pharmacological effects, such as antimitochondrial properties, could be confirmed additionally.

In this presentation, our most relevant findings on the medicinal chemistry of gold(I) organometallics will be summarised with a focus on important early preclinical parameters such as structure-activity-relationships, stability, protein binding, or cellular uptake.

Acknowledgments: Financial support by the DFG (Deutsche Forschungsgemeinschaft) is gratefully acknowledged.

References
Homoleptic gold dithiocarbamate TXNRD1 inhibitors prevent tumor recurrence in a small cell lung cancer mouse model

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Disulfiram (DSF), long used as a safe treatment for alcoholism, has recently received attention for its potential to be repurposed as an anti-cancer treatment. Currently, it is believed that DSF’s cancer-killing properties are the result of a chemical reaction in vivo, wherein the copper complex of DSF (CuET, 1) is the active agent.1 We have found that the combination of DSF with the FDA-approved drug aurothiomalate is highly toxic to small cell lung cancer (SCLC) cell lines, while showing low toxicity to other cancer cell types as well as to normal cells. Like copper salts and DSF, aurothiomalate and DSF undergo a chemical reaction, which produces the dinuclear dithiocarbamate gold complex 2 (DKFZ-608). As with other gold complexes, DKFZ-608 is a thioredoxin reductase 1 (TXNRD1) inhibitor; however, unlike some other gold complexes, it is devoid of activity on the glutathione system and the proteasome at concentrations cytotoxic to cells.

Targeting thioredoxin reductase 1 (TXNRD1) to kill cancer cells has long been discussed as a promising strategy for numerous reasons: (1) TXNRD1 over-expression was repeatedly identified as having a statistically significant association with poor prognosis. (2) It is suggested to be a key player in redox regulation, with a lower dependency in most normal adult cells and tissues than in cancer cells. (3) Inhibitors of TXNRD1 activity cause cytotoxicity in cancer cells. Despite this promising target profile, the success rate of TXNRD1 inhibitors has been disappointing in pre-clinical and clinical studies. One reason for this lack of success might be the physicochemical shortcomings of lead compounds, which pose liabilities in the area of bioavailability, stability, and dose-limiting side effects.

We find that DKFZ-608 (and analogs) may have the potential to overcome these liabilities and finally realize the clinical potential of TXNRD1 inhibitors. We present data describing the discovery of a lead compound and its optimization leading to improved formulation, on-target and anti-tumor activity. This compound class shows superior efficacy and selectivity for TXNRD1, and binds in competition with the TXNRD1 substrate thioredoxin-1 (TXN1). Consequently, low TXN1 expressers, like small cell lung cancer cells, are hypersensitive to the drug, while cells, which express higher levels of TXN1, are resistant. After treatment of cells with our inhibitors, we observe oxidative stress only in the cytoplasm, but not in mitochondria, contributing to a low general toxicity profile. Finally, in an SCLC animal model, DKFZ-608 eradicates residual tumor cells surviving first line therapy and completely prevents tumor recurrence.

Evidence-based value added to innovative health care by pharmacists

Chairs: H. Seidling, U. Jaehde

Evidence-based value added to innovative health care by pharmacists: Ambulatory care

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Over the last two decades, numerous studies have examined the incidence and prevalence of preventable drug related morbidity internationally as a means to characterize the quality of medicines management in the community. Systematic reviews estimate that approximately 5% of emergency hospital admissions are drug related with deficiencies in all key stages of the medicines use process, namely prescribing, monitoring and patient adherence (Howard et al). Aging populations and the parallel increase in chronic conditions, multi-morbidity and polypharmacy imply the risk of a further rise in the burden of preventable drug related harm and unrealized benefit. Pharmacists are increasingly recognized as an underutilized resource to reduce this burden, and a growing body of research literature evaluates the impact of pharmacist interventions in the ambulatory care setting, which is at least partly fueled by diminishing general practitioner resources in many countries, including Germany. A recent systematic review by the Cochrane Collaboration included 111 randomized controlled trials evaluating the clinical impact of pharmacist interventions targeting patients compared to care as usual (de Barra et al). The authors found overall “weak” evidence that the evaluated pharmacist interventions improved blood pressure control and “very weak” evidence that they improved diabetes control, while there was “weak” to “moderate” evidence for the lack of impact on adverse drug reactions, hospital admissions and mortality. The authors highlight substantial heterogeneity between interventions and outcome measures and the need for further research to examine which intervention components are effective in which context. Of note is, that none of the included studies were conducted in Germany (although one multi-centre study included a German site). Nevertheless, evaluations of pharmacist interventions in the German ambulatory care setting are increasing. For example, the West Gem study demonstrated in a cluster randomized stepped wedge trial that close collaboration between a study pharmacist and GPs can significantly improve the quality of prescribing as measured by the Medication Appropriateness Index in vulnerable older patients (Rose et al). The international research literature on pharmacist interventions highlights a number of challenges to the design of interventions and to evaluating their impact on clinical outcomes. Addressing these challenges in future evaluations of pharmacist services will be important to inform their wider implementation in the German health care setting.

References
Evidence-based value added to innovative health care by pharmacists - Hospital Setting

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In the last decades hospital pharmacy in Germany has made a big step forward. Being primarily a logistical unit to supply drugs to the ward level, pharmacists have become an integrated part in the interprofessional group of bedside health care professionals/experts. Clinical Pharmacy as the latest addition to the 4 examination subject chemistry, biology, pharmacology and technology has been shown to be an integral part in patient care. Hospital pharmacists fulfill several important tasks in patient care.

During the presentation, the way of the patient (and the patients medication) is followed and the several steps which can be supported by hospital pharmacists are highlighted and discussed in light of existing evidence in literature. Thereby, the current development in Germany will be presented in the context of international standards.
Evidence-based value added to innovative health care by pharmacists: Long-term care facilities

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Several studies have shown that the incidence of adverse drug reactions is particularly high in long-term care (LTC) facilities leading to severe consequences such as falls and hospitalization. In a prospectively designed cross-section analysis in North Rhine-Westfalia the incidence of adverse drug reactions was found to be 8 per 100 resident-months with a preventability rate of 60% (Jaehde & Thürmann 2012) indicating serious deficiencies in the health care of LTC residents. Various medication safety-enhancing interventions are currently being developed that have the potential to improve the health status of LTC residents and to reduce costs. A systematic review identified 27 randomized controlled trials describing interdisciplinary interventions in LTC facilities of which 18 demonstrated a statistically positive effect. Participation of primary physicians and/or a pharmacist were identified as success factors providing first evidence that pharmacists can play a major role in such interventions (Nazir et al. 2013).

Recently, we have developed an intervention in North Rhine-Westfalia involving community pharmacists supplying drugs to the LTC facilities in collaboration with a health insurance (AOK Rheinland/Hamburg) and the Pharmacists’ Association North Rhine. Based on prescription data of the AOK, the current medication and further information from the nursing home, the pharmacists performed a medication review for LTC residents aged at least 65 years and taking five or more drugs per day based on the patients’ medication only. Documented potential drug-related problems (DRPs) and the implementation rate of pharmaceutical interventions were evaluated descriptively. To assess the quality of the medication reviews, a corresponding reference system was developed based on the analysis of two experienced clinical pharmacists. 12 pharmacies performed medication reviews for 94 LTC residents. Overall, the pharmacists documented 154 potential DRPs (mean 1.6 per patient, SD 1.5) of which the most common were drug-drug interactions (40%) followed by potentially inappropriate medication (PIM) (16%) and inappropriate dosages (14%). 33% of the pharmacists’ interventions to solve DRPs were successfully implemented, mostly dosage adjustments. The identification of potentially severe drug-drug interactions and PIM showed the highest agreement (88% and 73%) with the reference system (Bitter et al. 2019).

In conclusion, there is increasing evidence that community pharmacists can identify and solve relevant DRPs in the medication of LTC residents. The reference system assessing the quality of medication reviews can contribute to its transparency and reveals the potential for its improvement. The community pharmacists’ knowledge of the LTC residents and their relation to the prescribers is crucial for providing successful medication reviews and hence contributing to medication safety.

References
3.9 Simulation in Pharmaceutic and Formulation Development

Chairs: D. Fischer, P. Langguth

Simulations in pharmaceutical technology

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Establishing and applying simulation tools in the field of pharmaceutical technology seems to be obvious in a quickly growing industry sector. However, the classical education of pharmacists and the urgent acceleration of the drug discovery and -development processes will support the investment in simulations in industry and academia only where convincing advantages exist. This presentation explains basic concepts in simulation and gives a series of examples demonstrating the gain in knowledge obtained. The industry perspective shows that investment and time required to deliver results must be carefully considered when selecting the simulation strategy. Apart from computer based numerical simulations, e.g. scaled analogue models may show sufficiently precise results. The following examples will be discussed:

- Mixing of liquids in a 800 L tank
- Operation of a static mixer [1]
- Air flow inside an inhaler [2]
- De-agglomeration of powder mixtures [3]
- Generating an aerosol for pulmonary treatment of horses [4]

Setting up the appropriate simulations requires a data-driven understanding of the relevant processes. In this respect the concept of “Quality by Design” is supported. Moreover, in the field of medical device development, the simulations are well suited to document and to explain design decisions in the design history file. An important step towards regulatory acceptance is research performed by FDA, especially the publication of a guidance for industry dealing with Computational Modeling [5].

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Pharmacokinetic Modelling and Simulation to Support Early Clinical Drug Development

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At the transition phase between late research and early clinical development, a strategy to efficiently explore the relationship between drug exposure and in vivo response in humans needs to be clearly outlined. This gives rise to questions on the selection of appropriate doses for the first-in-human studies and on the extrapolation of exposure/response relationships observed in vitro and in animals to the intended patient population, among others. Modelling and simulation can help to answer these questions, thereby having a direct impact on the design of early clinical studies and ultimately supporting strategic decision making.

Allometric scaling and physiologically based pharmacokinetic modelling are common tools to predict human pharmacokinetics based on preclinical and in vitro ADME properties. The risk of potential pharmacokinetic drug-drug interaction and food effects can be evaluated using modelling and simulation to support risk/benefit assessment and clinical study design. In addition, combining pharmacokinetic and pharmacodynamic modelling allow the early estimation of therapeutic doses for the intended indication.

The presentation will cover the general use of pharmacokinetic modelling and simulation in early drug development in a pharmaceutical industry setting. The applications and limits of pharmacokinetic modelling before first-in-human studies will be elaborated based on recent examples including efficacious dose estimation and prediction of drug-drug interaction.

**Figure 1.** Pharmacokinetic modelling to support early drug development.

PBPK – physiologically based pharmacokinetics. PK/PD – pharmacokinetic/pharmacodynamics

Acknowledgments: Examples presented here were discussed in dedicated project teams, in particular in close collaboration with colleagues from TMED and DMPK at Sanofi Frankfurt. Project-related collaboration with colleagues from UCB S.A. is also much acknowledged.
Simulation of pulmonary deposition during inhalation therapy


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Background: The type and design of an inhalation device determines the deposition of the inhaled drug in the lungs. We simulated the particle deposition of five commonly used inhaler aerosols which were generated by four devices in different lung models: tiotropium bromide (Respimat®), glycopyrronium bromide (Breezhaler®), aclidinium bromide (Genuair®), vilanterol and fluticasone (both Ellipta®).

Methods: Using realistic breathing patterns, we combined the output of an Alberta idealized mouth-throat model with four different virtual lung models: One self-constructed single-path model and three computed tomography-based models of the bronchial tree of a child, a middle-aged person and an elderly person to determine the amount of drug retained in the throat, deposited in the upper airways and in the periphery of the lungs.

Results: Despite geometric differences between the three CT lung models, we found similar regional deposition results with all evaluated devices; however, with the Genuair® aerosol, there was a trend towards higher drug deposition in the upper airways of the child lung model compared with the adult and geriatric lung model. The single-path model showed lower deposition in the upper airways of the lung than the CT models. The Respimat® inhaler showed the lowest throat deposition and at the same time the highest deposition in the whole lung and in the different lung regions compared to the other tested inhalers.

Conclusion: Human lung models that predictably simulate the amount of total drug delivered to the lungs, as employed here, could be helpful in the development of new drug formulation and devices, especially in children.

Figure 1. Simulated particle deposition patterns of tiotropium droplets in different lung models. From left to right: adult CT model, child CT model, elderly patient CT model, adult single-path model. The shown CT models were created using ITK-snap software[1].

Table 1. Particle deposition in different lung models: LD = in vitro lung dose = Alberta throat model output, UA = Deposition in the upper airways (trachea and the first 5-7 generations), P = Deposition in the peripheral lung airways (approx. generation 7 -23), SD = standard deviation. All results in [% of nominal dose]

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<th>LD (SD)</th>
<th>Child model</th>
<th>Adult model</th>
<th>Elderly model</th>
<th>Adult single-path model</th>
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<td>Respimat®</td>
<td>Tiotropium</td>
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<tr>
<td>Genuair®</td>
<td>Aclidinium</td>
<td>42 (1)</td>
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<td>7 35</td>
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Acknowledgments

Ralf Kröger (ANSYS Germany) provided help concerning CFD (computational fluid dynamics).

Exosome-Hydrogels as Machinery for Anti-Inflammatory Therapeutics’ Synthesis

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Extracellular vesicles (EVs) are natural lipid-based membranous particles produced by almost any cell. They may transfer nucleic acid and protein-cargoes selectively between cells locally and at distance, which has created excitement for both fundamental biology and in the drug delivery field [1]. Although initial clinical trials are ongoing, the use of EVs for therapeutic applications may be limited due to potential “dilution effects” upon systemic administration or undesired off-target activity which may affect their ability to reach their target tissues. To tap their full therapeutic potential in a localised manner, we created a biomedical hydrogel containing EVs designed to achieve local delivery of therapeutics [2]. Due to the challenge of incorporating EVs into hydrogels without compromising their biological constitution, such approach had not been developed yet.

EVs from human mesenchymal stem cells (MSC) were isolated and characterised regarding size distribution and yield, loaded with a glucuronidase enzyme using our established saponin-assisted technique [3]. Liposomes made from egg phosphocholine/cholesterol mol-60/40% were used for comparison. EVs and liposomes were incorporated into polyvinyl alcohol (PVA) hydrogel (12 wt%) and crosslinked with PEG (Figure 1). Incorporation of glucuronidase-loaded EVs or liposomes into biocompatible PVA hydrogels did not impact on hydrogel biomechanical properties and preserved the enzyme’s stability compared to free enzyme during two weeks of incubation at 37°C, as assessed using glucuronide-fluorescein.

To visualise EVs within hydrogels, uranyl acetate-labelled vesicles were imaged in carefully dehydrated gels by density-dependent colour scanning electron microscopy [4]. Taking advantage of density-sensitive backscattered imaging we spatially localised EVs within 3D-hydrogels. When incubating hydrogels with curcumin-glucuronide prodrug and murine RAW264.7 macrophages, all gels showed significant anti-inflammatory activities, as assessed by cell viability measurements. Recycling of gels after 7 d showed that this effect was lost for gels containing free enzyme but not when encapsulated into vesicles, indicating that both natural and synthetic vesicles may protect enzymes in the hydrogels during enhanced periods. Interestingly, without addition of curcumin-prodrug gels containing EVs showed an inherent anti-inflammatory effect, as detected by TNF-alpha gene expression analysis in bone marrow-derived primary macrophages. Our results indicate that hydrogels with MSC EVs are anti-inflammatory per se, which has not been reported to date. Our work on incorporation of EVs into a biomedical hydrogel may create an important basis for their therapeutic application.

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References
3.10 G-Protein-coupled Receptors

Chairs: P. Gmeiner, B. Wünsch

Dynamics of GPCR - G protein interactions as the key to understand coupling selectivity of GPCRs

Bünemann, M.

Abstract not available.
Bivalent ligands targeting heterodimers of dopamine receptors

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Besides their numerous physiological functions, dopamine D₂-like receptors are associated with severe neuropsychiatric disorders such as Parkinson’s disease and schizophrenia. In addition to their signalling as isolated entities, they transiently form homo-[1] and heterodimers [2] with other G protein-coupled receptors (GPCRs). Such interactions between GPCRs are known to affect ligand binding, signal transduction and receptor trafficking. In this context, the close relation and high co-localization of D₂R with the neurotensin receptor 1 (NTS₁R) is of substantial interest [3]. Bivalent ligands consisting of two pharmacophores tethered by an appropriate linker represent powerful tools to selectively address GPCR heterodimers. Driven by the binding energy of two recognition elements, a carefully designed bivalent ligand bridging the orthosteric binding sites of two adjacent protomers should exhibit extremely high binding affinity, and therefore high tissue selectivity between heterodimer-expressing cells and those that express only one individual receptor [4].

Starting from the crystal structure of NTS₁R and a D₂R homology model based on the D₃R x-ray structure, we have developed bivalent ligands consisting of two pharmacophores connected by an appropriate spacer to specifically address D₂R-NTS₁R heterodimers. These ligands possess subnanomolar binding affinity for the D₂R-NTS₁R heterodimer and exhibit over 1,000-fold selectivity over the D₂R monomer. Applying methods such as enzyme fragment complementation and radioligand binding, we study the formation of receptor heterodimers and we aim to further elucidate the signal transduction profile of the ternary ligand-protein assemblies. Our results indicate that the signaling behavior of the D₂R-NTS₁R dimer is mainly driven by NTS₁R. Importantly, bivalent ligands with appropriate spacer length strongly enhance β-arrestin-2 recruitment to the receptor dimer compared to a combination of two individual agonists, pointing towards a special mechanism of action [5].

Whether the D₂R-NTS₁R interaction is unique to the D₂R subtype, or if other dopamine receptors, such as D₃R, are able to interact with NTS₁R in a similar way, is one of our central research questions. We employ our bivalent ligands to study these putative D₃R-NTS₁R dimers in comparison to monovalent controls, using the previously established methods complemented with bioluminescence resonance energy transfer (BRET).

Acknowledgments:
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References:
Photopharmacology in Alzheimer research: Tools to investigate GPCRs

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In the last years the field of “photopharmacology” has emerged that incorporates units, such as azobenzenes and diarylethenes, into biologically active molecules, to obtain photoswitchable molecules that can reversibly change their structure and concomitantly their activity upon irradiation with light, normally UV light. Within our research efforts for anti-Alzheimer drugs targeting the human cannabinoid receptor subtype 2 (hCB2R [1] and the human muscarinic acetylcholine receptor subtype 1 (hM1R) [2], we have incorporated azobenzene units into hCB2R agonists and both dualsteric and bivalent hM1R agonists [3], the latter simultaneously address both the orthosteric and allosteric binding sites of the receptor.

Applying computational studies, such as molecular dynamics, as well as a portfolio of pharmacological assays, such as radioligand binding, functional studies, as well as FRET techniques, we were able to develop the first selective photoswitchable hCB2R “affinity on-switch” [4]. Furthermore, a dualsteric photoswitchable hM1R ligand was developed, the activity of which can be regulated by light as demonstrated in a set of FRET studies [5]. We also synthesized, characterized photophysically and pharmacologically bivalent hM1R ligands based on the agonist iperoxo and incorporated fluorine atoms to yield “red-shifted” ligands that show a considerably higher extent of photoconversion and can be switched by visible light [6]. We observed for the first time that “red-shifted” ligands can also differ significantly in their pharmacological activity [6]. Ongoing work on hM1R orthosteric agonists yielded "on- or off-switches” regarding efficacy and potency.

These findings show that photopharmacology can be successfully applied to various GPCR ligands, and the field is moving beyond the proof-of-concept, since it seems possible to specifically design GPCR ligands as “on- or off-switches”, and to compounds that are “affinity switches” and/or “efficacy switches”. This significantly expands the toolbox of GPCR investigation with specialized molecular tools supporting the investigation of the molecular basis of receptor function. The underlying principles seem generally applicable, since also a photoswitchable dualsteric hM1R ligand was developed and applied to optically control cardiac function, even in vivo [7].

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In Silico Pharmacology: A Mechanistic View on GPCR Modulation

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During the last decades, computational approaches have become essential for drug design and structural biology. For research on G protein-coupled receptors (GPCR), in particular, in silico methods and pharmacological experiments represent a strong and necessary combination for functional understanding being a prerequisite for rational and targeted drug design. Over the last decade, a multitude of GPCR-ligand complexes were determined by crystallography providing an indispensable structural view on this protein class, although these crystal structures only represent snapshots of a highly dynamic conformational ensemble. GPCRs are conformationally dynamic signaling machines, transferring information across membranes via multiple signaling pathways. Although about 30 % of all marketed drugs directly target GPCRs, little is known about the ligand-induced conformational changes that lead to intracellular signals. We combine molecular dynamics simulations and chemical interaction analysis (3D dynophores) to provide a more dynamic view on how binding of small organic molecules on the extracellular side of the receptor changes conformational behavior responsible for downstream signaling [1,2].

We will explain how computational approaches guided by pharmacological experiments iteratively unveil mechanisms of specific receptor functions [3,4]. A deep mechanistic understanding of GPCR functionality is essential for the rational design of tailor-made GPCR modulators. We will present case studies focusing on modulated receptor activation and biased signaling, which highlight the explanatory power of computationally-derived receptor models and their usage for prospective rational drug design.

**3.11 Radiopharmacy & Labeling**
Chair: W. Mier

**SL.37**

**Visualisation of cardiac fibrosis using \[^{68}\text{Ga}]\text{MHLL1} – A PET tracer for the fibroblast activation protein**

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**Objectives:** The fibroblast activation protein (FAP, also named seprase) is a serine protease with the ability to cleave Pro-X peptide bonds and exhibits endopeptidase activity with a preference for Ac-Gly-Pro motifs.\(^1\) Interestingly, the expression of FAP in healthy tissue is very limited, whereas high FAP-levels can be observed in over 90% of common human epithelial malignancies, fibrotic and inflammatory processes.\(^2\), \(^3\) This unique expression pattern predestines FAP as a potential diagnostic target for molecular imaging and therapy in various diseases. Therefore, we recently designed the gallium-68 labeled FAP-inhibitor \[^{68}\text{Ga}]\text{MHLL1}\(^4\) for imaging using positron emission tomography (PET). In this project, \[^{68}\text{Ga}]\text{MHLL1} was evaluated in vitro and in vivo in a preclinical myocardial infarction (MI) model to visualise fibrosis after tissue damage.

**Methods:** \(p\)-NCS-benzyl-NODA-GA was coupled via thiourea formation to a primary amino function of a pyrrolidine-based FAP-warhead, followed by HPLC-purification. The non-radioactive reference compound was realized by using \[^{68}\text{Ga}][\text{gallium(III)}]\text{chloride}. The \[^{68}\text{Ga}]-eluate was obtained from a \[^{68}\text{Ge}/^{68}\text{Ga}\]-generator and purified by anionic post-processing. In an automated synthesis (GRP-module 3V, Scintomics) 60 nmol precursor was labeled in HEPES at 100 °C for 20 min. Radiochemical yields were determined using a RP18 radioHPLC. Cell uptake assays were performed with different FAP(+) cell lines (3T3, MC-38). One week prior to imaging, the MI was induced via coronary ligation in C57BL/6 mice (n = 4). A dynamic PET-scan was performed for 60 min with 15.2±0.5 MBq \[^{68}\text{Ga}]\text{MHLL1}, followed by a 10 min static scan 20 min after injection of 15.2±3.8 MBq \[^{18}\text{F}][\text{FDG} and a fast CT scan for colocalization of signals. Binding specificity was assessed by pretreatment with the \[^{68}\text{Ga}\]-labeled reference (1mg/kg, iv, 5 min prior, n = 1). In a subset of animals, ex vivo autoradiography confirmed the signal localization with histologic comparison in adjacent sections.

**Results:** After HPLC-purification the precursor \[^{68}\text{Ga}]\text{MHLL1} was obtained with 28.5±16.8% chemical yield, the reference compound with 6%. The radiochemical yield was 40.0±5.4% with a radiochemical purity of 59.4±9.3%. Uptake assays proved specificity in two FAP-positive cell lines. In vivo PET-imaging of the myocardial infarction using \[^{68}\text{Ga}]\text{MHLL1} showed accumulation in the infarct territory at ~20 min p.i., with continuous washout. Tracer uptake was elevated in the infarct region compared to remote myocardium at 30-60 min after injection (1.7±0.3 vs. 1.2±0.2%ID/g, p=0.02). Ex vivo autoradiography displayed elevated activity in the infarct territory relative to remote myocardium. Histologic workup demonstrated fibrosis and tissue derangement typical of scar in the region of elevated \[^{68}\text{Ga}]\text{MHLL1} binding, proving tracer specificity for active fibrosis.

**Conclusions:** In a first in vivo study, the FAP inhibitor \[^{68}\text{Ga}]\text{MHLL1} presented specific uptake on fibrosis sites in myocardial infarct areas. Therefore, the novel tracer shows high potential as FAP-specific imaging agent, and thus, as a valuable tool in monitoring scar formation and fibrogenesis after infarct and further FAP(+) processes and diseases.
Acknowledgements: The authors would like to thank Petra Felsch and Alexander Kanwischer for their excellent technical support. The project was supported by the International Isotope Society – Central European Division.

References:
Development and validation of 2-nitroimidazole-furanoside based fluorine-18 labeled PET-radiopharmaceuticals for the in-vivo detection of hypoxic tissue in tumors


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Tumor hypoxia has proven to be a major negative predictive factor for therapy outcome. Positron emission tomography (PET) is a valuable tool for imaging of hypoxia, and radiolabeled 2-nitroimidazoles (azomycins) have been used as biomarkers for many years. $[^{18}F]$Fluoromisonidazole ($[^{18}F]$FMISO) can be seen as the most prominent example, which is incorporated into cells via passive diffusion. Another representative, $[^{18}F]$fluoro-azomycin-$\alpha$-arabinoside ($[^{18}F]$FAZA) may be regarded as $\alpha$-configuration nucleoside, but it is postulated that it also enters cells only via diffusion and is not transported by cellular nucleoside transporters. To mimic nucleosides more closely and thereby enhancing image contrast in comparison to $[^{18}F]$FAZA, our objective was to $[^{18}F]$radiolabel an azomycin-2´-deoxyriboside with $\beta$-configuration ($[^{18}F]$FAZDR, [1,2]) and comparatively evaluate it versus $[^{18}F]$FMISO, $[^{18}F]$FAZA and $\alpha$-$[^{18}F]$FAZDR.

First, for a deeper insight, we comparatively studied the interaction of FAZA, $\beta$-FAZA, $\alpha$-FAZDR and $\beta$-FAZDR with nucleoside transporters (SLC29A1/2 and SLC28A1/2/3) in-vitro. Precursors and non-radioactive standards for $[^{18}F]$FAZDR derivatives were synthesized from methyl 2-deoxy-D-ribofuranosides. The precursors were radiolabeled in an automated synthesizer. $[^{18}F]$FMISO, $[^{18}F]$FAZA or $[^{18}F]$FAZDR were injected in BALB/c mice bearing CT26 colon carcinoma xenografts. PET scans were performed after 1, 2 and 3 h post injection (p. i.).

Nucleoside transporter studies showed variable interactions of the compounds. The highest interactions being for $\beta$-FAZDR (IC50 124 ± 33 µM for SLC28A3), but also for FAZA with the non-nucleosidic $\alpha$-configuration, interactions were remarkable (290 ± 44 µM (SLC28A1); 640 ± 10 µM (SLC28A2)). $\alpha$-$[^{18}F]$FAZDR and $\beta$-$[^{18}F]$FAZDR were synthesized in reasonable radiochemical yields (15.9 ± 9.0% (n = 3), resp. 10.9 ± 2.4% (n = 4)), with radiochemical purities > 98% and molar activities > 50 GBq/µmol.

In small animal PET imaging tumor-to-muscle ratios (TMR) were significantly higher for $\beta$-$[^{18}F]$FAZDR at 1 h (2.76) compared to $[^{18}F]$FAZA (1.69, $P < 0.001$). In another set of experiments $\alpha$-$[^{18}F]$FAZDR was compared to $\beta$-$[^{18}F]$FAZDR and $[^{18}F]$FMISO. Highest TMR were again observed for $\beta$-$[^{18}F]$FAZDR at 1 h p.i. (2.52 ± 0.94, n = 4) in comparison to $\alpha$-$[^{18}F]$FAZDR (1.93 ± 0.39, n = 4) and $[^{18}F]$FMISO (1.37 ± 0.11, n = 5) with possible mediation by the involvement of nucleoside transporters. After 3 h p. i. TMR were not significantly different for all tracers (2.5 – 3.0).

In conclusion, syntheses of $\alpha$- and $\beta$-FAZDR could be developed (non-radioactive or $^{18}$F-labeled), allowing their further evaluation. First PET imaging results with both $[^{18}F]$FAZDR anomers showed advantages over $[^{18}F]$FAZA and $[^{18}F]$FMISO, regarding higher tumor contrast at earlier time points p. i., indicating their potential as PET hypoxia tracers. Differences in uptake behavior may be attributed to a potential variable involvement of transport mechanisms and need further investigation.

Conjugation of radiolabeled moieties or fluorophores to bioactive peptides, in particular via lysine, cysteine or the N-terminal amino acid, is a straightforward approach to obtain labeled peptidic molecular tools. However, the structural modification can impair the biological activity of the peptides and numerous bioactive peptides are devoid of lysine and cysteine. As some of those peptides contain arginine, so far neglected with respect to the preparation of peptide conjugates, we developed a widely applicable labeling strategy for peptides, based on the bioisosteric replacement of arginine by a functionalized $N^ω$-carbamoylated arginine, the latter enabling conjugation of the peptides to various moieties such as fluorescent dyes [1]. This concept was successfully demonstrated for peptidic agonists of neurotensin, angiotensin II and neuropeptide Y $Y_1$ receptors [1,2].

Likewise, the replacement of the guanidine group in the non-peptidic argininamide-type neuropeptide Y $Y_1$ receptor antagonist BIBP3226 [3] by an $N^ω$-carbamoylated guanidine moiety gave access to a radioligand ([$^3$H]UR-MK299) with very high $Y_1$ receptor affinity ($K_d = 0.044$ nM) [4]. Notably, also the 'cold' form of this radioligand proved to be a powerful molecular tool as it considerably promoted the crystallization of the $Y_1$ receptor [5].

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References:
Radiotherapy, in-vitro and in-vivo studies of $^{177}$Lu-labeled neurotensin receptor-1 antagonists as radiotracers for the therapy of pancreatic cancer

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Objectives: Pancreatic adenocarcinoma is the eighth leading cause of cancer death in the world with the poorest prognosis amongst all human malignant solid tumors [1]. The neurotensin receptor-1 (NTS1) is overexpressed in pancreatic tumor tissue [2], thereby it could be a molecular target for receptor-mediated radiotherapy. In this study two non-peptide $^{177}$Lu-labeled NTS1 antagonists were evaluated in vitro and in vivo to assess their suitability for NTS1-mediated radiotherapy.

Methods: $^{177}$Lu-FAUC 469 (1) and $^{177}$Lu-DOTA-CL 156 (2) were synthesized with > 98 % radiochemical yield, determined by radio-HPLC analysis of the crude product. The in-vitro evaluation was performed using AsPC-1 cells, a human pancreatic adenocarcinoma cell line. Saturation binding, internalization and efflux studies were accomplished by stability tests of the radiotracers in plasma. NMRI nude mice were used for in-vivo biodistribution studies (1.5 MBq ID/animal) and radiotherapy studies (25 MBq ID/animal, n = 6). The body weights and tumor volumes were determined five times per week. At the endpoint of therapy, the liver, spleen, kidney, heart and tumor tissue morphology were determined by HE, Masson-Goldner and PAS staining. Blood samples were analyzed for AST, ALT, creatinine, bilirubin, and gamma-GT to assess kidney and liver toxicity. A statistical permutation test using a two-sample t-statistic was used to analyze the growth curves of each therapy group.

Results: In-vitro characterization demonstrated high binding affinity to NTS1 (0.37 ± 0.13 nM (1) and 0.26 ± 0.06 nM (2), n = 3), fast cell uptake (82.2 ± 2.2 % for 1 and 76.4 ± 2.1 % for 2 after 240 min, n = 3) and high stability in human plasma for at least 96 h. Biodistribution data of 1 and 2 revealed excellent tumor uptake with 22.4 ± 3.3 %ID/g (1) and 34.2 ± 2.5 %ID/g (2) at 24 h p.i., together with low kidney (0.9 ± 0.5 %ID/g) and liver (1.5 ± 0.5 %ID/g) uptake of 2. Significant tumor growth inhibition was shown between day 11 and 21 after begin of treatment with 1 compared to control animals (adjusted p=0.049, n = 6). The histology of kidney and liver sections and the clinical chemistry data from blood samples did not show any toxic effects.

Fig. 1: Chemical structures of $^{177}$Lu-FAUC 469 (1) and $^{177}$Lu-DOTA-CL 156 (2)

In summary, $^{177}$Lu-FAUC 469 (1) represents a highly promising tracer for radiotherapy of NTS1-positive pancreatic carcinoma. The GMP-compliant radiosynthesis and first in-human-studies with $^{177}$Lu-FAUC 469 are ongoing.

Acknowledgments: ITG Garching is acknowledged for financial support of this study

References:
Protein degradation plays important roles in biological processes and is tightly regulated. Further, targeted proteolysis is an emerging research tool and therapeutic strategy. Mass spectrometry-based proteomics techniques enable the investigation of the causes and consequences of protein degradation in biological systems. We recently developed ‘multiplexed proteome dynamics profiling’, mPDP, combining dynamic-SILAC labelling with isobaric mass taggng for the multiplexed analysis of drug effects and stimuli on protein degradation and synthesis. When applied in combination with other quantitative proteomics approaches such as chemoproteomics and thermal protein profiling, mPDP provides unique insights in drug mechanism-of-action. The presentation will focus on the MoA of targeted protein degraders (PROTACs) and other compounds affecting protein stability.
Laser Capture Microdissection/Mass spectrometry- a promising tool in tissue-based clinical proteomics

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The advent of laser capture microdissection (LCM) techniques promise to overcome previous limitations in the isolation of specific cells from tissue samples. At the MPC we have successfully applied this approach in combination with mass spectrometry (MS) for specific characterization of cells and subcellular structures in different tissues such as skeletal muscle and brain.

In my talk I will give an introduction about the possibilities using LCM-MS and will present our results from skeletal muscle studies addressing e.g. myofibrillary myopathies (MFM).

The use of our highly sensitive LCM-MS approach helped to discover a number of new disease-relevant proteins accumulating in abnormal muscle fibers of MFM patients. This provides new insights into pathomechanisms of aggregate formation, one of the basic phenomena in the pathogenesis of the disease. The established significant differences between subtype-specific proteomic profiles may also be helpful in differential diagnostics of patients with protein aggregation myopathies.
The ubiquitous mould *Aspergillus fumigatus* can cause life-threatening disease in immunocompromised patients. Current diagnostics by biopsy and subsequent culture of the fungus remains slow and carries risks for the patient, while non-invasive approaches lack specificity and sensitivity. We propose a non-invasive method to diagnose this disease *in vivo* with high specificity by means of positron emission tomography (PET) using a $^{64}$Cu-NODAGA-labeled *Aspergillus*-specific antibody (JF5).

Murine JF5 [1] was humanized by grafting its complementarity-determining regions into a human IgG1 framework and produced in CHO cells according to Good Manufacturing Practice (GMP). The chelator p-NCS-Bn-NODAGA was synthesized according to GMP. We developed a cleanroom-compliant conjugation and diafiltration procedure, produced 150 mg of NODAGA-hJF5 and characterized it using validated procedures. $^{64}$Cu radiolabeling and quality control procedures of the tracer were developed and validated. Degree of chelator conjugation was quantified by mass spectrometry. High-performance size exclusion chromatography and ELISA were used to control the integrity and stability of intermediates and the final product. Repeated-dose-toxicity of NODAGA-hJF5 was assessed in rodents, crossreactivity was analyzed on human tissue arrays and peripheral blood mononuclear cells, and immunoactivation was tested in mixed lymphocyte reactions.

Performance of $[^{64}\text{Cu}]\text{Cu-NODAGA-hJF5}$ was assessed using small animal PET imaging in a mouse model of aspergillosis. *A. fumigatus* spores were deposited intra-tracheally in neutropenic C57BL/6 mice and the radiotracer (13 MBq) was injected intravenously. Simultaneous *in vivo* PET/MR was performed 3, 24 and 48 h after infection using a small animal PET insert within a 7T MRI (Bruker Biospin). *In vivo* results were validated using *ex vivo* biodistribution analysis (gamma counting), autoradiography and fluorescence microscopy.

hJF5 was found comparable to the murine antibody in immunoassays and was successfully produced in CHO cells under GMP. Diafiltration using a tangential flow filtration system was found to be suitable for buffer exchange and conjugation of larger antibody batches within our cleanroom environment. $[^{64}\text{Cu}]\text{Cu-NODAGA-hJF5}$ was successfully prepared adhering to GMP guidelines, with chelator:antibody ratios of ~2 and minimal loss of immunoreactivity. The required specifications for radiotracers were met, and *in vivo* and *in vitro* analysis showed a low toxicity profile.

The radiopharmaceutical revealed high uptake *in vivo* at sites of *A. fumigatus* infection, in particular 48 h after injection, and negligible accumulation in mock-infected animals. This was not observed with $[^{64}\text{Cu}]\text{CuCl}_2$ nor radiolabeled isotype antibody. $[^{64}\text{Cu}]\text{Cu-NODAGA-hJF5}$ was found to provide the best uptake ratio in mice 48 h after injection in the diseased lungs (17.1±2.5 %ID/cc) compared to saline-treated lungs (9.4±1.1 %ID/cc)) (2).

First human PET/MR imaging indicated a favorable toxicity profile, low unspecific binding to other pathologies of the respiratory tract, and enhanced focal uptake of the tracer in the lung of patients tested positive for *A. fumigatus*. First human PET/MR imaging indicated a favorable toxicity profile, low unspecific binding to other pathologies of the respiratory tract, and enhanced focal uptake of the tracer in the lung of patients tested positive for *A. fumigatus*. Taken together, $[^{64}\text{Cu}]\text{Cu-NODAGA-hJF5}$ proved to be a highly specific and sensitive tracer for immunoPET diagnosis of IPA, showing very promising results in preclinical models. Having established the complete production pipeline of this tracer under GMP and obtained encouraging results, we now aim to assess safety and performance of the radiopharmaceutical in clinical trials.

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**References:**
Inhibition of Cdk5 – A New Way to Improve the Standard Therapy of Hepatocellular Carcinoma

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Introduction: Patients diagnosed with advanced stage hepatocellular carcinoma (HCC) face a poor prognosis with very limited chemotherapeutic treatment options. The efficacy of the only approved multi kinase inhibitors – Sorafenib, Regorafenib and Lenvatinib – is impaired by low response rates and severe side effects. In the case of Sorafenib treatment a compensatory activation of growth factor receptors (GFRs) leads to treatment escape and progression of disease [2]. However, combining Sorafenib with specific inhibitors of individual GFRs has failed to improve the overall survival of HCC patients [3]. Therefore there is an urgent need for new treatment strategies for advanced stage HCC. Here, we investigated cyclin dependent kinase 5 (Cdk5) inhibition as a promising combination strategy to improve the response of HCC to the standard of care treatments.

Methods: Functional effects of the combination of Sorafenib/Regorafenib/Lenvatinib and Cdk5 inhibition were evaluated by applying genetic knockdown (siRNA/shRNA, CRISPR/Cas9) and clinically tested inhibitors of Cdk5. The mode of action of Cdk5 inhibition to improve Sorafenib response was judged by a LC-MS/MS-based proteomic approach. Intracellular receptor trafficking was investigated by confocal microscopy with live cell imaging and sophisticated analysis of vesicle properties.

Results: Combining Cdk5 inhibition with Sorafenib led to a synergistic impairment of HCC progression in vitro and in vivo, by inhibiting both tumor cell proliferation and migration. In response to Sorafenib HCC cells use a compensatory upregulation of the EGFR signalling pathway to evade Sorafenib treatment, which could be prevented by Cdk5 inhibition. This effect is mediated by a novel mode of action for Cdk5: Cdk5 inhibition interferes with intracellular trafficking which is crucial for GFR signalling, thereby leading to enlarged endosomal vesicles and accumulation of respective cargo proteins. Thus, GFRs are trapped in the endocytic system and are not available for further activation at the cell surface. First in vitro experiments show that this new, Sorafenib-independent mechanism for Cdk5 can be transferred to Regorafenib and Lenvatinib treatment of HCC cells.

Conclusion: The inhibition of Cdk5 offers an effective approach to enhance the response of the standard of care chemotherapeutics and prevent treatment escape. With Dinaciclib a clinically evaluated and well-tolerated Cdk5 inhibitor is readily available and our study provides evidence for clinically evaluating the combination of standard of care multi-kinase inhibitors and Dinaciclib to improve the therapeutic situation for advanced-stage HCC patients.

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References:
3.13 Microfluidics / Cell-based assays and organoids for drug discovery

Chair: S. Wölfli

Droplet Microfluidics in antibody discovery and personalized cancer therapy

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We have developed fully integrated droplet-based microfluidic platforms for the screening of therapeutic antibodies1, 2. In these systems tiny aqueous droplets (picoliter volumes) surrounded by oil serve as independent assay vessels. The technology allows the direct screening of several hundred thousand primary, non-immortalized murine or even human B-cells for the secretion of antibodies that do not just bind to a drug target, but functionally inhibit it. Furthermore, the technology can be used for genotypic and phenotypic characterization of blood cells at the single cell level3. We believe this opens the way for many new approaches in drug discovery, including personalized immunotherapy or the use of antibodies to control cellular pathways at will.

In parallel to this we have developed screening platforms enabling rapid identification of optimal drug cocktails for personalized cancer therapy4. Results are available within 24h after surgery at consumables costs of less than 150 US$ per screen. The power of this platform has been demonstrated using cancer cell lines, mouse models and even human tumor biopsies. We now envisage first clinical trials and assess further application fields, e.g. for the efficient stratification of patients for immunotherapies.

Analyzing toxicity and metabolic interactions with microfluidic liver-kidney-on-a-chip systems

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The development of new drug compounds is a challenging and time consuming process. Compounds identified by screening of small molecule libraries for disease specific molecular targets need to be further validated for target specificity and efficacy in complex biological systems in early stages of drug development.

Microfluidic tissue culture systems based on established cell lines, patient specific specialized cell types or even organoid structures can provide systems for the in vitro analysis of toxicity and metabolic interactions studies that can replace animal testing in early drug development, and open new approaches for the study of physiological and biochemical interactions occurring in humans in patient specific settings.

As an alternative to the reconstruction of complex organ and organoid like structures, we use a minimal, more simple approach focusing on tissue specific key metabolic properties that underlie drug metabolism and toxicity. To visualize the cellular responses we analyze metabolic adaptation and changes of the cells in the system and detect changes in gene expression in response to drug treatment using continuous monitoring and long term cultivation.

As an example of an optimized microfluidic tissue culture systems for drug testing the multi-chamber systems developed within the SysToxChip consortium [1] will be presented. This chip system can be used to explore liver dependent toxic effects of drugs in human cells [2,3,4]. In addition to drug testing in early drug development, microfluidic chip systems can be used in the clinical setting. In combination with iPSC technology these systems can be used for ex vivo drug testing and to model potential metabolic interactions between cells in the human body on an individual basis.

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Seeking for chemical OCT4 substitutes for the generation of human iPSCs

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Induced pluripotent stem cells (iPSCs) can be reprogrammed from terminally differentiated cells by ectopic expression of a core of transcription factors (OCT4, SOX2, KLF4 and MYC), which provides a promising source for autologous organ transplantation, drug discovery and disease modeling and opens a new era in the field of regenerative medicine. Despite of the great improvement in the generation of iPSCs, reprogramming of human somatic cells is still a time-consuming and inefficient process. Genetic and epigenetic abnormalities found in the iPSCs also raised safety concerns of clinical application. In comparison to genetic manipulation, small molecules show distinct advantages in the application and control and thereby have been intensively investigated. Recently, mouse chemically induced pluripotent stem cells (mciPSCs) have been successfully generated. However, the lack of sufficiently strong chemical substitutes for OCT4 makes the generation of human chemically iPSCs difficult. To solve this problem, we performed cell-based high-throughput screenings and identified a series of OCT4 inducing compounds (O4Is). We not only improved the activity of compounds by chemical optimization, but also demonstrated the benefit from our compounds in the maintenance and generation of human iPSCs. Moreover, we combined the chemical modification with various biological assessments, including DNA microarray, RNA-seq, proteomics, ATAC-Seq and knockdown/knockin technology, and successfully elucidated the mechanism of action of our chemical OCT4 substitutes.

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References:


3.14 New Research, New Researchers II

Chairs: A. Link, F. Hansen

Direct PPARγ activation by L-thyroxin and TETRAC links thyroid hormone and PPAR signaling

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Thyroid hormones (THs) operate numerous physiological processes on genomic level through activation of the nuclear thyroid hormone receptors (THR). Their signaling pathways were long considered well investigated but in the last decade, several further proteins including surface receptors and transporters were characterized as molecular targets of THs.[1,2]

We have discovered a direct peroxisome proliferator-activated receptor gamma (PPARγ) activation by THs as further component of TH signaling. PPARγ as the THR constitutes a ligand-activated transcription factor and is essentially involved in lipid and glucose homeostasis, adipocyte differentiation and inflammation.[3,4] We have observed remarkable activation of this nuclear receptor by several THs including T4 in cellular and cell-free environment. Amongst THs, the T4 metabolite 3,3’,5,5’-tetraiodothyroacetic acid (TETRAC) – considered as non-classical TH – turned out as most active PPARγ agonist with nanomolar potency in cellular PPARγ activation and cell-free co-activator recruitment. According to isothermal titration calorimetry and X-ray structural analysis (Figure 1), TETRAC binds to the canonical orthosteric PPARγ ligand binding site with nanomolar affinity. Moreover, TETRAC was identified as potent agonist of PPARγ’s heterodimer partner retinoid X receptor (RXR) leading to synergistic PPARγ:RXR heterodimer activation observed by markedly enhanced co-activator recruitment by the dimer. In hepatocytes and fibroblasts, THs robustly induced PPARγ regulated gene expression and THR knockdown in hepatocytes demonstrated THR independence of TH mediated PPARγ modulation. Activation of PPARγ regulated gene expression was also observed in mice indicating biological relevance of the TH-PPARγ:RXR interaction.

Our results characterize PPARγ:RXR as direct molecular target of THs and essential part of TH signaling wherein the non-classical TH TETRAC was discovered as most active endogenous PPARγ ligand known to date.

References:

Figure 1: X-ray structure of TETRAC bound to the PPARγ ligand binding domain
Targeting biosynthetic networks of the proinflammatory and proresolving lipid metabolome: a promising pharmacological strategy for intervention with inflammation

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Lipid mediators (LM) encompass proinflammatory prostaglandins (PG) and leukotrienes (LT) but also specialized proresolving mediators (SPM) which display pivotal bioactivities in health and disease. Pharmacological intervention with inflammatory disorders such as asthma and rheumatoid arthritis are commonly practiced with anti-inflammatory drugs that suppress LT or PG formation, which however, possess limited effectiveness and on-target-related side effects. Among the most prescribed drugs to treat inflammation are nonsteroidal anti-inflammatory drugs (NSAIDs) that interfere with the biosynthesis of arachidonic acid-derived proinflammatory PG and LT by targeting cyclooxygenases (COXs), 5-lipoxygenase (LOX), or the 5-LOX–activating protein (FLAP). However, these and related enzymes act in conjunction with marked crosstalk within a complex LM network where also proresolving SPMs are formed, with 15-LOX-1 being the key biosynthetic enzyme. So far, LM biosynthesis inhibitors (including NSAIDs) have been evaluated in cell-based studies with limited read-out, mainly addressing inflammation-promoting PGs and LTs in proinflammatory immune cells (i.e., neutrophils, monocytes, macrophage cell lines), whereas SPMs have not yet been essentially studied because they are the newest mediators uncovered with novel proresolving functions.

Here, we present how prominent LM pathways can be differentially modulated in human proinflammatory M1 and proresolving M2 macrophage phenotypes that, upon exposure to *Escherichia coli*, produce either abundant PGs and LTs (M1) or SPMs (M2) [1]. Targeted liquid chromatography–tandem mass spectrometry–based metabololipidomics was applied to analyse and quantify their specific LM profiles upon drug exposure. Besides expected on-target actions, we found that: 1) COX or 15-LOX-1 inhibitors elevate inflammatory LT levels, 2) FLAP and 5-LOX inhibitors reduce LT biosynthesis in M1 but less in M2 macrophages, 3) zileuton blocks resolution-initiating SPM formation whereas FLAP inhibition increases SPM levels, and 4) that the 15-LOX-1 inhibitor 3887 potently suppresses SPMs in M2 macrophages. Conclusively, interference with discrete LM biosynthetic enzymes in different macrophage phenotypes considerably affects the LM metabolomes with potential consequences for the inflammation-resolution pharmacotherapy [2].

Moreover, we discovered and characterized two novel benzoxanthene lignan structures that promote the above-described LM class switch from proinflammatory towards proresolving LM, not only in human macrophages but also in a zymosan-induced murine peritonitis model in vivo, suggesting their suitability as novel leads for pharmacotherapy of inflammatory disorders [3]. Together, our data may allow better appraisal of the therapeutic potential of these drugs to intervene with inflammatory disorders.

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Natural Products as Novel Lead Structures: Method Development and Biological Testing

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The treatment of infectious diseases is one of the challenges in drug development due to the increase of resistances of bacteria and viruses. [1] Isolation of natural products from plants, fungi, bacteria, marine organisms et cetera often led to novel drugs, which are today still important for the treatment of infectious diseases (e.g. erythromycin) [2]. Unfortunately, some promising natural products can only be isolated in small amounts from their natural sources limiting the screening for their potential biological activities. Furthermore, some of the natural products serve as lead structure for synthetic analogues with enhanced activity (e.g. amoxicillin). [3]

Total synthesis of natural products is a powerful tool to produce natural products for broader biological evaluation. An interesting example is the natural product class of the rubrolides. This marine-derived natural product family has an interesting portfolio of different biological activities [4]. In 2014, rubrolide R and S were isolated from the fungus Aspergillus terreus (OUCMDZ 1925), which was derived from barracuda intestines [5]. Both structures have been synthesised in a, dramatically short and protecting group free, sequence of three linear steps and were further evaluated in regard to their antiviral and antibiotic activities [6].

Another promising natural product was recently isolated from the endophytic fungus Phomopsis fukushii [7] has also been synthesised. The diaryl ether 2 showed significant anti-MRSA activity, which was further evaluated.

The so far unprecedented natural product class of the Scleropentasides bear an interesting β-C-acyl moiety.[8] Scleropentaside A 3 has been synthesised in only four linear steps starting from d-Glucose with perfect β-selectivity. The synthetic method allows synthesising β-C-acyl carbohydrates in only a few steps without using heavy metals.[9]

Small-molecule inhibitors of human coagulation factor XIIa: synthesis and anticoagulant activity

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Thrombosis is the formation of a potentially deadly blood clot inside a vein or artery which is associated with a high risk of mortality.1,2 Hospitalization is the most important risk factor for developing thrombosis resulting in approximately 10% of all hospital deaths.3 The venous thromboembolism-related annual healthcare cost range between $7594 and $16,644 per patient, which is equal to the total annual cost of $2-$10 billion for the patients in the U.S. only.4 Therefore, urgent measures should be undertaken to reduce the mortality rate and economic losses associated with thrombosis.

In recent years, a number of anticoagulants (e.g. 1-3) have been developed and proved to efficiently decrease the rate of stroke and other thrombosis-related disorders. Clinically used anticoagulants, however, exhibit unavoidable bleeding risk5 as they target the vital enzymes of the coagulation cascade affecting simultaneously thrombin generation contributing to thrombosis as well as thrombin formation required for hemostasis. Therefore, novel anticoagulants with a new mechanism of action are needed to control thrombosis without the risk of bleeding.

Recent studies have proved a fundamental role of Hageman Factor (FXII) in arterial and venous thrombosis. FXII knockout mice were protected from arterial thrombosis, ischemic stroke, and deep vein thrombosis. Despite its essential function in thrombosis, deficiency of FXII does not impair hemostasis in animals and humans.4,5 Therefore, Hageman Factor represent new, potentially safe, and promising target for thrombosis prevention.

To find new inhibitors targeting FXIIa, a series of aminotriazoles possessing quinoxalin-2-yl (5) and 6-phenylpyridin-3-yl (6) moieties were designed and synthesized from commercially available β-ketoesters 4. Utilizing chromogenic substrate, synthesized series of aminotriazoles 5 and 6 were subjected to enzymatic assay to evaluate their inhibitory activity towards human FXIIa. Some of the prepared aminotriazoles were found to be nanomolar inhibitors of human coagulation FXIIa (e.g. one of N-acylated derivatives of series 5 showed $K_i(\beta$-FXIIa) = 50 nM). Moreover, synthesized compounds significantly prolonged activated partial thromboplastin time (aPTT blood test) thereby showing compounds' ability to affect the intrinsic coagulation pathway. In contrast, prepared aminotriazoles showed little to no effect on prothrombin time (PT) indicating no influence on the extrinsic coagulation pathway. Synthesized FXIIa inhibitors represent a promising lead for further development of small-molecules targeting thrombosis.

Structure-activity study and molecular insights in the mode of action of complement C3 inhibitor Cp40

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The complement system serves in blood circulation as “first line of defense” against injurious stimuli and invaders. Upon activation, a series of cascading enzymatic reactions leads to an amplification of the response and to pathogen clearance and opsonic cell killing. Yet complement has also gained increasing interest as a potential drug target, since it may be inadvertently triggered on human cells or biomaterial surfaces, thereby contributing to clinical complications in the pathogenesis of various autoimmune, inflammatory and age-related diseases as well as transplant rejection. While the involvement of dysregulated complement activation in inflammatory and autoimmune diseases is now widely recognized¹, so far only one complement-specific drug has reached the market.

In this presentation we reflect on the development of the picomolar complement inhibitor Cp40, a next-generation derivative of the compstatin peptide. The C3 inhibitor compstatin was originally identified using a phage display approach² and several of its derivatives are currently in clinical development. In particular, the presented study is focused on recent structure-activity relationship investigations. Based on a novel co-crystal structure of Cp40 in complex with its complement target C3b, we used site-specific modifications/deletions, nonproteinogenic amino acids and tailor-made building blocks to elucidate the SAR in detail and identify key interaction determinants. By employing functional SPR experiments, we were able to further elucidate the molecular mode of action of Cp40. Building on this insight, we follow various strategies (e.g. prodrug approaches or bioconjugation) to improve affinity and pharmacokinetic properties for a use of this promising inhibitor class in a broad range of disease models.

3.15 The promise of cure - recent breakthrough innovation in cell- and gene therapy
Chair: H. Apeler

Cell and gene therapy developments: it is now or never!

André Berger, Matthias Renner and Klaus Cichutek, Paul-Ehrlich-Institut, Langen, Germany

It has now been almost 30 years since the first gene therapy trial was performed, intended for the treatment of severe combined immunodeficiency with retrovirally modified haematopoetic stem cells. Since then, as with any new medical approach, clinical translation initially led to a mix of encouraging and disappointing results. Advances in gene therapy vector systems and increasing clinical experience eventually paved the way for gene therapy to become a safe and efficacious treatment option for a variety of rare and common diseases.

To date, more than 2,000 gene therapy clinical trials have been or are being conducted with more in the planning. Currently, major clinical developments concern cancer, haemophilia, haemoglobinopathies, neurological disorders and ocular diseases. Mainly genetically modified cells and AAV-based vectors are used as the respective investigational medicinal products. Moreover, other types of viral vectors, plasmids, mRNAs as well as oncolytic viruses and genome editing tools are clinically explored.

In the recent years, special attention was given to the field of cancer immunotherapy, especially chimeric antigen receptor (CAR)- or T cell receptor (TCR)-based adoptive cell therapies. Two CAR T cell medicines, namely Kymriah and Yescarta, have received marketing authorisation in the EU in 2018. By now, six GTMPs (Glybera, Imlygic, Strimvelis, Yescarta, Kymriah, Luxturna) have been granted marketing authorization by the European Commission, since marketing authorisation of the first gene therapy medicinal product (GTMP) in 2012.

In the EU, marketing authorization applications of GTMPs as well as of all other advanced therapy medicinal products (ATMPs) are processed via the centralized procedure, which includes initial assessment by experts of two assigned EU member states’ competent authorities. A positive opinion of the Committee for Advanced Therapy (CAT) and the Committee for Medicinal Products for Human Use (CHMP) at the European Medicines Agency (EMA) leads to marketing authorization by the European Commission. In contrast, clinical trial authorization is granted by the national competent authority of the EU Member State, where the trial site is located.

GTMPs are considered most complex biomedicines, often initially developed in an academic setting. Thus, several activities, such as the ATMP action plan or the priority medicines program, were initiated at EU level to support the development of GTMPs and to help developers to comply with regulatory demands. Development of GTMPs generally requires close cooperation of basic scientist, manufactures, contract research organizations (CROs), the PI of the clinical trial, and independent review of the regulatory experts. Issues in manufacturing and control are high variations in cell starting material from individual patients in case of autologous genetically modified cells, non-clinical analyses issues concern obtaining data on the safety and efficacy of the product, in particular as suitable animal models are rarely available, and the design of the clinical study, for example regarding initial dosing or selection of appropriate control groups.

The cutting edge developments in gene therapy and the lack of respective guidance for these very novel products bear challenges not only for the developers, but also the regulatory experts assessing respective clinical trial applications. Such issues as well as potential solutions will be discussed in the presentation.
Hybrid retrovirus-transposon vectors

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A variety of different viral vector systems plays, namely derived from retroviruses such as Human Immunodeficiency Virus (HIV) and Murine Leukemia Virus (MLV) as well as Adeno-Associated Virus (AAV) a pivotal role as gene delivery devices in current somatic gene therapeutic strategies. While great progress has been achieved in the past decade to optimize these vector in regards of their safety profile and gene transfer efficiency, one of the major issues still to be solved are the high costs associated with vector production and purification currently hampering the clinical application of viral vector-based gene therapeutics at economically affordable prices. Thus, we utilized new strategies using transposon vectors to shorten the development time for the establishment of stable viral packaging cells (VPCs). MLV-based VPCs served as a proof-of-concept. The novel VPCs revealed also more than 20-fold higher productivity as compared to VPCs generated using classical approaches such as serial stable transfection with plasmid-based expression constructs. We showed that this advance productivity resulted from enhance particle count and vector fitness. These data shed a promising light on the future utility of this new hybrid-technology for vector systems derived also from other parental viruses.
Challenges in manufacturing and Quality Control of ATMPs

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Fraunhofer IZI operates, with more than 130 employees, three state-of-the-art clean room facilities for aseptic manufacturing of cell and gene therapy products. Due to the complexity of the manufacturing processes and quality controls for cell and gene therapies there are many challenges and potential pitfalls on the way to bringing such a product into the clinic. This starts with the enormous and mostly underestimated costs, which are related to the - mainly manual - labor-intensive manufacturing processes and the necessity of a high grade clean room environment. Other underestimated issues are the long timelines and intensive efforts involved in obtaining product-specific GMP-manufacturing authorization and the regulatory approval for a clinical trial. During process transfer or process development various setbacks could occur regarding selection of appropriate raw materials and the subsequent supplier qualification. This is of particular importance for materials of human, animal or recombinant origin that must comply especially with viral and TSE safety regulations. The high process complexity, the variations of the cellular starting material (especially in autologous settings) as well as the variety of different cell and gene therapy products is often related to difficulties in regard to a robust process validation. The same issue occurs for the validation of the highly sophisticated analytical methods, for which standard ICH guidelines or Pharm. Eur. are often not directly applicable. All these issues need special consideration and strategies that require frequent ongoing consultation and interaction with the regulators.
3.16 Natural Products – From Molecular Pharmacology to Clinical Applications
Chairs: M. Wink, T. Efferth

**Systems biology with natural products for individualized cancer therapy**

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To combat complex systemic diseases that harbor robust biological networks such as cancer, single target intervention is frequently ineffective. In such cases, network pharmacology approaches are highly useful, because they differ from conventional drug discovery by addressing the ability of drugs to target numerous proteins or networks involved in a disease. Pleiotropic natural products are one of the promising strategies due to their multi-targeting and due to lower side effects. Transcriptomics and proteomics became milestones to predict the response of tumors to standard chemotherapy and to identify the multifaceted modes of actions of synthetic and natural new drugs. The extraction of meaningful data from highly complex big data sets is a critical bottleneck, which requires sophisticated bioinformatical tools. The discourse between different disciplines is needed to develop strategies for effective decision making based on expression profiling and mutation analysis for precision oncology and drug discovery. Based on our own results, we discuss the application of network pharmacology for cancer drug discovery. We provide an overview of the current state of knowledge on network pharmacology, focus on different technical approaches and implications for cancer therapy (e.g. polypharmacology and synthetic lethality), and illustrate the therapeutic potential with selected examples from herbal mixtures, medicinal herbs and isolated phytochemicals. Finally, we present future perspectives on their plausible applications for diagnosis and therapy of cancer.

Selected papers:
Efferth T, Paul NW. Lancet Planet Health 2017;1:e301-e303.
Treatment of patients with cardiac AL amyloidosis with epigallocatechin-3-gallate (EGCG) from green tea - results of a clinical study and transcriptomics

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Systemic amyloid light-chain (AL) amyloidosis is a rare monoclonal B cell disorder with poor prognosis due to amyloid depositions in nearly all organs. Most patients die due to advanced heart involvement. The only evidence based therapy is cytoreduction of the underlying monoclonal gammopathy to stop the production of amyloid precursors. Promising in-vitro data show that epigallocatechin-3-gallate (EGCG), a polyphenol from green tea, can impair amyloid fibrillogenesis. Anecdotal clinical observations even suggest that EGCG has the potential to degrade amyloid from tissues in patients.

In the study, 38 patients with cardiac AL amyloidosis have been randomized in a 1:1 fashion after a successful chemotherapy. Major inclusion criteria were: complete or very good partial remission of the underlying plasma cell dyscrasia and cardiac involvement defined by a septum thickness > 12 mm and exclusion of other causes for myocardial hypertrophy. The time between last cycle of chemotherapy and study inclusion had to be 6 months maximum. The oral EGCG / placebo dosage was increased (month 1-3: 400 mg, months 4-6: 800 mg, month 6-12 1200 mg. EGCG levels were measured every 3 months (before and 2 h after the morning dosage) by HPLC.

Cardiac MRI was performed at start and end of the study to calculate left ventricular (LV) mass. Cardiac biomarkers as NT-ProBNP, 6 minute walk test and echocardiographic parameters were obtained every 3-6 months.

Out of 35 patients 8 patients stopped preterm (median 6,5 months after randomization, range 1-11,5) due to progression of gammopathy (n=5), side effects (n=2) or newly diagnosed breast cancer (n=1). Therefore, 27 patients can be analysed per protocol. No patient died during the study period. Treatment with EGCG capsules, even at higher dosage, had no severe side effects in 36/38 patients. The first analysis of study results showed no significant difference regarding LV mass, NT-proBNP values and 6-minute walktest. Therefore the primary and main secondary endpoints of the study have not been met. EGCG levels in the blood increased significantly when the dosage was increased to 2x 600 mg between months 6-12.

Blood samples were used to determine the EGCG levels. In parallel, we isolated mRNA from blood cells and generated transcriptomes by RNASeq using ILLUMINA sequencers. Substantial differences could be observed in differentially regulated genes in placebo and verum patients. After 12 months, 449 differentially expressed genes are significantly up-regulated und 445 genes are down-regulated in verum patients, while 265 genes are up-regulated and 426 genes are down-regulated in placebo patients compared to the beginning of the study. Between placebo and verum patients, 38 genes are up-regulated and 22 genes are down-regulated. Based on the gene ontology enrichment and molecular function analysis, these genes are enriched with pathways in B cell activation, B cell receptor signalling and blood coagulation. Our findings could suggest an involvement of EGCG in the regulation of amyloid fibril formation.

The failure of the clinical trial might have several reasons: too low EGCG levels between months 1-6, small study population, reduced compliance of the patients to withhold from green tea for one year. The EGCG effect might be not as strong as expected. However, the anti-amyloid effects of EGCG or other catechins should be further studied in AL amyloidosis patients.

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Targeting the mycobacterial membrane with photosensitizers

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Trehalose is a non-mammalian disaccharide found in the cell wall of mycobacteria. It is actively taken up by transport systems, modified and incorporated into the cell wall as trehalose monomycolate or dimycolate. We investigate the application range of several trehalose-conjugates and take benefit of the promiscuity of the extracellular enzyme complex Ag85, which incorporates trehalose tethered photosensitizers into the mycomembrane. Irradiation triggers singlet oxygen formation, killing mycobacterial cells more efficiently, as compared to photosensitizers devoid of trehalose conjugation. Our probes are potent anti-mycobacterial agents that are per se neither affected by permeability issues nor by detoxification mechanisms via drug efflux, and serve as interesting scaffolds for photo-induced therapy.

Dutta, Amit K.; Choudhary, Eira; Wang, Xuan; Záhorszka, Monika; Forbak, Martin; Lohner, Philipp; Jessen, Henning J.; Agarwal, Nisheeth; Korduláková, Jana; Jessen-Trefzer, Claudia: ACS Central Science, 2019, 5 (4), 644–650.
3.17 Bioanalytical Mass Spectrometry

Chair: M. Lämmerhofer

Spatial metabolomics in tissues and single cells

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Recent discoveries put metabolism into spotlight. Metabolism not only fuels cells but also plays key roles in health and disease in particular in cancer, inflammation, and immunity. In parallel, emerging single-cell technologies opened a new world of heterogeneous cell types and states previously hidden beneath population averages. Yet, methods for discovering links between metabolism, cell states, metabolic plasticity and reprogramming on the single-cell level and in situ are crucially lacking. First, I will present how the emerging technology of imaging mass spectrometry can be used for spatial profiling of metabolites, lipids, and drugs in tissues (Palmer et al., 2017). Second, I will present our method SpaceM for spatial single-cell metabolomics in situ (Rappez et al., 2019). We applied SpaceM to investigate hepatocytes stimulated with fatty acids and cytokines, a model mimicking the inflammation-associated transition from the fatty liver disease NAFLD to steatohepatitis NASH. We characterized the metabolic state of steatotic hepatocytes and metabolic plasticity associated with the inflammation. We discovered that steatosis and proliferation take place in distinct cell subpopulations, each with characteristic spatial organization and metabolic signatures. Overall, such methods open novel avenues for understanding metabolism in tissues and cell cultures on the single-cell level.

Very fast MALDI mass spectrometry (MS) is an emerging label-free technology for high-throughput enzyme assays that are devoid of photonic interferences. Recently, two types of cell-based MALDI MS assays have been presented: In target-agnostic phenotypic assays computational analysis of drug concentration-dependent changes of mass spectral patterns reveals lipids/metabolites such as Heme B that can be identified by ultra-high resolution MS and that can serve as response markers in drug discovery or chemical biology (1). Furthermore, cellular malonyl-CoA accumulation in response to fatty acid synthase (FASN) inhibition has been utilized for the characterization of potent FASN inhibitors in a mechanistic MALDI MS cell assay (2).

MALDI MS imaging (MSI) enables the label-free, spatially resolved analysis of lipids, metabolites, drugs or proteins at cellular resolution (“spatialomics”). For each pixel, it simultaneously measures the masses of hundreds to thousands of biomolecules (3). High molecular content datasets are statistically-computationally analyzed to reveal biomolecular patterns that correlate or colocalize with histopathology or other imaging modalities. New MSI trends will be discussed: Quantitative MS drug imaging as post-surgery control of drug disposition in human clinical samples (4), molecular analysis of drug-induced phospholipidosis or crystalline deposits in rodent kidney in toxicological pathology (3) and MS Imaging of endogenous tumor protease activity in fresh-frozen tissue sections (5). Most recently, combination of fast mid-infrared (MIR) imaging of naive tissue sections with MALDI MSI has suggested a new avenue towards fast detection and molecular analysis of tumor margins as well as focused analysis of computationally defined tissue morphologies by ultra-high resolution MSI (6).

References:
Sex bias in colitis yields a protective role of specialized pro-resolving mediators in female mice

Pace S.1, Troisi F.1, Bilancia R.1,2, Gerstmeier J.1, Schädel P.1, Ialenti A.2, Rossi A.2, Serhan, C.N.3, Borrelli F.2, Werz O.1

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The incidence, prevalence and severity of various chronic immune-mediated diseases with unresolved inflammatory component are sex-biased and regulated by sex hormones. Crohn’s disease and ulcerative colitis, the most common forms of inflammatory bowel diseases (IBD), are characterized by chronic intestinal inflammation caused by inflammatory signals including cytokines, chemokines, proteases, and lipid mediators (LM), associated to mucosal injury, increased epithelial permeability, and marked neutrophil recruitment. Accumulating evidence implicate sex differences in the onset and progression of IBD and sex hormones affect the susceptibility to IBD as well as disease severity and progression. Here, we demonstrate that the sex dimorphism in experimental colonic inflammation correlates to different levels of specialized pro-resolving mediators (SPM) in the colon of male versus female mice.

Oral dextran sodium sulfate (DSS) administration caused more severe colon inflammation in male versus female mice along with higher colonic myeloperoxidase activity and plasma chemokine levels during the acute phase. Targeted liquid chromatography-tandem mass spectrometry-based metabololipidomics (LC-MS/MS) of colonic tissues revealed superior amounts of SPM (i.e., resolvin (Rv)D2 and RvD5, protectins, and maresin-1) and their biosynthetic lipoxygenases in healthy female versus male mice. However, SPM levels were similar in the acute inflammatory phase between the sexes and even inverted in the resolution phase. Gonadectomization of male mice elevated colonic SPM without alterations in females. Intrarectal application of RvD2, RvD5 and maresin-1 prevented DNBS-induced colon inflammation and chemokine production in male mice, and thus abolished the sex bias.

Taken together, the biosynthetic pathway of SPM is sex-biased in the colon of mice with superior colonic SPM levels in female versus male animals. Our data indicate that the lower susceptibility of female mice to develop colitis versus males in experimental IBD models coincides with higher colonic SPM levels in females under healthy conditions that eventually protect against the inflammatory disease. Since resolution pharmacology is emerging as an alternative and attractive strategy to intervene with excessive and chronic inflammation, our findings may help to improve established pharmacotherapeutic concepts of IBD, in a gender-tailored manner.
3.18 Challenges and opportunities in model based dose adaptation  
Chairs: C. Kloft, T. Lehr

**Therapeutic drug monitoring in oncology – Case examples**

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Kinase inhibitors (KI) play a pivotal role in the molecular targeted therapy of a variety of malignancies. Unfortunately, therapeutic success is often associated with relevant adverse effects leading to a substantial loss of quality of life or even to discontinuation of treatment. Furthermore, a relapse of the disease is regularly observed in patients treated with kinase inhibitors after initial therapeutic response. Supra- or subtherapeutic drug exposure is a potential cause for these observations. Many KIs are characterized by a large interindividual variability in pharmacokinetics (PK) and several studies have already depicted a correlation between drug exposition on the one hand and adverse effects and/or therapeutic effectiveness on the other hand for some substances. These findings can for instance be attributed to oral administration (dependent on concomitant food uptake, gastrointestinal absorption and intragastric pH-value), metabolism by cytochrome P450 enzymes, extensive comedication, albumin dependant distribution, and adherence [1].

Many KIs are substrates of CYP3A4, induction or inhibition of this isoenzyme might be of clinical relevance. An example for a potent CYP inductor is mitotane. The orphan drug is used for the treatment of adrenocortical carcinoma (ACC) in adjuvant setting and metastatic disease. Due to the limited response rate, there are many efforts trying to replace mitotane treatment by modern KIs and new cytotoxic regimens. Small studies and case series investigating erlotinib, gefitinib and imatinib, all substrates of CYP3A4, yielded disappointing results [2, 3, 4]. It is worth noting, that mitotane exhibits very unfavourable pharmacokinetics with elimination half-lives ranging from weeks to months. Clinically relevant enzyme induction might still be observable months after cessation of drug intake. This could have led to subtherapeutic exposure in those studies. Thus, therapeutic drug monitoring (TDM) will be crucial in future trials investigating CYP3A4 substrates for the treatment of ACC when the patients were pre-treated with mitotane.

TDM of KIs is associated with several obstacles: Sample collection is difficult, due to the fact, that most patients receiving treatment are outpatients. For many substances, there is a lack of a validated PK target or proposed PK-targets refer to area under the concentration-time curve (AUC) which is not easily assessable in routine drug monitoring, due to sparse sampling. Very limited data on the pharmacokinetics of KIs in patients with special conditions e.g. dialysis or hepatic impairment are available and there are substances with a daytime dependent bioavailability (e.g. Nilotinib) complicating the interpretation of through levels. Model supported therapeutic drug monitoring could remove the blind folds and even allow for having a glimpse at the future by incorporation of prior knowledge and simulations. Through levels could be derived from a sample collected at any, well-documented time point and an estimated AUC based on the pharmacokinetic model could be used to evaluate target attainment. Furthermore, new techniques enabling the patients to collect TDM samples themselves could be crucial to obtaining useful and enough data for the model supported TDM of orally administered KI.

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**References:**

Model-based precision dosing of tamoxifen therapy

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Background & Objectives: Patients diagnosed with an oestrogen receptor (ER)-positive breast cancer are commonly treated with the selective-oestrogen modulator tamoxifen. This oral anticancer drug is administered at a standard dose of 20 mg once daily (QD), with long treatment durations in the adjuvant setting (>5 years). Since the discovery of the 100-fold more active metabolite endoxifen, tamoxifen is considered a prodrug. Large variabilities in tamoxifen and endoxifen pharmacokinetics (PK) have been observed between patients treated with 20 mg tamoxifen daily, which has been partly attributed to variations in patients’ CYP2D6 enzyme activity. A large clinical study identified an association between endoxifen and treatment success suggesting a therapeutic target concentration (6 ng/mL [1]). Whether and how to individualise tamoxifen treatment is in focus of ongoing intense discussions. Here we compare three different dosing strategies and propose a more appropriate one which potentially maximises clinical efficacy in the individual patient applying clinical trial simulations.

Methods: A large clinical target population was simulated (n=10,000; CYP2D6 poor (PM): intermediate (IM): normal/ultrarapid (NM) metaboliser: 5%:15%:80%). Using a population PK model [2] three dosing strategies were investigated:

(1) Standard dosing (20 mg/day)
(2) CYP2D6-adapted dosing
(3) Model-informed therapeutic drug monitoring dosing

For strategy (3) initial dosing was based on (2) and was revised after two weeks QD dosing considering a “virtual” blood sample using Bayesian forecasting. The strategies were compared with respect to PK target attainment (%patients at risk) and remaining variability in exposure at steady-state in the CYP2D6 subgroups and the population after QD dosing.

Results: 97% PM and 52% IM were at risk of subtherapeutic concentrations after standard treatment (strategy 1). Daily doses of 40 and 80 mg for IM and PM were appropriate to reduce the risk to <10% and to obtain similar concentrations to those of NM (20 mg) (strategy 2). However, the variability in endoxifen concentrations across (64% vs. 53% CV in strategy 1 vs. 2) and within the subgroups (both >40% CV) remained large. With strategy (3) the target concentration was met with doses from 5-120 mg/day showing a narrower endoxifen concentration range within the sub-/population. Moreover, target concentrations were achieved with lower doses (as compared to strategy 2) in a considerable number of NM (5 or 10 mg: 38%), IM (10 or 20 mg: 42%) and PM (40 or 60 mg: 79%).

Conclusions: With the model-informed dosing strategy (3) the PK target was met in almost all patients with the lowest individually required dose, significantly decreasing the variability in endoxifen concentrations across patients. Strategy (3) has further advantages: (i) In the long-term therapy (adjuvant or palliative setting) it might be beneficial to avoid unnecessary high concentrations reducing the risk for adverse events. (ii) By adapting the dose early in treatment the time to target attainment was reduced, which might be of importance in the neoadjuvant and metastatic setting. Prerequisites to perform strategy (3) comprise: CYP phenotype and plasma concentration determinations as well as an experienced interdisciplinary team of pharmacists/clinicians to predict individual doses. Model-based precision dosing might be the best strategy for the individual patient, however, for feasibility in practice a quick and easy-to-use tamoxifen dose decision tools ought to be available.

References:
Precision dosing software using pharmacometric models to integrate prior information on the pharmacokinetics of a drug has the merit to streamline the therapeutic drug monitoring (TDM) process. Advantages of model-based dose adaptation include the possibility to derive individualized dosing regimens already before reaching steady state and even before the first dose through probabilistic simulation. Moreover, less reliance on fixed sampling times, a lower number of total required TDM samples and potentially increased precision in the derived individual PK estimates and calculated doses make precision dosing software appealing for clinical implementation. On the other hand, a number of scientific and implementation challenges remain to be addressed in order to benefit most from the merits of model-based TDM, of which the following will be discussed:

How is the impact of uncertainty in sampling time in model-based TDM? When is TDM sampling most informative? A simulation study with the antibiotic meropenem, for which TDM is increasingly done, will be presented in which the impact of typically clinically observed uncertain recording of infusion duration (varied from ±7.5 min to ±22.5 min, standard deviation [SD]) and sampling times (varied from ±5 min to ±30 min, SD) on determined the population and individual parameters will be shown. This case study demonstrated that undocumented uncertainty of only ±5 min (SD) could substantially increase the imprecision of pharmacokinetic parameters on both the population as well as individual level of short half-life drugs such as meropenem. Moreover, if an erroneous dataset was used for the development of a pharmacometric model, more TDM samples were required to determine the individual profile accurately due to inflation of the residual variance.

Which pharmacometric model shall be used as prior information in model-based dose adaptation? Case studies with vancomycin and coagulation factor VIII will be presented, in which strategies for a fit-for-purpose evaluation of pharmacometric models in model-based dose adaptation will be outlined. However, in some situations when patients are unstable or not directly assignable to a specific population, the a priori choice of a single underlying pharmacometric model remains challenging. For this purpose, a recently developed ‘learning’ model-averaging algorithm will be presented that continuously evaluates a number of candidate models during the TDM process and selects the most suitable model for an individual patient over the therapeutic course. Using a simulation study, it was shown for the antibiotic vancomycin that the algorithm was in particular useful to discriminate the pharmacokinetic profile from patients originating from substantially different populations, e.g. critically ill or obese patients and improve the predictive performance in these ‘outlier’ patients.
4 POSTERS
4.1 Medicinal Chemistry and drug design

Synthesis and structure-activity relationship studies on indolylalkyl-substituted piperidine-1-carboxylates as inhibitors of fatty acid amide hydrolase and monoacylglycerol lipase

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Anandamide (AEA) and 2-arachidonoylglycerol (2-AG) are the main endocannabinoids. The biological effects of these endocannabinoids include analgesic and anti-inflammatory activities. AEA and 2-AG are produced on demand and are rapidly inactivated, AEA predominantly by fatty acid amide hydrolase (FAAH) and 2-AG mainly by monoacylglycerol lipase (MAGL). Because inhibition of the degradation of the endocannabinoids prolongs their beneficial effects, inhibitors of FAAH and MAGL are regarded as new agents for the treatment of pain and inflammatory diseases.

Recently, we have found that certain indolylalkyl-substituted phenyl piperidine-1-carboxylates, such as compound 1, are potent inhibitors of FAAH that do not inhibit MAGL [1]. In literature, it has been reported that replacing the aryl group of aryl pyrrolidine-1-carboxylate inhibitors of FAAH and MAGL by an 1,1,1,3,3,3-hexafluoropropan-2-yl substituent shifts activity from FAAH towards MAGL inhibition [2]. In order to also obtain compounds with MAGL inhibitory potency, we have started investigations on indolylalkylpiperidine-1-carboxylate derivatives, which contain this particular group. In the course of structure-activity relationship studies among other variations the length of the alkyl spacer was altered and various substituents were introduced into the indole heterocycle. These structural variations led to potent dual FAAH/MAGL as well as selective MAGL inhibitors.

References


Synthesis and evaluation of pharmaceutical relevant surface modifiers

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The antimicrobial functionalization of surfaces plays a decisive role in the improvement of hygiene in the personal and clinical environment. Much used surfaces for instance handrails, balustrades or doorknobs present a big risk potential, because multi drug resistant germs for instance MRSA (Methicillin-resistant Staphylococcus aureus) or 4-MRGN (multidrug resistant Gram-negative bacteria, e.g. Acinetobacter Baumanii) can be spread via those. Because the percentage of resistant germs is rising and lack of new innovative antibiotics, there is a need for new methods to fight bacteria. Soluble cationic antiseptics stands out against classic antibiotics, because they have a differing mode of action and there are little to no resistances known against them. The downside of the soluble cations is that the only provide a temporary protection of the surface. By attaching them permanently to a surface, a more permanent solution is attainable. The biocidal effect of simple ammonium cations (e.g. trimethylalkylammonium) has been shown in the past. As anchoring group, phosphonic acids have good capabilities to form strong bonds to different metals.

Herein we present the synthesis of other more complex cationic compound. A modular synthetic approach is used to allow easy adaptation to increase coating capabilities and antimicrobial properties. Synthesized molecules were coated on titanium nanoparticles to evaluate the coating properties of the compound. Afterwards the molecules were coated on titanium disk to evaluate the antibiotic activity.

References


The orphan nuclear receptor Nurr1 is responsive to non-steroidal anti-inflammatory drugs

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Nurr1, as a member of the nerve growth factor-induced (j) subfamily of orphan receptors [1], is a neuroprotective transcription factor mainly found in dopaminergic neurons. Levels of Nurr1 are diminished in Parkinson’s Disease (PD) patients and dopamine neuron development in midbrain is Nurr1 dependent. Moreover, Nurr1 knock-out in mature dopamine neurons resembled the progressive pathology in early stage of PD [2]. Therefore, Nurr1 appears as promising target in PD treatment. Originally, Nurr1 was considered as ligand-independent nuclear receptor due to its closed ligand-free conformation and its high constitutive activity [1]. To date, no endogenous ligand has been clearly identified but recently, crystal structures of Nurr1 ligand binding domain in complex
with two different prostaglandin A (PGA) forms were published [3,4]. Both structures reveal a binding site entirely different from nuclear receptors canonical binding site. Compared to the Nurr1 ligand binding domain (LBD) apo crystal structure [1], intermolecular stabilization of activation function 2 located in the C-terminal helix 12 seems to be responsible for Nurr1’s intrinsically active conformation. As ligand binding causes outward movement of helix 12 these interactions disappear and space for a new pocket is created suggesting a possibility for bidirectional modulation of Nurr1 activity (Figure 1).

Figure 1: X-ray structures of Nurr1 LBD in apo (PDB: 1OVL [1], grey) and PGA1 (purple) (PDB: 5Y14 [3], petrol) bound state.

Inspired by PGA bound Nurr1, non-steroidal anti-inflammatory drugs (NSAIDs) as drugs interfering with prostaglandin metabolism were screened for Nurr1 modulatory activity. In our Gal4 hybrid based cellular test system, Nurr1 responded to diverse NSAIDs with agonistic and inverse agonistic behavior: Isothermal titration calorimetry (ITC) confirmed binding of the active hits to recombinant Nurr1 protein and we observed marked effects of NSAIDs on nuclear receptor coactivator 1 (NCor1) recruitment status to Nurr1 in cell-free environment. The most active NSAIDs concerning Nurr1 modulation were selected for structural optimization where we discovered optimization potential (ECIC50 ≤ 39 μM) in early SAR evaluation supporting the suitability of NSAIDs as leads for Nurr1 ligand discovery. Our results clearly indicate that Nurr1 is druggable and provide NSAIDs as promising tool compounds for Nurr1’s activity understanding as well as starting points for ligand development.

References:

Identification of a new class of broad spectrum Metallo-β-lactamase inhibitors

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Increasing number of resistant pathogens generate a great risk to public health as they cause diseases that are impossible to cure. Metallo-β-lactamases, the most common cause of bacterial resistance, are a diverse set of zinc-containing enzymes that catalyze the hydrolysis of β-lactam drugs [1, 2]. The major defense mechanism against these multi-resistant enzymes is to develop β-lactamase inhibitors, which have no bactericidal effect but can maintain the usefulness of existing β-lactam antibiotics.

Recently, we identified a series of thiol-containing inhibitors of highly prevalent metallo-β-lactamases that restore the function of the β-lactam antibiotics [3]. Encouraged by these results, we screened our in-house thiol-containing compound library and found a highly potent broad-spectrum inhibitor of class B1 type β-lactamases, NDM-1, VIM-1 and IMP-7 (IC50: 0.08-1 μM).

In order to further investigate the structure-activity relationship, a series of derivatives were synthesized which led to an improvement in the in vitro activity against these MBL’s. In view of overcoming potential solubility and permeability issues, various chemical groups were introduced on the core structure. In addition to this, co-crystallization studies are ongoing to obtain a complex structure, providing the basis for structure-based optimization.


POS.5

Synthesis and structural activity relationships of novel dihydroisoindol-based anti-infective agents against Trypanosoma brucei rhodesiensi

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Sleeping sickness (Human African Trypanosomiasis - HAT) remains one of the most public health problems in Africa. The disease is caused by two subspecies of trypanosoma brucei: gambiense and rhodesiense. While T. b. gambiense causes a chronic illness with the onset of symptoms after a prolonged incubation period, T. b. rhodesiense causes a more acute illness with much more aggressive effects, with the onset within a few days or weeks after the bite of infected tsetse flies. Existing medicines for the treatment of these diseases are insufficient, and without treatment the disease is invariably fatal. The need for new anti-HAT drugs continues to persist and aiming into this issue; we have prepared a large number of new heterocyclic compounds having different central rings [1-2], that were further assayed against different trypanosoma subtypes. Among the most promising was the dihydroisoindol core ring. Herein we report the optimization of a novel class of dihydroisoindol ureas, which showed selectively in vitro potency against one species of trypanosoma: T. b. rhodesiense. Employing traditional medicinal chemistry approaches we have modified two portions of the central core based on suitable modifications of R1 and R2 (Figure 1). The compounds are fused ring ureas bearing various substituents at the 2-position of the isodindole portion of the backbone (R1) and we evaluated the SAR based on the effect of halogenation, methylation as well as the introduction of various other substituents at the aromatic ring. In addition, the internal core of the molecule was modified by insertion of a nitrogen atom at the X- and Y-positions. Several compounds of this series exhibited an in vitro EC50 ≤ 1μM against T. b. rhodesiense and no detectable activity against other parasites, like. T. b. gambiense, T. cruzi amastigotes or L. infantum. All potent compounds were furthermore tested for toxicity against MRC-5 and PMM cell lines. The compounds exhibited no significant toxicity to both cell lines. The solubility studies were performed on selected compounds and they showed excellent solubility in the tested conditions. A relatively facile synthetic pathway has been amenable to a large number of functional groups, giving rise to a structurally diverse set of analogs. This family of compounds thus shows significant promise for trypanosomiasis drug discovery.
The liver harbors an enormous regenerative capacity as answer to injury, but after a certain degree of serious illness this regenerative ability can collapse with mostly a fatal outcome for the patient. The Mitogen-activated Protein (MAP) Kinase 4 (MKK4) was found to be a major regulator of liver regeneration and could be a valuable drug target helping to reawake this intrinsic capability to regenerate.[2,3] Vin et al. underlined the effect of PLX4720 (Vemurafenib) as a paradoxical-activator of ERK-signaling by inhibition of off-target kinase(s). One of these off-target kinase(s) was determined to be MKK4. Inhibition of MKK4 could be useful for the treatment of liver failure and/or inducing hepatocyte regeneration and preventing hepatocyte apoptosis.[4] In 2013 Wüstefeld et al. proved that silencing of Mitogen-Activated Protein Kinase Kinase 4 (MKK4) in mice is substantially involved in liver regeneration. [1] At the same time, FDA-approved BRAF-inhibitor Vemurafenib showed an off-target-effect on MKK4. [2] With this knowledge analogues of Vemurafenib could be used as a tool for fluorescence-based assay systems. Finding new small molecules that inhibit MKK4 may represent a possible starting point of treating acute and chronic liver diseases. Fluorescence polarization provides fast details about inhibitor-protein interaction and can be adopted for high-throughput screenings of compound libraries. Structure analysis of the binding mode of Vemurafenib to BRAF showed, that the p-chloro substituent is solvents exposed and not necessary for binding at the ATP-site.[3] The same should apply for the binding side of MKK4. Thus, the attachment of the bulky fluorophore S-Carboxytetramethylrhodamin (S-Tamra) through an alkyl linkage is possible without losing binding affinity. Chemical variation and optimization led to a selection of derivatives with different linker lengths, which possess higher binding affinities than Vemurafenib itself and promising IC50 values. Therefore, the optimized tracers meet the demands of a Fluorescence Polarisation assay to identify new small-molecule inhibitors of MKK4.

In the past years, Fluorescence imaging has become an important tool for full-body preclinical imaging, providing spatial resolutions of cellular processes in the range of nanometers. Especially the development of fluorescent systems absorbing and emitting fluorescent radiation in the visible to near-infrared electromagnetic spectrum are of interest, due to reduced scattering coefficients of biological tissues at wavelengths in the 650 nm - 1600 nm region and the absence of autofluorescence in 1200 nm - 1800 nm range enhancing the signal-to-noise ratio [1,2].

Fluorescence quantum yield can further be enhanced by the establishment of a Förster resonance energy transfer system (FRET-system), incorporating the quantum dot as donor which absorbs light at a defined wavelength and subsequently transfers energy in a non-radiative manner onto a suitable acceptor such as lanthanides. The transfer depends on the distance between both chromophores, which led to the development of stable lanthanide-chelates immobilised onto nanoparticles [4,5]. With regard to its excellent chelating properties, the macrocyclic chelator 1,4,7,10-Tetra-azaacyclododecan-1,4,7,10-tetra-acetic acid, referred to as DOTA, is suitable for nanoparticle surface modification and can easily be tuned via its analogue 1,4,7,10-Tetra-azaacyclododecan-1,4,7,10-tetra-acetic acid (DOTAZA) comprising side-chain azide-groups, which can be functionalised in a Cu-mediated Click reaction [6,7]. Since phosphonates possess good metal surface binding properties, the modification of DOTAZA with alkyn phosphonates represents a promising approach for the establishment of efficient DOTA-based FRET-systems (Figure 1) [8]. In this work, the...
synthesis and analysis of DOTAZA-phosphonate based lanthanide-chelates immobilised onto nanoparticle is carried out.

Figure 1: DOTAZA based FRET-system.

References:

POSTERS

Exploration of fragments as starting points for multi-target drugs

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Introduction: Multi-target drugs are in the focus of pharmaceutical research and often exhibit superior efficacy and safety profiles. However, the identification of starting points for multi-target drug discovery is tedious. Fragments were proposed to be ideal starting points for multi-target drug design [1], but prospective applications of this technique are rare [2, 3]. In this study we systematically explore the possibilities to identify fragments as starting point for multi-target drug design.

Material and Methods: Five proteins, the soluble epoxide hydrolase (sEH), the S-lipoxygenase (5-LO), the leukotriene A4 hydrolase (LTA4H), the retinoid X receptor (RXR), and the farnesoid X receptor (FXR) were identified as starting points for multi-target drug design. A differential scanning fluorimetry (DSF) screening was used. In this approach, a shift in the melting point of the protein indicates a binding. As the retinoid X receptor (RXR), and the farnesoid X receptor (FXR) were cross validated using orthogonal activity assays. Five proteins, the soluble epoxide hydrolase (sEH), the S-lipoxygenase (5-LO), the leukotriene A4 hydrolase (LTA4H), the retinoid X receptor (RXR), and the farnesoid X receptor (FXR) were identified as starting points for multi-target drug design. A differential scanning fluorimetry (DSF) screening was used. In this approach, a shift in the melting point of the protein indicates a binding. As the retinoid X receptor (RXR), and the farnesoid X receptor (FXR) were cross validated using orthogonal activity assays.

Results: The DSF prescreen led to the identification of 22 fragments that were proposed to be ideal starting points for multi-target drug design. The fragments that showed a 1°C or higher shift in the melting point, indicating a binding, on more than one protein were then cross validated using orthogonal activity assays.

Acknowledgements: This study demonstrates the opportunities and limitations of a fragment based approach to identify novel starting points for multi-target compounds. Further studies are required to demonstrate fragment-to-lead optimization considering potency and efficacy.

References:
Overexpression of histone deacetylases (HDACs) is frequently observed in a wide variety of tumor diseases. Due to their repressive effect on gene transcription and their essential influence on drug resistance mechanisms of tumor cells, HDACs advanced to become interesting targets for the epigenetic therapy of cancer.[1] Glucocorticoid receptor multiform (GBM) represents one of the most common and aggressive primary brain tumors, with high resistance against standard therapies. The use of alkylating agents, such as temozolomide (TMZ), in combination with radiotherapy represents the current standard treatment of GBM after surgical resection of the tumor.[2] Recently, the first-in-class hybrid alkylating histone deacetylase inhibitor, tinostamustine, has been reported. Tinostamustine showed superior in vivo activity compared to bendamustine, temozolomide and radiotherapy as well as the potential to overcome drug resistance.[3]

In this study, we designed a scaffold containing a DNA-damaging cap group, different linker types and a hydroxamic acid moiety as warhead to aim at dual targeting. Notably, our parallel synthesis approach offers a straightforward and efficient procedure for the synthesis of HDAC-based multi-target drug libraries. Our preloaded resins are easy to handle and stable at least for six months stored at 4 °C. Furthermore, this approach circumvents the common difficulties of handling hydroxamic acids through the protection with our functionalized resin. The subsequent testing for in vitro activity against different HDAC isoforms showed the great potential of several compounds as HDAC1 and/or HDAC6 inhibitors with promising anticancer activities. Notably, our solid phase supported method yielded a hit compound with promising pan-HDAC activity and improved cytotoxicity in comparison to TMZ, miltefosine (MTZ) and chlorambucil. To the best of our knowledge, no hybrid DNA-alkylating HDAC inhibitors containing TMZ or MTZ have been reported so far. Thus, this series of compounds represents a solid basis for further evaluation of DNA-alkylating HDAC inhibitors and for gaining deeper knowledge of their anticancer properties.[4]

References:

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Gold(I/II) complexes with N-heterocyclic carbene ligands as antibacterial agents

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With a slow but steady increase of resistance in bacteria against current antibiotics there is a strong demand to identify new modes of action and to develop new and unconventional antibacterial agents.[1] Organometallic gold compounds have demonstrated promising properties as anticancer and antibacterial agents. Recently, organometallic complexes consisting of gold(I) and a N-heterocyclic carbene (NHC) ligand have been reported to exhibit high activity against the enzyme thiorodoxin reductase (TrxR), both in its bacterial and mammalian form.[2,3] The inhibition of bacterial TrxR is in particular detrimental for many Gram-positive bacteria based on the lack of glutathione, which can compensate for TrxR inhibition.[4]

The formation of class-A 4-hydroxyquinoline-G-Protein coupled receptor (GPCR) d- and oligomers has been proven after a long controversy.1 The existence of the D1-H3 receptor heteromer was published by Rafael Franco and his group. They could prove its existence in artificial cell systems as well as in the rat striatum and cortex, using different pharmacological techniques like co-immunoprecipitation, BRET-assays and bimolecular fluorescent complementation (BiFC). Furthermore, they found a heteromeric complex consisting of the D1-, H3-receptor and subunits of the NMDA-receptor.2,3 By inhibiting this complex with the H3R antagonist thioperamide the dopamine, NMDA and β1-α-Amyloid induced cell death could be reduced.4 These results show the neuroprotective potential of this complex. Therefore highly selective bivalent D1-H3 receptor ligands could be useful in the therapy and for the comprehension of neurodegenerative diseases such as Alzheimers Disease (AD). For the design of the first bivalent ligands we used the known H3R antagonist JNJ-5207852 and the D1R antagonist SCH-23390 as lead structures (Fig. 1).1-4 In a three-step synthesis derivatives of JNJ-5207852 with an additional terminal amino group or carboxylic acid were synthesized. A seven-step synthesis was established to yield derivatives of SCH-23390 with a variety of functional groups added. These additional functional groups allow the coupling of a linker, which is a crucial part of the bivalent ligand, because it must possess the correct properties in length, flexibility and solubility to allow both pharmacophores to bind to their respective binding pocket simultaneously. Starting from commercially available polyethylene glycol glyoxylic derivatives of different lengths, highly flexible and soluble linkers were synthesized. Furthermore, we prepared structures, that are rigidized by multiple aromatic and non-aromatic ring-structures. These linkers are coupled via amide coupling reactions or click chemistry to the pharmacophores to give the final bivalent ligands.
Synthesis started with the derivatization of the known D2R antagonist L-pharmacophore was connected to the centre part of the molecule. A cells.2 However, the functional consequences of this intricate heteromerization. Furthermore, it could also turn out to be a promising alternative for treating drug addiction.3 It is believed, that the H2R is involved in the transmission of histamine and other neurotransmitters (e.g. acetylcholine) in the central nervous system (CNS). Due to this assumption the development of CNS-penetrating H2R agonists is of great interest as they are probably going to stimulate postsynaptic H2Rs, which could have similar effects to treat cognitive disorders (e.g. Alzheimer diseases) as already reported H2R antagonists. The prototype of highly potent H2R ligands of the guanidine class is the weak partial agonist 3-[1H-imidazol-4-yl]propylguanide (SK&F-91486).4 Further development of this structure led to the highly potent ligands imipramine and amipramine.5 However, they showed a lack of receptor subtype selectivity and a poor bioavailability. In order to increase receptor subtype selectivity, especially towards the H2R and H3R, the imidazolyl head group was bioisostERICally replaced by a 2-amino-4-methylthiazolyl group. The alteration of alkylated guanidines via acylated guanidines to structural analogs has been doubted. Yet their existence has been proven and many heterodimers (Hets) have been found and investigated in recent years.1 The group of Rafael Franco detected a strong and selective D2-H3 receptor interaction at the membrane level of reserpinized mice and announce that preliminary results indicate promising effects on mice regarding to their anxiety behavior. Taking this compound as a lead structure, we tried to further improve pharmacological properties at the dopamine D3 receptors. This is attributed in particular to the 2-specificity towards other receptor family’s (e.g. dopamine). Preliminary assays will characterize the first heterobivalent ligands, which will soon be synthesized after linking the two pharmacophores previously mentioned by conducting click chemistry.


POS.14
Synthesis and pharmacological characterization of bivalent ligands for the D4-H2 receptor heteromer

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For many years the ability of rhodopsin-like class A G-Protein-coupled receptors (GPCRs) to form homo- or heterodimers and oligomers has been doubted. Yet their existence has been proven and many heterodimers (Hets) have been found and investigated in recent years.1 The group of Rafael Franco detected a strong and selective D2-H3 receptor interaction at the membrane level of reserpinized mice and could also provide evidence for D3-H4-Hets in co-transfected HEK-293 cells.2 However, the functional consequences of this intricate heteromerization are not fully understood but seem to play a crucial role concerning drug addiction. Therefore, the development of heterobivalent ligands is important to further explore the importance of receptor-heteromerization. Furthermore, it could also turn out to be a promising alternative for treating drug addiction.3

Synthesis started with the derivatization of the known D2R antagonist L-741,626 using 5 steps (Fig. 1).4 After alkylation of 5-hydroxyindole with 1-bromo-3-chloropropane followed by chloro substitution with azide the pharmacophore was connected to the centre part of the molecule. A derivative of the non-imidazole H2R antagonist JNJ-5207852 was obtained in a nine-step synthesis (Fig. 1).5 An aminofunctionality in para-position of the piperidine moiety was appended and coupled with chloroacetylethylation. The next step was another chloro/azide exchange. The centre part was synthesized from isothiophenyl dichloride and propargylamine and will be linked to the pharmacophores by forming triazole moieties.

**Fig.1 Structures of L-741,626 and JNJ-5207852**

Pharmacological characterization of the bivalent ligands will be performed by radioligand binding assays on Sf9 cell membranes for all histamine receptor-subtypes which are already established. Moreover, functional assays for histamine and for the dopamine receptors using biosensors (e.g. HaloTag and Nanoluc as well as split luciferase complementation techniques) are designed and will be used. These

DOCKING AND SYNTHESIS OF GluN2A SELECTIVE NMDA RECEPTOR ANTAGONISTS

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NMRA Receptors are heterotetrameric ion-channel glutamate receptors that require glutamate and glycine for activation. When activated it allows the positively charged ions to flow through the cell membrane. It controls synaptic plasticity and memory function. GluN2A containing NMDA receptors have been found to be involved in anxiety, depression and schizophrenia. The exact role of the NMDA receptor with GluN2A subunit in the disease process is still unclear. To better understand this role, potent and selective GluN2A inhibitors were designed and synthesized, hereby the ring B of TCN-201 was replaced by electron-rich 1,3-thiazole ring and 1,3-oxazole ring. In 2016, the X-ray crystal structure of the GluN2A subunit containing NMDA receptor with TCN-201 was solved. TCN-201 binds to the receptor at the interface between the GluN1 and GluN2A subunits by adopting a hair-pin or U-shaped conformation. To lock TCN-201 in this conformation, the rings A and B were replaced by a [2,2]Paracyclophane system. Docking studies suggested that compounds 1 and 2 should highly interact with the GluN2A receptor. The synthesized molecules will be tested soon.

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POS.17
Targeting LSD1 in Cancer Cells by Nitroreductase-mediated Prodrug Activation

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The lysine-specific demethylase 1 (LSD1 or KDM1A) has emerged as a highly promising therapeutic target in human malignancies and several drugs are already under current investigation in clinical trials. LSD1 is also established as a key effector of the differentiation block in acute myeloid leukaemia (AML), which may be selectively targeted by inhibitors, leading to a pronounced therapeutic effect. To increase therapeutic effectiveness and decrease side effects during treatment, we aimed to develop a specific targeting of leukemic cells with irreversible LSD1 inhibitors.

To achieve target-specificity, pharmacologically inactive and nontoxic forms of LSD1 inhibitor 1a with a nitro-aromatic system, so-called bioreductive prodrugs, are designed, synthesized and tested against LSD1 activity in-vitro and on cultured AML THP1 cells. As prodrug-activating enzyme, the E. coli Nitroreductase NfsB (NTR) was selected, that is introduced in leukemic cells using a virus-mediated transduction. By reduction of the nitro-aryl bioreductive system by the NTR, the active tritylcyproamine-based LSD1 inhibitor is subsequently released and forms a covalent adduct with the cofactor FAD leading to an irreversible inhibition of LSD1.

We identified promising prodrug/drug pairs by measuring the expression of CD86 surface marker and by performing colony-forming unit assays with THP1 cells. Several prodrugs are converted into the active parent drug by the NTR, which is solely expressed in transfected tumour cells. Depending on the nitro-aryl system, different activation patterns can be observed both in vitro and in vivo. By applying different targeting techniques such as antibody-directed enzyme-prodrug therapy (ADEPT) and gene-directed enzyme-prodrug therapy (GDEPT), these prodrugs provide a direction for more selective anti-cancer drugs. [3]

References

POS.18
Is a selective virtual screening possible?

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The Dual Leucine Zipper Kinase (DLK), also known as MAP3K12, is expressed in the peripheral and central nervous system and is associated with neurodegeneration and axon regeneration. Additionally, DLK is inhibiting insulin-gene transcription and secretion in beta cells. Therefore, inhibiting DLK represents an interesting concept for the treatment of diabetes.[7]

In our project, we strive for finding diverse potential ATP competitive inhibitors with novel scaffolds by using structure-based virtual screening. In this context, the question arose, whether our developed virtual screening protocol was able to find truly selective DLK inhibitors. To investigate this question, we used our screening protocol on another diabetes relevant kinase (GSK-3β)[2] as the (anti)target.[2]

Therefore, all screening steps were done with the most promising GSK-3β conformations. A dataset of 216 known active DLK inhibitors and about 16000 decoys (potential inactive compounds) were first docked by HTvS conformations. A consensus score was generated and enrichments were calculated for both datasets.
more different kinases (FGFR4, TTK, MAPKAPK2 and 3) [4] covalent engagement of this moiety by an electrophilic residue, a so-called "warhead" [5] might not only be exploited to achieve selectivity against p70S6Kα, but also within the kinase.

Amongst the aforementioned kinases, only FGFR4 has been covalently targeted so far. The irreversible FGFR4 inhibitor BLU9931 [6] makes use of an ortho-phenylenediamine-linked acrylamide warhead to trap the GK+2 cysteine. In order to selectively address p70S6Kα, we sought to combine the scaffold of the p70S6Kα inhibitor PF-4708671 [3] with the linker and warhead moiety of BLU9931.

For the preparation of reactive PF-4708671 analogs, we started with different 2,4-dichloropyrimidine derivatives and introduced N-protected piperazine in the 4-position via nuclophilic aromatic substitution (SNAr). The ortho-phenylenediamine linker was installed as the (substituted) 2-nitroaniline precursor by means of Buchwald-Hartwig-arylation. After reduction of the nitro group, acylation of the primary amine with acryloyl chloride was used to generate the acrylamide warhead. Finally, deprotection of the piperazine followed by the introduction of the N-substituents by nucleophilic substitution furnished a series of putatively covalent p70S6Kα inhibitors.

References:
[6] Haarer, L. 1; Laufer, S.A. 1 and Gehringer, M.1

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Comparison of the results revealed, that the known DLK inhibitors were highly enriched by using the DLK target structures, while using the GSK-3β target structures, no enrichment could be observed (Fig. 1).

To validate these findings, a dataset of known selective GSK-3β inhibitors were screened against both targets. We found, that GSK-3β selective inhibitors were not enriched in the DLK, but only in the GSK-3β target structures.

Fig. 1: Frequency distribution of the ligands in DLK (left) and GSK-3β (right); the consensus score is plotted on the x-axis with low values meaning high screened compounds; frequency of DLK- and GSK3β-inhibitors are shown enlarged 10 times.

References:
[6] Haarer, L. 1; Laufer, S.A. 1 and Gehringer, M.1

Design and Synthesis of Potential Covalent Inhibitors of the Protein Kinase p70S6K

Haarer, L. 1; Laufer, S.A. 1 and Gehringer, M.1

The mTOR (mechanistic target of rapamycin) signaling pathway is a key regulator of cell proliferation and growth, and inhibition of this pathway has been used in the treatment of cancer and immune disorders. [1] However, some of these signaling network remain poorly elucidated. The protein kinases p70S6Kα and β belong to the downstream effectors of the mTOR-complex mTORC1. While the physiological role of p70S6Kα is comparably well characterized, the specific functions of p70S6Kβ are less clear. A redundant functional profile of p70S6Kα and β was initially assumed, however, it is becoming increasingly apparent that these enzymes play distinct roles in mTOR signaling. [2]

So far, the characterization of p70S6Kβ-specific functions has been hampered by the lack of suitable chemical probes. While specific protein kinase inhibitors (PKIs) targeting p70S6Kα have already been developed, [3] no such tool compounds are available for p70S6Kβ. Achieving specificity for p70S6Kα constitutes a considerable challenge since the latter kinases share a virtually identical ATP binding site differing only in a single amino acid. As a distinctive feature, p70S6Kα possesses a tyrosine (Tyr-151) at the gatekeeper+2 (GK+2) position in the so called "hinge region" and this moiety is replaced by cysteine (Cys-150) in p70S6Kβ enabling covalent targeting approaches. Since an equivalently positioned cysteine is present in only four other, structurally

POS.19

Design, Synthesis and Optimization of Novel Tricyclic Janus Kinase 3 (JAK3) Inhibitors

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The four-membered family of Janus kinases (JAK1, JAK2, JAK3 and TYK2) plays a vital role in immune function, inflammation and hematopoiesis. This enzyme family belongs to the class of cytosolic tyrosine kinases, that are involved in various signalling cascades initiated by cytokines and growth factors mediating immune response and proliferation via DNA transcription in the nucleus. [1] While the expression of JAK1, JAK2 and TYK2 is ubiquitous, JAK3 is limited to hematopoietic tissues and has a key function in the maturation of lymphocytes. Therefore, JAK3 represents an interesting target for the development of immunosuppressive drugs. Tofacitinib, Pfizer’s pan-JAK inhibitor was approved for the treatment of rheumatoid arthritis by the FDA in 2012.2

Tofacitinib competively binds to the ATP pocket of JAK3 forming two hydrogen bonds from its heterocyclic towards the hinge region. The aliphatic side chain fills the ATP pocket towards the p-loop, while the exocyclic chiral methyl group of the piperidine side chain binds to a small
Several new synthetic routes of the new scaffold have been developed to enable derivatezation of different residues that allow for a more detailed SAR exploration. Derivatives with substituents at the C-3 position of the bicyclic core (R') addressing the hydrophobic region II of JAK3 as well as hydrophilic substituents in order to improve solubility have been prepared. Also, the influence of the chiral methyl group at the C-2 position (R') of the aliphatic side chain (X = C, N) on the inhibitory activity and selectivity in presence of small substituents at the C-3 position of the bicyclic core (R') has been investigated. In our attempt to characterize the new set of compounds as JAK3 inhibitors cellular data of chosen compounds was generated. In addition, an X-ray crystallography structure of JAK3 in complex with a nitric-substituted phenyl derivative could be achieved.

References:

In vitro studies on TLX druggability assessment and early ligand discovery

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Recent reports suggest that the nuclear receptor (NR) TLX (homologue of the Drosophila tailless gene, NR2E1), exclusively expressed in small areas of the hippocampus, might act as a master regulator of neurogenesis[1]. Disrupted TLX expression caused aggressiveness[2], mental illness[4] and brain tumor formation[5] in mice. Thus, TLX may hold promise as target to treat neurodegenerative diseases and brain tumor formation.

To date, no endogenous ligands are known for the orphan receptor TLX and the limited data on few putative synthetic TLX modulators is inconsistent. To assess the druggability of TLX, we have studied the receptors molecular function with several assay setups in vitro. We observed a remarkable inhibitory activity of TLX on several other NRs suggesting that TLX acts as transcriptional repressor.

To reflect this characteristic in an in vitro test system for TLX ligand discovery, we established a hybrid reporter gene assay involving VP69-Gal4 as a potent inducer of reporter transcription and TLX as repressor to enable a bidirectional modulation of TLX activity. Screening of a commercial fragment library revealed TLX ligands which exhibit positive in vitro test system for TLX ligand discovery. By expanding these results to structurally more intricate natural products and approved drugs, we identified TLX modulators with low micromolar potency that appear suitable for structural optimization towards potent TLX ligands.

Our preliminary results characterize TLX as repressor of various ligand-activated transcription factors in vitro and suggest that the receptor is druggable with small molecules either promoting or disturbing its transcriptional repressor activity.

References:

An interesting class of KOR agonists are compounds with an ethylenediamine substructure. U-50,488 was firstly synthesized in 1981 and is a highly selective KOR agonist (Ki = 0.51 nM). It consists of a tertiary amine embedded in a pyrroldine ring and a second N-atom acylated with a 2-((3,4-dichlorophenyl)acetyl) moiety. Both N-atoms are attached to a cyclohexane ring in trans-configuration ([1S,2S]-configuration). Further studies showed that besides the cyclohexane scaffold, a piperidine or piperazine structure can be used in which the ethylenediamine structure is partly embedded. In our work group, piperazine containing compound 1 was synthesized and the (S,S)-isomer showed the highest KOR affinity (Ki = 0.31 nM). In order to investigate the bioactive conformation, a bicyclic ethylene-diamine derivative 2 with a bridgehead N-atom was synthesized. By comparing the dihedral angles (N-C-C-N) of the endo- and exo-configured isomers, the endo-isomer with a dihedral angle of 58.3° was quite potent (Ki = 73 nM), while the exo-isomer with an angle of 168° was nearly inactive (Ki ≥ 1 µM). Still, with a Ki-value of 73 nM, endo-2 is less active than (S,S)-1 (Ki = 0.31 nM), we postulate that a dihedral angle of 58.3° is not the preferred angle of (S,S)-1 in its bioactive conformation. Our next goal is to change the dihedral angle by substituting the bridgehead nitrogen atom with a carbon atom and changing the ring size of the second ring. Moreover, the polarity can be changed by using different residues in 3-position of the bicyclic system.

References:

Design, Synthesis and Characterization of Novel Irreversible JAK3-Inhibitors with High Isoform Selectivity Based on a Tricyclic Hinge-Binding-Motif

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Strong analgesics are used to reduce the perception of pain by activation of classical opioid receptors MOR, KOR and DOR. Since centuries alkaloids in poppy seeds, which mainly activate MOR are used as pain killers and anaesthetics. However, they can cause severe side eects like euphoria, physical dependency, respiratory depression and inhibition of gastrointestinal motility. Therefore, in the last decades KOR agonists were developed as alternative analgesics. Besides their analgesic effect, KOR agonists are potent neuroprotective and antihyperalgesic agents. They do not interfere with the respiratory system, however, depression, diuresis and sedation can occur. Since KOR is not only located in the CNS but also in the periphery, activation of peripherally localized receptors can be used to treat visceral pain, itching skin and inflammatory diseases.

An interesting class of KOR agonists are compounds with an ethylenediamine substructure. U-50,488 was firstly synthesized in 1981 and is a highly selective KOR agonist (Ki = 0.51 nM). It consists of a tertiary amine embedded in a pyrroldine ring and a second N-atom acylated with a 2-((3,4-dichlorophenyl)acetyl) moiety. Both N-atoms are attached to a cyclohexane ring in trans-configuration ([1S,2S]-configuration). Further studies showed that besides the cyclohexane scaffold, a piperidine or piperazine structure can be used in which the ethylenediamine structure is partly embedded. In our work group, piperazine containing compound 1 was synthesized and the (S,S)-isomer showed the highest KOR affinity (Ki = 0.31 nM). In order to investigate the bioactive conformation, a bicyclic ethylene-diamine derivative 2 with a bridgehead N-atom was synthesized. By comparing the dihedral angles (N-C-C-N) of the endo- and exo-configured isomers, the endo-isomer with a dihedral angle of 58.3° was quite potent (Ki = 73 nM), while the exo-isomer with an angle of 168° was nearly inactive (Ki ≥ 1 µM). Still, with a Ki-value of 73 nM, endo-2 is less active than (S,S)-1 (Ki = 0.31 nM), we postulate that a dihedral angle of 58.3° is not the preferred angle of (S,S)-1 in its bioactive conformation. Our next goal is to change the dihedral angle by substituting the bridgehead nitrogen atom with a carbon atom and changing the ring size of the second ring. Moreover, the polarity can be changed by using different residues in 3-position of the bicyclic system.
The Janus kinase (JAK) family includes four cytosolic non-receptor tyrosine kinases that play important roles in crucial physiological processes like cell proliferation, differentiation, migration, hematopoiesis and regulation of the immune system. While three of the four family members (JAK1, JAK2 and TYK2) are ubiquitously expressed, JAK3 fulfils its important tasks only in cells of the immune system like B-, T- and NK cells.[1]

Recently approved unselective JAK-inhibiting drugs such as Tofacitinib, Ruxolitinib, Baricitinib or Pfeliitinib were used for immune-mediated diseases, like rheumatoid arthritis, inflammatory bowel diseases, autoimmune skin disorders or transplant rejection. However, these drugs suffer from frequent side effects resulting from unselective targeting of multiple JAK-pathways, which include increased infection rates, anemia and a risk of cardiovascular diseases and malignancies.[2, 3] In order to avoid this therapeutic difficulty, highly selective JAK3-inhibition is considered as a valuable strategy to reduce these adverse side effects.

Within our medicinal chemistry program for developing novel JAK3-selective inhibitors, we identified a tricyclic core scaffold with favourable properties for further optimization. In a small sub-project, we tried to address a non-conserved cysteine near the ATP binding pocket of JAK3, that cannot be found in other members of the JAK-family. Different substitution patterns were investigated to identify a suitable position of a phenylacrylamidewarhead to access the cysteine in an optimal angle for covalent bond formation. While the first derivatives A and B only showed moderate inhibition of JAK3, compound C exhibited a high potency at low nanomolar concentrations. In stark contrast, a close analogue of C containing a non-reactive propionamide displayed an about 2500-fold weaker potency on JAK3 and therefore strongly supports the hypothesis of covalent inhibition by C.[4, 5] Further derivateisation of this lead structure allowed to explore the SARs of this inhibitor class and even resulted in additional selectivity within the JAK-family. Two key compounds were tested for their selectivity within a set of kinases, that are carrying cysteines in similar positions in the hinge binding pocket. Furthermore, an in-silico model was created to show a possible binding mode of structure C.

Janus kinases (JAKs). This kinase family, consisting of four closely related isoforms (JAK1, JAK2, JAK3 and TYK2), is a key player in the regulation and homeostasis of the immune system.[1] Since JAK3 is the only family member, which is exclusively operating in immunocompetent cells, it earned a special attention in the last years, as a promising target for the development of a new class of immunosuppressants.[2]

Due to the high structural similarity of the four JAKs, the development of isoform specific inhibitors is a challenging task. However, another unique feature of JAK3 is the presence of a non-conserved cysteine residue (C909) nearby the ATP binding site, which is replaced by a serine in the other three family members. A high JAK3 selectivity can be therefore achieved by the targeting of C909 with electrophilic inhibitors, a strategy that has already been successfully applied by our group as well as others.[3-5]

In a new approach to develop irreversible JAK3 inhibitors, we postulated that an alteration of the commonly observed hinge-binding pattern of purine isosteres would favor the 3-position of the five-membered pyrrol ring as possible attachment point for electrophilic warheads to target C909. We developed a modular synthetic route which allowed us to prepare a small set of 7-Azaindoles carrying an electrophilic warhead in 3-position. Some of the new inhibitors showed high potency on JAK3 together with a highly increased isoform selectivity. The proof-of-concept was verified by XRay crystallography showing both, the alternative orientation of the hinge binding motif as well as the covalent bond formation between the inhibitor and C909 of JAK3. A moderate activity and target engagement in a cellular setting was also shown for these early compounds, which can be further optimized in ongoing optimization cycles. The low molecular weight of this novel core scaffold offers possibilities for further modifications in order to modulate physicochemical properties or even further increase potency and selectivity. Therefore, these compounds can serve as a promising starting point for the development of a novel class of covalent JAK3 inhibitors.

References:

**POS.25**

Dual Inhibition of STS and 17β-HSD1: A Novel Drug-Prodrug Approach for the Treatment of Endometriosis

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Estrogens, especially the most active one estradiol (E2), are well known to play a crucial role in the development of estrogen-dependent diseases like breast cancer [1] and endometriosis [2]. Local E2 biosynthesis in endometriosis is essentially affected by the key enzymes steroid sulfatase (STS) and 17β-hydroxysteroid dehydrogenase type 1 (17β-HSD1) (Fig. 1). Both enzymes are overexpressed in endometriotic tissue and lead to a strong local E2-production and thus push the progression of the disease. Consequently, inhibition of STS and 17β-HSD1 is an attractive concept for the treatment of endometriosis [3, 4]. 17β-HSD2 is the physiological counterpart of the type 1 enzyme and should not be inhibited. Recently our group published the first dual STS/17β-HSD1 inhibitors (DShHIs) [5] which show strong inhibition of both enzymes.

**POS.24**

Flipping the Hinge-Binding-Motif: 3,5-disubstituted 7-Azaindoles as Promising Scaffold for the Development of Covalent JAK3 Inhibitors

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The physiologically important processes of proliferation, differentiation and maturation in haematopoietic cells are mainly driven by cytokines like interleukins (ILs), interferons (IFNs), and growth factors. The signal pathways initiated by these extracellular stimuli are often mediated by the
These compounds bear a sulfamate group for STS inhibition and a phenolic OH-group entailing 17β-HSD1 inhibition (Fig. 2, A). The fact that the sulfamate group is cleaved upon interaction with STS and non-enzymatic hydrolysis releasing the corresponding phenol gives rise to an intriguing design concept for inhibition of the two target enzymes (drug-prodrug approach). We aimed at synthesizing compounds in which the OH group is masked as a sulfamate group (Fig. 2, B). These compounds should be drugs for inhibition of STS and, at the same time, produgs for 17β-HSD1 inhibition. Eight sulfamate compounds were synthesized, of which five showed strong inhibition of STS in a cellular assay (IC50 = 13–28 nM). The corresponding phenols were also synthesized and displayed potent 17β-HSD1 inhibition in cellular (IC50 = 5–20 nM) as well as high selectivity over 17β-HSD2. Stability measurements in buffer and DMEM revealed that the sulfamate compounds (STS inhibitors) hydrolyzed to the corresponding phenols (17β-HSD1 inhibitors), with half-life times ranging from minutes to hours depending on the substitution pattern of the compounds. In an enzymatic assay which in a time dependent manner monitors the classical hydrolysis of the sulfamates to phenols and increase in 17β-HSD1 inhibition, we found a strong correlation between phenol formation and enzyme inactivation. These results show that the drug-prodrug concept was successfully implemented. Thus, the novel compounds are potential therapeutics for the treatment of endometriosis.

Figure 1. The sulfatase pathway of local E2 biosynthesis.

Figure 2. Design rationale (drug-prodrug approach).

Development of RPA Protein-Protein Interaction Inhibitors as Novel Therapeutic Agents for the Treatment of Pancreatic Cancer

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The replication protein A (RPA) is an essential protein, which is able to bind to ssDNA in human cells. It is involved in diverse DNA metabolism pathways such as replication, repair, recombination, cell cycle regulation and DNA damage checkpoints [1]. It is inhibition of RPA leads to genome instability and, in case of high proliferating cell lines such as cancer cells, to replication catastrophe followed by cell death. On the other hand, in healthy transgenic mice shRNA mediated suppression of RPA is well tolerated and opens a therapeutic window up to nine days. Therefore, RPA inhibition represents a promising therapeutic target for the treatment of proliferative disorders [2] (unpublished data).

Structurally, RPA is a heterotrimetric complex consisting of three subunits RPA1 (70kDa), RPA2 (32kDa) and RPA3 (14kDa). Henricksen et al. showed that the subcomplex of RPA2 and RPA3 is a precursor in the assembly of the complete RPA complex [3]. Inhibition of the subcomplex formation can consequently disrupt the RPA complex’s overall function.

Fig. 1. Proposed interaction of a leading compound (RPAi) within the RPA’s hot spot

We identified a hot spot in the RPA2-RPA3 subcomplex crystal structure, which we exploited for in silico small molecule screening for new inhibitors (Fig. 1). We were able to identify a hit compound with a low µM activity in vitro EMSA assays. Following a classical structure optimization workflow, supported by molecular modelling, we developed a series of highly potent compounds. These inhibitors showed good efficacy against pancreatic cancer cell lines with IC50 values in the double-digit nM range and favorable off-target activity shown by EMSA assays. Additional target engagement evidence was further corroborated by 2D-NMR protein binding studies [4] with the best suitable compound of the series (unpublished data).

Since the target activity is established, the further development of our lead compound focuses on the optimization of the ADME-properties to finally create an initial clinical candidate for the treatment of pancreatic cancer.

Acknowledgements:

The authors would like to thank Khalil Samarah for the support in chemical synthesis and characterization of final compounds during his master thesis.

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POS.26

Targeting the Osimertinib-Resistant L858R/T790M/C797S EGFR Mutant: Design, Synthesis, Biochemical and Crystallographic Evaluation of Low Nanomolar Inhibitors

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The treatment of non-small-cell lung cancer (NSCLC) with epidermal growth factor receptor (EGFR) inhibitors is made challenging by acquired resistance caused by somatic mutations [1]. Third generation EGFR inhibitors (WZ4002, Osimertinib) have been designed to overcome resistance, mediated by the T790M mutation, through covalent binding to the Cys797 residue of the enzyme. These inhibitors are effective against most clinically relevant EGFR mutations, however their high
Based on a selectivity screening of a highly potent reversible p38 inhibitor [4], we identified EGFR inhibition as an off-target effect of this compound. High potency, as well as moderate physicochemical properties and cellular activity against p38, led us to pick this compound as a lead structure for further improvements in terms of mutant EGFR inhibition.

With this concept, we have successfully developed highly potent reversible and irreversible T790M EGFR inhibitors that showed picomolar IC_{50}-values in an enzyme assay and down to 14 nM EC_{50} in a cellular assay against p38, which led to tumour relapse in initially responding patients [2,3].

In contrast to classic third generation EGFR inhibitors, some of these compounds showed high inhibitory activity in the low nanomolar range against the therapy-resistant L858R/T790M/C797S EGFR triple mutant [5-8]. Moreover, to further support the concept of the binding modes and enlighten specific interactions we determined two X-ray crystal structures for both a reversible and an irreversible compound.

Ligands for the bromodomain and extra-terminal domain (BET) family of ligands for the bromodomain and extra-terminal domain (BET) family of histone demethylases.

Fe(II)- and 2-oxoglutarate-dependent Jumonji C domain-containing histone demethylases (JmC KDMs) play an important role in the regulation of epigenetic processes by oxidatively removing methyl marks from distinct lysine residues. As their aberrant expression is implicated in numerous diseases, particularly cancer, KDM-inhibitors possess a promising therapeutic potential [1,2]. Aiming for the development of new compounds led to significant H3K36 hypermethylation indicating on-target activity by in-vivo inhibition of KDM4.

Figure 1 Deferasirox docked to KDM4A

We thank the DFG (CR3962) for funding.


POS.29

Synthesis and evaluation of phosphonic acids for the BET bromodomain ligands: Probing the WPF shelf to improve BRD4 bromodomain affinity and metabolic stability

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Ligands for the bromodomain and extra-terminal domain (BET) family of histone demethylases are promising therapeutic agents for treating a range of cancers and inflammation [1,2]. We were able to show that our previously developed 3,5-dimethylisoxazole-based BET bromodomain ligand (OXFBD02) [3] inhibits the interactions of BRD4(1) with the RetA subunit of NF-κB, in addition to histone H4. This ligand showed a promising profile in a screen of the NCI-60 panel but was rapidly metabolised ([t_{1/2} = 39.8 min]). Structure-guided optimisation of compound properties led to the development of the 3-pyridyl-derived OXFBD04. Co-crystallization of OXFBD04 with BRD4(1) in combination with molecular dynamics (MD) simulations and NMR titration experiments assisted our understanding of the role played by an internal hydrogen bond in altering the affinity of this series of molecules for BRD4(1). OXFBD04 shows improved BRD4(1) affinity (K_{D} = 126 nM), optimised physicochemical properties (LE = 0.43; SFI = 5.96), and greater metabolic stability ([t_{1/2} = 388 min]) [4].

![Image](image-url)


POS.28

Synthesis and Biological Evaluation of derivatives of Deferasirox as Inhibitors for JumonjiC-domain containing Histone Demethylases


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OXFBD04 shows improved BRD4(1) affinity ([K_{D} = 126 nM]), optimised physicochemical properties ([LE = 0.43; SFI = 5.96]), and greater metabolic stability ([t_{1/2} = 388 min]). Structure-guided optimisation of compound properties led to the development of the 3-pyridyl-derived OXFBD04. Co-crystallization of OXFBD04 with BRD4(1) in combination with molecular dynamics (MD) simulations and NMR titration experiments assisted our understanding of the role played by an internal hydrogen bond in altering the affinity of this series of molecules for BRD4(1). OXFBD04 shows improved BRD4(1) affinity ([K_{D} = 126 nM]), optimised physicochemical properties ([LE = 0.43; SFI = 5.96]), and greater metabolic stability ([t_{1/2} = 388 min]).

![Image](image-url)

Improved high-yield synthetic strategy of the dibenzosuberone scaffold for p38α MAP kinase inhibitors

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The p38α MAP kinase (MAPK) plays a crucial role in the regulation of the biosynthesis of pro-inflammatory cytokines like TNF-α, IL-1β and ATF-2. These cytokines are involved in the pathology of several diseases, such as cancer or autoimmune disorder. Hence, p38α MAPK inhibitors are potential candidates for the treatment of these severe diseases. Our group has previously developed the potent and highly selective p38α MAPK inhibitor Skepinone-L (fig. 1), which is currently in use as high-quality chemical probe and clinical candidate 1,2,3.

Figure 1: Based on the dibenzosuberone scaffold, decorations moved away from ethers to esters and amides.

The latest published synthesis of the dibenzosuberone scaffold consists of six steps with a limited yield of 37% 1,4. In addition, this synthetic route has a drawback of unstable reagents, which is especially problematic for a larger scale synthesis. To address this, an improved synthesis is required. In our contribution we describe a new synthetic route with reduced steps starting from commercially available starting material and improved overall yield.

Our initial trials to facilitate the dibenzosuberone synthesis consisted of a three-step process involving Heck coupling, reduction of the double bond and consequently cyclization via Eaton’s reagent resulting in an improved overall yield. In combination with its localization in the lysosome, Rhodesain is indeed crucial for cell growth and the parasite viability. [5,6,7]

For further optimization of these inhibitors, it is essential to get insights into drug-target interactions regarding thermodynamics, kinetics and structure-activity-relationship. Therefore, we developed an optimized method for recombining expression of catalytically active Rhodesain. In this method, the enzyme is expressed as an inactive precursor containing a N-terminal pro-domain. Besides protein trafficking and proper folding, this pro-domain works as active-site directed inhibitor. The crystal structure of pro-Rhodesain was recently solved by our group. By taking advantage of the inactive Rhodesain pro-form that occurs during the protein purification, we were able to selectively label an additionally introduced cysteine residue of the proenzyme without addressing the much more reactive active site cysteine. The thus biotinylated enzyme was immobilized and used in SPR experiments, giving insights into the binding kinetics of our compounds. Furthermore, we used ITC experiments to investigate the thermodynamic parameters of the binding events.

References:
Design and synthesis of a radiotracer for ecto-5'-nucleotidase (CD73) – a novel target for the immunotherapy of cancer

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Ecto-5'-nucleotidase (CD73) is a member of the ecto-nucleotidase family, which catalyzes the hydrolysis of nucleoside monophosphates, mainly AMP, producing the signaling molecule adenosine. Further ecto-nucleotidases include the nucleoside triphosphate diphosphohydrolases (NTPDases; subtypes 1, 2, 3 and 8), the nucleotide pyrophosphatases/phosphodiesterases (NPP1-4) and the alkaline phosphatases (APs).[1] CD73 is often co-localized with adenosine receptors. CD73 inhibitors reduce extracellular adenosine levels, which results in an indirect blockade of adenosine receptor activation. Many tumor cells over-express ecto-nucleotidases, which metabolize pro-inflammatory ATP into anti-inflammatory, immunosuppressive, tumor growth-stimulating, and angiogenic adenosine.[2] Therefore, ecto-nucleotidases possess great potential as novel drugs for the (immuno)therapy of cancer and infections. The ADP analogue α,β-methylene-ADP (AQPCP, \( K_i = 88.4 \) mM, human CD73) has recently been used by our group as a lead structure for the development of potent and selective competitive CD73 inhibitors.[3-5] One of these compounds, PSB-12651 (\( K_i = 1.23 \) mM, rat CD73), was selected as a lead structure to develop a tritium-labelled radioligand for CD73. [HPSB-17230] represents a high-affinity tracer which is anticipated to become a useful tool for biological studies, drug screening, and diagnostic applications.

References:

MALDI-TOF Mass Spectrometry Profiling of Bacteria Coupled with Machine Learning Techniques Identifies Mode of Action of Antibacterial Drugs

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There is a growing unmet medical need for new antibiotics to battle the increased frequency of resistance among bacterial pathogens (1). Despite years of target-oriented approaches, few new drug classes or drugs with novel modes of action have entered the antibiotics market. Meanwhile, the main antibiotic targets remain cell wall synthesis proteins, bacterial DNA gyrase, and the bacterial ribosome (2). The main problem associated with the failure of biochemical assays to deliver new drugs is that they do not take membrane permeability into account. This is especially a problem in the case of Gram negative pathogens. (3) In recent years, the shortcomings of biochemical target-based assays led to a renewed interest in phenotypic screening procedures (4, 5). For antibacterial activity, growth inhibition of bacterial cell cultures provides a straightforward readout, but offers no further information with respect to which target sites are engaged and is limited to compounds with relatively high activity (2, 5).

We present a protein mass fingerprinting method using MALDI-TOF MS in combination with data-dependent, supervised machine learning methods, to identify specific phenotypic response signatures generated in bacterial cell cultures upon exposure to well-characterized antibiotics at sub-lethal concentrations. The approach, denoted PhenoMS-ML, proves useful in the elucidation of mechanism of action in a drug screening setting. We show that the classification models generated with the method identify and classify the mode of action of unknown antibacterial agents in wild-type Escherichia coli and Staphylococcus aureus with over 90% accuracy. The method allows for the sensitive,
label-free, and high-throughput identification of drug target mechanisms at sub-lethal concentrations in a biologically relevant, phenotypic setting.

This work was funded by the basic government funding of Heidelberg University (Baden-Württemberg, Germany).

(1) O’Neill: Review on Antimicrobial Resistance (May 14th 2015)

Conformational states of the flaviviral protease

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Drug development is often complicated in the case of multiple enzyme conformations that affect the binding site, as it is for the flaviviral protease. Infections by flaviviruses, such as dengue, Zika, West Nile, and Japanese encephalitis viruses, represent a global health burden, with no drugs currently available. The protease is an attractive target, since it is responsible for the processing of the viral genome during replication into structural and non-structural proteins (NS).1 The enzyme is a trypsin-like serine protease, composed of NS2B and NS3.2 The first X-ray structures released showed two states, open and close based on the relative position of NS2B with respect to NS3.3 We perform an analysis covering the entire repertoire of 45 crytal structures in the protein data bank for flaviviral proteases, focusing on structures released in the past three years.4 The study reveals an array of conformations for this protein, beyond the reported open and close forms. We pinpoint the main criteria to distinguish different conformations such as the position of NS2B and the orientation of the oxyanion hole, and identify the impact of changes in the folding of NS3 on the spatial distribution in the active site. The array of conformations of the protease appears as different stages in a dynamic movement of the NS3 conserved residues 151-153. We show in one conformation a subpocket extending from the catalytic aspartate 75 to a pocket at the back of the protein, where inhibitors show an unusual binding mode. The finding provides a different view of the flaviviral protease. It is particularly of value for researchers concerned with the study of the protein mechanism of action, drug optimization by molecular modeling and docking studies, and strategies for inhibitor design and synthesis in medicinal chemistry.

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References

Dimeric Strychnine Oxime Ethers as Bivalent Ligands Targeting Glyine Receptors

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The most pronounced pharmacological action of strychnine, the major alkaloid from Strychnos nux vomiaca, is an antagonistic activity at glycine receptors (GlyRs). GlyRs are chloride channels composed of five subunits (α or β) and linked to hyperpolarisation and inhibition of neuronal firing.1 The crystal structure of human α3 GlyR in complex with strychnine revealed five equivalent strychnine binding sites located at the interfaces of the subunits.2 Recently, our groups have become interested in a bivalent ligand approach for targeting GlyRs. In particular, a (E)-oxime ether group at C-11 of strychnine has been identified as a possible linker to be incorporated into strychnine-based bivalent ligands.3 Oxime ethers of (E)-11-isotrotoxostrychnine – C=H=CR with R = Me, nPr, nBu, and n-pentyl were only up to 3-fold less potent antagonists compared to strychnine at homocerc α1 and heterocerc α1β GlyRs.4 Unfortunately, numerous attempts to isolate a first dimeric ligand from a reaction of (E)-11-isotrotoxostrychnine with 1,6-dibromohexane in different ratios, using different solvents and bases failed. Here, we describe an alternative approach towards dimeric strychnine oxime ethers. Briefly, (E)-11-isotrotoxostrychnine was O-alkylated using Br-(CH2)n-NHBoc and, after Boc-deprotection, the resulting amines were coupled with dicarboxylic acids HOOC-(CH2)n-COOH. The final compounds were pharmacologically evaluated on homocerc (α1), and heterocerc (α1;β) GlyRs in a functional fluorescence-based and a whole-cell patch-clamp assay, and their antagonist potencies were compared to those of strychnine.

Acknowledgments: Deutscher Akademischer Austauschdienst, Bundesministerium für Bildung und Forschung

References:

Exploiting Synergies in NASH

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Non-alcoholic fatty liver disease (NAFLD) occurs as the first manifestation of the metabolic syndrome in the liver with an alarming prevalence of 14 – 27% of the industrialized world’s population.[1] Even worse, 5 – 20% of the patients develop an irreversible steatohepatitis (NASH) that can progress to fibrosis, cirrhosis and hepatocellular carcinoma if untreated. Hence, there is a strong demand for novel anti-steatotic and anti-inflammatory drugs to treat the condition at early stage. The nuclear peroxisome proliferator-activated receptors (PPAR, NR1C1-3) and the farnesoid X receptor (FXR, NR1H4) modulate lipid metabolism as well as inflammation and their therapeutic efficacy as targets in NASH has been demonstrated in clinical trials.[2-5] While FXR agonist oclacitabolin[6] demonstrates anti-steatotic and anti-fibrotic effects in liver,[8] PPARα (e.g. activated by elobraban) regulates fatty acid oxidation in key metabolic tissues such as skeletal muscle thereby improving liver health indirectly.[7] Furthermore, PPARα activation has anti-inflammatory properties. The extrahepatic effects of PPARα and the hepatic FXR activation promise synergistic efficacy. The validity of dual pharmacotherapeutic approaches has recently been evidenced in mouse models[8,10] We have identified antrhronic acid derivatives as excellent lead structures for dual PPARα/FXR modulator development (pEC50 up to 7.2 for PPARα and pEC50 up to 5.6) [11] In the present study, we desire to yield nanomolar dual PPARα/FXR agonists that will be evaluated in pre-clinical in vivo studies for their ability to cure NASH. The structure-activity relationship of the acidic head, the antrhronic acid core, and the lipophilic tail revealed several molecules boosting the potency independently for either PPARα (pEC50 up to 7.2) or FXR (pEC50 up to 6.8) and introducing selectivity over PPARα and
PPARγ. These structural motifs can now be recombined in one molecule to come up with a balanced, nanomolar dual PPARα/FXR agonist with enhanced solubility and might evolve as a clinical candidate for NASH treatment.

Acknowledgements. P. Hetti gratefully acknowledges financial support by the Else Kröner-Fresenius-Stiftung, Translational Research Innovation - Pharma (TRIP).

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POS.38

Carbamoylguanidines as CNS-penetrating H2R agonists and their physiological role in the brain: Potential drugs for neurodegenerative diseases?

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In the last four decades the development of histamine H2 receptor (H2R) ligands led to various classes of potent agonists. Coming from 3-(1H-imidazol-4-y)propylguanidine (SK&F 81468), a weak partial agonist, imidazole-containing dianilaylated guanidines like imipramine and armodipine turned out to be highly potent full agonists at the H2R.2,3 Beside their poor characteristics with respect to bioavailability, both compounds showed up a lack of selectivity towards the H1,3,4-R. Bioisosteric exchange of the imidazole by 2-amino-4-methylthiazole eliminated the selectivity drawbacks, while amendment of the acylated guanidine group to acylated guanidines was a successful approach to improve bioavailability and blood-brain barrier (BBB) penetration.4,5 As the acylguanidines showed up with stability problems due to hydrolytic cleavage further development to carbamoylaguanyldes brought highly stable, selective and potent H2R ligands.6

Figure 1: Structures of the carbamoylated guanidines targeting the histamine H2 receptor.

Most recently around one hundred compounds, including bivalent ligands, were synthesized in our group to further investigate structure-activity relationships for the histamine H2 receptor.7,8 In current projects we focussed on the development of CNS-penetrating H2R agonists (Figures 1), to get more insight about the physiological role of the H2R in the brain. Since the H2R is also described to be involved in the brain histaminergic neurotransmission and other central neurotransmitters (e.g. acetylcholine), H2R agonist could have similar effects to treat cognitive disorders (e.g. Alzheimer’s disease) as already reported H3R antagonists. The polarity of known H2R agonists is the major challenge in the search of candidates with suitable properties to cross the BBB, while maintaining selectivity towards the H1,3,4-R subtypes. Our approach resulted in UR-Po563, an enantiopure (ee > 99%) highly potent and selective agonist at the H2R (pK(α) (H2R) = 7.60; pEC50 = 8.12, Emax = 0.95 (pGlu2-HR-atrium)), which is currently tested in vivo on several behavioral models including anxiety-related tests at mice. As of now, preliminary data exhibit very promising effects of UR-Po563 in the ‘Tail Suspension Test’. We are currently testing the abrogative effect of multiple antagonists (CNS-penetrant H1 and H2R antagonists) on the effects provided by UR-Po563. In addition, experiments are underway to investigate and possibly treat peripheral side effects on the heart and stomach.

References:

POS.39

Abstract withdrawn.

POS.40

Pyridinylimidazole-based inhibitors of CNS kinases.

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Protein kinases can be considered as the drug targets of 21st century. Since the approval of imatinib in 2001, more than 40 other protein kinase inhibitors have been launched to the market. The main focus of pharmaceutical research, however, is the development of protein kinase inhibitors for the treatment of cancer (> 85% of approved drugs).

Although there is strong evidence in the literature that inhibition of certain protein kinases expressed in the brain may result in neuroprotective effects and slow down the progression of the neurodegenerative disease, the development of CNS-penetrant kinase inhibitors has only been promoted in recent years.

Using rational approaches, we developed novel, potent, metabolically stable (and brain penetrant) inhibitors of different CNS kinases based on our pyridinylimidazole-derived p38α MAP kinase inhibitor LN9504,5 (Fig. 1).

References:
Despite the fact that the drug flupirtine has been on the market for many years (1986-2018), there is still incomplete understanding concerning its metabolism. It was approved as a non-opioid analgesic with a unique mechanism of action, namely the opening of voltage-gated potassium channels (Kv7.2/3) in the central nervous system. However, the reoccurrence of rare but severe liver injuries associated with the drug’s intake led to the recent withdrawal of flupirtine. It has been postulated that a quinone diimine, a reactive metabolite formed via oxidative metabolism of flupirtine, is responsible for the hepatotoxicity. After oral administration of flupirtine, the main metabolites found in human urine were the N-acetylated analog D13223, 4-fluorohippuric acid, mercapturic acid derivatives and unchanged drug. For D13223 the metabolic pathway has been elucidated whereas the pathway for the biological inactive 4-fluorohippuric acid is still unclear. We have initiated in vitro investigations by using HPLC to clarify if 4-fluorohippuric acid can be formed by the proposed metabolic pathway described below. First, the carbamate group of flupirtine (1) is cleaved by hepatic esterases. Next, facile oxidation of the product (2) to quinone dimines (3 and 4) is proposed. Through hydrolysis of the imine (4), produced by tautomeration of quinone diimine, 4-fluorobenzaldehyde (5) could be formed. The latter is further oxidized by aldehyde dehydrogenase (ALDH), resulting in the formation of 4-fluorobenzoic acid (6), which can be enzymatically coupled with glycine to yield 4-fluorohippuric acid (7). This poster will present results of these studies, which indicate that the proposed pathway is possible.

Gold organometallic compounds have been extensively investigated as potential anticancer metallodrugs and have shown a high potential regarding antiproliferative effects [1,2,3,4]. Enhanced stability is a driving argument for the design of gold(I)(NHC) based drugs, the more surprising is the lack of methods for pharmaceutical analytics about their stability and solution chemistry. Such analytical methods are important key elements for future metabolomic investigations and will help to ensure a better understanding of their biological behavior [5]. We selected complexes of the type of alkynylgold(I)(NHC) for detailed investigations of the metabolic pathway. Investigations of the metabolic pathway of flupirtine metabolite 4-fluorohippuric acid in vitro by HPLC

**POS.41**

**Basal Histamine H4 Receptor Activation: Agonist Mimicry by the Diphenylalanine Motif**

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Histamine H4 receptor (H4R) orthologs exhibit species-dependent basal (constitutive) activity. In contrast to mouse H4R (mH4R), human H4R (hH4R) shows an extraordinarily high degree of basal activity. In a previous molecular-pharmacological study, the mutation F169V significantly decreased the high basal activity of hH4R, and the basal activities of the hH4R-F168A and hH4R-F169V+S179M mutants were even comparable to that of mH4R [1,2]. In contrast, the basal activity of hH4R was maintained in the S179M mutation. Driven by such promising results, we aimed at further investigating the molecular mechanism of basal H4R activation by performing 2 µs molecular-dynamics simulations of six H4R variants (hH4R, hH4R variants S179M, F169V, F169V+S179M, F168A, and mH4R), each revealing a different degree of basal activity. Most interestingly, during the MD simulations, F169V ECL2.55 dips into the orthosteric binding pocket only in the case of hH4R, thus adopting the role of an agonist and contributing to the stabilization of the active state. Strikingly, the overall distances between the Cα atoms of D94 3.32 and A2986.30, a measure of TM VI outward movement and GPCR activation, significantly decreased the high basal activity of hH4R, and the basal stabilities of TM VI, rigidity analysis was able to correctly predict the basal activation and from rigidity analysis provide a molecular explanation for differential basal activities of H4R variants. Moreover, this study has provided novel insights into molecular mechanisms of basal H4R activation that are also of importance for other GPCRs.

**POS.42**

**Investigations of the metabolic pathway of flupirtine metabolite 4-fluorohippuric acid in vitro by HPLC**

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Despite the fact that the drug flupirtine has been on the market for many years (1986-2018), there is still incomplete understanding concerning its metabolism. It was approved as a non-opioid analgesic with a unique mechanism of action, namely the opening of voltage-gated potassium channels (Kv7.2/3) in the central nervous system. However, the reoccurrence of rare but severe liver injuries associated with the drug’s intake led to the recent withdrawal of flupirtine. It has been postulated that a quinone diimine, a reactive metabolite formed via oxidative metabolism of flupirtine, is responsible for the hepatotoxicity. After oral administration of flupirtine, the main metabolites found in human urine were the N-acetylated analog D13223, 4-fluorohippuric acid, mercapturic acid derivatives and unchanged drug. For D13223 the metabolic pathway has been elucidated whereas the pathway for the biological inactive 4-fluorohippuric acid is still unclear. We have initiated in vitro investigations by using HPLC to clarify if 4-fluorohippuric acid can be formed by the proposed metabolic pathway described below. First, the carbamate group of flupirtine (1) is cleaved by hepatic esterases. Next, facile oxidation of the product (2) to quinone dimines (3 and 4) is proposed. Through hydrolysis of the imine (4), produced by tautomeration of quinone diimine, 4-fluorobenzaldehyde (5) could be formed. The latter is further oxidized by aldehyde dehydrogenase (ALDH), resulting in the formation of 4-fluorobenzoic acid (6), which can be enzymatically coupled with glycine to yield 4-fluorohippuric acid (7). This poster will present results of these studies, which indicate that the proposed pathway is possible.

Gold organometallic compounds have been extensively investigated as potential anticancer metallodrugs and have shown a high potential regarding antiproliferative effects [1,2,3,4]. Enhanced stability is a driving argument for the design of gold(I)(NHC) based drugs, the more surprising is the lack of methods for pharmaceutical analytics about their stability and solution chemistry. Such analytical methods are important key elements for future metabolomic investigations and will help to ensure a better understanding of their biological behavior [5]. We selected complexes of the type of alkynylgold(I)(NHC) for detailed stability studies by HPLC-DAD-MS, in comparison to the well-known antineoplastic agent Auranofin. A RP-based chromatographic method
New pathoblockers: highly potent and selective inhibitors of Clostridial collagenases


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Microbial infections are still a constant threat to public health and since daptomycin (a cyclic lipopeptide), which was first reported in 1987 and introduced as a drug in 2003, no new classes of antibiotics have been approved.[1] Even towards this new compound, first resistances occurred just a few years after introduction onto the market, which shows the urgent need for more antibiotics with new modes of action.[2]

Virulence factors are known to play a major role in infections and are therefore an attractive new target. So-called ‘pathoblockers’ address those virulence factors instead of the survival factors of bacteria. As the bacteria are not killed but “disarmed”, a pathoblocker exerts reduced selection pressure; accordingly, bacteria are then less prone to the development of resistances.[2]

The extracellular zinc metalloprotease collagenase H (ColH) from the myonecrotic Clostridium histolyticum is such a prominent virulence factor for the progression of Clostridia-associated diseases. This protease destroys the host’s connective tissue, thus leading to improved host invasion and colonisation. Also, nutrition supply as well as the spread of toxins into the damaged tissue are promoted.[3] Besides the major role of ColH in disease development, its extracellular localisation makes it a starting point for further optimisation.[4] ESI (+) and (-) ionisation with a quadrupole analyser was used for mass spectrometry. The first results indicate that alkynylgold(I)(NHC) complexes with acetylcysteine are stable in the analysed solvents with no significant changes in their AUCs [Fig 1].

It is possible to dissect the hit compound into four parts (see Fig. 2): an aryl moiety, pointing into a CoLH-specific binding pocket that is crucial for selectivity, a linker with possible side-chain attachments, and a zinc-binding group (ZBG), being pivotal for high binding affinity. According synthetic variations have been performed to give a detailed structure-activity relationship (SAR), and first promising results from these studies will be presented.

References:

Figure 1: Co-crystal structure of ColH with an inhibitor [4]

Figure 2: Structure of ColH inhibitors

It is possible to dissect the hit compound into four parts (see Fig. 2): an aryl moiety, pointing into a ColH-specific binding pocket that is crucial for selectivity, a linker with possible side-chain attachments, and a zinc-binding group (ZBG), being pivotal for high binding affinity. According synthetic variations have been performed to give a detailed structure-activity relationship (SAR), and first promising results from these studies will be presented.

References:

Syntethsis of mursaymycin analogues with variations in the nucleoside moiety

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Muraymycins are a class of naturally occurring nucleoside antibiotics showing promising antibacterial activity against many Gram-positive and few Gram-negative bacteria. [1,2] Their mode of action is based on the inhibition of the bacterial enzyme MraY, thereby blocking an essential step in the early stages of bacterial peptidoglycan synthesis. [1,2] With respect to their structural complexity, simplified muraymycin analogues were designed based on the results of SAR studies of the natural products and semisynthetic derivatives. Their facilitated synthetic accessibility allows further structural variations for more detailed SAR insights.[1,3,4]

Reference:
5’-defunctionalized muraymycin analogues with variations in the nucleoside moiety were synthesized and evaluated for their in vitro inhibition of MraY. A 5’-defunctionalized muraymycin analogue simplified in the urea dipeptide moiety, that had been previously synthesized in our group [3], was used as reference compound. [4] The structure of the nucleobase was then modified via synthesis of the 5’-fluorouridine-based derivative 1 (see figure), thereby altering the electron density of the heteroaromatic system. The thymidine-containing analogue 2 was synthesized to evaluate the effect of increased steric demand of the nucleobase. To investigate the influence of the 2’-hydroxy group, the 2’-deoxy analogue 3 was prepared. The synthetic route followed a modular approach. The synthesis of the 5’-deoxy nucleosyl amino-acid building blocks had previously been developed in our group [5] and was adjusted for the synthesis of the modified analogues. The urea dipeptide building block was prepared via a solid phase-supported method. Evaluation of in vitro MraY inhibition was performed via a fluorescence-based assay. [6, 7] The synthetic work and results for in vitro MraY inhibition of 1-3 will be presented.

References:

Cationic peptide conjugation to vancomycin leads to improved pharmacokinetics and overcomes all types of vancomycin resistance

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The increasing number of drug resistant bacterial strains is a major problem in modern medicine. It is perceived by the WHO in a list of “high priority pathogens” such as vancomycin-resistant enterococci or staphylococci. The limitation of the treatment of those pathogens by common drugs is a tremendous problem in clinics today. The resistance against vancomycin can be differed in three main types in accordance with their resistance gene called vanA-, vanB- or vanC-type [1]. The vancomycin-resistance can be explained by mechanisms that are related to the cell wall components. Resistant bacteria change the binding epitope resulting in a massive loss in affinity of vancomycin. Strategies of modifying vancomycin specifically for this structural change were successful but chemically extremely complex [2]. In this study we were able to overcome all common vancomycin-resistances by a new technology of conjugation of cationic peptides to vancomycin (Fig. 1A). The conjugates were not only able to overcome the resistance by an up to 1000-fold decrease of the Minimal Inhibitory Concentration but also attain improved pharmacokinetics in comparison to vancomycin as they redirect the nephrotoxic vancomycin to the liver. FUO02, the actual lead candidate furthermore shows no cytotoxic effect in vitro and in vivo. The good in vivo tolerability and the good in vitro activity allowed testing the compound in a murine infection model. The drugability was proven by a significant reduction of colony-forming units of a vancomycin-sensitive Staphylococcus aureus strain in the liver (Fig. 1B).

Today, approximately 15 million chronic hepatitis B (HBV) patients are superinfected with the hepatitis D virus (HDV) and have no specific treatment options [1]. As HDV is the most aggressive form of viral hepatitis, there is a high need for novel drugs. Since the discovery of the entry receptor (NTCP) of HBV/HDV, the transporter has become a major target for a whole group of upcoming drug candidates. The most promising one is Myrcludex B, a peptide consisting of 47 amino acids and a myristoylated N-terminus. The amino acids 9-15 (NPLGFFP) of Myrcludex B represent the essential inhibitory sequence. The amino acids between position 30 and the C-terminus form an accessory domain that contributes to the binding efficiency [2]. While major changes in the essential site lead to abrogation of the inhibitory capability of the peptide [2], the exact role of the accessory domain is yet to be fully determined.

In this study derivatives of Myrcludex B were synthesized with solid phase peptide synthesis employing the Fmoc/Bu strategy and tested for HDV infection inhibition in cell culture models. The derivatives have alanine exchanges in distinct positions: replacement of positions 32 and 33, positions 32-37 and 16-21 in combination with positions 34-39. All derivatives show profoundly reduced inhibitory efficacy against HDV infection.

To further analyze the binding affinity of the derivative with alanine substitutions in positions 16-21 and 34-39 in comparison to Myrcludex B, both peptides were labelled with the fluorescent dye atto565 via an NHS-ester to a lysine side chain. The alanine derivative showed a significantly weaker and unspecific binding, which could be confirmed in a FACS assay. In conclusion, it could be shown that changes in the accessory domain can result in severe reduction of inhibitory efficacy and binding affinity.


Amino Acid-based allosteric Inhibitors of Zika- and Dengue-Virus NS2B/NS3-Proteases

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Hepatitis B and D: The importance of accessory domains in peptidic entry-inhibitors

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POS.47

POS.46

POS.48
Since the 1950s, the Zika virus spread through Africa and South Asia, but during the last years, it emerged into the Pacific region and came to the Americas. After being transmitted by mosquitoes like Aedes albopictus, the virus releases its positive, single-stranded RNA inside the cell, from which a polyprotein is translated containing the whole virus proteinome. To cleave this polyprotein into its functional parts the viral NS2B/NS3-protease is essential. Therefore, this protease is a valid target for the development of drugs.[1]

In previous work we developed inhibitors of the Dengue virus NS2B/NS3-protease.[2] Due to the high sequence and structure similarity of flaviviral proteases these inhibitors also display similar affinities to the Zika virus protease. The inhibitors show a non-competitive behaviour at both targets, but unfortunately lack sufficient water solubility. To obtain inhibitors with better logD-values while keeping the overall compound geometry, the ortho-substituted aromatic ring system was exchanged to a proline residue and other proteinogenic and non-proteinogenic amino acids. This led to a new and potent generation of inhibitors of flaviviral proteases. Structure-activity relationship was explored and inhibitors with further structural variations were synthesized. The compounds were also tested in cell-based assays, revealing cell-permeable prodrugs. Metabolism and metabolites of these prodrugs were studied in in-vitro assays supported by LC-MS.

References:

POS.49
Dopamine D2 receptor signaling: toward deconvolution of the holistic readout obtained by dynamic mass redistribution

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Dopamine receptors, belonging to the super family of G-protein coupled receptors (GPCRs), are classified according to their preferred G-protein coupling, i.e. the G-coupled receptors D1 and D2 (D2 family) and the G-coupled subtypes D3, D4 and D5 (D2 family). Since the first cloning of the human D2long and D2short receptor in 1989 [1], various conventional functional assays, such as cAMP and arrestin assays, as well as label-free ‘non-invasive’ methods (dynamic mass redistribution (DMR)) have been used for the investigation of D2 receptor-mediated cellular signaling. However, it is well known to which extent distinct signaling pathways contribute to the holistic responses (cytoskeletal and cell morphological changes) measured by DMR.

Aiming at a deconvolution of D2 receptor-mediated holistic responses, we applied the DMR technique to the human dopamine D2long receptor using genetically engineered CHO-K1 cells stably expressing the long isoform of the dopamine D2 receptor. The contribution of individual signaling pathways to the optical DMR signals was explored by measuring DMR traces upon agonist stimulation in the absence and in the presence of pharmacological tools accounting for a specific silencing of signaling pathways (e.g. pertussis toxin: inhibition of G; gallein: inhibition of Gq/11).

The potencies of the investigated D2 receptor agonists (e.g. dopamine and pramipexole), determined in the DMR assay, were higher compared to D2 receptor affinities obtained from competition binding studies with [3H]-methylspiperone at genetically engineered HEK293T cells. Inhibition of the G-dependent pathway with pertussis toxin led to a marked decrease in the DMR signal obtained upon quinpirole-mediated D2 receptor activation. This suggested that the holistic response, measured by DMR, is mainly mediated by Gs and not by Go or Gi. Inhibition of the interaction of Gβγ-subunits with its downstream effectors by pre-treatment of the cells with gallein resulted only in a slight decrease in the DMR response compared to the control experiment in the absence of gallein indicating a minor contribution of Gβγ-subunits to the signal detected by DMR.

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References:

POS.50
Elucidation of the Mechanism of Action of allosteric Dengue- and Zika-Virus NS2B/NS3 Protease Inhibitors

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In recent years, the Dengue virus, a mosquito born member of the flaviviridae family, has emerged a serious health issue as its main vectors, namely Aedes albopictus and Aedes aegypti have spread due to climate change and increasing globalization from their tropical origins to more tempered climate zones such as Mediterranean and even middle European countries. [1,2,3] The Dengue virus is a positive, single stranded sense RNA virus, whose genome consists of three structural (capsid, membrane precursor, envelope) and seven non-structural (NS-) proteins. All of them are expressed as one precursor polyprotein. [4] For virus maturation, correct processing of the polyprotein to the functional proteins is crucial. This is conducted by the hosts proteases signalase and furase and by the viral NS2B/NS3 protease complex (figure 1). [4] Inhibition of this protease suppresses viral maturation and leads to loss of pathogenicity. [5] The NS2B/NS3 protease comprises two different conformations: an inactive “open” and an active “closed” form where the NS2B cofactor domain is wrapped around NS3 and partially forms the S3 pocket of the active site. [6]

Previously, potent allosteric inhibitors of the NS2B/NS3 proteases were developed. [7] While molecular docking studies indicate binding in the open conformation of the protease, the detailed mechanism of action remains largely unresolved.

Therefore, we applied various methods to achieve deeper insights into the mechanism of inhibition. In contrast to our enzymatic cleavage assay and MST measurements which showed low micromolar IC50 and Kd values, ITC experiments did not show enthalpy-driven binding. MST measurements and DRX SwitchSENSE sizing experiments indicated conformational changes upon inhibitor binding. These findings point to a
mostly entropic-driven binding of the inhibitors. Herein we report different approaches based on cysteine conformational traps, DRX2 thermodynamic measurements and competitive ITC experiments to overcome these – so far – apparent contradictory results.

2 WHO Regional Office for Europe: Fact sheet 2015. 

POS.51
The Novel Histone Methyltransferase KMT9: an Assay Platform for Inhibitor Screening
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Epigenetic marks established on histone tails by specific enzymes are important regulators of gene expression. There are three classes of these so-called epigenetic proteins, firstly writer enzymes setting a specific mark, secondly erasers which are removing it and thirdly reader proteins, which are mediating a specific effect based on the post-translational modification. Recently the spectrum of histone modifying enzymes was expanded by the discovery of the novel, heterodimeric seven-ß-strand histone methyltransferase KMT9, which is capable of monomethylating lysine 12 of histone H4 (H4K12me1). The H4K12me1 mark is mostly located at promoter sites of cell cycle regulating genes. Prostate cancer cells, both hormone-sensitive and castration-resistant, are sensitive to substrate by potential inhibitors. Sinefungin as well as the natural molecular target for the treatment of cancer. With its chemical property as a soft Lewis acid, gold(I) has a high affinity to thiols and selenols, as a soft Lewis acid, gold(I) has a high affinity to thiols and selenols, mostly entropic-driven binding of the inhibitors. Herein we report different approaches based on cysteine conformational traps, DRX2 thermodynamic measurements and competitive ITC experiments to overcome these – so far – apparent contradictory results.


POS.52
Indirect MYC targeting via conformation changing Aurora Kinase A inhibitors
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Hepatocellular Carcinoma (HCC) – the second most frequent cause of cancer-related death – has a great need for better therapeutic strategies since the treatment options for advanced HCC are limited. Furthermore, the rapid emergence of resistance against existing treatments demonstrates the necessity for novel therapeutic approaches. [1-2] In previous publications and preliminary work, it was shown, that transient systemic MYC inhibition serves as a suitable treatment option for HCC. [3] However, due to its lack of binding cavities MYC cannot be targeted directly by small molecules. By using a direct in vivo shRNA screen, recent findings showed that liver cancer cells bearing mutations in the gene encoding tumor suppressor protein p53 (Tps3 in mice and TPS3 in humans) and that are driven by the oncoprotein NRAS become addicted to MYC stabilization via a mechanism mediated by aurora kinase A (AURKA). [4] Therefore, an indirect MYC targeting becomes possible. Our work focuses on the design and synthesis of small molecules that prevent MYC/AURKA interaction by inducing a conformational shift in the kinase. In silico predictions propose that the crucial interaction for complex formation is located in close proximity to the kinases hydrophobic spine. Hence we make use of the already in our group successfully applied concept of type 1/ß inhibitors. Guided by in silico approaches and further mechanistic studies we aim to develop tool compounds providing structural features that are able to prevent MYC/AURKA complex formation and ultimately degrade MYC.

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POS.53
Alkynylgold(I) NHC Complexes as Thioredoxin Reductase Inhibiting Potential Antitumor Agents
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In consequence of spread and severity of cancer there is need for novel drugs. Thioredoxin reductase (TrxR) has been identified as a potential molecular target for the treatment of cancer. With its chemical property as a soft Lewis acid, gold(I) has a high affinity to thiols and selenols, which are present in the active site of TrxR. [1,2] Inspired by the disease-modifying antirheumatic drug Auranofin, several types of ligands have been used to form gold(I) complexes as TrxR inhibitors [2]. Beside halide, thiolate and phosphine based ligands, the focus has more recently shifted towards organometallic complexes due to their higher chemical stability. Recent published results show that alkynes and N-heterocyclic carbenes (NHCs) as ligands to gold(I) display both described effects (forming stable complexes and good activity against TrxR). [1-3] NHC-gold(I) complexes demonstrated their potential as strong inhibitors of TrxR in combination with cytotoxic activity against several tumor cell lines, such as MCF-7 (breast adenocarcinoma) and HT-29 (colon adenocarcinoma) cells [3,4]. Alkynyl-gold(I)-phosphinophosphons have shown a similar strong activity against the same cell lines with additional angiogenic effects in zebrafish embryos [5]. A synthesis and characterization procedure for complexes with an alkynyl and a NHC ligand was developed [Figure 1]. Ongoing biological tests deal with the cytotoxicity and TrxR inhibition of this type of gold organometallics. First experiments confirmed strong cytotoxicity against several tumor cell lines and efficient inhibition of TrxR. The current results of this ongoing project will be presented on the poster.

Figure 1: Synthesis procedure for an example of an alkynyl-gold(I)-NHC-complex: a) alkylation with ethyl iodide, b) reaction with AgO2 and transmetalation with SMe2AuCl, c) reaction with phenylacetylene under basic conditions.
POS.54

Novel Synthetic Pentathiepins Mediate the Inhibition of GPx1, Cell Viability, Cell Proliferation and Increase Intracellular ROS Levels

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The discovery of the natural product pentathiepin Varacin brought the focus on research into the wide range of biological activity that these interesting compounds possess, including antifungal, cytotoxic and DNA-damaging activity [1, 2]. Studies suggest that these properties are mainly mediated by the pentathiepin ring system and the generation of reactive oxygen species (ROS) in the presence of thiols [3, 4]. Interestingly, we have recently discovered that some synthetic pentathiepins are potent inhibitors of the glutathione peroxidase 1 (GPx1) [5], an important enzyme for maintaining the intracellular redox balance. In this project, we analysed five novel pentathiepins with regards to their potential to inhibit the bovine erythrocyte GPx1 as well as the viability and proliferation of nine different human cancer cell lines in vitro. In selected cell lines the influence of the compounds on the intracellular ROS level was evaluated. Chemical reactions between various pentathiepins and thiols were also investigated. To address these aims, enzyme- and cell-based microplate assays were performed, half maximal inhibitory concentrations (IC50) calculated and the ROS sensor H2-DCFDA analysed via flow cytometry. All tested pentathiepins were potent and specific GPx1 inhibitors, as well as capable of inhibiting the viability and proliferation of all nine cancer cell lines at very low micromolar concentrations. Moreover, four out of five compounds significantly increased the intracellular ROS levels. Differences between the IC50 values of the pentathiepins and their varying capabilities to generate ROS suggest that not only the pentathiepin ring mediates these biological effects but also that the distinct ring substituents can have a modulating influence.

Fig. 1: Structures of (R)-(+)- and (S)-(-)-DABN-PtCl2

Fig. 2: General structure of the tested Pt(II) complexes

References:

Since the approval of cisplatin in 1978 by the U.S. FDA, relatively few platinum complexes have been approved for the treatment of cancer worldwide. Thus, the design of new Pt cytostatics is a challenging task for medicinal chemists. On the one hand many types of cancers are not sensitive to Pt anticancer agents (e.g. breast cancer) while on the other hand treatment with cisplatin often leads to acquired resistant, which can be cross-resistance to other platinum complex e.g. Carboplatin. Over the years it has become clear that the nature of the diamine ligand strongly influences both the innate selectivity towards different types of cancer as well as the development of resistance to Pt(II) complexes. Surprisingly, the biological activities of the few known Pt(II) complexes with the 2,2’-diaminobiphenyl ligands had never been tested. These ligands are of interest due to the possibility of atropisomerism when the phenyl rings are further substituted in the 6 positions with bulky groups.

In our previous work we studied the cytotoxic effects of (R)-(+)- and (S)-(-)-DABN-PtCl2 (fig. 1) on human cancer cells and investigated their cellular uptake and the DNA binding. We found that the (R)-Pt(II) complex is taken up by cells better than the (S)-Pt(II) complex but that the (S)-Pt(II) complex is more potent as a cytotoxic agent. In addition, both complexes had greater potency than cisplatin on human cancer cells [1].

On the basis of this previous work, we have synthesised seven new platinum(II) complexes with various 6,6-disubstituted, 2,2’-diaminobiphenyl ligands (fig. 2) and studied the cytotoxic effect on four different human cancer cell lines. A cisplatin resistant cell line was also used to evaluate if there is any cross-resistance of the new complexes with cisplatin. Due to their structural similarity we further investigated for SAR, which could be useful for further analogue design and a rational drug design.

References:

POS.55

Synthesis and biological evaluation of platinum(II) complexes with 2,2’-diaminobiphenyl ligands

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Folates are essential cofactors in the de novo biosynthesis of pyridine and pyrimidine [1]. Moreover, antifolates are key components in cancer therapy [2]. Targeting tumor specific cell surface epitopes (tumor markers) with small molecules can lead to improved tools for cancer diagnosis and therapy. Elevated levels of prostate specific membrane antigen (PSMA) are used as a tumor marker for prostate cancer [3].
PSMA is a glycosylated type-II membrane protein that is present in high density on the surface of malignant prostate cancer cells. Its expression increases with clinical stage, thus making it an extremely useful tumor marker.[4] Pseudopeptidomimetics like GPI can be used as modular ligands for the targeting of prostate cancer [5]. GPI binds with nanomolar affinity to PSMA and permits conjugation of effector molecules (e.g. fluorophores, chelates) without altering the binding properties [6]. However, GPI has suboptimal binding properties in vivo and needs to be improved for imaging applications in animals or humans. HDAC has been developed as a transition state analogue of the native PSMA substrate N-acetyl-aspartylglutamate (NAAG). In addition, PSMA has been found to act as a folate hydrolase and does thus recognize folypolyglutamates in the same binding pocket as NAAG [7]. We have combined properties of the known ligand GPI with structural elements of folate to design improved modular PSMA ligands. These ligands should be attached with a conjugation site for effector molecules (in our case DOTA). We have established an interesting compound library with which we would like to evaluate the impact of different aromatic and hydrophobic moieties as pteridine analogues, their spacing from GPI and the role of geometry and aromaticity of the spacer via in vitro assays using LNCaP (PSMA positive) and PC3 (PSMA negative) cell lines [8].

Among other epigenetic modifiers, histone deacetylases (HDACs) have been established as valuable drug targets for single-target and combination therapies against non-solid cancers.[3] Apart from their potential to modify the chromatin structure via histone modifications, several of the eleven HDAC isoforms and particularly HDAC6 possess a broader substrate spectrum, including α-tubulin and the chaperone protein Hsp90.[2] HDAC6 further regulates the aggresome formation as the second major cellular protein degradation pathway alongside the 20S proteasome. Simultaneous blockade of both synergistic targets therefore leads to accumulation of misfolded proteins resulting in apoptosis of fast-growing malignant cells.[1] In consequence, the combination of panobinostat, a non-selective HDAC inhibitor (HDACi), and bortezomib, a proteasome inhibitor (PI), has already been approved for the treatment of multiple myeloma while further combinations of HDACi and PI are currently being investigated at different clinical stages.

Due to the distinct shapes of the enzymatic targets, HDAC6 inhibitors and proteasome inhibitors also qualify for the polypharmacological approach that uses one drug to interact with multiple targets. In 2018, our group reported the discovery of RTS-V5 as the first-in-class dual HDACi and PI.[3] The binding modes of RTS-V5 were confirmed by X-ray crystal structures of RTS-V5 in complex with both HDAC6 and the 20S proteasome. Additional biological assays further indicated blockage of the cell cycle, proliferation, colony formation and aggresome accumulation as well as the induction of apoptosis, thus proving the anticipated synergistic effect resulting from the inhibition of both targets. In comparison to the HDAC6-preferential drug candidate ricolmostin, RTS-V5 also showed promising anticancer activities against selected hematological cancer cell lines as well as therapy refractory primary patient-derived leukemia cells.[3]

In summary, these initial results suggest RTS-V5 as a good candidate for further optimisation. However, it became clear that the HDAC6 inhibitory activity needed to be increased. In this study, we therefore focused on modifying the HDACi part of the drug scaffold (Figure 1) by introducing different linker moieties and zinc binding groups. As a result, we present new RTS-V5 analogues with increased inhibitory activities against HDAC6.

Acknowledgments: We want to gratefully acknowledge the financial support from the NIH (P01-Grant).

References:

Figure 1. RTS-V5, the first-in-class dual HDAC and proteasome inhibitor.

References:

POS.57

Design and synthesis of dual histone deacetylase-proteasome inhibitors

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Acknowledgments: We want to gratefully acknowledge the financial support from the NIH (P01-Grant).

References:

POS.58

Balancing HDAC Inhibition and Drug-likeliness - Biological and Physicochemical Evaluation of Class I Selective Histone Deacetylase Inhibitors

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Epigenetic modifications and their crucial role in the regulation of gene expression have been widely studied and many enzymes have been identified that interact with the chromatin structure. For instance, via histone lysine acetylation and deacetylation epigenetic marks are either attached resulting in open, transcriptionally active chromatin or removed resulting in condensed, transcriptionally repressed chromatin. [1] Histone deacetylases (HDACs) remove acetyl groups from histone and non-histone proteins and hereby influence cell cycle progression, cell proliferation and differentiation. HDACs are over-expressed in several cancer types and inhibition of HDAC function can result in various anti-cancer effects. [1] As a consequence, some HDAC inhibitors (HDACi) like vorinostat, romidepsin, belinostat and panobinostat are now established, FDA-approved anti-cancer drugs for the treatment of T-cell lymphoma or multiple myeloma. The HDAC family encompasses eleven zinc-dependent enzymes (HDAC1-11) classified in 4 groups: class I, class IIa, class IIb, and class...
IV. Class I consists of the nuclear enzymes HDAC1-3 and HDAC8. [1] HDAC1 and HDAC2 are among others associated with prostate, gastric, breast and hematopoietic cancers. [1,2] Additionally HDAC2 seems to be critically involved in cognitive processes like learning and memory [3] and HDAC3 was shown to play an important role in inflammation and neurodegenerative diseases. [4] From a structural point of view, the differences within class I isoforms are mainly based on an internal cavity the so called “foot-pocket”. Ortho-aminooanoilides like tacedinaline (CI-994) are HDAC1-3 selective inhibitors. When the aminoanilide group additionally bears a bulky substituent such as phenyl ring to occupy the “foot-pocket”, the HDAC1/2 binding affinity and selectivity is strongly increased. [1] However, introducing bulky, lipophilic substituents increases the molecular weight and lipophilicity considerably and worsens solubility. As a consequence, HDAC1 that target the HDAC1/2 foot-pocket are not necessarily drug-like substances.

Figure 1: CI-994-like fragments were synthesized and their solubility, lipophilicity and HDAC inhibition was evaluated. Based on our results we selected drug-like candidates for further investigation.

To balance the clear advantage of increased HDAC inhibition and selectivity via targeting the “foot-pocket” and the disadvantages of these substances, we synthesized a small set of HDACi fragments closely related to CI-994 (Fig. 1) and evaluated their solubility, lipophilicity (log D7.4) and inhibition of selected HDAC isoforms. In this work, we report the structure-activity and structure-physicochemical properties relationships of a series of novel class I selective ortho-aminooanoilides.

Acknowledgements: Experimental support from A. Schöler is gratefully acknowledged.

References:

was shown in the NCI-60 DTP human tumor cell line screening (Gleevec: 49 nM) and in in vitro and in vivo studies on gynecological tumors [3,4].

Methods: The potential of P8-D6 treatment to reduce proliferation and induce apoptosis in various established ovarian cancer cell lines, breast cancer cell lines and ex vivo primary cancer cells were analysed by emission spectroscopy, flow cytometry and microscopy. For better comparison, cells were treated with different concentrations of P8-D6 and standard therapeutics for 48h. Likewise, the effects on non-cancer cells were analysed and hepatotoxicity of P8-D6 was investigated. The expression levels of topoisomerase II were measured by western blot. Fluorescence microscopy showed whether P8-D6 gets into the nucleus to its target. Moreover, P8-D6 was tested in an ovarian cancer xenograft mouse model as an in vivo study to determine the therapeutic efficiency.

Results: The results indicate a significantly increase of apoptosis in gynecological cancer cells by P8-D6 than its references. Non-cancer cells were slightly effected. No hepatotoxic effect in in vitro studies was seen. By staining, P8-D6 was detected in the nuclei. In the in vivo study P8-D6 showed promising results.

Conclusion: P8-D6 has promising antitumor properties in in vitro studies on ovarian cancer and breast cancer. It has fewer side effects on normal cells than references and no hepatotoxic effect. P8-D6 is a strong and rapid inducer of apoptosis.

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POS.59
Targeting gynaecological cancer with the new dual topoisomerase inhibitor P8-D6

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Introduction: The development of innovative cytotoxic compounds with suitable physicochemical properties has a high clinical need in cancer therapy. In gynaecological oncology, breast cancer is the most common and ovarian cancer the most aggressive tumor [1, 2]. Resistance developments and severe side effects are the reasons for lack of treatment success. P8-D6 is an effective inducer of apoptosis by acting as a dual topoisomerase inhibitor. Its outstanding antitumoral property

POS.60
Synthesis of Gallic Acid Esters and Their Evaluation as Anticancer Drug Candidates

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Gallic acid is a naturally occurring, non-toxic polyphenolic compound known to have various pharmacological activities. Besides antifungal, antibacterial, antilucer and other properties, gallic acid can prevent carcinogenesis by its antioxidant behaviour as well as induce apoptosis of tumour cells due to prooxidative properties, which increase oxidative stress within the cell. Other antioxidants with anticancer properties can be found among phenolic structures such as eugenol, vanillin or cinnarcarol, but also molecules like cinnamaldehyde are known to prevent cancer and have a potential for use in the chemotherapy of different cancer types. With the intent to combine the beneficial properties of gallic acid with those of other (phenolic) anticancer agents, we aimed to combine gallic acid with each of the other compounds in one molecule. The different ester compounds were obtained in a five-step synthetic pathway. The pharmacological properties of the gallic acid esters have been tested in an MTT assay using HER-2 breast cancer cells showing promising IC50 values.
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References:

Immunotherapy addressing the PD-L1 receptor with checkpoint inhibitors is a promising therapy strategy for cancer patients. However, in average only 30% of patients respond to checkpoint inhibitor immunotherapy. Therefore, it is important to select those patients prior to therapy who will likely respond. Molecular imaging techniques such as PET and SPECT have the advantage of providing a whole body scan and are able to fully address the issue of heterogeneous PD-L1 expression. Low-molecular weight compounds such as peptides are favourable as imaging agents, especially peptides have the advantage of providing a whole body scan and are able to fully address the issue of heterogeneous PD-L1 expression. PET imaging probes to visualize and quantify PD-L1 upregulation in cancer tissue will serve as a basis for the development of PET imaging probes to visualize and quantify PD-L1 upregulation in cancer tissue.

References:

POS.61

Imaging of PD-L1 for immunotherapy monitoring
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Background: Epigenetic modifications in cancer cells can cause poor prognosis, development of cancer relapse and malfunction of anticancer therapy. Thereby gene expression with regard to epigenetic modulations can be affected by histone acetylation, histone deacetylation, phosphorylation, and DNA transcription. Histone deacetylases (HDACs) are one of the key mediators of epigenetic regulation, and are classified into four groups. The class—I, −II and −IV containing a zinc ion in the active site whereas the class−III, so called sirtuins, are NAD+-dependent protein lysine deacetylases.

Results and conclusion: The focus of this current research is to determine the protein expression profiles of various HDACs in a set of 17 human cancer cell lines from different tumor sources. First results indicate correlations between the expression profiles of various HDACs such as Sirt1 with Sirt3 and HDAC4. Furthermore we found indications for correlations between HDAC isoenzyme expression and the sensitivity of cell lines towards certain anticancer drugs, such as HDAC4 with podophyllotoxin, methotrexate and 5-fluorouracil or Sirt5 with colchicine. In combination studies of HDAC inhibitors with anticancer drugs, interesting effects on the proliferation of cancer cells were identified. TheIC50 values of anticancer drugs were determined in combination with HDAC inhibitors at non-toxic concentrations and compared with single agent cytotoxicity. Therefor unspecific HDAC inhibitors such as vorinostat and trichostatin-A as well as newer azo-based Sirt2 inhibitors were tested. A highly significant enhancement of cisplatin and lomustine cytotoxicity in some cell lines was found when combined with an HDAC inhibitor, indicating a potential synergistic effect in anticancer therapy.

References:

POS.62

Studies on histone deacetylase protein expression profiles of various cancer cells and the effect of histone deacetylase inhibition on anticancer drug cytotoxicity
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Background: Histone deacetylases are enzymes that remove acetyl groups from histones and other proteins, leading to changes in gene expression. In cancer, the expression and activity of HDACs can be altered, affecting the sensitivity of cancer cells to treatments such as histone deacetylase inhibitors.

Results and conclusion: This study aimed to investigate the expression profiles of HDACs in various cancer cell lines and the effect of HDAC inhibition on drug cytotoxicity.

References:

POS.63

Preliminary studies of fluorescence- and 68Ga-labeled anti-miR-21
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Objectives: MicroRNAs (miRNAs) are short, non-coding RNAs that post-transcriptionally regulate gene expression by binding to mRNA and thereby play a significant role in many biological processes and diseases, in which they represent a promising new biomarker in the diagnosis. Using radiolabeled anti-miRNA oligonucleotides, which bind highly specifically and with high affinity to the complementary miRNAs, have the potential to assess miRNA levels in vivo. Molecular imaging can
be performed by positron emission tomography (PET) using positron emitting radionuclides such as $^{68}$Ga and $^{18}$F. miR-21 is involved in the development of cardiac fibrosis, which leads to myocardial dysfunction [1,2]. An anti-miR-21 oligonucleotide labeled with short-lived radionuclides, $^{68}$Ga or $^{18}$F, would be valuable for the diagnosis of pathogenic changes in miR-21 levels by non-invasive molecular imaging, especially in the early stage of a disease. The aim of this project is to develop radiolabeled oligonucleotides targeted to miR-21 to evaluate potential as diagnostic tools in cardiac fibrosis.

Materials and Methods

Anti-miR-21 was functionalized with a hexyl-disulfide moiety and locked nucleic acids (LNA). The disulfide group was reduced with diithiothreitol and coupled with the fluorescent dye ATTO 647N, the complexing agent TEP and NODAGA by maleimide thiol coupling to yield ATTO647N-anti-miR-21, NODAGA-anti-miR-21 and TEP-anti-miR-21, respectively. Analytics and mass confirmation were performed by HPLC-ESI-MS (TOF) and purification by Sephadex G-25 in PD MidiTrap and PD-10 desalting columns. ATTO647N-anti-miR-21 was incubated in mouse 3T3 fibroblasts for 24 h. qPCR was used to measure antisense effects and the transfection efficiency (LNA) was determined by FACS. NODAGA-anti-miR-21 and TEP-anti-miR-21 were labeled with galium-68 in first test runs. Approximately 200 MBq of gallium-68 eluate was added to the corresponding precip in water at room temperature for THP and at 95 °C for NODAGA. The pH was adjusted to 6 for THP and to 4 for NODAGA. Radiolabeling was monitored and analytics were performed by radioHPLC.

Results

Three novel anti-miR-21 derivatives were successfully synthesized: ATTO647N-anti-miR-21, NODAGA-anti-miR-21 and TEP-anti-miR-21. Based on the qPCR (miR-21 knockdown) and FACS data (LNA transfection efficiency) we see that ATTO647N-anti-miR-21 (100nM) is absorbed by mouse 3T3 fibroblasts with a prolonged inhibitory effect over 24h. Initial radiolabeling demonstrated feasibility, though low absorbed by mouse 3T3 fibroblasts with a prolonged inhibitory effect over 24h.

Conclusions

ATTO647N-anti-miR-21 displays selective reduction of as well as binding to miR-21 in mouse 3T3 fibroblasts. The precursor NODAGA-anti-miR-21 and TEP-anti-miR-21 were labeled with galium-68. These studies form the foundation for non-invasive imaging of miR-21 levels in cardiovascular disease. In further studies the radiolabeled derivatives will be evaluated in vivo by PET imaging.


**POSTERS**

**POS.64**

**18F-Labeled PET Tracers Derived from Angiotensin II Receptor Antagonists Ibosartan and Valsartan**

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Ibosartan (A) is an anti-inflammatory compound that was discovered in a pharmacoepo-based virtual screening approach at the University of Innsbruck. It represents the first dual inhibitor of 5-lipoxygenase-activating protein (FLAP) and soluble epoxide hydrolase (sEH), both targets within the arachidonic acid cascade. The compound decreases the formation of pro-inflammatory leukotrienes by inhibiting FLAP (IC50 = 0.2 μM), a protein, that transports arachidonic acid form the membrane to the 5-lipoxygenase. Simultaneously, it increases the concentration of anti-inflammatory epoxyeicosatrienoic acids by avoiding their sEH-catalyzed degradation (IC50 > 20 mM). Diflapolin shows high target specificity in biological in vitro assays and in vivo experiments confirmed the anti-inflammatory properties. [1, 2]

However, a major disadvantage of diflapolin is the very low water solubility. Therefore, structural optimizations are needed to increase solubility and bioavailability, while retaining or improving the dual activity.

Diflapolin (A) is an anti-inflammatory compound that was discovered in a pharmacoepo-based virtual screening approach at the University of Innsbruck. It represents the first dual inhibitor of 5-lipoxygenase-activating protein (FLAP) and soluble epoxide hydrolase (sEH), both targets within the arachidonic acid cascade. The compound decreases the formation of pro-inflammatory leukotrienes by inhibiting FLAP (IC50 = 0.2 μM), a protein, that transports arachidonic acid form the membrane to the 5-lipoxygenase. Simultaneously, it increases the concentration of anti-inflammatory epoxyeicosatrienoic acids by avoiding their sEH-catalyzed degradation (IC50 > 20 mM). Diflapolin shows high target specificity in biological in vitro assays and in vivo experiments confirmed the anti-inflammatory properties. [1, 2]

However, a major disadvantage of diflapolin is the very low water solubility. Therefore, structural optimizations are needed to increase solubility and bioavailability, while retaining or improving the dual activity.

These results demonstrate the strategy of deriving novel AT-R PET tracers directly from clinically used sartans with the least structural modification. Both 18F-ibosartan and 18F-valsartan may serve as tracers for renal imaging, inflammatory assessment, and cancer diagnosis by providing information regarding the distribution of AT-R and RAAS function under pathological conditions.

Gratitude is expressed to the International Doctorate Program “Receptor Dynamic” of the Elite Network of Bavaria. The German Academic Ment Foundation is gratefully acknowledged for awarding Matthias Hoffmann a PhD scholarship. This project was supported by the German Research Council (Deutsche Forschungsgemeinschaft (DFG) grants CH 15160/1-1 and HI 1789/3-3).


**POS.65**

**Novel diflapolin derivatives with benzimidazole subunit as sEH/FLAP inhibitors**

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Diflapolin (A) is an anti-inflammatory compound that was discovered in a pharmacoepo-based virtual screening approach at the University of Innsbruck. It represents the first dual inhibitor of 5-lipoxygenase-activating protein (FLAP) and soluble epoxide hydrolase (sEH), both targets within the arachidonic acid cascade. The compound decreases the formation of pro-inflammatory leukotrienes by inhibiting FLAP (IC50 = 0.2 μM), a protein, that transports arachidonic acid form the membrane to the 5-lipoxygenase. Simultaneously, it increases the concentration of anti-inflammatory epoxyeicosatrienoic acids by avoiding their sEH-catalyzed degradation (IC50 > 20 mM). Diflapolin shows high target specificity in biological in vitro assays and in vivo experiments confirmed the anti-inflammatory properties. [1, 2]
Diflapolin can be divided into five parts, the heteroaromatic core (I), the spacer unit (II), the methyl substituted para-phenylene (III), the urea moiety (IV), and the 3,4-dichlorophenyl substituent (V). In a previous SAR study, derivatives with benzimidazole as the heteroaromatic core have been synthesized and biologically evaluated. Results show that small modifications of subunits II, III and V have a remarkable effect on inhibitory activity on both targets.

Here, we present the multi-step synthesis and biological activities of a novel series of compounds (i.e. compounds of type B) that harbours a benzimidazole as the heteroaromatic core. Additional modifications were conducted on the urea substructure (IV), on the substitution pattern of benzothiazole core have been synthesized and resolved in 0.9 % saline solution. 20 minutes static PET images of BCG infected (3 months prior to study) mice were acquired from 30 to 50 minutes after i.v. bolus injection of 5 to 8 MBq $^{68}$Ga-DOTA-Pur followed by a spoiled GRE 3D MRI sequence. Use of pre-concentrated $^{68}$Ga resulted in high labeling yields (68 ± 2.6 %) and RCP (≥ 99% after Strata-X purification). MicroPET/MRI images (figure 1) clearly visualized in both modalities widespread infection on the right upper torso originating from the infection induced in the armpit. BCG foci in lung (2nd infection site) and liver (systemic infection as result of lung infection) also could be clearly identified. High signals in kidneys and bladder confirmed prior findings on main excretion pathway via renal clearance. PET/MRI findings were confirmed by ex vivo histological analysis of harvested tissues.

We demonstrated for the first time microPET/MRI-imaging of BCG infection in mice with a puromycin based radiopharmaceutical. Based on these results we conclude that $^{68}$Ga-DOTA-Pur is a promising tracer for imaging of bacterial infections.

**References:**

**Figure 1:** Structure of diflapolin (A) and general structure of synthesized diflapolin analogues (B).

**References:**

**111-Aminostyrchnine and (Strychnine-11yl) propionamide: Synthesis, Stereochemistry, and Pharmacological Evaluation at Glycine Receptors**

Zlotos, D. P.; Mohsen, A. Y.; Mandour, Y. M.; Marzouk, M. A.; Breitinger, U.; Villmann, C.; Breitinger, H-G; Schreiber, C.; Jensen, A. A.; Holzgrabe, U.

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Styrchnine, the major alkaloid from Strychnos nux vomica, is a highly potent antagonist of glycine receptors (GlyRs). GlyRs are chloride channels composed of five subunits (α or β), which are linked to hyperpolarisation and inhibition of neuronal firing.\(^1\) The crystal structure of human α3 GlyR in complex with styrchnine revealed five equivalent styrchnine binding sites located at the interfaces of the subunits.\(^2\) Recently, our groups have become interested in a bivalent ligand approach for targeting GlyRs.\(^3\) In order to examine whether a C-11 amide linkage is suitable for styrchnine-based bivalent ligands for GlyRs, (11S)-11-aminostyrchnine 1, and the corresponding propionamide 2 were synthesized and characterized as antagonists of homomeric α1 and heteromeric α1β GlyRs in a functional fluorescence-based and a whole cell patch-clamp assay, and in [3H]styrchnine binding studies. The absolute configuration at C-11 of 1 was determined based on vicinal coupling constants and NOESY experiment. Docking studies to the styrchnine binding site from the crystal structure of the α3 glycine receptor showed a styrchnine-analogous binding mode of compound 2, explaining its high antagonistic potency. The findings provide a valuable extension of SAR of styrchnine at GlyRs and identify the C-11 amide linkage to be suitable for future development of styrchnine-based bivalent ligands targeting glycine receptors.

**References:**

**POS.67**

**MEDICINAL CHEMISTRY AND DRUG DESIGN**
The continuously growing threat from multi-resistant bacterial strains makes a renewed focus on antibiotic development more urgent than ever before. There are many different pathways that can be targeted by antibiotics, for example bacterial protein biosynthesis. Amongst others, RNA polymerase (RNAP) as an essential enzyme of bacterial protein biosynthesis is considered as a promising target. [1,2] In 2017 MAFFOLI et al. extracted and discovered pseudouridimycin (PUM) 1 which showed inhibitory activity against RNAP of Gram-negative and Gram-positive bacteria. [3] They described the novel natural product with this new mode of action and a promising structure together with some first semi-synthetic modifications.

In our work we plan to conduct a detailed SAR study, in order to synthesize new antibiologically effective derivatives of PUM. First, the exchange of pseudouridine with uridine at the deoxy-pseudouridimycin structure 2 was aspired, thus leading to target structure 3. Furthermore, we intend to investigate the contribution of the guanidine moiety and therefore designed additional candidate structures 4 and 5. Some of the corresponding synthetic work as well as first results on RNAP inhibition will be presented.

Acknowledgments: Landesforschungsförderprogramm Saarland.

References:

POS.68

Synthesis of acyl-β-C-glycosides

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In 2012, Scleropentasides have been isolated from the leaves and twigs of Scleropyrum pentandrum. Scleropentasides are β-C-glycosidic furan-2-carbonyl compounds of glucose. These kind of C-glycosidic compounds have not been isolated from natural sources before and are unique in terms of their structural pattern so far. In terms of biological activities only the antioxidative properties of Scleropentasides have been investigated so far.[1] A synthetic approach to the Scleropentaside natural product family would help to obtain more material for further biological evaluation which is necessary to evaluate this interesting natural product class and derivatives thereof in detail.

A β-selective type of C-glycosylation to yield acyl C-glycosidic compounds 1 was achieved. The reaction of thiilalated dithianes 2, which were firstly introduced by Corey and Seebach as nucleophilic carbonyl synthons,2 with glycosyl halides 3 as electrophiles yielded C-glycosidic compounds 4 in good yields with excellent β-selectivity. Scleropentaside A (Ar = 2-furyl) 1 was synthesized by this approach in just three linear steps starting from D-glucose. Also a number of derivatives with different glycosyl or aryl units were synthesized by this method.[3]

References:

POS.69

Pseudouridimycin (PUM) and its derivatives as potential new antibiotics

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The worldwide emerging antibiotic resistance has become an imminent problem of our time. Therefore it is necessary to develop new potential antibacterial drug candidates. A new promising class of antibacterial substances are the naturally occurring muraymycins. These nucleoside antibiotics are capable of inhibiting the translocase I (MraY') enzyme, which is part of bacterial peptidoglycan biosynthesis. They display a good target interaction, but on the downside show deficient bacterial cellular uptake.[1]

In our approach we aim to conjugate muraymycin analogues to other antibiotics which already show an intrinsic ability to permeate the bacterial cell wall. These new muraymycin conjugates should be able to utilize the transport mechanisms of the conjugated molecules and furthermore act as dual inhibitors. As a first model substance to mediate cellular uptake streptomycin was chosen. It was previously reported that after a modification at the aldehyde function the activity of streptomycin furthermore act as dual inhibitors. As a first model substance to mediate cellular uptake streptomycin was chosen. It was previously reported that after a modification at the aldehyde function the activity of streptomycin...
Two general types of linkers will be used for these conjugates: a stable triazole conjugation achieved via CuAAC and an intracellularly cleavable disulfide, which releases the two parent drugs.[3] The synthesis of the muraymycin analogues is carried out using a solid phase-supported synthesis, as well as a synthesis in solution. The modification of streptomycin with the azide or the thiol linker, respectively, was achieved via an oxime ligation. First results from these studies will be presented.

References:

Biological Evaluation of halogenated Rubrolide Analogs

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In 1991, the first rubrolides have been isolated from the marine fungus Ritterella rubra. This natural product class has shown various pharmaceutical relevant activities such as moderate inhibition of protein phosphatases 1 and 2A, antibacterial, and antiviral effects.[1] In 2014, Rubrolide R1 and S2 have been isolated[2] and in 2017, the total syntheses of both compounds have been published.[3] These two natural Rubrolides revealed biological activity against pH1N1 and H3N5 and Rubrolide S2 has also shown activity against the tobacco mosaic virus.[4] In order to increase the biological activity we investigated different halogenation patterns of the Rubrolide scaffold. Promising results were observed in the relation between substitution patterns of the synthesized Rubrolide analogs and the ability of virus inhibition. A trend towards higher brominated Rubrolides with a chloride atom at the furanone core was observed.[5]

References:
5. de Vries, J., Assmann, M., Schützenmeister, N., manuscript in preparation.

Figure 1: Plaque Inhibitions Assay of Rubrolide Analogs 4 – 6 und Ribavirin with influenza A virus H3N2 on MDCK cells. Sample concentrations: 100 µM, 10 µM und 1 µM. Positive control: Ctr 1 (no substance) und Ctr 2 (0.1 % DMSO). Fixation of cells with 4 % PFA and visualization of viable cells with crystal violet. Results of three independent experiments. n.d. – no plaques detectable.[3]

Synthesis of a modular system for adenosine receptor probes

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G protein-coupled receptors (GPCRs) represent the largest family of integral membrane proteins.[1] Adenosine receptors (AR) are GPCRs activated by the nucleoside adenosine. They represent novel drug targets, e.g. in immuno-oncology and for neurodegenerative diseases.[2] The labeling of drug targets in native tissues is required for research and diagnostic applications.[3,4] Antibodies are frequently used for this purpose, however, specific antibodies for membrane proteins such as GPCRs are difficult to obtain.[5]

The aim of our study has been the synthesis of a modular system for AR probes attaching a variety of tags via a linker to functionalized selective high-affinity ligands. For this purpose, a highly selective antagonist was selected for each AR subtype, and derivatives were synthesized containing a functional group located at the part of the binding site that is oriented towards the extracellular receptor domains. The designed compounds were synthesized in multi-step procedures, and linkers of different lengths were subsequently attached. In a next step, the AR affinities and selectivities were determined. An A2AR antagonist modified in such a way displayed high affinity (K_i value of 0.6 nm at the human A2AR) and high selectivity (K_i value > 1000 nm at human A1, A3 and A2BRs). The compound was consequently coupled with a Bodipy 493-503 derivative as a first tag. In the future, different fluorophores, DNAs for proximity ligation assays, and antibodies for antibody-drug conjugates (ADCs) will be considered for attachment to this and further AR ligands.

References:

Synthesis and evaluation of phosphonic acids for the antimicrobial coating of metal surfaces

References:
5. de Vries, J., Assmann, M., Schützenmeister, N., manuscript in preparation.
The increasing emergence of multiple drug resistance (MDR) in bacteria demands the development of new antimicrobial strategies [1]. As a matrix for the proliferation of bacteria, surfaces in hospitals or other public places pose a huge infection risk [2]. Bacterial growth often occurs via the formation of a protective biofilm within the bacterial colony, which renders treatment other than mechanical removal futile [3]. A promising step to combat this problem is the coating of metal surfaces with molecules that prevent the formation of biofilms. This approach seems particularly appealing when it comes to the biofilm formation on invasive tools, such as surgery equipment, catheters or implants [4]. Passive and active antifouling strategies differ depending on the point of interaction within the biofouling process. While passive approaches suppress the reversible binding of biological matter to the surface, thereby impeding bacterial growth due to a lack of nutrients, active approaches aim at directly killing microbes [5].

Self assembled monolayers of sulfobetaine zwitterions have already shown a drastical reduction of the unspecific protein adsorption in human blood serum [6]. Quaternary alkyl ammonium salts are effective contact biocides destroying the bacterial membrane through various ionic interactions and replacement of cations [7]. They present a direct method of killing bacteria, interacting before the protective matrix of the colony can be formed which can increase their efficacy compared to antibiotics administered after the biofilm formation. As anchoring group, phosphonic acids exhibit many favourable properties such as robust synthetic approaches, formation of strong bonds to numerous metals, pH independency as well as high stability [8].

Herein we present the synthesis of cationic as well as zwitterionic phosphonic acids. The modular synthetic approach allows easy modification and access to a library of different materials in a low number of steps from cheap and easily available starting materials. The synthesized molecules are coated on different medicinally relevant materials such as titanium or stainless steel. Afterwards, we present the results of the biological testing of the antimicrobial activity against different strains of bacteria. The results show a promising antimicrobial activity against many gram positive and negative bacteria.

**Fig. 1:** (A) Dose-response curves of functional P2X7R YO-PRO-1 uptake assay of agonist BzBzATP (EC50 = 2.1 nM) and two novel antagonists AZD9056 (IC50 = 14 ± 0.7 nM), JNJ47965567 (IC50 = 13 ± 0.8 nM) and two novel antagonists (IC50 = 16 ± 1.4 nM) and (C) antagonists AZD9056 (IC50 = 14 ± 0.7 nM), JNJ47965567 (IC50 = 13 ± 2.1 nM) and two novel antagonists 3 (IC50 = 16 ± 1.4 nM) and 4 (IC50 = 134 ± 9.6nM), n ≥ 5.

# Pharmacological Evaluation of Novel Compounds for their Activity at P2X Receptors

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Adenosine triphosphate (ATP) is known as the molecule that provides the energy for many different processes in living cells, but ATP also acts as an extracellular ligand on receptors in the field of purinergic signaling such as P2Y and P2X receptors [1]. The P2X receptors are non-selective trimeric ion channels activated through the binding of extracellular ATP. The molecular architecture of this ion channel protein family is build up from homo- or heterotrimeric complexes of seven different subunits (P2X1-7). Each subunit stoichiometry determines particular functional properties of the P2X receptor subtype. An increasing ATP release by e.g. damaged tissues and dying cells promotes activation of P2X ion channels and causes the influx of extracellular cations, including calcium and sodium ions [2]. The homotrimeric P2X7 receptors (P2X7Rs) are involved in the pathologies of various diseases, including autoimmune diseases such as rheumatoid arthritis, neurodegenerative diseases, neuropathic pain and cancer [3]. Therefore, P2X7Rs represent interesting and promising targets for the development of new therapeutic agents. The aim of this project is the pharmacological evaluation of known and novel compounds targeting P2X7Rs with different functional assays. The compounds are characterized in three different functional P2X7R assays (influx of Ca2+-ions, the uptake of YO-PRO-1 and the release of interleukin 1β (IL-1β)) for their activation or inhibition of particular intracellular signaling pathways. The derived structure-activity relationships are used to guide the development and optimization of new P2X7R ligands as future therapeutics for cancer and inflammatory diseases.

**Fig. 1:** (A) Dose-response curves of functional P2X7R YO-PRO-1 uptake assay of agonist BzBzATP (EC50 = 152 ± 23 nM), two novel agonists 1 (EC50 = 53 ± 22 nM) and 2 (EC50 = 160 ± 243 nM) and (C) antagonists AZD9056 (IC50 = 14 ± 0.7 nM), JNJ47965567 (IC50 = 13 ± 2.1 nM) and two novel antagonists 3 (IC50 = 16 ± 1.4 nM) and 4 (IC50 = 134 ± 9.6nM), n ≥ 5.

**References:**

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**POS.75**

**Insertion of NanoLuc into extracellular loops enables BRET-based binding assays at the neuropeptide Y Y1 and Y2 receptor**
Recently, real-time BRET-based binding assays were reported for several G-protein coupled receptors (GPCRs), which were N-terminally fused to the very brightly blue-emitting NanoLuciferase (NanoLuc) ($\lambda_{max} = 460$ nm) [1]. However, our efforts to apply this strategy to GPCRs with comparatively long N-termini (> 25 amino acids), e.g., members of the neuropeptide Y (NPY) receptor family, did not give concentration-dependent BRET. Therefore, we intended to explore whether insertion of the NanoLuc into extraacellular loops of NPY receptors represents a strategy enabling BRET-based binding assays for such GPCRs. Based on the recently resolved crystal structure of the NPY Y1 receptor [2], we inserted the NanoLuc in an unstructured region in ECL2 of the Y1 receptor (intraLuc1192) and the Y2 receptor (intraLucD197), and stably expressed these constructs in HEK293T cells. As the pyrylium/pyridinium dye Py-5 shows ideal spectral properties ($\lambda_{max}$ (em.) = 470 nm, $\lambda_{max}$ (ex.) = 650 nm) for a combination with NanoLuc bioluminescence, we synthesized a Py-5-labelled fluorescent Y1 receptor ligand (UR-CM138), derived from UR-MK299 [2, 3], and a Py-5-labelled Y2 receptor ligand (UR-JB264), derived from the standard Y2 receptor antagonist BIIE0246 [4], as molecular tools for our BRET assays.

![BRET assay](image)

Our strategy resulted in a concentration-dependent increase in BRET ratio for both receptors, i.e., binding of UR-CM138 at the Y1 receptor and of UR-JB264 at the Y2 receptor was saturable. Both fluorescent ligands exhibited high receptor affinities with equilibrium dissociation constants (Kd) of 0.24 nM (UR-CM138, Y1R) and 20 nM (UR-JB264, Y2R), which were in good agreement with binding data from radioligand ($K_i$) and flow cytometry ($K_b$) binding assays as well as with functional data ($K_i$) from $\beta$-arrestin recruitment assays. We also performed NanoBRET competition binding experiments with standard Y1 and Y2 receptor ligands, yielding $K_i$ values being in accordance with reported data from radioligand competition binding experiments.

In conclusion, we present a novel approach to establish BRET-based binding assays for GPCRs, which are incompatible with an N-terminal luciferase tagging in terms of BRET measurement.

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POS.76

Novel $^{18F}$-labeled D$\alpha$-receptor ligands

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The role of the dopamine D$\alpha$-receptor subtype in the development of neurodegenerative diseases such as schizophrenia has been discussed for several decades. Specific radiotracers for more detailed preclinical and clinical investigations are still missing so far. The objective of this study was to develop D$\alpha$-selective radioligands for positron emission tomography (PET). The selected lead structures exhibit high D$\alpha$ subtype selectivity and a suitable LogP value, rendering them attractive for the development of a D$\alpha$-selective radiogand for PET-imaging [1]. Two D$\alpha$-ligands, known from literature [2,3], were selected for labeling with fluorine-18 ($t_{1/2}$=108 min), the most ideal positron emitting nuclide.

D$\alpha$R-radiogand $[^{18F}]$ was synthesized from 2-$^{[18F]}$fluorophenylpiperazine 2, obtained by an alcohol enhanced Cu(II)-mediated radiofluorination [4] of the Boc-protected precursor 1 followed by deprotection of the radiolabeled intermediate with TFA. Subsequently, $[^{18F}]$ was obtained by reductive amination with 3 in a total isolated radiochemical yield of 7 % within 2 h. Three independent autoradiographic studies with molar activities up to 90 GBq/μmol showed high content of non-specific binding that covers any possible specific binding.

The chiral D$\alpha$R-radiogand $[^{18F}]$ was also obtained by alcohol enhanced Cu(II)-mediated radiofluorination [4] of the boronic acid precursor 4 in a RCE of 66±5 % within 60 min after isolation by semi-preparative HPLC. Preliminary in vitro autoradiographic study indicates specific binding of $[^{18F}]$ in areas with D$\alpha$-expression, consistent with results published earlier [5].

References:

POS.77

Determination of inhibitory potency and target residence time of p38 $\alpha$ MAPK inhibitors

![MAPK inhibitors](image)
p38 α mitogen-activated protein kinase (MAPK) is a serine/threonine kinase that plays a crucial role in regulating cellular responses to external stress stimuli, making it a promising target for treatment of inflammatory diseases. Up to date no p38 α inhibitor has been launched to the market. The development of novel p38 α inhibitors requires appropriate testing systems to identify and characterize potential inhibitor candidates. Selected compounds from an in house p38 α MAPK inhibitor library were subjected to a sequential screening approach. Firstly, inhibitory potency was determined by a direct ELISA assay previously reported by Goettert et al [1].

This ELISA assay had to be adapted due to stockout of the initially used detection antibody resulting in the challenge of choosing an equivalent antibody. As a result, the previously used antibody directed against the dual phosphorylated pThr461/ATF-2 protein was replaced by an anti-ATF-2 pThr2 antibody. The suitability of the newly introduced antibody was proven by conventional assay validation. With this new setup IC50 values of the selected compounds were determined.

Secondly, these compounds were analysed regarding their target residence time in a commercially available fluorescence polarization assay kit as reported by Kumar and Lowery [2]. Target residence time was calculated based on the recovery of kinase activity after formation of an inhibitor kinase complex at saturating inhibitor concentrations and subsequent dilution. Results were compared to already existing data obtained by surface plasmon resonance. The rationale behind this comparison was to find a technique more suited for high throughput screenings, featuring a better cost efficiency and higher convenience for application as a standard laboratory procedure.

References:

POS.78

Encapsulation of the dual FLAP/mPGES-1 inhibitor BRP187 in biodegradable polymers improves its bioavailability in human whole blood

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Leukotrienes (LTs) and prostaglandins (PGs) play pivotal roles in the initiation and maintenance of inflammation [1]. To prevent their production from the substrate arachidonic acid (AA), a new pharmacological approach was designed, that is, the dual inhibition of microsomal prostaglandin E2 synthase-1 (mPGES-1) and the 5-lipoxygenase-activating protein (FLAP) [2]. Such dual FLAP/mPGES-1 inhibitors are proposed to exhibit lower risk of side effects versus non-steroidal anti-inflammatory drugs (NSAIDs). The lipophilic and acidic BRP-187 [(4-(4-chlorophenyl)-5-[4-(quinolin-2-ylmethyl)phenyl]isoxazol-3-carboxylic acid) has a high tendency to bind plasma proteins and therefore displays low bioavailability in human whole blood (HVB) and in vivo experiments. To overcome this problem, we encapsulated BRP-187 in biocompatible polymers (i.e., acetylated dextran (AcDex) and poly(lactic-co-glycolic acid) (PLGA) via nanoprecipitation. Dynamic light scattering (DLS) showed that the produced particles were spherical and have a hydrodynamic diameter (d) around 200 nm. The encapsulation efficiency was determined by UV-VIS spectroscopy to 60-80% and the different particles with and without drug were tested on their ability to inhibit the 5-lipoxygenase product formation in polymorphonuclear leukocytes (PMNL). The potency of free BRP-187 and drug-loaded nanoparticles (NPs) was comparable in pretreatment periods of 15 min to 2 h. However, an extended preincubation time of 5 h impaired the efficiency of free BRP-187, while AcDex- and PLGA-based NP containing BRP-187 retained its potent inhibitory effect, indicating that encapsulation improves the availability of BRP-187 in PMNL. Cellular uptake studies were designed using fluorescent NPs containing BRP-187, which showed temporal enrichment of the drug in PMNL. M1 or M2 macrophages when analyzed by confocal microscopy. Finally, we will provide first evidences that BRP-187 NPs are operative in human whole blood assays.

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POS.79

Studies on the cellular uptake of nucleoside antibiotics

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Nucleoside antibiotics are representatives of antibacterially active natural products with versatile structures and mode of action. They include, amongst others, the family of muraymycins which were first isolated in 2002 from Streptomyces sp. [1,2] Members of the antimicrobially active muraymycin family contain a uridine-derived core structure and can be divided into four groups (A-D) due to different peptide structures. [1] For instance, members of series A such as 1 comprise lipophilic side chains which are α-functionalised with a guanidine or hydroxyguanidine-function, whereas muraymycins of series C such as 2 include an unfunctionalised L-3-hydroxyeucine. The antibiotic potency of muraymycins is based on the inhibition of the essential bacterial protein translocase I (MraY). [1] As key enzyme, MraY is involved in the intracellular synthesis of peptidoglycan biosynthesis. Therefore, muraymycins are attractive candidate structures for antimicrobial drug development and thus, several artificial analogues of muraymycins have been synthesized in our group.

Due to the location of the active site of MraY at the cytosolic side of the cellular membrane, inhibitors of MurA are required to cross the bacterial cell wall. [1,3] In Gram-positive bacteria, such as Staphylococcus aureus, antibiotics only have to pass through one membrane whereas Gram-negative bacteria like Escherichia coli are composed of two membranes, one inner and one outer membrane. An essential challenge for the development of muraymycins towards drug candidates is their limited uptake into bacterial cells. With the objective to investigate the cellular uptake in Gram-positive and Gram-negative bacteria, an appropriate assay was established in our group. This assay is based on the incubation of a defined amount of bacterial cells with the candidate compound. The cellular uptake was then quantified by LC-MS after cell lysis. For Gram-negative bacteria a differentiation between periplasmic and cytosolic uptake has also been achieved. Some first results regarding the cellular uptake of several synthetic muraymycin derivatives in different types of bacteria will be presented.

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References:

Dynamic pharmacophore models unveil binding mode ensembles of partial GPCR agonists

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Given the essential role of G protein-coupled receptors (GPCRs) in many physiological processes, more than 30% of currently marketed drugs deploy their therapeutic effect by targeting GPCRs. However, the molecular understanding of how ligands translate their chemically encoded information to intracellular signaling is still incomplete, which renders novel methodologies for studying GPCRs highly necessary for rational drug design 1,2.

In this talk we will focus on a novel computational approach, which combines classical three-dimensional pharmacophores with MD-based sampling in a fully automated fashion. The resulting dynamic pharmacophores (dynophores) provide information about the interaction pattern in space and time 3,4. This enables us to mechanistically understand protein-ligand complexes as dynamic entities.

We will demonstrate the descriptive power of dynamic pharmacophores on a challenging task in GPCR research, the mechanistic basis for partial receptor activation. Using muscarinic receptors as a model system we show the existence of binding mode ensembles for classical partial muscarinic agonists like picrocine or arecoline 5. By a comparison with the full agonist iperoxo and the inverse agonist QNB, we can link distinct binding modes with receptor activation and deactivation.

Furthermore, we discuss the concept of binding mode ensembles in the context of crystallographic data and recent NMR studies, which indicate that binding mode ensembles might play an important, but underestimated role for ligand-dependent pharmacological effects at GPCRs and other drug target classes.

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5. M. Bermudez et al.: manuscript in preparation

Synthesis and biological activity of aminotriazoles targeting human coagulation factor XIIa

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Thromboembolic conditions are one of the leading reason for mortality worldwide claiming the lives of more people than AIDS, breast cancer, and car accidents combined. The high death toll of thrombosis is explained by its connection to the top three cardiovascular killers: heart attack, stroke, and venous thromboembolism. Considering a high thrombosis-related mortality rate, urgent measures should be undertaken to reduce death rate, disability, and economic losses caused by this disorder.

Anticoagulants are life-saving drugs, which are used to counteract thrombosis. However, all currently used anticoagulants (e.g. 1-3) exhibit a life-threatening side effect of internal bleeding 6,7 as they target vital enzymes of the coagulation cascade. Whereas mainstream research in this field is focused on ‘well-established’ drug targets (thrombin and factor Xa), we strive to develop novel inhibitors of Hageman Factor (FXIIa, one of a key player in thrombosis development) controlling internal bleeding risk 8,9.

To disrupt the intrinsic coagulation pathway and prevent thrombosis (without affecting extrinsic pathway), FXIIa proteolytic activity should be blocked by an appropriate inhibitor. For this purpose, after careful analysis of FXIIa’s crystal structure, two series of small molecule inhibitors (6-7) possessing aminotriazole scaffold were designed and synthesized starting from commercially available carboxylic acids 4 and anhydrides 5.

Synthetically obtained compounds were tested for their ability to inhibit the whole blood and plasma coagulation using in vitro coagulation assays (activated partial thromboplastin time (aPTT), prothrombin time (PT)) and compared to the activity of anticoagulants 1-3. Some of the prepared aminotriazoles 6-7 significantly prolonged coagulation time in aPTT tests showing compounds’ ability to affect the intrinsic coagulation pathway, which is initiated by FXIIa. In contrast, synthesized compounds were found to have little effect on PT showing thereby little to no influence on the extrinsic coagulation pathway. This is an indirect indication of their selectivity towards the serine proteases from the intrinsic coagulation pathway. Based on the biological activity data structure-activity relationships were established. Currently, we study specific inhibitory activity and selectivity of synthesized aminotriazoles 6 and 7 towards FXIIa.


Improvement of bioanalytical sensitivity and COX-2-selective antitumor activity of cobalt alkyne complexes due to ligand fluorination

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[\text{[\text{Prop-2-ynyl}}-\text{2-acetoxybenzoate}]\text{dicobalt} \text{hexacarbonyl} \text{(Co-ASS)}] \text{is a metal complex that demonstrated promising growth-inhibitory potency against certain tumor cell lines [1]. The inhibition of both cyclooxygenase isoenzymes (COX-1 and COX-2) is assumed as probable mode of action. However, the selective inhibition of COX-2 is aimed because it plays a significant role in carcinogenesis, while COX-1 mainly regulates homeostatic functions [2].}

With the objective of generating COX-2-selective compounds, the approach to introduce a fluorine substituent within the aromatic moiety of Co-ASS was followed as a frequently applied strategy in medicinal chemistry [3]. Moreover, it was intended to exploit the fluorination for the purpose of bioanalytical labeling.

The influence of the fluorine substituent on the cytotoxic and the antimitotic properties against colon (HT-29) and breast cancer cell lines (MDA-MB-231, MCF-7) was determined. The inhibition of both cellular and isolated COX as well as the induction of the apoptosis-related caspases 3/7 were evaluated. Additionally, cellular uptake studies were conducted based on the sequential analysis of both cobalt and fluorine performing high-resolution continuum-sources atomic respectively molecular absorption spectrometry (HR CS AAS / MAS).

The compounds exhibited a remarkably less activity in the COX-1/2-negative MCF 7 cell line, while they reduced the cell biomass in the COX-
Based on these results, virtual and synthetic fragment growing will be promising fragments used to identify a potent inhibitor of DXS as a potential new agent against tuberculosis essential for medically relevant pathogens (e.g., Plasmodium falciparum).[2] Wang, X.; Dowd, C. S.: [3] The WHO reports that tuberculosis is among the ten top causes of death worldwide with 1.6 million estimated deaths and 10.0 million new infections in 2017. An alarming number of 558,000 people developed a drug-resistant form of tuberculosis (82% of those were also multidrug-resistant).[4] Manuscript under review.

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The WHO reports that tuberculosis is among the ten top causes of death worldwide with 1.6 million estimated deaths and 10.0 million new infections in 2017. An alarming number of 558,000 people developed a rifampicin-resistant form of tuberculosis.[1] In this dangerous situation, identification of new drug candidates is of highest priority to overcome the drug resistance problem. Several inhibitors of DXS have been published to date. While they are very potent in vitro, they often show only weak activity in cell based assays.[2] To improve selectivity and potency, more research into drug resistance Pathway inhibitors is urgently needed.[3] To improve selectivity and potency, more research into drug resistance Pathway inhibitors is urgently needed.

A ligand-based virtual screening afforded three structurally unrelated hit compounds. Adapted from an established assay platform a suitable fluorescence assay for HDAC10 was designed and validated.[2] Fluorescence of the deacetylated product (2) is quenched by derivatization with naphthalamine-2,3-dicarboxylic acid (NDA). Measuring the remaining fluorescence allows to determine the HDAC10 activity. A strong effect on HDAC10 was recently shown for the "HDAC6 selective" inhibitor Tubastatin A. Using the newly developed assay system we were able to confirm HDAC10 inhibition of Tubastatin A and other common HDAC inhibitors. This data implicates new chances for inhibitor design.

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produce adverse and dangerous side effects like euphoria, respiratory depression, constipation, tolerance, and physical dependence. However, centrally active KOR agonists produce centrally mediated side effects such as sedation, dysphoria and diuresis. Peripherally restricted KOR agonists can avoid these CNS side effects and might be useful for the treatment of visceral and neuropathic pain, pruritus, and inflammation. Therefore, research has been focusing on KOR agonists with peripheral restriction.

Since KOR affinity and selectivity are strongly dependent on the stereochemistry, we developed conformationally restricted KOR agonists derived from GR-89696. It is a high-affinity and very potent KOR agonist but contains a rather flexible pyrrolidin-1-ylmethyl residue. In order to analyze the bioactive conformation, the ethylenediamine pharmacophore was conformationally fixed by incorporation into a bicycle framework.

On the poster the synthesis of the four stereoisomers of 1 is presented. The (1S,5S,6R)-stereoisomer 1a shows the highest KOR affinity of the four stereoisomers. The N-benzyl moiety outside the pharmacophoric ethylenediamine system allows further chemical modifications (e.g. passage of the blood brain barrier).

References:

### POS.86

**PPARγ modulators as add on agents to resensitize imatinib-resistant CML cells**

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Recent studies examined the achievement of deeper molecular response in the treatment of chronic myeloid leukemia (CML). A new strategy is based on the combination of the tyrosine kinase inhibitor imatinib with Peroxisome Proliferator-Activated Receptor gamma (PPARγ) ligands [1]. Pioglitazone, a full agonist of PPARγ, has been screened for the therapy of resistant CML and revealed the gradual elimination of the residual CML stem cell pool in combination with imatinib [2]. The partial PPARγ agonist telmisartan (Figure 1) was studied concerning its effect in cancer therapy as well [3]. It is preferable to pioglitazone due to its lower potency on PPARγ and thus having less side effects. Our group showed for the first time that telmisartan, when combined with imatinib, can circumvent the resistance of the CML cells more effectively than the full PPARγ agonist pioglitazone.

These findings induced us to modify telmisartan leading to two series of compounds (Figure 1, X = H, F, Br, OH, NH2). The carboxylic acid series (R = OH) was aimed to activate PPARγ comparably to telmisartan, while the series bearing a carboxamido moiety (R = NH2) was assumed to show lower activation. Hence, the correlation between the impact on PPARγ and the potency of the compounds on resistant CML cells was intended to be understood.
Pioneering human induced pluripotent stem cell (iPSC)-based preclinical studies have raised safety concerns and pinpointed the need for safer and more efficient approaches to generate and maintain patient-specific iPSCs. One approach is searching for compounds that influence pluripotent stem cell reprogramming using functional screens of known drugs. Our high-throughput screening of drug-like hits showed that imidazopyridines—analogs of zolpidem, a sedative-hypnotic drug—are able to improve reprogramming efficiency and facilitate reprogramming of resistant human primary fibroblasts. The lead compound (464) showed a remarkable OCT4 induction, which at least in part is due to the inhibition of H3K4 demethylase (KDM5, also known as JARID1). Experiments demonstrated that KDM5A, but not its homolog KDM5B, serves as a reprogramming barrier by interfering with the enrichment of H3K4Me3 at the OCT4 promoter. Thus, our results introduce a new class of KDM5 chemical inhibitors and provide further insight into the pluripotency-related properties of KDM5 family members.
via a process that was designed to be minimally irritating to the candidates and that took constructive alignment and the necessary change management respectively into consideration.

The major outcome characteristics (average grades and failure rates) and their consistencies have been compared between the periods before ("1st period") and after ("2nd period") the implementation of the new concept.

**Aim:** Subject of the retrospective data analyses were the outcomes for the 4 examination modules of the 1st pharmacy state exam and the comparison between the examination performance before and after the changes in the legal regulations (2nd AAppO-ÄndV, 2000).

**Methods:** For this purpose the average performance and the failure rates of these examination modules have been analyzed separately for the periods before and after the changes in the legal regulations (2nd AAppO-ÄndV, 2000). Moreover, trend tests were performed to detect significant differences between these 2 time periods in focus.

**Results:** The arithmetical means of examination grades calculated since 1984 for (I) – (IV) amount to (I) 3.23, (II) 3.13, (III) 3.10, (IV) 3.13. The average failure rates were (I) 15.4 %, (II) 18.4 %, (III) 14.9 %, and (IV) 13.3 %.

The arithmetical means of the grades were fairly stable for each of the analyzed periods. Neither positive nor negative trends were detected (trend test, n.s.). Yet, for each of the submodules average grades indicate a decay in performance within the new concept (2nd period): (I) 3.14 → 3.25, (II) 2.97 → 3.25, (III) 2.91 → 3.30, (IV) 2.96 → 3.28.

**Conclusion:** The introduction of new subjects -apparently- increases the complexities of each of the respective MCO submodules (and, hence, their difficulties) as well as the complexity of the whole 4 independent submodules. However, failure rates have remained largely unchanged. It can be concluded that the change process was smooth and successful; yet, the character of the examination modules have changed to some extent.

**Stammsitz der Berliner Apotheker – Chronik der 128jährigen Geschichte des Hauses in der Carmerstraße 3**

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BAV-S14-1978-1982; Geschäftsbericht 1981 und VHS- Videos im BAV.


Norbert Bartlitzko zum 50jährigen Kreistag; zitiert in 50-Jahr-Abenteuer.de/zeitschriften und 50jahre-abenteuer.de/kalender/kammernstandorte

A. Adlung: Der Berliner Apotheken-Verein in seiner geschichtlichen Entwicklung (1932), S. 11.

**Aim:** Retrospective analyses of the 2 out of a total of 4 exam days include a survey of the exam contents, potentially favored topics within the blueprint, of the test question performance parameters, and of the candidate performance, from year 2006 onwards.

**Other Topics**

**Strengthening of the pharmacy-specific focus of the first state examination through the AAppO revision of 2000:** More than a decade of MCO state exams with human biology and basic pharmacuetics

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In other countries than Germany the pharmacy curriculum may determine that pre-pharmacy studies cover the students’ education in basic sciences. In Germany, this basic part is integrated into the pharmacy curriculum and supposed to be pharmacy relevant. According to the AAppO, in general, exam objectives (as derived from and agreed within constructive alignment) need to be of pharmaceutical relevance. The two entirely new subject fields introduced into the exams (via a revision of the AAppO in the year 2000) are human biology (clinical anatomy and physiology) as well as pharmacuetics with focus on drug formulation (compounding), and both of them are of high intrinsic pharmaceutical relevance. The first part of the state examination consists of 4 independent submodules (= 4 separate MCO exams = 4 subject groups); (I) pharmaceutical chemistry, (II) pharmaceutical biology, biochemistry/biotechnology, human biology, (III) physics, physical chemistry, drug formulation, (IV) pharmaceutical analysis and QC. The candidates’ performance within these new subject fields is characterized via the analyses of their contribution to the overall outcome in the respective exam submodules, (III) for "biology"=day 2 and (II) for "pharmacy"=day 3.

**Aim:** Retrospective analyses of the 2 out of a total of 4 exam days include a survey of the exam contents, potentially favored topics within the blueprint, of the test question performance parameters, and of the candidate performance, from year 2006 onwards.
Methods: The test question analyses include the calculation of average performance parameters (r- and p-values) for each exam, i.e., a total of 24 performance parameters resulted, since two exam dates per year have been analyzed. The r-value indicates the difficulty of an item, the r-value the discriminatory power of an exam item.

(i): “Biology” consists of residual pharmaceutical biology (RPB) + human biology (HB), 73 vs. 27 test items;

(ii): “Physics” consists of physics and physical chemistry (PPC) + physical pharmacy and formulations (PPF), 60 vs. 20 test items.

Results: With respect to human biology as well as pharmaceutical compounding, the selected topics of items are evenly distributed over the respective catalogue with some enhanced focus on the (central) nervous and the cardiovascular systems.

(ii): Within-exam average r-values yield arithmetical means 0.27 for HB and 0.29 for RPB (ranges, 0.23 – 0.30 for HB and 0.25 – 0.31 for RPB), average p-values amounts to 60.4 % for HB (range, 48.0 % - 65.7 %) and 60.1 % for RPB (range 55.2 % - 64.6 %).

(iii): Arithmetical means of the respective average r-values for physics to drug formulation amount to 0.22 for PPF and 0.27 for PPC (range, 0.17 – 0.25 for PPF and 0.25 – 0.30 for PPC), p-values were 64.9 % for PPF (range, 50.8% - 73.3%) and 58.4 % for PPC (range, 51.4% - 61.5%).

Conclusions: The retrospective analyses indicate that - for candidates - human biology is as difficult as residual pharmaceutical biology and physical pharmacy and drug formulation appears less difficult than physical and pharmaceutical chemistry. No significant trend of r-p-values was detected in any of the sequences (trend-test).

POS.92

His-Tyr-based heme-binding peptides: From heme binding to catalytic function

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Transient heme binding to proteins is known to change their structure and consequently may alter their function and corresponding physiological and/or cellular responses. [1] In addition, in some cases (e.g. Alzheimer’s disease) it has been shown that interaction with heme could also lead to the development of a catalytic activity of the complex similar to the action of peroxidases. [2,3]

In an approach to classify and evaluate motifs for heme binding to short, surface-located peptide stretches in proteins - so-called heme-regulatory motifs - a concept was derived to discriminate different binding modes and to predict potential heme-regulated proteins and their heme-binding sites. [4] This classification was based on the existence of different iron-coordinating amino acids as well as distinct surrounding amino acids in a short nonapeptide sequence. Extensive studies on more than 150 heme-peptide complexes have been carried out in order to understand their interactions with heme. [4] Peptides containing histidine or tyrosine as the iron-coordinating amino acid were studied in more detail earlier. [5] In further attempts to understand heme binding to these peptides we then focused on specific subclasses of such histidine- and tyrosine-based motifs. In addition, we examined how these peptides may influence the peroxidase-like activity of heme. In a recent approach we have shown that peptides other than amyloid-beta may possess such a catalytic activity and that these may differ in length and amino acid composition. [2] It turned out that the most promising results were obtained for peptides containing histidine or tyrosine as the coordinating amino acid, however, not all of them showed a peroxidase-like activity despite a high affinity for heme binding. This motivated us to have a closer look at a selection of app. 50 peptides of different sequence compositions to understand requirements for the development of a peroxidase-like activity in non-covaletly linked heme-peptide complexes. Moreover, such complexes would not only be physiologically relevant to better understand disorders such as Alzheimer’s disease or lipid oxidation, yet could also represent a starting point for the development of a new class of mimics for proteins such as cytochrome P450 as diagnostic tools to determine the metabolic pathway of a drug/prodrug.

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POS.93

Towards scaffold-diverse DNA-encoded small molecule libraries

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Compound libraries barcoded with DNA, called DNA-encoded libraries (DELs), offer an efficient, high-throughput platform for target-based screening. [1] DELs translate molecular biology techniques for molecular evolution such as DNA barcoding, selection, DNA amplification, and DNA sequencing to combinatorial compound synthesis and identification. [2] We developed computer-based statistics tools to design DNA barcodes, and to deconvolute screening data for screening outcome interpretation. The productivity of any screening library depends not only on chemical space coverage. Access to diverse compound structures depends on a combination of available starting materials and synthesis methodology. Solution-phase DEL synthesis requires chemical methods that show a combination of DNA-convertibility, water-tolerance and fast kinetics to operate on highly diluted DNA-tagged reactants. Ideally, they are operationally simple and furnish target compounds from diverse starting materials with high yields. Only a very few chemical reactions meet all these demands, access to encoded heterocyclic chemistry is even rare. In order to expand methodology for encoded compound synthesis, we are developing DEL coding strategies that are initiated with solid phase-bound DNA strands. [3,4] Solid phase synthesis enables the use of a broad scope of organic solvents for target compound synthesis, and harsher reaction conditions because the DNA is constantly protected. A solid-phase-based DEL strategy allowed for translation of several chemistries for encoded library synthesis, among them cycloaddition reactions and isocyanide MCR chemistry (Figure 1a). [5] In a second strategy, we develop bespoke catalysts for encoded library synthesis based on oil-in-water micelles. Micellar catalysis is an attractive principle for DEL synthesis due to both reaction acceleration and DNA shielding. Amphiphilic block copolymers formed spherical nanoreactors and immobilized a catalyst in the DNA-inaccessible hydrophobic core. Sulfonic acid-substituted micelles converted DNA-tagged aldehydes by Povarov and Groebke reactions to diverse substituted tetrahydroquinolines and imidazopyridines without noticeable DNA degradation, and facilitated Boc group removal from amines (Figure 1b). [6] Furthermore, a Cu(I)-immobilizing micelle mediated the DNA-compatible oxidation of DNA-tagged aldehydes by Povarov and Groebke reactions to diverse substituted tetrahydroquinolines and imidazopyridines without noticeable DNA degradation, and facilitated Boc group removal from amines (Figure 1b). [6]

Figure 1: Expanding the chemical space of DNA-encoded compound libraries. a) Exploring the scope and limitations of solid phase-based synthesis approaches to encoded libraries. b) A micelle-based reaction system for encoded synthesis.

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6. submitted
Coenzyme A (CoA), synthesized from its precursor pantothenic acid (Vitamin B5), is a molecule with versatile functions, mainly renowned as a metabolic key factor throughout all organisms. It essentially participates in the anabolism and catabolism of fatty acids, sugars and amino acids as well as in the aerobic energy production. Furthermore, its unique carboxylic acid activation in the form of thioesters is used for the acetylation of small molecules, i.e. for the physiological production of the neurotransmitter acetylcholine or the phase II biotransformation of drugs like sulfonamides. Biomolecules like histones are acetylated as well, resulting in a flexible epigenome. Current research suggests not only an acetyl transfer to proteins, but additionally a redox-dependent modification of cysteine residues by a disulfide bridge with free CoA itself, termed CoAlation.

All mentioned examples rely on the reactivity of the terminal thiol of CoA, while its nucleoside part seemed to be redundant until recently. In 2009, Ribonucleic acids (RNAs) modified with the cofactors nicotinamide adenine dinucleotide (NAD) and CoA at their 5′-end were identified in bacteria in addition to the canonical triphosphate. Further efforts on the characterization of NAD-RNAs propose that this cap structure also exists in eukaryotes, in parallel to the mRNa cap structure 7-methylguanylate (m7G). No successful progress was reported for CoA-RNAs, though. The development of specific analytical methods for CoA-RNAs will reveal their functional scope in comparison with CoA and other RNA 5′-ends in vitro as well as their relevance in vivo. 

Biosynthetic strategies to access 3′-dephospho-Coenzyme A (dCoA), dCoA thioesters and other analogs were established and resulting products are incorporated into model RNAs by in vitro transcription. Functional studies are then performed, i.e. the decapping of CoA-RNAs by pyrophosphatases to yield monophosphorylated 5′-ends, and analyzed by thiol-mercury affinity electrophoresis. In vivo experiments address CoA capping rates of e. coli RNAs during different growth conditions on the one hand. Therefore, RNAs are digested and analyzed by liquid chromatography-tandem mass spectrometry (LC-MS/MS). On the other hand, CoA-RNA specific sequencing protocols aim to identify highly CoA-modified RNA sequences, which are examined in detail by customized Northern blot analyses in a next step. The outcomes will disclose a new perspective on both, the cap and RNA cap structures.

References:

As thiol modified silica is a commonly used material in a wide range of chromatographic areas, e.g. adsorption and separation of catecholic ions, protein separation by thiol-disulfide interchange of sulfhydryl containing proteins in covalent chromatography and in development of stationary phases as basic material for further modification, this work reports on a study conducted on the synthesis of thiol modified silica gel by thermally induced and photo-induced polymerization reactions. Thereby, poly(3-mercaptopropyl)methylsiloxane (PMPMS) was coated onto vinyl modified silica by radical thio-ene click polymerization reaction forming a crosslinked layer on its surface. According to literature such bonding chemistry showed significant better stability in comparison to brush-type analogs with bifunctional siloxane bonding to the silica. Aiming a sulfhydryl group rich, thin PMPMS layer on the surface of the silica several factors such as quantity of PMPMS, radical starter and solvent were investigated. In addition, reaction time and temperature were studied as well. In thermally induced polymerization reactions 2,2′-azobisisobutyronitrile (AIBN) was used as radical starter, in photo-induced reactions 2,2-dimethoxy-2-phenylacetophenone (DMPA) was used instead. As the thickness of the PMPMS layer correlates with the total amount of incorporated PMPMS, it was determined indirectly by the sulfur content measured by elemental analysis. For the purpose to assess the amount of unreacted sulfhydryl groups a chemical reaction with 2,2′-dipyridyl disulfide (DPDS) was carried out. Here, accessible and reactive sulfhydryl groups react quantitatively with DPDS to generate equimolar amounts of 2-gyridyl thiol, which was determined by HPLC-UV/Vis. The estimation of the sulfhydryl group content is necessary, as an adequate amount is needed for chromatographic or further modification issues such as implementation of selective ligand molecules on the silica surface. Consequently, suitable reaction conditions were figured out in order to generate a thin PMPMS layer on the silica surface containing a sufficient amount of unreacted sulfhydryl groups.

Development of stable bond thiol modified silica with high loading density

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Soil-transmitted helminth infections are one of the most common infections worldwide. Deprived communities in tropical and subtropical areas show an increased prevalence, in particular in sub-Saharan Africa [1]. However, anti-infective drugs like anthelmintics are highly affected by pharmaceutical counterfeiting [2]. Due to the incomplete data on substandard and falsified medicinal products, in total 42 batches of anthelmintic drugs, including albendazole (ABZ), mebendazole (MBZ) or praziquantel (PZQ), were collected in the northwest of Tanzania from randomly selected local suppliers to determine the content of the active pharmaceutical ingredient (API) by HPLC-UV. In addition, 54 batches were collected in West Africa (Côte d’Ivoire, Ghana and Burkina Faso). The method used for the determination of the content of ABZ, MBZ and PZQ are related to the assay described in the International Pharmacopoeia [4] and European Pharmacopoeia [5] respectively.

Determination of the content of albendazole, mebendazole and praziquantel in pharmaceutical preparations available in Northern Tanzania

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4.3 Analytics and toxicology

Analytical Toolbox for the Characterization of Coenzyme A-capped Ribonucleic Acids

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POS.95
Meaningful size characterization of nanoparticles is a major prerequisite for the successful development and use of nanoparticles in pharmaceutics. Size characterization of nanoparticles involves not only the determination of the physical stability during storage but should also be used to ensure sufficient stability of the particles during in-vivo studies [1]. Today, most frequently techniques include microscopic techniques, dynamic light scattering and laser diffraction. However, these techniques are not always predictive and thus other techniques might be necessary to yield discriminative and meaningful results.

Methods:
In this study the Coulter-Counter principle (Multisizer 4e, Beckmann-Coulter, Germany) was used for the characterization of three different nanocarrier systems (nanocrystals, lipid nanoparticles, solid lipid nanoparticles). Results obtained were compared to the results obtained by the classical methods, i.e. dynamic light scattering, laser diffraction and light microscopy. Measurements were performed in water and in isotonic sodium chloride solution. In addition, for all samples, zeta potentials were analysed in each dispersion medium.

Results:
Classical size characterization of the nanoparticles yielded mean particle sizes of about 150 - 200 nm for the lipid nanoparticles and of about 400 nm for the nanocrystals. In contrast, much larger sizes were observed, when the particles were analysed with the Multisizer. Reasons for this are the need to analyse the particles in isotonic sodium chloride solution, which led to a strong decrease in zeta potentials (decrease > 25 mV) and thus to a partial agglomeration of the formulations. The destabilizing effect of the sodium chloride solution was not reliably detected by dynamic light scattering and laser diffractometry measurements. Reasons for this might be the formation of rather loose and low amounts of large agglomerates which were too large to be detected by dynamic light scattering and/or were destroyed by the stirrer of the laser diffractometer.

Conclusion:
Classical methods for particle size characterization, e.g. dynamic light scattering and laser diffraction, yield reproducible results when samples are analysed in water. However, large aggregates can be overlooked. In this study, destabilization of the samples due to the addition of electrolytes was only reliably detected by utilization of the Coulter-Counter principle. In fact, if size characterization is done to predict the physical stability of samples in isotonic solutions, e.g. cell culture media, impedance measurements utilizing the Coulter-Counter principle are advantageous.

Acknowledgments: University of Würzburg: Klaus Schilling and Sebastian Schmidt.

References:
4. POS.98
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Utilization of the Coulter-Counter principle for size analysis of nanoparticles

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Figure 1: Boxplot of all tested samples divided into API-classes

Figure 2: Results of the batch-analysis of the tested drug products (● ABZ; ■ MBZ; ♦ PZQ) with at least one sample with API content out of specification limits (dotted lines)

Depending on the availability, one to six samples per batch were examined. So far, 94 samples out of 33 batches from Tanzania were tested. Unravelling seven samples out of five batches did not complied with specifications of the current pharmacopeias.
orientation of the cofactor nicotinamide ring in their bound conformations. This coincides with a switch of the sign of the most prominent band in the associated CD spectra.[4]

Figure 1. Effect of cofactor binding on the circular dichroism (CD) spectrum.

References:

**POS.99**

New approaches for (Smartphone-) Imaging of Lateral Flow Immune Assays (LFIA). Enhancing the capability for point of care diagnostics (POCTs).

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Lateral Flow Immune Assays (LFIA) are easy-to-use sensing systems for home monitoring of e.g. drugs and biomarkers. Nanoparticle dyes, like gold nanoparticles or fluorescent quantum dots can be used to increase sensitivity, multiplexing potential or allow for imaging and quantification by smartphone. We introduce an imaging system for monitoring of cardiac glycoside digoxin using a smartphone as readout device and includes a customized Shiny App for data processing based on a gold-nanoparticle labelled LFIA [1]. This approach is now extended to a quantum dot labelled duplex LFIA using an improved version of our smartphone readout device in combination with data processing software to develop a portable and fast assay for sepsis biomarkers: C-reactive protein (CRP) & Interleukin 6 (IL-6). We compare imaging hardware and probes composed by different nanoparticles for the design of easy to use-, low cost LFIA point of care devices matching the need for fast diagnostics in precision medicine.

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References:

**POS.100**

The development of a second dimension for the 2D-LC separation of oligonucleotides

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In the recent years, the use of biopharmaceuticals has become increasingly important. Between January 2014 and July 2018, 47% of genuinely new approved drugs in the US were based on biopharmaceuticals. Additionally, 40% of the 6,000 or more products in clinical development worldwide are biopharmaceuticals. Besides monoclonal antibodies (mAbs), which remains the dominant branch of biopharmaceuticals, other gene- and nucleic acid-based products like antisense oligonucleotides (ASOs) have also been established [1]. From Fomiviren as the pioneer ASO treating cytomegalovirus (CMV) retinitis to Zolgensma for a new gene therapy by spinal muscular atrophy (SMA), the list of such nucleic acid-based approvals is very long. Hence, a better understanding of these complex synthetic oligonucleotides is essential and demands suitable analysis methods. Conventionally, the characterization of oligonucleotide is performed by ion-pair reversed-phase liquid chromatography (IP-RP-LC) coupled to a mass spectrometry (MS) detection. But the use of ion-pairing reagents like triethylamine (TEA) causes significant ion suppression which decreases the sensitivity of MS [2]. A possibility to avoid this problem is to add a second chromatographic dimension featuring a complementary separation mechanism without any MS-incompatible additives in the mobile phases. Recently, our group has reported a LC-method using mixed mode chromatography (MMC) to separate oligonucleotides which could be considered as the first chromatographic dimension in a 2D-LC setup [3]. In this work, we want to develop an appropriate complementary LC-method for the second dimension with a focus on the hydrophilic interaction liquid chromatography (HILIC-LC) which is expected to be an effective alternative to IP-RP for LC-MS analysis of oligonucleotide. This method should then be combined with the above mentioned HPLC method as 1D in a 2D-LC setup.

References:
Ovarian carcinoma is one of the most lethal gynaecological malignancies. Cisplatin is well established as cytostatic agent in the therapy of ovarian carcinoma. However, since there is a high proportion of advanced stage cases at diagnosis, the overall curative rate is less than 40% across all stages. Although most ovarian cancer patients respond initially to front-line Pt-based chemotherapy, resistance to cisplatin is commonly observed during treatment, which massively restricts the therapeutic regime. Circumventing cisplatin resistance, therefore, remains a critical goal for cancer treatment and considerable efforts have been taken to solve this problem throughout the past years. Aiming to better comprehend the functional diversity of ovarian tumor cell lines we pursue this strategy characterizing a new ovarian carcinoma cell line W1, or the cisplatin resistant subtype W1CR, which presents different resistance features, such as collagen overexpression [3]. The aim of this study was to provide insight into the molecular mechanisms of cisplatin chemoresistance and the elucidation of potential targets for sensitization strategies.

To evaluate CAM-DR as target for sensitization strategies, we analyzed cytotoxicity (MTT-assay) of cisplatin in both cell lines in absence or presence of COL1 and the associated signaling pathways at the protein level (SDS-PAGE/WB, proteome profiler array) or by RNA microarray. To further focus on the role of integrin on cellular resistance, a β1 integrin knockdown was performed in W1/W1CR cells by a lentiviral approach.

MTT data confirmed that the cisplatin resistant subline W1CR displayed a 3- to 10-fold higher EC50 value of cisplatin cytotoxicity compared to W1 cells. If subjected to integrin activating treatment (COL1), both W1 and W1CR cells showed a significant increase in resistance to cisplatin, associated with the upregulation of various signaling pathway components, which finally refer to an impact of adhesion mechanisms on resistance. Furthermore, inhibition of integrin-linked kinase (ILK) or focal adhesion kinase (FAK) led to a higher gain in sensitivity, especially in cells grown on COL1. In line with these data, COL1 lost its resistance triggering effect in the W1 β1-integrin knock-down cells, while the resistant W1CR β1-integrin knock-down cells behave differently, indicating a more rapid rewiring of the resistance signaling in the W1CR cells.

The data provide evidence for a potential link between integrin activation and tumor cell resistance against cytotoxic drugs and thus sheds new light on CAM-DR as novel therapeutic targets in oncology. Further work is needed to investigate and simulate the underlying mechanisms of CAM-DR, such as transferring into a 3D cell culture model that can better reflect the in vivo setting of tumors than conventional monolayers (2D) cell culture. In addition, further signaling pathways and other cellular contacts with the microenvironment components should be investigated as potential targets.

References:

**Insight into the cisplatin resistance of W1CR ovarian carcinoma cells to decipher novel targets for sensitization**

**4.4 Cancer**

**POS.101**

**Insight into the cisplatin resistance of W1CR ovarian carcinoma cells to decipher novel targets for sensitization**


Ovarian carcinoma is one of the most lethal gynaecological malignancies. Cisplatin is well established as cytostatic agent in the therapy of ovarian carcinoma. However, since there is a high proportion of advanced stage cases at diagnosis, the overall curative rate is less than 40% across all stages. Although most ovarian cancer patients respond initially to front-line Pt-based chemotherapy, resistance to cisplatin is commonly observed during treatment, which massively restricts the therapeutic regime. Circumventing cisplatin resistance, therefore, remains a critical goal for cancer treatment and considerable efforts have been taken to solve this problem throughout the past years.

Tumor cell binding to microenvironment components such as collagen type 1 (COL1) attenuates the sensitivity to cytotoxic drugs like cisplatin, referred to as ‘cell adhesion mediated drug resistance’ (CAM-DR) [1,2]. Aiming to better comprehend the functional diversity of ovarian tumor cell lines we pursue this strategy characterizing a new ovarian carcinoma cell line W1, or the cisplatin resistant subtype W1CR, which presents different resistance features, such as collagen overexpression [3]. The aim of this study was to provide insight into the molecular mechanisms of cisplatin chemoresistance and the elucidation of potential targets for sensitization strategies.

To evaluate CAM-DR as target for sensitization strategies, we analyzed cytotoxicity (MTT-assay) of cisplatin in both cell lines in absence or presence of COL1 and the associated signaling pathways at the protein level (SDS-PAGE/WB, proteome profiler array) or by RNA microarray. To further focus on the role of integrin on cellular resistance, a β1 integrin knockdown was performed in W1/W1CR cells by a lentiviral approach.

MTT data confirmed that the cisplatin resistant subline W1CR displayed a 3- to 10-fold higher EC50 value of cisplatin cytotoxicity compared to W1 cells. If subjected to integrin activating treatment (COL1), both W1 and W1CR cells showed a significant increase in resistance to cisplatin, associated with the upregulation of various signaling pathway components, which finally refer to an impact of adhesion mechanisms on resistance. Furthermore, inhibition of integrin-linked kinase (ILK) or focal adhesion kinase (FAK) led to a higher gain in sensitivity, especially in cells grown on COL1. In line with these data, COL1 lost its resistance triggering effect in the W1 β1-integrin knock-down cells, while the resistant W1CR β1-integrin knock-down cells behave differently, indicating a more rapid rewiring of the resistance signaling in the W1CR cells.

The data provide evidence for a potential link between integrin activation and tumor cell resistance against cytotoxic drugs and thus sheds new light on CAM-DR as novel therapeutic targets in oncology. Further work is needed to investigate and simulate the underlying mechanisms of CAM-DR, such as transferring into a 3D cell culture model that can better reflect the in vivo setting of tumors than conventional monolayers (2D) cell culture. In addition, further signaling pathways and other cellular contacts with the microenvironment components should be investigated as potential targets.

References:
The strong responses to Sestrin 2 over-expression indicate treatment potential of targeting Sestrin 2 functional role. Nevertheless, cell line specificity of this over-expression suggests that targeting Sestrin 2 is highly contextual and depending on the genetic background of cancer as well as on its metabolic phenotype.

**4.5 Clinical Pharmacy**

**GLICEMIA 2.0 – a randomized, controlled trial in secondary and tertiary prevention in a type 2 diabetic population: preventive care in pharmacies, Germany**

**Methods:** GLICEMIA 2.0 is a randomized, controlled trial in pharmacies the impact of an intensive pharmaceutical care on the glycaemic control developing this disease [2]. The aim of GLICEMIA – a primary prevention program. GLICEMIA significantly reduced the risk for type 2 diabetes patients in pharmacies. This program is based on the concept of GLICEMIA – a primary prevention program. GLICEMIA significantly reduced the risk for type 2 diabetes patients who had a high probability developing this disease [2]. The aim of GLICEMIA 2.0 is now to evaluate the impact of an intensive pharmaceutical care on the glycaemic control of type 2 diabetes patients.

**Methods:** GLICEMIA 2.0 is a randomized, controlled trial in pharmacies located in Bavaria, Germany. Eligible participants were type 2 diabetes patients with among others, a minimum HbA1c of 7 % and at least 18 years of age. The pharmaceutical care in the intervention group consisted of personal consultations and group meetings with a focus on lifestyle intervention and medication management. The control group received written materials and the usual care. Assessments took place at baseline, after six and after twelve months. As primary outcome the study examines if there is a statistically significant difference in HbA1c – values (Point-of-Care) between intervention and control group. As secondary outcome further laboratory data (fasting level of blood glucose, weight, blood pressure), physical activity, life quality, adherence, number of medications and drug related problems are evaluated. Furthermore, a cost analysis takes place.

**Results:** 26 pharmacies took part in GLICEMIA 2.0 and recruited 198 type 2 diabetes patients in total. Therefore, 183 fulfilled the inclusion criteria. 30 patients quit prematurely. During the study period of one year, the mean HbA1c values ± SD in the intervention group (n=96) was reduced from 8.3 ± 1.3 % to 7.3 ± 1.0 % [median: from 8.0 % (IQR: 7.5 % - 8.6 %) to 7.3 % (6.5 % - 7.9 %)]. The control group (n=86) only showed a reduction from 8.1 ± 1.0 % to 7.8 ± 1.2 % [median: from 7.9 % (IQR: 7.3 % - 8.6 %) to 7.6 % (IQR: 7.1 % - 8.2 %)]. Including all eligible patients the difference adjusted via linear mixed model with several co-variables between intervention and control group (n=181) in mean change of HbA1c from baseline was statistically significant (intervention effect: -0.5 %, p=0.0007). No statistically significant difference in fasting plasma glucose levels could be proven. 23% of patients in the intervention group could reduce their weight by 5 % or more. 91 % of the intervention group evaluated participating in GLICEMIA 2.0 as beneficial.

**Conclusion:** Summarizing the final results of GLICEMIA 2.0, the study resulted in a significant reduction of HbA1c-values compared to the control group. Almost one in four participants of the intervention group reached the target of reducing his or her weight by 5 % or more. A beneficial effect of pharmaceutical care in type 2 diabetes patients has already been identified. The influence on physical activity, life quality, adherence as well as medication management is still under evaluation and once completed, will conclude the results of the study.

**References:**

**A physiologically-based pharmaco kinetic model of the CYP1A2 substrate theophylline and its application for the prediction of drug-drug interactions with fluvoxamine, rifampicin and smoking**

**Background and Objectives:** CYP1A2 accounts for about 13% of the cytochrome P450 (CYP) content in the liver and is involved in the metabolism of 15% of all therapeutic drugs [1,2]. The US Food and Drug Administration (FDA) recommends theophylline as a sensitive CYP1A2 substrate (fraction metabolized of 0.7 [3,4]), fluvoxamine as a strong CYP1A2 inhibitor, rifampicin as a moderate and smoking as a strong CYP1A2 inducer, for drug-drug interaction (DDI) studies [5]. The objectives of this study were to establish a whole-body physiologically-based pharmacokinetic (PBPK) model of theophylline and to apply this model for the investigation of theophylline DDIs with fluvoxamine, rifampicin and smoking.

**Methods:** PBPK modeling was performed with PK-Sim® and MoBi® (version 7.3.0., www.open-systems-pharmacology.org). Theophylline drug-dependent parameters (e.g. logP, solubility) and theophylline plasma concentration-time profiles (37 clinical studies, dosing range 125 – 426.6 mg as intravenous and 79 – 500 mg as oral dose) were gathered from literature and used for model development. Model evaluation was performed by comparison of predicted plasma concentration-time profiles to observed data of theophylline in clinical DDI studies with fluvoxamine (2 studies), rifampicin (3 studies) and smoking (5 studies).

**Results:** The established theophylline model incorporates metabolism via CYP1A2 and CYP2E1 as well as glomerular filtration with reabsorption in the renal tubule. The metabolic pathways were implemented as saturable Michaelis-Menten kinetics. The theophylline fraction metabolized via CYP1A2 and the plasma concentration-time profiles of theophylline are well described by the model, with 34/37 of the predicted area under the plasma concentration-time curve (AUC) values and 36/37 of the predicted peak plasma concentration (Cmax) values within two-fold of the observed values. To predict the impact of smoking on the theophylline pharmacokinetics, the mixed inhibition of CYP1A2 by fluvoxamine was modeled using inhibition constants (Kic and Klu) of 10 nmol/l [6]. During co-administration with fluvoxamine, the observed theophylline DDl AUC ratio (AUC DDI / AUC control) and Cmax ratio (Cmax DDI / Cmax control) were 2.55 (n = 2) and 1.13 (n = 2), respectively. Fluvoxamine-theophylline DDI modeling predicts a theophylline DDI AUC ratio of 2.63 (n = 2) and a DDI Cmax ratio of 1.11 (n = 2). To predict the impact of rifampicin on the theophylline pharmacokinetics, the induction of CYP1A2 and CYP2E1 was modeled using a half-maximal induction concentration (EC50) of 0.34 μmol/l for both CYP enzymes and maximal induction effects (Emax) of 0.65 for CYP1A2 and of 0.8 for CYP2E1 [6,7]. During co-administration with rifampicin, the observed theophylline DDI AUC ratio and Cmax ratio were 0.84 (n = 3) and 1.05 (n = 2), respectively. Rifampicin-theophylline DDI modeling predicts a theophylline DDI AUC ratio of 0.78 (n = 3) and a DDI Cmax ratio of 0.94 (n = 2). To predict the impact of smoking on the theophylline pharmacokinetics, the induction of CYP1A2 was modeled using a 5.4-fold higher CYP1A2 enzymatic activity. Theophylline plasma concentration-time profiles of smokers can be successfully predicted with 5/5 of the predicted AUC values and 5/5 of the predicted Cmax values within two-fold of the observed values.

**Conclusion:** A whole-body PBPK model of theophylline has been developed, that accurately describes and predicts the plasma concentration-time profiles of theophylline for the entire range of reported doses and administration protocols. Furthermore, this model was successfully applied to predict the effects of fluvoxamine, rifampicin and smoking on the pharmacokinetics of theophylline.

**Acknowledgments:**
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**References:**
A physiologically based pharmacokinetic/pharmacodynamic (PBPK/PD) parent-metabolite model of nicotine including its chronotropic effect and CYP2A6/CYP2B6 metabolism

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Introduction and objectives: Since nicotine is the pharmacologically active substance in tobacco responsible for addiction it also is a proximate cause of smoking-induced diseases such as chronic obstructive pulmonary disease (COPD) and lung cancer [1]. Hence, a detailed understanding of the pharmacokinetics and pharmacodynamics of nicotine is desirable to derive new strategies of smoking cessation and to get a better understanding of the role of nicotine in pathophysiological processes. Our goal was to develop a whole-body physiologically based pharmacokinetic/pharmacodynamic (PBPK/PD) model to investigate the fate of nicotine including its main metabolite cotinine after intravenous (i.v.), oral (p.o.), transdermal and pulmonary administration in nonsmokers and smokers.

Methods: A parent-metabolite PBPK model of nicotine and cotinine was built in PK-Sim® and Mobi® (version 7.4.3) [2]. Physicochemical model input parameters as well as plasma profiles and nicotine-induced chronotropic effect data were obtained from published literature. For PBPK model building, 63 plasma profiles from 27 clinical studies were split into an internal training (12 plasma profiles) and an external test (51 plasma profiles) dataset. Initially, a cotinine model was established by fitting unknown model parameters to plasma profile training data from i.v. administration in healthy volunteers. Subsequently, the model was complemented by the parent compound nicotine using i.v. plasma concentration-time profiles. Model evaluation was performed by comparing observed plasma profiles from the external test dataset with predicted profiles. In addition, nicotine clearance was adjusted for smokers and the model extrapolated to p.o., transdermal and pulmonary application of nicotine. Transdermal and p.o. absorption kinetics were estimated by fitting relevant model parameters. Conclusively, a PBPK/PD heart rate-tolerance model was implemented using heart rate data from 7 different studies.

Results: The model includes nicotine metabolism to cotinine via CYP2A6 and CYP2B6 which accounts for about 75% of nicotine elimination. The corresponding Michaelis-Menten constants Vm and Km were fitted within the range of literature values to 31 µM and 820 µM, respectively, whereas the catalytic rate constant km was fitted to 13 min-1 both for CYP2A6 and CYP2B6. Additionally, nicotine is cleared by an unspecified hepatic clearance (first order kinetics) and renal clearance (GFR fraction of 1.00). The elimination routes are consistent with published literature [3]. For cotinine, the model contains an unspecified hepatic metabolism (first order kinetics) and a glomerular filtration with tubular reabsorption (GFR fraction of 0.07). The final model was capable to precisely describe and predict all i.v. profiles of the internal and external dataset. Geometric mean fold errors (GMFEs) of predicted and observed areas under the plasma concentration-time curve (AUC) were 1.07, 1.30 and 1.06 for nicotine, cotinine metabolite and administered cotinine profiles, respectively. According to published data, nicotine clearance in smokers appears to be about 15% lower compared to nonsmokers [4] resulting in higher AUC values. By adjusting the model to a lower nicotine metabolic capacity, we were also able to predict all plasma profiles including the concomitant increase in AUC after i.v., p.o., transdermal and pulmonary administration in smokers (GMFEs: 1.07, 1.20, 1.13 and 1.27 for nicotine, cotinine metabolite, 1.30, 1.15 and 1.16 for i.v., p.o. and transdermal administration). The PBPK model was complemented by a heart rate-tolerance model including circadian rhythm to describe the chronotropic effect of nicotine. The tolerance model proved superior to a classical Emax model and was able to predict heart rate peaks occurring rapidly after pulmonary and i.v. nicotine intake.

Conclusion: We successfully developed a whole-body PBPK/PD model of nicotine and its main metabolite cotinine to predict nicotine and cotinine plasma profiles in smokers and nonsmokers for different dosing regimens and various application routes in an excellent way. Furthermore, the model successfully predicts the chronotropic effect of nicotine. This may help improving strategies of smoking cessation in order to decrease tobacco addiction and providing insights into the involvement of nicotine in pathophysiological processes.

Reference:
2. Gissane T. et al. Front Physiol. 2011; 2; [F1].

Dose adjustment of risperidone in Cytochrome P450 2D6 intermediate- and poor metabolizer based on physiologically-based pharmacokinetic model

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Background: The genetic polymorphism of Cytochrome P450 2D6 (CYP 2D6) plays an influential role on the appearance of positive and adverse drug reactions to different antipsychotics, especially in risperidone (RIS). Due to non-functional alleles of the CYP2D6 gene, the poor metabolizer (PM) phenotype appears to be associated with more adverse drug reactions, going along with frequent discontinuation of the therapy [1]. Based on that large inter-individual variability in RIS’s and 9-Hydroxyrisperidone’s (9-OH-RIS) plasma concentrations [2], a successful development of a physiologically-based pharmacokinetic (PBPK) model provides a powerful tool to calculate the optimal dose for different CYP2D6 phenotype. The objective of the study is to optimise phenotype-related dosing of RIS, using a whole-body PBPK approach.

Methods: A PBPK model for RIS and 9-OH-RIS was successfully developed to predict the pharmacokinetics on genotyped healthy volunteers treated with single-dose RIS, considering relevant CYP2D6 and -3A4 related changes in the different CYP2D6 phenotypes: extensive metabolizer (EM), intermediate metabolizer (IM), PM and ultra-rapid metabolizer (UM) [3]. Based on the developed PBPK models, steady-state conditions with the administration of 3 mg RIS twice a day were simulated. This simulated dosage of 6 mg/day corresponds to the most recommended clinical dose for schizophrenia patients. For those CYP2D6 genotypes in which plasma concentration of the active moiety (RIS plus 9-OH-RIS) was outside the therapeutic range (20-60 µg/L), a dose adjustment was performed [4].

Results: Based on the previously developed PBPK models, steady-state conditions were simulated. Here, steady-state trough plasma concentration of PM was +100% higher than those of EM (25.3 µg/L in EM and 52.4 µg/L in PM) at the same dose. Even peak plasma concentration of PM was significantly higher (+53.1%) in PM than in EM (50.5 µg/L in EM and 77.4 µg/L in PM). This was also evident in the CYP2D6 IM type. Here, trough plasma concentration was +62.3% higher than those of EM (25.3 µg/L in EM and 41.1 µg/L in IM), as well as peak plasma concentration which was +38.5% higher in IM than in EM (50.5 µg/L in EM and 70.0 µg/L in IM). Consequently, RIS’s dose was reduced by -10% for IM and by -25% for PM. After dose adjustment trough and peak plasma concentrations of the active moiety were within the therapeutic reference range and showed similar plasma concentrations like those of EM with 6 mg RIS.

Conclusion: PBPK modelling can provide a valuable tool to predict RIS’s and 9-OH-RIS’s pharmacokinetics in subjects genotyped for CYP2D6, taking CYP3A4 into account. These models are able to ultimately support decision making regarding dose optimization strategies, especially for subjects showing lower CYP2D6 activity. Therefore, dose adjustment during steady-state that compensate for genetically caused differences
in active moiety’s plasma concentrations amounts to -10% for IM and -25% for PM in comparison to EM, respectively.

References:

Stability studies with tigecycline in bacterial growth medium and impact of stabilizing agents: A prerequisite for in-vitro PK-PD experiments

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Tigecycline, as a representative of the class of glycyclines, is a last resort, broad-spectrum antibiotic. It shows activity against Gram-positive and Gram-negative, anaerobic and aerobic bacteria including strains, that are resistant to many other antibiotics. Currently, tigecycline is indicated for patients with complicated skin and intra-abdominal infections, as well as community-acquired pneumonia. The manufacture recommends a 100 mg loading dose, followed by 50 mg twice a day as a standard treatment protocol [1]. Previously published in-vitro infection models showed, that a bacterial regrowth occurs in particular at the standard dosing protocol [3], indicating the need to optimise and potentially revise the standard dosing regimen. Tigecycline is known to be sensitive to light and oxygen, requiring its use without delay after reconstitution [2,4]. To test new dosing regimens incl. intensified infusion durations in Hollow Fiber infection models, tigecycline’s stability must be enhanced. A novel formulation identified tigecycline to be stable up to one week in 0.9% saline solution by adding 3 mg/mL ascorbic acid and 60 mg/mL sodium-pyruvate as oxygen-reducing agents [2]. Due to inconsistency in minimal inhibitory concentrations (MIC), it was revealed, that there is a medium-age related effect on susceptibility, affected by the amount of dissolved oxygen in medium [4].

The objective of these stability studies was (i) to quantify the degradation process in cation-adjusted Mueller Hinton Broth (ca-MHB) and evaluate the transferability of the previously mentioned antioxidant reagents in fresh and aged broth, and (ii) to evaluate the potential impact of the antioxidants on bacterial growth. To improve the stability of tigecycline in in-vitro infection models, it’s degradation in fresh and aged ca-MHB were compared to stabilized fresh and aged ca-MHB containing 3 mg/mL ascorbic acid and 60 mg/mL sodium-pyruvate. The stabilizing components were tested alone and in combination. The stability studies were conducted at 37 °C, ambient air, pH 7, protected from light, for 24 hours relating to typical conditions used in the Hollow-Fiber infection model. High-pressure liquid chromatography (HPLC) was applied for concentration analysis and time-kill studies were used to evaluate the potential impact on bacterial growth. The measurements revealed a different degradation rate regarding the age of ca-MHB and presence of stabilizing agents in ca-MHB: In aged, non-stabilized ca-MHB, we observed a rapid variable degradation larger than 24 % within 24 h. In contrast to that, in fresh, non-stabilized ca-MHB more than 80% of tigecycline remained. Addition of both antioxidants to ca-MHB resulted in tigecycline concentrations of more than 91% after 24 h, regardless of the ca-MHB age. The time kill curve experiments with Staphylococcus aureus (ATCC29213) showed no change in bacterial growth by adding 0.3% ascorbic acid, and 0.09 % pyruvate, adjusted to pH 7. This reduced concentration of antioxidant was calculated as pyruvate’s concentration, diluted to the central compartment of later planned Hollow Fiber experiments. More time kill curves will be conducted to test if tigecycline’s antibiotic activity and antibiotic-free bacterial growth, is unaffected by adding even higher amounts of antioxidants.

To conclude, we confirmed the stabilizing effect of ascorbic acid and sodium-pyruvate on tigecycline in solution. We revealed, that the recommended concentrations of oxygen reducing agents stabilized ca-MHB to a similar extent as described for saline solution. These results show the importance of using freshly prepared ca-MHB for every experiment and the need for stabilizing agents to determine exact outcomes of tigecycline’s effect in in vitro experiments. Using freshly prepared, stabilized ca-MHB for experiments with tigecycline should be implemented in lab routines and guidelines. The stabilized tigecycline solution will be used in upcoming Hollow Fiber infection model experiments to evaluate alternative dosing regimens for tigecycline.

Physiologically-based pharmacokinetic modeling of trimethoprim-drug interactions with the CYP2C8 victim drugs repaglinide and pioglitazone

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Background and Objectives: Trimethoprim, an inhibitor of bacterial folate acid metabolism, is used for treatment of bacterial infections. It is applied as monotherapy or in combination with sulphonamides. Trimethoprim is one of the most commonly prescribed antibiotics in Germany, ranking fifth after penicillines, cephalosporines, macrolides and fluorochinolones (10th standard units of trimethoprim were prescribed in Germany in 2010) [1]. The antibiotic is a weak inhibitor of cytochrome P450 (CYP) 2C8 and a potent inhibitor of multidrug and toxin excretion (MATE) 1 and 2-K [2]. Due to the frequent prescription of trimethoprim, investigation of its drug-drug interaction (DDI) potential is clinically relevant. Co-administration of trimethoprim increases the area under the concentration-time curve (AUC) of repaglinide and pioglitazone [2,3], which are both predominantly metabolized by CYP2C8 and are therefore recommended by the U.S. Food and Drug Administration as CYP2C8 victim drugs in DDI studies [4,5]. Polymorphism of the gene encoding for CYP2C8 also affects the pharmacokinetics of repaglinide and pioglitazone (drug-gene interaction, DGI); expression of the CYP2C8*3 allele leads to decreased repaglinide and pioglitazone AUCs compared to wild type CYP2C8 [3,6]. Physiologically-based pharmacokinetic (PBPK) modeling is a valuable tool to describe and predict the pharmacokinetics of drugs and to investigate DDIs, DGl and even DDIGs (drug-drug-gene interactions). The objectives of this study were to develop a whole-body PBPK model of the CYP2C8 perpetrator drug trimethoprim and to predict the DDIs of trimethoprim with repaglinide and pioglitazone, as well as the DDGI of trimethoprim with pioglitazone in carriers of the CYP2C8*3 allele [3].

Methods: A whole-body PBPK model of trimethoprim was built and evaluated using PK-Sim® and MoBi® (version 7.4.0, www.open-systems-pharmacology.org). Evaluation of the trimethoprim model was performed by comparison of predicted to observed plasma concentration-time profiles, AUC and peak plasma concentration (Cmax) values and calculation of geometric mean fold errors (GMFEs). To predict the DDIs with repaglinide and pioglitazone, an inhibitory constant (K) describing the competitive inhibition of CYP2C8 was added to the trimethoprim model and the model was subsequently coupled to previously developed models of repaglinide and pioglitazone [7]. The performance of the DDI models was evaluated by comparison of predicted to observed victim drug plasma concentration-time profiles, DDI AUC ratios (AUC DDI / AUC control) and DDI Cmax ratios (Cmax DDI / Cmax control). For the DDGI model, comparison of DDGI AUC ratios, DDGI Cmax ratios and calculation of GMFEs was performed.

Results: The final trimethoprim model applies transport via P-glycoprotein, an unspecific hepatic clearance process, tubular secretion via MATE1 and passive glomerular filtration. Comparison of predicted and observed trimethoprim AUC and Cmax values show low GMFEs of 1.21 (range 1.00-1.88, n=27) and of 1.19 (range 1.00-1.82, n=27), respectively, demonstrating the good model performance. Application of the newly developed trimethoprim model for DDI prediction results in plasma concentration-time profiles of the victim drugs (repaglinide and pioglitazone) during trimethoprim co-administration that adequately match the observed profiles. The trimethoprin-repaglinide DDI model
predicts DDI AUC and Cmax ratios of 2.10 and 1.64, respectively, which is in good agreement with the observed DDI AUC and Cmax ratios (1.60 and 1.42, respectively) [2]. The trimethoprim-pioglitazone DDI model predicts DDI AUC and Cmax ratios of 1.79 and 1.18, respectively, which also is in good agreement with the observed DDI AUC and Cmax ratios (1.42 and 1.18, respectively) [3]. Predicted vs. observed DDI AUC ratios and DDGI Cmax ratios show GMFEs of 1.29 (range 1.21-1.38, n=3) and of 1.05 (range 1.02-1.08, n=3) for the trimethoprim-pioglitazone DDGI (taking CYP2C8*3 homozygous-, heterozygous- and non-carriers into account), indicating a good predictive performance of the PBPK models. Conclusion: A whole-body PBPK model of the CYP2C8 inhibitor trimethoprim has been successfully established. The model precisely describes the plasma concentration-time profiles of trimethoprim observed in clinical trials and accurately predicts the co-administration of trimethoprim with repaglinide or pioglitazone. In future studies this PBPK DDI network can be extended with models of MATE victim drugs.

8. Pos.110

Model based dose finding in Hemophilia A: Evaluation of the predictive performance of ten published pharmacometric models for Factor VIII

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Hemophilia A, an x-linked inherited disorder with a deficiency of the coagulation glycoprotein FVIII, is one of the most common bleeding disorders. To prevent and treat possible life-threatening bleedings the substitution of the aforementioned protein is key element in the management of this disease. Current issues of hemophilia A are high interpatient variability in the pharmacokinetics (PK) of factor VIII, the variable product response and high treatment costs. Nonetheless, dosing according to instruction inserts only takes into account the body weight of the patient and the therapeutic indication of factor VIII treatment (e.g. prophylaxis, surgery, heavy bleeding). Hence, therapeutic drug monitoring (TDM) facilitated by population PK models has gained interest to individually tailor factor VIII dosing. Regarding the prophylactic treatment the time spent below a plasma concentration of 1 IU/dL should be as short as possible as this strategy leads to fewer bleedings and haemarthroses. Thus, especially the precise prediction of trough levels is important. The objective of the present study was to investigate the predictive performance of population PK models in this crucial step. For the evaluation, a clinical dataset was provided comprising 35 hemophilia A patients receiving five different FVIII-products with a total of 209 one stage assay observations. Ten published population PK (pop PK) models were recreated and processed using NONMEM 7.4. The models were evaluated regarding their predictive performance, i.e. scenario (i): a priori estimation based on dosing and covariate information; and scenario (ii): Bayesian forecasting, i.e. dosing and covariate information incl. a single previously measured trough sample to predict the factor VIII concentrations in a richly sampled occasion (n=5 measurements). The results were graphically examined using goodness of fit plots and visual predictive checks. Furthermore, forecasting metrics were compared including relative bias (rBias) and relative root mean square error (RMSE).

None of the population PK models were two-compartment models, and one was a one-compartment model. The statistical sub models included interindividual variability (IIV) on the clearance (CL) or on multiple parameters, while only four accounted for interoccasion variability. Besides the inclusion of body weight the covariates differed as well as the handling of endogenous factor VIII levels and the underlying drug product: four accounted for the patients age, three distinguished the applied drug type and four included an endogenous factor VIII activity. The accuracy and precision of the prediction based on scenario (i) varied substantially resulting in rBias values between -16 and 102 (RMSE 46 - 263). In general most of the models overpredicted trough levels and underestimated peak levels. In scenario (ii) the predictive performance improved in all models (except the model of Nestorov et al. 2015 [1] and the one-compartment model, Karafollidou et al., 2009 [2]). The rBias was reduced from 37 to 22 on average (RMSE 143 to 87).

The models with the smallest metrics (rBias <15 + RMSE <65) in scenario (ii) accounted for body weight on CL and V1, a baseline of <1 IU/dL or stratified on the severity of the disease; and the inclusion of IV on CL and baseline. The best predictive performance was displayed using the model by Abrantes et al., 2017 [3] and Zhang et al., 2017 [4] in particular regarding trough level prediction. Despite these levels are most critical in prophylaxis treatment we emphasize the two models may not be best in every purpose, i.e. reaching target levels in on-demand or perioperative treatment. After further investigation suitable models will be implemented into the TDMx software (<www.TDMx.eu>, [5]).

POS.111

Bioanalytical method validation for the quantification of voriconazole in microdialysate: An assay for small sample volumes and low concentrations.

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Objectives: Voriconazole (VRC), a broad-spectrum triazole, is frequently prescribed as prophylaxis and used as first-line therapy of invasive fungal infections. After standard dosing highly variable exposure is observed, demanding for a better understanding of the influential factors to guide optimised dosing [1]. For this, knowledge of target-site pharmacokinetics is an essential prerequisite. These can be assessed by microdialysis, a minimally invasive method to sample unbound drug concentrations directly at the site of action. As concentrations in microdialysate are low and sample volume is limited, developing and validating an assay with a low limit of quantification (LLOQ) requiring as little microdialysate volume as possible was aimed for.

Methods: A LC-MS/MS assay was developed using an Agilent LC system that was connected to a triple quadrupole MS/MS system. All samples were prepared by diluting 5 µL of microdialysate with 195 µL water containing the internal standard (VRC-D3) at a concentration of 0.5 ng/mL. For chromatographic separation, an InfinityLab Poroshell 120 Phenyl Hexyl column (RP, 2.1x100 mm, 2.7 µm, Agilent Technologies, Waldbronn, Germany) was used and temperature controlled at 30°C. The injection volume was set to 2 µL. As eluents, methanol and ultra-pure water were utilised both containing 0.1% [VIV] formic acid to improve the ionisation process. Elution and separation of analytes was achieved by a gradient method with increasing organic solvent over time using a flow rate of 0.350 mL/min. Total run time was 6.4 min. Ionisation in the MS system was accomplished by an electrospray ionisation source (ESI) and ion acquisition was performed in multiple reaction monitoring (MRM). Thereby, two transitions were monitored for the internal standard VRC-D3 (m/z 281, 224, 127) , respectively.

Results: The assay was successfully validated for selectivity, carry-over, LLOQ, calibration function, accuracy, precision, matrix effect and stability according to the European Medicines Agency (EMA) guideline on bioanalytical method validation [2]. Assay linearity was observed across a large concentration range (5 – 5000 ng/mL), indicated by a coefficient of
Optimizing 5-fluorouracil chemotherapy with regard to DPD drug-gene interactions and circadian effects utilizing a physiologically-based pharmacokinetic (PBPK) modelling approach

Background and Objectives: The intravenously administered cytotoxic drug 5-fluorouracil (5-FU) is used as a first line agent in the treatment of colorectal cancer and further tumours including breast, head and neck cancer [1]. Due to its poor oral bioavailability and high toxicity 5-FU is administered as a continuous infusion over 22 to 46 hours [2]. Dihydropyrimidine dehydrogenase (DPD) catalyses the rate-limiting process in the inactivation of fluoropyrimidine based drugs such as 5-FU [3]. However, DPD’s enzyme activity is highly variable depending on example polymorphisms in DPYD, the encoding gene for DPD or circadian rhythms. Changes in DPD’s enzyme activity can affect 5-FU plasma levels and ultimately increase the risk for severe toxicities. Thus, it is important to quantify the extent of circadian expression patterns as well as DPD polymorphisms to optimize 5-FU therapy regimens and consequently limit the occurrence of adverse drug effects [4]. Hence, the objective of this work was to build a physiologically-based pharmacokinetic (PBPK) model for 5-FU and its metabolites 5,6-dihydrouracil, α-fluoro-β-ureidopropionic acid and α-fluoro-β-alanine and furthermore, develop dose recommendations for the clinically relevant DPYD gene variants c.1905G>A (DPYD*2A), c.1676T>G (DPYD*13), c.2846A>T (DPYD*9B), and c.1129-5923C>G (HpB3) [5].

Methods: PBPK model development was performed with PK-Sim® and Modfit® v.7.4 as part of the Open Systems Pharmacology Suite [6]. Data for model development were extracted from literature, including physicochemical parameters and plasma concentration-time profiles for all compounds and for various DPYD genotypes. Data were separated into a training and test dataset for model development and evaluation, respectively. Additionally, an uracil (U) model was developed and used for the evaluation of the genotype implementation, in which the enzyme activity of genetic variants of DPYD are described through the ratio of U and its first metabolite dihydrouracil (DHU). The final models were used for dose optimization by simulating a 400 mg/m² 5-FU bolus injection followed by a 600 mg/m² continuous infusion over 22 hours for wildtype patients. The resulting exposure described as the area under the plasma concentration-time curve (AUC) was used as reference value. Exposure for different genotypes were simulated and their corresponding dose was adapted stepwise until matching exposures compared to wildtype were reached.

Results: Whole-body PBPK models for 5-FU and its three metabolites, as well as for U and DHU were developed. The compiled data consist of 36 5-FU studies (as bolus injection, continuous infusion or peroral solution in 226 patients) and two peroral U studies (as peroral solutions). The model precisely predicts the PK of 5-FU in wildtype patients and heterozygous patients for the gene variant *2A. Continuous infusions showed a circadian pattern in plasma concentration which was accurately described by implementing a time-dependent sine function for enzyme activity of DPD. Ratio of predicted to observed AUC values for wildtype patients was 1.1 for 5-FU, DHU ratios for the four gene variants as well as predicted and observed DGI ratios (AUC heterozygous/AUC wildtype) for the most prominent gene variant DPYD*2A lied within the two-fold acceptance limits. Dose recommendations were derived for combinations of hetero- and homozygous variants. Model predicted dose reductions were 25% for DPYD*2A for the initial bolus injection, and 50% for the following continuous infusion. The current CPIC guideline recommends a 50% reduction of the 5-FU dose altogether [5].

Conclusion: A comprehensive set of PBPK models for 5-FU and its metabolites were successfully developed. The models captured the important impact of drug-gene interactions and circadian rhythms and could play an important role in decreasing the occurrence of potential life threatening adverse drug effects by deriving alternative dosing regimens for DPD deficient patients.

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References

POS.113

A survey on continuing education of pharmacists: Status quo, perspectives and outlook

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Background and Objectives: Continuing education refers to post-secondary learning programs directed to professionals to preserve or extend knowledge. In Pharmacy, new active pharmaceutical ingredients are approved to the markets regularly. About half of all systematic reviews are not up to date anymore after five years [1]. Comparably, medical guidelines are updated every third year averagely. Due to that, pharmacists are obliged to educate themselves further. In Germany, this requirement is written down in the professional code of conduct of every federal state’s chamber of pharmacists. However, a uniform catalog requirement is written down in the professional code of conduct of every federal state’s chamber of pharmacists. In Germany, continuing education refers to post-secondary learning programs directed to professionals to preserve or extend knowledge. In Pharmacy, new active pharmaceutical ingredients are approved to the markets regularly. About half of all systematic reviews are not up to date anymore after five years [1]. Comparably, medical guidelines are updated every third year averagely. Due to that, pharmacists are obliged to educate themselves further. In Germany, this requirement is written down in the professional code of conduct of every federal state’s chamber of pharmacists. However, a uniform catalog requirement is written down in the professional code of conduct of every federal state’s chamber of pharmacists. Pharmacists may apply to a voluntary certificate; an extent of 150 points (one point equals 45 min of secondary learning programs directed to professionals to preserve or extend knowledge. In Pharmacy, new active pharmaceutical ingredients are approved to the markets regularly. About half of all systematic reviews are not up to date anymore after five years [1]. Comparably, medical guidelines are updated every third year averagely. Due to that, pharmacists are obliged to educate themselves further. In Germany, this requirement is written down in the professional code of conduct of every federal state’s chamber of pharmacists. However, a uniform catalog requirement is written down in the professional code of conduct of every federal state’s chamber of pharmacists. Pharmacists may apply to a voluntary certificate; an extent of 150 points (one point equals 45 min of...
Methods: An extensive literature search was performed to look for studies on the impact of continuing education, its scope and extent for both pharmacists and other professions such as physicians in Germany and abroad. It includes an analysis of the various professional codes of conduct for pharmacists. Each federal chamber of pharmacists was asked to provide the number of voluntary certificates existing concerning the overall count of pharmacists. The online survey was conducted with the tool SoSci Survey [3] and was disseminated in specialist pharmaceutical news media as well as to pharmaceutical organizations such as the DPhG to share it with their members and forward them to this survey. It was directed to both pharmacists (community and hospital pharmacists) and future pharmacists (students of Pharmacy, pharmacists in practical internship) to include their expectation as well. Concerning one’s profession, the survey addressed the knowledge about the duty to continuing education, the necessary extent, the benefit, and the motivation or demotivation. Pharmacists also were asked to their extent of continuing education, their workspace, the extent of medication-related services, integration in practice and time of proficiency. In advance, a pretest was completed successfully.

Results: The literature search revealed wide variability in the phrasing to continuing education in the professional codes of conduct. A proof of burden, including consequences for failing, only exists in one pharmacist chamber’s district so far. The voluntary certificate of the chambers is not a significant factor yet. Studies on physicians’ performance evidenced a benefit of continuing education on their skills and patient outcomes [4] proving the value, function, and requirement of continuing education. However, opportunities to pharmacists to educate themselves widely vary among districts. Online tutorials are still of minor importance; a nationwide network does not exist. The online survey revealed broad interests among pharmacists. Overall, 1011 datasets were completed. As 128 participants did not meet the inclusion criteria, 883 valid cases were included (698 licensed pharmacists, 136 students, and 49 pharmacists in internship). Participants from every pharmacist chamber’s district took part in the survey. Among licensed pharmacists, 58.6% (n = 698) worked in a community pharmacy, and 41.4% in a hospital pharmacy. First results show that those working in hospital were more engaged in continuing education (median: 45 points vs. 30 points per year). Asked for the minimum yearly extent of continuing education mandatory, licensed (community and hospital) pharmacists deemed 30 points (median) on average appropriate, students and those in practical internship called for less (median: 24 points). A control mechanism of one’s dedication to continuing education was favored by the majority of the students (77.2%, n = 136). Among licensed pharmacists, the majority (51.1%, n=698) rejected that. In support of such a mechanism were 37.1%. Acceptance was slightly higher among hospital pharmacists (42.6% vs. 33.3%).

Conclusion: The combination of literature search and an online survey directed to pharmacists allows drawing a holistic image of the status quo of pharmacists’ continuing education in Germany. A strength is that this study also includes the perspectives, motivation, and requests of colleagues, and those still in training, referring to continuing education, which allows identifying pitfalls to overcome. In mind of the political discussion of further pharmacists’ competencies, such as medication management-related services, it will be crucial that pharmacists ensure to maintain their position as the drug expert. Therefore, harmonization of pharmacists’ activities and dedication to continuing education in Germany is necessary.

References:
Antioxidative Activities of Phylloxanthobilins, Abundant Natural Products Derived from Chlorophyll

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In the course of the last three decades, the abundant bilin-type products of Chlorophyll breakdown, named phylloxybilins, have come to our attention. Considered 'mere' left overs of a controlled biological Chlorophyll detoxification original, phylloxanibles are now believed a new opportunity for discovering relevant bioactivities contributing to health promoting effects. Phylloxanibles are abundant in leaves, fruit and in some vegetables. Recently, yellow phylloxanibles, the phylloxanobilins, were shown to occur in unprecedented abundance and diversity in Echinacea purpurea, a plant with undoubted high importance in phytotherapy. Even in fresh green leaves - without any 'obvious' chlorophyll breakdown - phylloxanobilins were discovered. Many different pharmacological activities have been demonstrated for Echinacea purpurea, nevertheless, neither a single compound nor a compound class has been identified that can account for all of the efficacies. Caffeic acid derivatives are considered a relevant class of constituents with anti-oxidative activity. We are able to demonstrate a high anti-oxidant potential of the phylloxanobilins isolated from Echinacea, superior to caffeic acid in in vitro experiments and comparable to caffeic acid in cellular approaches. A structurally different type of phylloxanobilin has recently been identified in de-greened leaves of savoy cabbage. We show that the newly identified phylloxanobilin closely resembles bilirubin, an established strong antioxidant that plays a role in the prevention of various diseases. Savoy cabbage, too, is known to have beneficial effects on human health, attributed to its high content in antioxidants.

The natural product Neocarzilin A (NCA) was discovered decades ago and despite its potant cytotoxic effects no mode of action studies were performed up to date. Synthesis of neocarzilins A, B, C and a stereoisomer of NCA provided insights into structural preferences as well as access to probes for functional studies. NeCA pertained as the most active member and was not only effective against cell proliferation but also migration, a novel and so far overlack activity. The potent anti-migratory effects could also be confirmed in in vivo models using a mouse model. To decipher the molecular mode of action, we applied chemical proteomics for target discovery and revealed that NCA targets cancer cell migration via an irreversible binding to the largely uncharacterized synaptic vesicle membrane protein VAT-1. A corresponding knockout of the protein confirmed the phenotype and pull-down studies showed the interaction with an intricate network of key migration mediators such as Talin-1. Overall, we introduce VAT-1 as a promising novel target for the development of selective migration inhibitors with the perspective to limit toxicity in absence of anti-proliferative effects.

Vioprolide A reduces pro-inflammatory processes in human endothelial cells – inhibition of protein translation as central mechanism?

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Inflammation is a biological process with the purpose of protecting the body against pathogens and altered cells. Leukocyte recruitment towards the site of inflammation and activation of pro-inflammatory signalling pathways are essential for the elimination of the inflammatory stimuli. After successful elimination of the trigger, inflammation must be resolved for rebuilding homeostasis. However, through defects which prevent the body from resolution and rebuilding homeostasis, inflammation becomes chronic and causes the emergence of diseases such as chronic respiratory diseases or rheumatoid arthritis. Vioprolide A, a cyclic peptide derived from the myxobacterium Cystobacter violaceus, was identified to influence TNF-induced ICAM-1 expression during the screening of a myxobacterial compound library. ICAM-1 is an adhesion molecule that promotes the tight adhesion of leucocytes to the endothelium which is a key step in the extravasation of leucocytes. We aimed to investigate the effects of vioprolide A on inflammatory processes in the endothelium and to get insights into the underlying mechanisms of action. Initial data revealed that vioprolide A reduced TNF-induced leucocyte adhesion to primary human umbilical vein endothelial cells (HUVECs) after 24 h. Intravital microscopy of postcapillary venules of the cremaster muscle in mice confirmed a reduction of firm adhesion and transmigration through the vascular endothelium in vivo. Leukocyte adhesion is promoted by adhesion molecules like E-selectin, ICAM-1 and VCAM-1. As examined by flow cytometry, vioprolide A concentration-dependently (0.3–10 nM) reduced the TNF-induced cell surface expression of these molecules after short (4 h) as well as long (24 h) treatment periods. The expression of these adhesion molecules is mainly regulated via the NF-κB signalling pathway. In order to test if the reduced adhesion molecule cell surface expression was caused by an effect on gene transcription, mRNA expression of these adhesion molecules was investigated. Although mRNA expression decreased after long (16 h) treatment periods, short (6 h) treatment periods surprisingly revealed elevated mRNA levels. This is in contrast to the reduced cell surface expression after 4 h and indicated that vioprolide A might rather interfere with protein translation than mRNA expression. Hence, general protein synthesis upon vioprolide A treatment was examined showing a concentration dependent (0.3–10 nM) and time dependent impairment with a maximum effect after 16 h. Since the activation of the pro-inflammatory NF-κB signalling pathway relies on the binding of TNF to its cell surface receptor (TNFR1), its protein expression was analysed. Interestingly, vioprolide A reduced the protein expression in a time-dependent manner showing a maximum effect after 16 h corresponding to the findings of general protein synthesis analysis. After 24 h TNFR1 protein expression started to rise again. qPCR experiments verified that the reduced TNFR1 protein expression upon vioprolide A treatment (10 nM) was due to an impact on protein level and not mRNA expression as there was no reduction over time in TNFR1 mRNA levels. Since the effect on protein synthesis was

The natural product Neocarzilin A (NCA) was discovered decades ago and despite its potent cytotoxic effects no mode of action studies were performed up to date. Synthesis of neocarzilins A, B, C and a stereoisomer of NCA provided insights into structural preferences as well as access to probes for functional studies. NeCA pertained as the most active member and was not only effective against cell proliferation but also migration, a novel and so far overlack activity. The potent anti-migratory effects could also be confirmed in in vivo breast carcinoma mouse model. To decipher the molecular mode of action, we applied chemical proteomics for target discovery and revealed that NCA targets cancer cell migration via an irreversible binding to the largely uncharacterized synaptic vesicle membrane protein VAT-1. A corresponding knockout of the protein confirmed the phenotype and pull-down studies showed the interaction with an intricate network of key migration mediators such as Talin-1. Overall, we introduce VAT-1 as a promising novel target for the development of selective migration inhibitors with the perspective to limit toxicity in absence of anti-proliferative effects.

Vioprolide A reduces pro-inflammatory processes in human endothelial cells – inhibition of protein translation as central mechanism?

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Inflammation is a biological process with the purpose of protecting the body against pathogens and altered cells. Leukocyte recruitment towards the site of inflammation and activation of pro-inflammatory signalling pathways are essential for the elimination of the inflammatory stimuli. After successful elimination of the trigger, inflammation must be resolved for rebuilding homeostasis. However, through defects which prevent the body from resolution and rebuilding homeostasis, inflammation becomes chronic and causes the emergence of diseases such as chronic respiratory diseases or rheumatoid arthritis. Vioprolide A, a cyclic peptide derived from the myxobacterium Cystobacter violaceus, was identified to influence TNF-induced ICAM-1 expression during the screening of a myxobacterial compound library. ICAM-1 is an adhesion molecule that promotes the tight adhesion of leucocytes to the endothelium which is a key step in the extravasation of leucocytes. We aimed to investigate the effects of vioprolide A on inflammatory processes in the endothelium and to get insights into the underlying mechanisms of action. Initial data revealed that vioprolide A reduced TNF-induced leucocyte adhesion to primary human umbilical vein endothelial cells (HUVECs) after 24 h. Intravital microscopy of postcapillary venules of the cremaster muscle in mice confirmed a reduction of firm adhesion and transmigration through the vascular endothelium in vivo. Leukocyte adhesion is promoted by adhesion molecules like E-selectin, ICAM-1 and VCAM-1. As examined by flow cytometry, vioprolide A concentration-dependently (0.3–10 nM) reduced the TNF-induced cell surface expression of these molecules after short (4 h) as well as long (24 h) treatment periods. The expression of these adhesion molecules is mainly regulated via the NF-κB signalling pathway. In order to test if the reduced adhesion molecule cell surface expression was caused by an effect on gene transcription, mRNA expression of these adhesion molecules was investigated. Although mRNA expression decreased after long (16 h) treatment periods, short (6 h) treatment periods surprisingly revealed elevated mRNA levels. This is in contrast to the reduced cell surface expression after 4 h and indicated that vioprolide A might rather interfere with protein translation than mRNA expression. Hence, general protein synthesis upon vioprolide A treatment was examined showing a concentration dependent (0.3–10 nM) and time dependent impairment with a maximum effect after 16 h. Since the activation of the pro-inflammatory NF-κB signalling pathway relies on the binding of TNF to its cell surface receptor (TNFR1), its protein expression was analysed. Interestingly, vioprolide A reduced the protein expression in a time-dependent manner showing a maximum effect after 16 h corresponding to the findings of general protein synthesis analysis. After 24 h TNFR1 protein expression started to rise again. qPCR experiments verified that the reduced TNFR1 protein expression upon vioprolide A treatment (10 nM) was due to an impact on protein level and not mRNA expression as there was no reduction over time in TNFR1 mRNA levels. Since the effect on protein synthesis was
stronger after 16 h than after 24 h, leukocyte cell adhesion as well as adhesion molecule cell surface expression were re-analysed after 16 h. In both cases vioprolide A (10 nM) had a stronger effect after 16 h than 24 h, indicating that the effects on leukocyte migration and adhesion molecule expression might be connected to the observed impairment of translation.

Taken together, vioprolide A may inhibit pro-inflammatory mechanisms such as leukocyte adhesion by impeding the expression of cardinal proteins such as TNFR1 caused by an impairment of translation. The underlying mechanism of translational regulation is currently studied.

**POS.117**

**Enrichment of bioactive phytochemicals from natural extracts by Centrifugal Partition Chromatography using polarity adjusted solvent regimes**

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Plant extracts contain various types of bioactive compounds and their isolation is a major challenge for the identification and characterization of the biological activity.\(^1\) The aim of this work was to develop a polarity adjusted solvent fractionation to predict the required parameters for the isolation of the biological active substances from a complex natural extract using Centrifugal Partition Chromatography. Selected phytochemical standards with different polarity were chosen to obtain information for the required solvent systems and the prediction of retention times by partition coefficients. In the first step suitable solvent mixtures and suitable concentrations were identified, which enabled not only an enlargement of the required design space but also a baseline separation for all reference substances. In the next step the operating conditions were transferred to a natural plant extract containing the phytochemicals used above for defining the corresponding design space. The results show that the retention times of the phytochemicals in the plant extract corresponded with those of pure substances with high purity. In fact, by using Centrifugal Partition Chromatography, we were able to separate the main phytochemicals of a complex natural extract by developing a polarity adjusted fractionation protocol and predicting optimal operation conditions. In the next steps, bio activity tests of the purified phytochemicals will be performed in comparison to the reference substances. Furthermore, the efficiency of this approach will be transferred to further plant extracts and other phytochemicals.

\(^1\) Handa, S. et al.: Extraction Technologies for Medical and Aromatic plants (ICS-UNIDO) 2008
4.7 Inflammation

POS.118

NHC Gold compounds suppress immune responses by inducing the AHR-TGF β1 signalling pathway in vitro and in scurfy mice

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The immunosuppressing activity of certain gold complexes has long been identified and broadly studied. Nevertheless, the precise mechanisms remain unclear. Using a novel liver-on-a-chip system we found that gold complexes, containing the planar N-heterocyclic (NHC) carbene moiety as ligand, are potential ligands of the Aryl Hydrocarbon Receptor (AHR). Our leading compound MC3 has been further studied and was found to activate the TGF-β1 signalling pathway, resulting in the suppression of CD4+ T-cell activation in vitro, in human and mouse T-cells. Genetic knockdown, chemical antagonists of the AHR or inhibition of the of the TGF-β1 signalling pathway decreased the activity of MC3. Treatment with MC3 on Scurfy mice, a mouse model of the human IPEX syndrome, reduced the autoimmune response and prolonged the lifespan to up to 60 days. In conclusion, these results demonstrate that a NCH-gold (I) complex has immunosuppressive effects in scurfy mice; suggest that the immunosuppressive effect of gold complexes in enhanced using planar NCH moieties as ligands to activate AHR-related pathway; and open a new clinically potential target and treatment of autoimmune diseases.

Acknowledgments: Thank others for any contributions. Funding: This work supported by the SFB grant program (CH 160/3-1) and the BMBF grant programs Drug-IPS (FKZ 0315398A-FKZ 0315398B) and SysToxChip (FKZ 031A303A-FKZ 031A303E).

References:
X. Cheng et al.: NHC Gold compounds suppress immune responses by inducing the AHR-TGF β1 signalling pathway in vitro and in scurfy mice (under revision)

POS.119

The role of the carbazole derivative C81 in the inflammatory activated vascular endothelium

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During inflammation leukocytes migrate through the activated endothelial barrier into the underlying tissue where they differentiate into distinct macrophage phenotypes amplifying the inflammatory response. In the healthy organism this process is physiologically terminated, whereas the pathophysiological situation of chronic inflammatory diseases, such as psoriasis or rheumatoid arthritis, is characterized by ongoing leukocyte infiltration resulting in severe tissue damage. Therefore, there is a great need for the discovery of new drug leads and targets for pharmacotherapeutical treatment of these disorders.

Results of a thermal shift assay revealed that the carbazole derivative C81 shows the highest binding affinity to a number of kinases such as the BMP-2-inducible kinase (BMP2/BIKE), the adaptor-associated kinase 1 (AAK1) as well as to the CDC-like kinase 1 and 4 (CLK1/4) and the proto-oncogene, serine/threonine kinase PIM3. Since the vascular endothelium crucially regulates inflammatory processes, we hypothesized that these kinases might play a pathophysiological role in the inflammation-activated endothelium. The actions of these kinases have not been characterized in the vascular endothelium so far. Therefore, we aimed to analyze the pharmacological potential of C81 and to investigate the role of these kinases in inflammatory processes in vascular endothelial cells.

To analyze the potential involvement of the aforementioned kinases in an in vivo cell adhesion assay using human umbilical vein endothelial cells (HUVECs) and the monocytoid-like THP-1 cell line was performed. Here only endothelial cells were treated with respective kinase inhibitors. The inhibition of AAK1, CLK1/4 or PIM3 in endothelial cells did not impair the adhesion of THP-1 cells onto a TNF-activated HUVEC monolayer. Of note, RNAi silencing of BMP2K in HUVECs reduced the adhesion of THP-1 cells significantly.

Initial experiments using C81 show that only high concentrations of the compound affected the viability of HUVECs after 24 hours of treatment (IC50: 171 μM).

In vivo results of intravital microscopy in the murine cremaster muscle demonstrated that the adhesion of leukocytes was significantly reduced after C81 treatment. In an in vivo cell adhesion assays C81 treatment significantly decreased the adhesion of THP-1 cells onto inflammatory-activated endothelial cells under static and under flow conditions, which better mimic the physiological situation. Importantly, also the transmigration of THP-1 cells through a TNF-activated endothelial monolayer in the direction of a chemotactic gradient was significantly reduced by C81. As the interaction of leukocytes and the endothelium is mainly mediated by cell adhesion molecules (CAMs), the effect of C81 on their expression was analyzed (western blot, flow cytometry, qPCR) in HUVECs. The total and the surface protein expression of ICAM-1, VCAM-1 and E-selectin as well as their mRNA levels were strongly decreased after C81 treatment. Of note, mRNA levels and the surface expression of CAMS were not attenuated in BMP2K knockdown cells. Moreover, C81 significantly reduced the TNF-induced activation of the MAPK JNK, while the phosphorylation of p38 was not impaired. Interestingly, western blot analysis revealed that C81 significantly decreased the total protein expression of the TNF receptor 1, while its mRNA level remained unaffected. Initial results of the analysis of effects of C81 on the inhibition of the de novo protein synthesis indicate that the compound might affect the translation of proteins with short half-life resulting in a reduced expression of the TNF receptor 1 after C81 treatment.

We provide first insights into the anti-inflammatory action of the carbazole derivative C81 in vitro and in vivo. Since the inhibition of BMP2K seems to be responsible only for some of the pharmacological actions of C81 and the involvement of kinases predicted by the thermal shift assay can be excluded for the anti-inflammatory actions of the compound, we will further investigate the role of other potential targets. The precise role of BMP2K in inflammatory endothelial processes as well as the involved pathways during BMP2K silencing and C81 treatment will be further elucidated.

POS.120

Beyond leukotriene formation-Influence of 5-lipoxygenase on gene expression.

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5-lipoxygenase (5-LO) is the key enzyme in the biosynthesis of leukotrienes and specialized proresolving lipid mediators (SPMs) [1, 2]. It is mainly expressed in leukocytes and is part of the innate immune system. 5-LO can shuttle between the cytoplasm and the nucleus. Upon cell activation the protein translocates from soluble cellular compartments to the nuclear membrane. Besides the 5-LO activating protein (FLAP) which
is required for cellular leukotriene and SPM formation, 5-LO interacts with other proteins like coactosin-like protein (CLP) [3], Dicer [4], β-catenin [5] and p53 [6].

Here, we give an insight into the role of 5-LO in the regulation of cell proliferation and differentiation and its biological functions apart from leukotriene and SPM formation. We present data, how 5-LO may act as a STRaND (shuttling transcriptional regulator and non-DNA binding) influencing the gene expression of target genes. Therefore, we introduced a 5-LO knockout (KO) in the myeloid cell line MonoMac6 and compared the mRNA expression levels of target genes between KO and wild type cells.

References:
Modelling a coextrusion blow molding process with a neural network

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Introduction: Classically, the goal of pharmaceutical development is to design a quality product and its manufacturing process in such a way that the defined performance of the product (QTPP - quality target product profile) is consistently achieved (ICH Q8). This approach is called QbD - Quality by design. Following the approach, the effects of material and process parameters on the product CQAs are determined. This functional connection between input and output can be determined by means of prior knowledge, experimental data, the control strategy or a combination thereof [1].

Objective: Establish a model, which can be found via an analytical solution, a simulation or a parameter study. [2] A standard method might be DOE (Design of Experiments) which would provide a linear or a quadratic approximation, at best. Usually, a DOE requires a set of experiments [3], which should be avoided and instead, pre-existing data should be used.

Background: Boehringer Ingelheim developed and produces the Respimat, a propellant-free soft mist inhaler. This consists of a device and a drug-filled cartridge. The latter consists of a double-walled plastic container inside an aluminum casing. The plastic container consists of outer and inner foils and is produced by means of a coextrusion blow molding process. The production of such a container is dependent on many, partly mutually influencing, parameters. [4]

Environment variables:
- temperature
- humidity
- air pressure

Input variables:
- mechanical settings
- process parameters

Output variables:
- process controls
- production efficiency

Acknowledgements: Boehringer Ingelheim, Johannes Gutenberg-Universität Mainz, Dr. Herbert Wachtel

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Figure 1: Black box of the functioning of the simulation program

Experimental: A simulation software was programmed to describe the blow molding process of the plastic container. This makes it possible to estimate the influence of modified input variables on the CQAs. In addition to the 26 process parameters, the environmental variables (absolute air pressure and air humidity) are also taken into consideration (see figure 1). The simulation uses a five-layer neural network (multilayer perception) and was trained with data from production monitoring. The simulation program is linked to several databases of the company Boehringer Ingelheim and enables an easy operation due to its interactive structure.

Results: Connections between the machine settings/ process parameters/ environment variables on the output variables are thus intuitively visible and quantitatively documented. In addition, the results of the simulation can be used for process control as they solve the multi-dimensional optimization problem.

Modelling a coextrusion blow molding process with a neural network
Influence of PEG spacer selection on active targeting of trastuzumab-modified nanoparticles

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Despite new and innovative cancer therapeutics being approved for clinical use in the last years, side effects are still a central problem in conventional chemotherapeutics. Therefore, therapy options specifically targeting tumor tissue are a desired approach. One promising idea to overcome this obstacle is using nanoparticulate drug vehicles in combination with ligands (e.g. antibodies) which address specific target structures on the tumor [1]. The effect of this active targeting has been investigated for many ligands and their targets for cancer treatment in the last years [2]. In this context, attractive targeting structures for nanoparticulate drug delivery systems are receptors which are overexpressed in tumor tissue. In the present study nanoparticles (NP) modified with the humanized monoclonal antibody trastuzumab were used to target the human epidermal growth factor receptor 2 (HER2) on HER2 overexpressing breast cancer cells. NP based on human serum albumin were prepared using a desolvation method and stabilized with glutaraldehyde. Subsequently the surface of the NP was modified with a bifunctional polyethylene glycol (PEG) spacer, using two different molecular weights (5 kDa and 10 kDa). The antibody trastuzumab was thiolated with 2-Iminothiolane and covalently linked to the PEG spacer. Cell culture studies on active targeting were performed using the HER2 overexpressing breast cancer cell line BT-474 [3]. To investigate the cell association of the NP and the influence of the different PEG spacers on this process, different methods, including flow cytometry, life cell imaging and fluorescence microscopy, were used. In conclusion stable NP modified with trastuzumab using two different PEG spacers varying in molecular weight could be prepared. Active targeting of HER2 overexpressing cells was achieved, and therefore a receptor mediated uptake of the NP into the cells with both tested PEG spacers was proven.

References:

Surface modification of PLGA nanoparticles with cysteine for oral drug delivery: Analysis of a modified stabilizing agent as an example for polyvinyl alcohol modification via divinyl sulfone.

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Oral application is the most favorable route of administration respecting patient compliance. However, many active pharmaceutical ingredients (API) show limited oral bioavailability due to poor solubility or permeability at the gastrointestinal barriers. Embedding the API into nanoparticles (NP) is a promising opportunity to overcome these obstacles. A functionalized NP with the ability to pass the intestinal membrane can be used as carrier for several different API to increase their oral bioavailability. Poly(lactic-co-glycolic acid) (PLGA) is a promising starting material for such NP as its in vivo application is already approved. M-cells, as a discussed transport region for unmodified PLGA NP in vivo, are less numerous than e.g. enterocytes in the intestine [1]. Therefore, surface modification of NP is necessary to increase the permeability by addressing specific transportation mechanisms. Chemical modification of PLGA is difficult since the functional end group, a carboxylic acid, is not available in some versions of PLGA, limiting the applicability of modifications at this position. In this study, a novel method of surface alteration [2] via modification of polyvinyl alcohol (PVA) with divinyl sulfone (DVS) is varied, analyzed and tested for preparation of PLGA NP. PVA represents a common stabilizer for preparation of PLGA NP. Since PVA is located at the surface of the NP after preparation [3] the modification of PVA leads to a functional surface of the NP. PVA 4-68 (MW: 25-20 kDa) was coupled with DVS (MW: 118.15 g/mol), leading to a product containing a reactive vinyl group (PVA-VS). Reaction time was controlled by adjusting pH value (Fig.1). After purification via dialysis (membrane: regenerated cellulose, MWCO: 3.5 kDa) analysis via $^1$H-NMR spectroscopy proved the presence of the vinyl group.

![Figure 1: Reaction of PVA with DVS.](image)

PVA-VS can be versatilely used for reaction with substances containing nucleophilic structure characteristics (e.g. $-\text{NH}_2$, $-\text{SH}$). L-Cystein (CYS; MW: 121.16 g/mol) was used due to its high

The optimal required lecithin concentration was identified to enable stabilised particles in a sodium chloride solution imitating physiological condition. Furthermore, for targeting issues the amphiphilic apolipoprotein E (ApoE) was added after preparation with the aim of an adsorptive binding to the amphiphilic lecithin without affecting the protein’s functionality [1]. The bound amount of ApoE was determined by SDS-PAGE and correlates well with the nanoparticle yield. The particle cores were labelled with the fluorescent dye Lumogen® F Red 305 enabling tracking in cell culture experiments as well as to simulate drug embedment. All characteristics were preserved during this procedure, which proved the capability of this system to possibly deliver an embedded drug. The abilities as drug delivery system were tested by analysing the nanoparticles in cell culture studies at endothelial cells and at an in vitro blood brain barrier (BBB) model, because ApoE modified lipid nanoparticles are a promising strategy to overcome the BBB [1, 2]. In conclusion, a new, biocompatible drug delivery system based on cholesterol ceteate, which mimics physiological lipoproteins, has been successfully established.
nucleophilicity of the thiol group. Cystein modified NP are plausible for oral application, since the physiological transport mechanisms for amino acids can be addressed. The product of the reaction of PVA-VS with CYS (PVA-VS-CYS) was purified via dialysis, structural analysis via 1H-NMR spectroscopy showed that no unreacted vinyl groups were left. The amount of bound CYS was quantified indirectly analyzing the quantity of unreacted CYS via reaction with Ellman’s reagent and photometric analysis. Variation in reaction time of PVA with DVS (30 s, 90 s, 120 s) led to different amounts of vinyl groups in the product PVA-VS which was verified by an increased amount of covalent bound CYS in the product PVA-VS-CYS.

PLGA NP were prepared with unmodified PVA as well as with PVA-VS-CYS and analyzed via photon correlation spectroscopy (PCS). NP prepared with PVA or PVA-VS-CYS led to systems with comparable physicochemical characteristics. Modification of PVA before using it as stabilizer for preparation of PLGA NP allowed a quantitative and qualitative analysis of the product and ensured covalent binding of the ligand. The results clearly show that preparation of stable, surface modified PLGA NP via PVA-VS modification is possible.

Conclusion:
Results prove that tablets made from paper are not only feasible for an individualized drug therapy with a manual processing of smartFilms®, e.g. in an official pharmacy, but can also be produced in large-scale by the production of pellets made from paper, which are subsequently transferred into tablets. Results demonstrate again, that tablets made from paper are a real alternative to classical powder tablets, especially for the formulation of actives with poor aqueous solubility and low oral bioavailability.

References

ANDROGENETIC ALOPECIA (FIO) is a common disease, which often is accompanied with a major loss of life quality. One of its main causes lies in a genetic hypersensitivity for dihydrotestosterone of the hair follicles. An established treatment for this malady are 5-alpha-reductase inhibitors, like finasteride. Unfortunately, the common oral way of application leads to severe side effects, like constant loss of libido, continuing sexual dysfunction and severe depression [1]. To still provide the patients with the benefits of finasteride and dramatically reduce the severity of the side effects, it is of major importance to find alternative ways to the common oral application route.

Recent studies have shown, that such a promising alternative might be a local application of the active and preferentially a targeting to the hair follicles, where it can act as a depot [2]. Due to the poor aqueous solubility of finasteride, a formulation as nanocrystals seems to be feasible [3]. Therefore, the aim of this study was to develop a nanocrystal suspension of finasteride for the development of dermal applicable formulations.

For this study, state-of-the-art NanoWitt LAB-100 milling equipment (Frewitt fabrique de machines S.A, Fribourg, Switzerland) was used. Finasteride suspensions were prepared with 1% (w/w) finasteride and 1% (w/w) Brij L23 as stabilizing non-ionic surfactant. The circulation of the suspension was set to 500 ml/min for 120 minutes. Samples were taken after 15 min, 30 min, 45 min, 60 min, 90 min, and 120 min, respectively. The rotor of the milling chamber was adjusted to 1500 rpm, with a bead to suspension-ratio of 60:40 (V/V). Suspension temperature was monitored during the hole milling procedure and was constantly kept between 15 to 20°C. Three different sizes (small: 100 µm, medium: 300 µm and large: 400 µm) of yttria stabilized zirconia beads (SLSBeads Type ZY-E, Sigmund Lindner GmbH, Switzerland) were used. Size measurements were performed by dynamic light scattering (DLS) using a NanoSIZER ZS (Malvern Panalytical, Germany). To determine the optimal production conditions the Dv(0.95) values obtained by SLS, and the average sizes (hydrodynamic diameter and polydispersity index) obtained by DLS were compared for the different production conditions. For the medium sized beads, the average sizes were 200 nm (90 min) and 200 nm (120 min). With sizes of 195 nm (90min) and 185 nm (120min) for the small sized beads, there were no relevant differences in size with respect to bead size or time. For the large beads the average sizes were slightly larger (280 nm (90min), 270 nm (120 min)), but also here no relevant difference in size between the two milling times could be detected.

The previously shown DLS data were consistent with the SLS measurements, since no relevant differences in the measured sizes could be detected between 90 min and 120 min of processing time, for the small and medium sized beads. Compared to an initial Dv(0.95) of 10 µm, every condition applied led to a pronounced reduction in size. After 60 min of processing time the small sized beads showed a Dv(0.95) of 0.51 µm whereas the medium sized beads showed a Dv(0.95) of 0.49 µm.
POS.128

Ex Ovo Shell-less Hen’s Egg Test on the Chick Area
Vasculosa as a Test Model for Inhalable Formulations
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Lung infections can be efficiently treated with inhalable formulations like antibiotics, e.g. for patients with cystic fibrosis with tobramycin (Tb) [1]. Especially nebulized nanoparticles show suitable properties for pulmonary applications avoiding exhalation and aggregation but also overcome obstacles of mucus or biofilm [2]. Nevertheless, there is a lack of test models to eliminate the limitations of animal tests in mice and rats or higher species. Therefore, in the present study the ex ovo shell-less hen’s egg test on the chick area vasculosa (HET-CAV) was investigated as alternative test system to examine antimicrobial activity and biocompatibility under nebulization conditions. Tobramycin loaded nanoparticles (NP) as model drug were prepared by a double-emulsion evaporation method using poly(lactic-co-glycolic acid) (PLGA) with and without poly(ethylene glycol) (PEG) [3]. Physicochemical characteristics of the particles were determined by dynamic light scattering (DLS) confirming a hydrodynamic diameter of 200 nm. Lyophilized NP were reconstituted in 0.9% NaCl solution for nebulization by vibrating-mesh technology (H50, Beurer, Germany) and investigated regarding aerodynamic characteristics for lung delivery with a mass median aerodynamic diameter between 4.6 µm to 5.4 µm and 50% fine particle fraction. In vitro biocompatibility studies showed no cytotoxicity in the human lung cell line A-549 up to a concentration of 1 mg/mL after 24 h incubation. The susceptibility towards bacterial spreading was analyzed using infected HET with aliquots of diluted bacteria suspensions and antibiotic solutions both pipetted into an O-ring placed on the CAV. Decreased spreading of Pseudomonas aeruginosa on the egg surface with an antibiotic treatment over 24 h incubation was visualized using bacterial autofluorescence imaging (Moleculight® IX). Additionally, stability and efficacy of released Tb after vibration-mesh nebulization were confirmed on the ex ovo hen’s egg model using a tailor-made setup utilizing with a 3D printed cone and P. aeruginosa infected eggs. Toxicological profiles of the applied particles confirmed the biocompatibility on a complex biological surrounding displaying no toxic effects like haemorrhage, vascular lysis or thrombosis. In conclusion, we demonstrated that polyester NP displayed excellent properties as biocompatible drug delivery systems for antibiotics. The nebulization of NP offers a highly suitable approach to deliver NP efficient to the deep lungs. HET-CAV offers an alternative test system with beneficial conditions especially for bacterial growth in a complex biological surrounding. Ultimately, this ex ovo model is applicable for inhalative formulations with a modified setup which confirmed antimicrobial efficacy and biocompatibility of tobramycin loaded nebulized NP.

Acknowledgments
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POS.129

Second-Skin SmartLipids® for advanced Corneotherapy
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Corneotherapy is a dermal treatment principle with the aim to maintain, strengthen or repair the skin barrier [1]. The aim of this study was to investigate the skin carrying properties of second-skin - smartLipids® (S.L.) on healthy and impaired skin. S.L. belong to the third generation of the lipid nanoparticles and are composed of a mixture of various liquid and solid lipids. The special feature of the S.L. is their lipid matrix which is composed of lipids that mimic the intercellular lipid layer of the stratum corneum, e.g. ceramides, free fatty acids and cholesterol [2]. The skin carrying properties of the S.L. were assessed by determination of the biophysical skin properties, e.g. transdermal water loss (TEWL) and skin hydration (Corneometer values) prior to and after the dermal application of the S.L. After dermal application lipid nanoparticles form an invisible patch, which was described earlier [3] and was again proven in this study by Frictionmeter measurements. The film formation resulted in an increase in skin hydration but had no influence on the TEWL, indicating that the S.L. were able to form a protecting but breathable film on the skin, which results in a pleasant skin feel upon dermal application and skin carrying and protecting properties at the same time. Results provide first evidence for the skin carrying properties of second-skin - smartLipids® (S.L.) and the suitability of their use in advanced corneotherapy.

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References:

POS.130

Lipid-polymer hybrid nanoparticles for mRNA delivery in Dendritic cells: Impact of lipid composition
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Vaccination is considered as an effective way to prevent the outbreak of a disease. Vaccines are often injected in the muscles or into the dermis. To overcome syringe-associated limitations like safety issues, patient compliance and logistic constraints, needle-free vaccination delivering antigen to the abundant antigen presenting cells (APCs) of the skin is a promising option. Our aim is to reach these immunocompetent cells without harming the stratum corneum via the transdermal route [1] by minimally invasive methods [2]. We are developing a lipid-coated polymeric nanocarrier loaded with mRNA encoding for the antigen. Such changes have gained considerable attention in recent research due to their good safety profile, the high flexibility in exchanging targeted diseases and the easy and fast production compared to other vaccine

References:
types. Another advantage is the amplification of the signal during the translation in the cells compensating the low bioavailability. The particulate carriers should protect the mRNA and deliver it into cutaneous APCs, in particular dendritic cells, and, in ideal case, co-deliver an adjuvant to increase and tailor the immune responses. The translated protein antigen is processed and presented in a MHC-restricted manner to lymphocytes in the draining lymph node. Such activation pathway enables potent immune responses including humoral and cellular immune responses.

To use a non- or minimally-invasive delivery route to the skin in a sufficient way in vivo the applied nanoparticles must meet certain requirements, which are most importantly mRNA protection and high transfection efficiency. As an in vitro model, we use a murine dendritic cell line DC2.4 to select the optimal particle composition. Starting from a recently developed cationic lipid-coated polymer particle (DOTMA-PLGA)[3], we investigated the influence of adding specific ratios of a second, pH-dependently charged lipid for coating. First, we assessed colloidal properties such as hydrodynamic size, zeta potential, colloidal stability and the preparation method. All tested ratios achieved suitable size (≤ 240 nm), homogenous size distributions (PDI ≤ 0.14), and sufficient colloidal stability. Then biological parameters like cytotoxicity by live-/dead-staining, cellular uptake into DCs and the transfection efficacy technological aspects of all particles were comparable, the cell-based assays revealed a non-linear relationship between lipid composition and ≤ 0,14) and together with transfection efficiency assays leads to the selection of the most promising candidates for in vivo testing in the mouse model.

Diflapolin is the first dual inhibitor of the 5-lipoxygenase-activating protein (FLAP) and the soluble epoxide hydrolase (sEH) and demonstrated a synergistic anti-inflammatory effects on lymphocytes in the draining lymph node. Such activation pathway were assessed on polymorphonuclear neutrophils (PMNL) and in a human whole blood CAV). The anti-inflammatory effects were assessed on polymorphonuclear neutrophils (PMNL) and in a human whole blood CAV). The anti-inflammatory effects were assessed on polymorphonuclear neutrophils (PMNL) and in a human whole blood CAV). The anti-inflammatory effects were assessed on polymorphonuclear neutrophils (PMNL) and in a human whole blood CAV). The anti-inflammatory effects were assessed on polymorphonuclear neutrophils (PMNL) and in a human whole blood CAV). The anti-inflammatory effects were assessed on polymorphonuclear neutrophils (PMNL) and in a human whole blood CAV). The anti-inflammatory effects were assessed on polymorphonuclear neutrophils (PMNL) and in a human whole blood CAV). The anti-inflammatory effects were assessed on polymorphonuclear neutrophils (PMNL) and in a human whole blood CAV). The anti-inflammatory effects were assessed on polymorphonuclear neutrophils (PMNL) and in a human whole blood CAV). The anti-inflammatory effects were assessed on polymorphonuclear neutrophils (PMNL) and in a human whole blood CAV).

The drug load of the NPs was characterized by photon correlation spectroscopy, laser Doppler anemometry and transmission electron microscopy (TEM). The drug load of the NPs was determined by high performance liquid chromatography with UV/VIS detection, and in vitro drug release kinetics were investigated. Biocompatibility of the NP was tested in vitro in cell-based assays and ex ovo in a shell less hen’s egg test on the chick area vasculosae (HET-CAM). The anti-inflammatory effects were assessed on polymorphonuclear neutrophils (PMNL) and in a human whole blood assay by lipid mediator metabololipodismics using UPLC-MS/MS to evaluate inhibition of 5-LOX product formation. All NPs showed a hydrodynamic diameter about 250 nm. The zeta potential indicated a stable nanoparticle dispersion (-15 to -27 mV). TEM visualisation showed spherically shaped particles. All NPs showed an excellent biocompatibility after systemic injection in the dynamic blood flow of the hen’s egg test. As a proof of concept, the human whole blood assay demonstrated a stronger decrease of pro-inflammatory 5-LOX products (e.g. l-LETB4, LTB4) for the diflapolin-NPs in comparison to the free compound. Conclusively, the NPs may protect the diflapolin from plasma protein binding resulting in an enhanced efficiency.

In conclusion, polymer-based NPs are an excellent and biocompatible drug delivery system for safe, efficient and anti-inflammatory treatment with the dual FLAP/sEH inhibitor diflapolin.

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References:

POS.123

Development of tailor-made quercetin nanocrystals for target-oriented dermal drug delivery

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Nanocrystals are a simple and efficient formulation principle for active pharmaceutical ingredients (APIs) to increase the aqueous solubility and thus the bioavailability of poorly water-soluble drugs (BCS class II and IV) [1]. Recent studies suggest that, besides an enhanced bioavailability after oral administration, nanocrystals also exhibit many positive effects after dermal application, i.e., an excellent skin adhesiveness, improved dermal penetration and a depot effect due to uptake of the nanoparticles into the hair follicles [2]. Recent research suggests that nanocrystals of approx. 200 nm promote the penetration of an API through the stratum corneum, whereas sizes from 400 to 600 nm are preferentially located in the hair follicle, where they act as depot [1,3]. Hence, the production of nanocrystals with tailor-made size is desirable to pursue a target-oriented dermal drug delivery.

The aim of this study was the development of tailor-made quercetin nanocrystals with a state-of-the-art NanoWitt LAB-100 milling equipment (Frewitt fabrique de machines S.A, Fribourg, Switzerland) under industrial conditions. Quercetin was chosen as model drug due to its prominent antioxidative activity, which is beneficial for prevention of photocaging of the skin [4,5]. Suspensions were prepared with 5% (w/w) quercetin and 1% (w/w) D-α-tocopheryl polyethylene glycol succinate (TPGS) as stabilising surfactant. The milling was carried out with NanoWitt LAB-100 milling equipment in continuous mode configuration. Circulation of the suspension was set to 500 ml/min for 120 minutes. The rotor of the milling chamber was adjusted to 1500 rpm, with a bead/suspension-ratio of 60/40 (V/V). Suspension temperature was constantly kept between 15 to 20°C. Three different sizes (small: 100 μm, medium: 300μm and large: 400 μm) of zirconia coated beads (SiLbeads Type ZY-E, Sigma Lindner GmbH, Switzerland) were used to control the resulting particle size distribution over time. The volumetric particle size distribution of the nanoparticles was determined by static light scattering (SLS) using a Mastersizer 3000 (Malvern Panalytical, Germany). The span (Dv(0.90)-Dv(0.10))/Dv(0.50) was calculated as a parameter for the broadness of the particle size distribution.

Results showed, that the smallest bead size is suitable for a drastic size reduction. After 30 min, the Dv(0.95) value reached 330 nm with a span of 3.11. Further 90 min milling reduced the Dv(0.95) to 216 nm with a span of 2.64, indicating a relatively narrow particle size distribution. This would make the manufacturing process suitable for nanosuspensions with an enhanced skin penetration of the active through the stratum corneum. The medium and large bead size were able to achieve nanosuspensions with a more suitable particle size for hair follicle targeting with Dv(0.95) values of 390 nm (medium, 60 min) and 450 nm (large, 90 min), respectively. The calculated span of the large bead size
was slightly higher with a value of 3.57, compared to 3.42 for medium bead size. Finally, the D(v)0.95 after 120 min were 338 nm (large) and 268 nm (medium) with a span(large) = 3.24 and a span(medium) = 2.94. Thus, the largest bead size resulted in the broadest particle size distribution.

In conclusion, tailor-made querctin nanocrystals for target-oriented dermal drug delivery can be easily and reproducibly manufactured using different bead sizes, utilized in the milling process. Nevertheless, future investigations have to clarify the skin penetration properties of the nanosuspensions.

Acknowledgments

The authors kindly acknowledge the support of Frewitt fabrique de machines S.A., Switzerland to provide the NanoWit LAB-100 milling equipment. The study was partly supported by ZIM-Project No. 274414/002267.

References:


POS.133

Mimicking nature to solve the problem of poor water solubility of alkaloids by means of salt formation with large organic acids

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Most of the drugs on the market are suffering from low aqueous solubility, even though the drug development process tries to take care of this property. To overcome the problem, the formation of an appropriate salt may help to improve the poor solubility and, hence, typically salt screenings are performed. In the case a drug substance forms a well-organized lattice characterized by strong lattice forces, it is difficult to achieve sufficient water solubility. Using large counterions, which are able to interfere with the lattice, may be an alternative to the classical salt formation with small ions, such as chlorides, bromides, mesylates, and sodium and potassium, respectively. Interestingly, nature provides a large number of stentially ‘demanding’ anions, perhaps because in the plant vacuoles a lot of basic secondary metabolites have to be dissolved for emergency reasons. However, to the best of our knowledge it is not well understood, whether the large counterions prevent the crystallization of the metabolites and what is the physical background. In order to study the influence of natural organic acids on the solubility of alkaloids we have chosen opium as a model system. It consists of easily available papaverine and morphine on the one hand and citric acid, malic acid, tartaric acid and meconic acid on the other hand. In order to collect information about aggregation, solubility, zeta potential, and dissolution rates the formed salts were investigated by means of XRPD, DSC, and IR in solid state as well as NMR spectroscopic methods, photometrical and potentiometric solubility measurements, DLS and zeta potential measurements in solution. In the series studied here the papaverine as a citrate salt showed an enhanced water solubility by factor of 3 in comparison to papaverine hydrochloride. An explanation for the increased water solubility of the citrate salt is the amorphous nature of the salt as well as the colloidal dispersion of the citrate solution in comparison to the crystalline papaverine hydrochloride. This is an important step forward in our efforts to make use of the principles of nature for solubility enhancement.

POS.134

From in-vitro assays to in-vivo application studies – new cationic lipoplexes in comparison

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Cationic lipids in combination with a helper lipid complex therapeutic DNA and are an efficient system to transport nucleic acids into cells. In the last decade cationic lipids are an often used transfection agent because of their high loading capacity, low cytotoxicity, low immunogenicity and absence of oncogenic risk. After an extensive physico-chemical analysis, the biocompatibility of newly designed lipoplex formulations get into focus of our research activity. What will happen after systemic application of the lipoplexes? Therefore various in-vitro and in-vivo assays regarding their hemocompatibility, biodistribution and pharmacodynamic activity are necessary. For evaluating the interactions of the cationic lipoplexes with blood components we performed hemolysis assay, particle size measurement and erythrocyte aggregation assay.

For in-vivo studies an animal model gives the opportunity to estimate the behaviour and toxicity of the lipoplexes in the organism. The zebrafishembryo as an in-vivo model has many advantages because of their small body size and optical transparency for studying the delivery of nanomedicines [1]. Especially the optical transparency allows observing the biodistribution direct in the living animal by microscopic observation. Besides that, the zebrafish has a high genomic and molecular similarity to humans and the discoveries in zebrafish experiments can give hints on biocompatibility in humans [2]. In addition, the experiments on...
Zebrafish embryos do not count as animal testing as long as they are completed before day 5 after fertilization. The presented experiments show the systemic administration of lipoplex formulations, which show excellent in-vitro results. The in-vivo biodistribution and transfection efficiency of these formulations were evaluated in the zebrafish embryo. The in-vitro and in-vivo analysis of the lipoplexes showed no hemolysis, low cytotoxicity and an efficient transfection.


Figure 1: Schematic representation of the polyelectrolyte multilayers (PEMs) with embedded lipoplexes using layer-by-layer technique. The PEMs consist of alternating layers of hyaluronic acid (HA) and chitosan (CHI). Polyethyleneimine (PEI) is used as starting layer.

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References:

**POS.135**

DNA-loaded polyelectrolyte multilayer scaffolds for local transfection in regenerative medicine

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Therapeutic treatment of insufficient bone regeneration is a challenging problem and a topic of on-going research for novel treatment strategies. A promising approach to treatment and improvement utilizes non-viral gene delivery using the bone morphogenetic protein-2 gene (BMP-2) to achieve local and sustained expression of this growth factor [1]. The introduction of DNA vectors encoding for therapeutic genes makes DNA-loaded material films suitable for stimulating bone formation. Particularly useful for application in implantation for regenerative medicine is a nucleic acid delivery-system with controlled release of DNA from biocompatible systems [2].

In this study, we have developed a multi-layered polyelectrolyte film, that permit both, the immobilization and controlled release of DNA from the surface of glass cover slips. Our approach makes use of the layer-by-layer method for the assembly of nanostructured thin films consisting of alternating layers of hyaluronic acid (HA) as polyanion and chitosan (CHI) as polycation. Here, lipid/DNA complexes (lipoplexes), consisting of novel cationic lipids in combination with a helper lipid are embedded within polyelectrolyte multilayers (PEMs) (Figure1). Thus, a multilayer system is to be produced, which enables localized, surface-based transfection. We focused on the development of methods to achieve effective loading of the PEMs with DNA and on the intensive surface characterization using confocal fluorescence microscopy, ellipsometry, atomic force microscopy and zeta potential measurements. Interactions between C2C12 myoblasts with the functionalized PEMs were investigated using confocal microscopy.

We have successfully established a system that allows a loading of DNA in the PEM-film, and which is also capable of transfecting C2C12 cells. Ellipsometric measurements were used to monitor the thickness growth of the PEMs after treatment with cationic lipoplexes. In addition, further cell studies on C2C12 cells showed focal adhesion to extracellular matrix. First in-vitro experiments were carried out, in which a good transfection could also be achieved with our established system.

**POS.136**

PVM/MA-MCT nanocapsules – overcoming hydrolysis of nanoparticles polyanhidride nanoparticles for oral delivery.

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Poly(methyl vinyl ether-alt-maleic anhydride) - PVM/MA is a commercially available polymer that has been described as a functional excipient for drug delivery during the last years. Due to the ability to develop non-specific adhesions to mucous membranes, PVM/MA in its native or cross-linked form, is considered to be suitable as a polymeric matrix for nanoparticles for oral applications [1,2]. PVM/MA has a moderate chemical reactivity due to the presence of the anhydride bonds, allowing grafting of the polymer backbone with primary amines and alcohols without the use of complex chemical reactions [3]. In an aqueous environment, the maleic anhydride group is hydrolyzed into two carboxylic groups, yielding the water-soluble free acid. The hydrolysis will change the microenvironment and leads to the dissolution of nanoparticles or the formation of nanohydrogels (for cross-linked nanoparticles). The hydrolysis of plain and cross-linked PVM/MA nanoparticles (NP and NP-CL, respectively), was investigated over time at relevant physiological pH values (1.2, 5.0, and 7.4). The dense polymeric nanoparticles are solubilized at different rates that are influenced not only by time, but also by the pH of the medium. Dynamic light scattering (DLS), nanoparticle tracking analysis (NTA), auto-litration, and ATR-FTIR results showed that at pH 7.4, the nanostructures were completely solubilized in less than 20 minutes, while the cross-linked structure formed a mesh with hydrogel character (Fig. 1). Hence, the instability of PVM/MA nanoparticles in neutral pH hinders its application as a useful drug delivery system. To circumvent PVM/MA-nanoparticle drawbacks, enhancing the drug loading, encapsulation efficiency, and keeping the ability of PVM/MA to develop adhesiveness with the mucosa along the GIT, nanocapsules composed of PVM/MA and medium-chain triglycerides (PVM/MA-MCT NC) were developed and characterized. Plain and cross-linked PVM/MA-MCT NC were produced without the need for any type of stabilizers. The stability of plain and cross-linked PVM/MA-MCT NC was also evaluated at different physiological pH conditions. Particle size distribution was determined by laser diffraction (LD), and dynamic light scattering (DLS). Particle morphology was revealed by cryo- and FF-TEM (Fig. 2). The results showed the pH of the medium did not change the particle size over time, although an effect on the PDI of samples at pH 1.2 was observed. Thus, for the first time PVM/MA-MCT nanocapsules prepared employing only acetone as an organic solvent and without stabilizers were described and characterized, being a promising drug delivery system for oral administration.
Hydrogel-forming materials of both natural and synthetic origin are materials of great interest for applications in drug delivery, tissue engineering and regenerative medicine. Our group developed an anhydride-containing oligomer platform for cross-linking of amine-containing hydrogel forming materials like collagenous peptides and gelatin, which are derived from naturally occurring collagen and elastin. Gelatin, which is derived from naturally occurring collagen and elastin, is used in research and development of medical products in a variety of ways, for instance with chitosan-based hollow tubes that are polymer is used in research and development of medical products in a variety of ways, for instance with chitosan-based hollow tubes that are

The production of chitosan-containing 2K-hydrogels requires changes of their biochemical and biophysical properties by variation of the molecular weight distribution. The chemically characterized oligomers can be isolated by repeated precipitation and vacuum drying. Prior to application of the material the anhydride groups can be hydro- or aminol yzed in order to obtain polyanionic structures for interaction with minerals or metals. Via partial derivatization of the anhydride groups the oligomers can be made functionalized with additional (bio)functional groups to impart specific biological functionality.

Based on our expertise with maleic anhydride (MA) containing oligomers,[3] we began to synthesize derivatives with enhanced biological functionality.

The need for improved ways of drug delivery is growing along with the identification and development of highly selective or DNA editing API's, especially for the treatment of cancer, autoimmune or neurological diseases.[1] Polymeric or polymer coated nanostructures are of special interest as their physico-chemical properties can be tuned precisely during synthesis.[2]

We hypothesize that amphiphilic anhydride group (red boxes) containing oligomers represent a versatile group of polymeric material to stabilize metal, metal oxide or mineral NP’s via colloidal association and ionic interactions. The incorporation of anhydride groups in oligomers has several advantages in this context. Anhydride groups are neutral which supports oligomer polymerization in hydrophilic organic solvents without solubility problems. The oligomers can be purified and isolated by repeated precipitation and vacuum drying. Prior to application of the material the anhydride groups can by hydro- or aminol yzed in order to obtain polyanionic structures for interaction with minerals or metals. Via partial derivatization of the anhydride groups the oligomers can be decorated with additional (bio)functional groups to impart specific biological functionality.

Based on our expertise with maleic anhydride (MA) containing oligomers,[3] we began to synthesize derivatives with enhanced amphiphility by incorporating oligo(ethylene glycol) (PEG) alcohols ([14] to the get terpolymers of the o14PEGMA type. Expansion with a functionally inert filler monomer like 4-acryloylmorpholine (MO) is optional. The pristine anhydride-containing oligomers were characterized for chemical composition and comonomer incorporation as well as molecular weight distribution. The chemically characterized oligomers were converted into water-soluble salts that already proved suitable for calcium phosphate NP stabilization. We strive to develop functionalized NPs for sRNA delivery particularly for application to the brain.

Formulation of 2-component-hydrogels from anhydride-containing oligomers and chitosan

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Hydrogel-forming materials of both natural and synthetic origin are materials of great interest for applications in drug delivery, tissue engineering and regenerative medicine. Our group developed an anhydride-containing oligomer platform for cross-linking of amine-containing hydrogel forming materials like collagenous peptides and gelatin, which are derived from naturally occurring collagen and inherently biocompatible, degradable and cell adhesive [1, 2]. The resulting 2-component-hydrogels (2K-hydrogels) feature adjustability of their biochemical and biophysical properties by variation of the component ratio, oligomer composition and cross-linking conditions. Reacting part of the anhydrides in the oligomer with other amine-containing molecules allows for covalent modification of the hydrogels [3, 4]. The 2K-hydrogels can be employed for different applications, for instance for the generation of nerve regeneration structures by manual fabrication or 3D-printing.

In this work, the use of chitosan as the amine-containing component in the 2K-hydrogels is investigated. Chitosan is an aminopolysaccharide and is derived from naturally occurring chitin by deacetylation (DD) is a lot higher than in the collagenous peptides (0.4 μmol/g). With the oligomer oPNMA-10 (oligo(ethylene glycol) diacrylate monomaleate (PEDAS)-co-N-isopropylacrylamide-co-maleic anhydride) with 10 MAeq per PES, 7% in dimethylformamide (DMF), stable hydrogels could be generated with different volumes of chitosan solutions containing between 1% and 2% chitosan (DD 80%) dissolved in acetic buffer with pH 3.0 and 4.76. No gelation was observed on addition of DMF without oligomer. Depending on the ratio between oligomer and chitosan, as well as pH, component incorporation between 75% and 100% could be achieved. Rheological characterization gave storage moduli between 11.8 kPa and 32.5 kPa. Water content of the generated 2K-hydrogels ranged from 81.3% to 94.7%, with the highest storage moduli being found for the hydrogels with the lowest water contents. With these results, the general suitability of chitosan as the amine-containing component in 2K-hydrogels could be shown. The low pH values of the employed buffer system means that the hydrogels need to be prefabricated and washed to exchange the acidic reaction medium for a more physiological buffer, which makes them not suitable for direct cell incorporation. Nevertheless, the high adaptability of this system and the attractive components for tissue engineering/regenerative medicine makes this material platform highly attractive for 3D-printing applications.

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Anhydride-containing amphiphilic oligomers for nanoparticle stabilization and functionalization

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The need for improved ways of drug delivery is growing along with the identification and development of highly selective or DNA editing API’s, especially for the treatment of cancer, autoimmune or neurological diseases.[1] Polymeric or polymer coated nanostructures are of special interest as their physico-chemical properties can be tuned precisely during synthesis.[2]

We hypothesize that amphiphilic anhydride group (red boxes) containing oligomers represent a versatile group of polymeric material to stabilize metal, metal oxide or mineral NP’s via colloidal association and ionic interactions. The incorporation of anhydride groups in oligomers has several advantages in this context. Anhydride groups are neutral which supports oligomer polymerization in hydrophilic organic solvents without solubility problems. The oligomers can be purified and isolated by repeated precipitation and vacuum drying. Prior to application of the material the anhydride groups can by hydro- or aminol yzed in order to obtain polyanionic structures for interaction with minerals or metals. Via partial derivatization of the anhydride groups the oligomers can be decorated with additional (bio)functional groups to impart specific biological functionality.

Based on our expertise with maleic anhydride (MA) containing oligomers,[3] we began to synthesize derivatives with enhanced amphiphility by incorporating oligo(ethylene glycol) alcohols ([14] to the get terpolymers of the o14PEGMA type. Expansion with a functionally inert filler monomer like 4-acryloylmorpholine (MO) is optional. The pristine anhydride-containing oligomers were characterized for chemical composition and comonomer incorporation as well as molecular weight distribution. The chemically characterized oligomers were converted into water-soluble salts that already proved suitable for calcium phosphate NP stabilization. We strive to develop functionalized NPs for sRNA delivery particularly for application to the brain.

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The use of liposomes as drug vehicles can be an option to overcome the blood-brain barrier [2].

The human blood-brain barrier (BBB) is a highly selective cellular structure which separates central nervous system (CNS) and circulating blood stream. It plays a very important role for the protection of the CNS structure which separates central nervous system (CNS) and circulating blood stream. It plays a very important role for the protection of the CNS and therefore prevents various pharmaceutical substances from reaching the brain. Furthermore, a variety of diseases of the CNS are associated with dysfunctions of the BBB.

In order to investigate barrier functions of the BBB and drug transport under physiological and pathophysiological conditions, the SensorTransBBB system was developed. The microphysiological chip in microwell format, produced by micro-injection molding, consists of ten parallel culture channels which can be perfused with cell culture medium. Porcine brain capillary endothelial cells (PBCECs) were cultivated on the surface of a synthetic hydrogel matrix thus forming a barrier between a fluidic culture and the perfusing fluid. The viability of PBCECs was assessed by calcein stainings and cell layer integrity was determined by a permeability assay using FITC dextran. Furthermore, tight junction protein ZO-1 was present at the cell boarders as visualized by immunostainings, indicating a functional BBB.

In this study hCMEC/D3, a well-characterised cell line with good blood-brain barrier properties [4], was used as an in vitro BBB model. PEGylated liposomes, mainly consisting of 1,2-Distearoyl-sn-glycero-3-phosphocholine (DSPC), with the addition of cholesterol and a rhodamine-conjugated lipid for fluorescent detection, were produced using dual asymmetric centrifugation [5] and loaded with a hydrophilic small drug molecule. Peptide-linked DSPE-PEG(2000)-maleimide was incorporated into the liposomes for either cell-penetrating and/or targeting purposes.

To study cellular uptake, a modified version of a previously established liposome uptake assay was used [6]. Cells were incubated with fluorescent liposomes in different concentrations for 2 hours. Concentration of liposomal components in lysed cells after incubation and washing and the corresponding uptake efficiency were determined using fluorescent spectroscopy. Uptake was also visualised using confocal laser scanning microscopy. First results show a significantly higher uptake of the tested surface-modified PEGylated liposomes than of non-modified PEGylated liposomes. The highest uptake efficiency generally occurred at a liposome concentration of 0.125 mM total lipids. Confocal microscope images confirm abovementioned results.

In further studies, modified liposomes are to be tested in transwell models to elucidate not only uptake into but also permeation across brain capillary endothelial cell monolayers. Studies with isolated and functional intact rodent brain capillaries as well as in vivo pharmacokinetic studies with drug-loaded liposomes will complete this study.

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References:

SensorTransBBB – Establishment of a Microfluidic Chip Model of the Blood-Brain-BARRIER

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The human blood-brain-barrier (BBB) is an epithelial barrier that prevents the egress of substances from the blood into the brain. Microfluidic systems are simple tools to mimic physiological or pathological conditions and can be used to study barrier functions and drug transport.

In order to investigate barrier functions of the BBB and drug transport under physiological and pathophysiological conditions, the SensorTransBBB system was developed. The microphysiological chip in microwell format, produced by micro-injection molding, consists of ten parallel culture channels which can be perfused with cell culture medium. Porcine brain capillary endothelial cells (PBCECs) were cultivated on the surface of a synthetic hydrogel matrix thus forming a barrier between a fluidic culture and the perfusing fluid. Furthermore, tight junction protein ZO-1 was present at the cell boarders as visualized by immunostainings, indicating a functional BBB.

The project was funded in part by the BMBF and the Baden-Württemberg Stiftung.

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by ultrasonic treatment, then heated up to 70 °C for four hours in a water bath, mixed with the gelatin solution and then treated again with ultrasound at 70 °C for two hours.

Freshly prepared samples were measured with an oscillatory HAAKE MARS III rheometer (Thermo Scientific, Germany). A linear cooling ramp from 70 to 25 °C with a temperature gradient of 0.5 °C/min was applied, followed by a 90 min ageing period at 25 °C. The storage modulus (G') and the loss modulus (G'') during cooling and isothermic ageing at 25 °C were measured in controlled shear deformation mode with 1 % deformation and a frequency of 1 Hz.

Results

Rheological measurements in the temperature range between 70 and 25 °C revealed significantly higher values of the loss modulus (G'') for the G/P blends than for the gelatin formulation at the same solid content. In the gel state (T = 25 °C), the storage modulus (G') was not significantly different for the G/P blends and the gelatin formulation with a 35 % solid content. In contrast G/P formulations with 40 % solid content showed a significantly higher G' compared to pure gelatin samples. This clearly implies that the impact of pectin on G' strongly depends on the solid content in the formulation.

Conclusion

Oscillating rheology is a promising approach to gain a better understanding of the temperature dependent viscoelastic properties of G/P blends. The study revealed that the viscosity increase associated with the addition of pectin to the gelatin in the sol state is a great challenge for the Rotary Die machinability and narrows the formulation window necessary to achieve gastroresistant capsule shell properties.

POS.142

Influence of Biorelevant Media on Membrane Fluidity of Tetraether Liposomes

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The stability of liposomes in gastric and intestinal fluids is of central interest for oral application of liposomal preparations. Encapsulated substances can display various instabilities towards the surrounding media as well as insufficient permeability through the gut wall when liposomes become leaky. Only through maintaining liposomal integrity, the payload can be successfully delivered to possible regions of interest along the gastrointestinal tract (GIT). It has been demonstrated that membrane fluidity is directly correlated to the permeability of low molecular solutes and protons [1]. Therefore, the objective of this study was to assess the influence of simulated gastric (FaSSGF) and intestinal fluid (FaSSIFv2) on liposomal membrane fluidity. Fluidity was measured using anisotropy of diphenylhexatriene (DPH), a fluorescent probe that shows different polarization depending on its microenvironment. Liposomes with different compositions were tested: All liposomes consisted of either egg phosphatidyl choline (EPC) or dioleoylphosphatidylcholine (DOPC) as main lipid with varying added concentrations of cholesterol. 10 mole-% of main lipid was then substituted. The obtained liposomal formulations were then characterized regarding size, size distribution and zeta-potential. The well-known cell penetrating peptide (CPP) Penetratin was used to compare with the novel CPP-decorated vesicles. Liposomes were generated using dual centrifugation. The standard lipid compositions contained a ratio of 40:60 cholesterol : egg phosphatidylcholine (EPC). When CPP-linked lipid was added to the formulation in concentrations of 0.1 mole-%, 0.5 mole-% and 1 mole-%, the adequate amount of EPC was substituted. The obtained liposomal formulations were then characterized regarding size, size distribution and zeta-potential. The effect of the CPPs on the liposomal membrane fluidity was assessed, using fluorescence anisotropy of diphenylhexatriene (DPH). In addition, the toxicity of the novel formulations at the highest CPP concentration was tested. CaCo-2 cell monolayers were exposed to the formulation for 4 hours at different concentrations, after which an AlamarBlue® assay was performed, testing the viability of the treated cells.

As expected an increasing CPP concentration results in larger liposomes with a higher positive charge. Two out of the four formulations have an effect on the viability of the cells, which is why they have been ruled out as potential candidates for our formulation.

In the following steps the effect of the formulation on the membrane fluidity of a CaCo-2 cell monolayer will be evaluated. The most promising formulation will be tested in an animal experiment, using a model cargo API.

Acknowledgments: Liposol GmbH (Ludwigshafen Germany) for the Lipids

POS.144

EPR spectroscopy as a tool to evaluate poly-anhydride nanostructures – how polymer hydrolysis changes the nanoevironment?

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Poly(methyl vinyl ether-alt-maleic anhydride) – PMVMA was proposed to be the polymeric matrix of nanoparticles for oral and topical administration considering its ability to develop adherence with mucosa and moderate chemical reactivity due to the presence of the anhydride bonds[1,2]. In an aqueous environment, the maleic anhydride group is hydrolyzed into two carboxylic groups, yielding the water-soluble free acid. The hydrolysis will change the microenvironment and leads to (i) the dissolution of nanoparticles or (ii) the formation of nanohydrigelts (for cross-linked nanoparticles). At pH 7.4, the nanostructures were completely solubilized in less than 20 minutes (Fig. 1), while the cross-linked structure formed a mesh with hydrogel character. It was described that a nanocapsules system made with PVM/MA and medium-chain

References:

Acknowledgments:
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POS.143

Characterization of Cell Penetrating Peptides as Enhancers of Oral Liposome Delivery

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Liposomes still present a promising nano-sized drug delivery system due to the biocompatibility of their components and their variability in functionality and targeting. While the internalisation mechanism of liposomes via the gastrointestinal route is still not completely clear, the use of uptake enhancers seems to be inevitable in order to gain reasonable permeability through the gastrointestinal epithelial barrier.

The aim of this study was to generate arginine rich liposomes, characterize them and test them for possible toxicity in cell tissue culture (CaCo-2 cells).

Four different poli-L-arginine structures were synthesized and coupled to a linker lipid which was then incorporated into the liposomal formulation. The well-known cell penetrating peptide (CPP) Penetratin was used to compare with the novel CPP-decorated vesicles. Liposomes were generated using dual centrifugation. The standard lipid compositions contained a ratio of 40:60 cholesterol : egg phosphatidylcholine (EPC). When CPP-linked lipid was added to the formulation in concentrations of 0.1 mole-%, 0.5 mole-% and 1 mole-%, the adequate amount of EPC was substituted. The obtained liposomal formulations were then characterized regarding size, size distribution and zeta-potential. The effect of the CPPs on the liposomal membrane fluidity was assessed, using fluorescence anisotropy of diphenylhexatriene (DPH). In addition, the toxicity of the novel formulations at the highest CPP concentration was tested. CaCo-2 cell monolayers were exposed to the formulation for 4 hours at different concentrations, after which an AlamarBlue® assay was performed, testing the viability of the treated cells.

As expected an increasing CPP concentration results in larger liposomes with a higher positive charge. Two out of the four formulations have an effect on the viability of the cells, which is why they have been ruled out as potential candidates for our formulation.

In the following steps the effect of the formulation on the membrane fluidity of a Caco-2 cell monolayer will be evaluated. The most promising formulation will be tested in an animal experiment, using a model cargo API.

Acknowledgments: Liposol GmbH (Ludwigshafen Germany) for the Lipids
triglycerides (MCT) can circumvent PVM/MA-nanoparticle drawbacks, enhancing the polymer stability in aqueous medium, the drug loading capacity and encapsulation efficiency [3]. Electronic paramagnetic spectroscopy (EPR) is a non-invasive technique that permits the quantitative measurement of microviscosity, microviscosity and, using special probes, it is also possible to quantify microacidity and oxygen content inside a sample. Stable free radicals, such as nitroxides, are widely employed as model drug reporting the microenvironment of pharmaceutical formulations [4]. In this study EPR was used to evaluate the microenvironment of the nanostructures and their ability to retain the spin probe TEMPO-Benzoate (Fig.1). The presence of immobilized spin probe at the first-time point shows that the nanoparticles are dense polymeric structures. The signal of this immobilized spin probe decreased over 10 h, at intrinsic pH, and after 1 h at pH 7.4, suggesting the dissolution of the nanoparticle protective environment. The results also demonstrated the presence of two distinct milieus in the nanoparticles formulation (TB I and TB II). First one with polarity and viscosity equal to that observed for pure MCT, and the second with intermediate viscosity and polarity, suggesting the presence of an interfacial area. This interfacial area was affected by the environmental pH presenting reduced mobility of the spin-probe at pH 1.2. Although partially hydrolyzed, the amphiphilic polymer stabilizes the interface of administration.

Fig. 1 –EPR spectra of (A) TB in water, MCT and dry PVM/MA, (B) NP- TB at 5 min and 10 h after preparation. NP-TB diluted (1:1) in PBS pH 7.4 at 1 hour after preparation. (C) NC-TB at 1 h and 1 day, and of NC-TB after 24 h of dialysis against buffered solutions at pH 7.4 or at pH 1.2.


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smartLipids 2nd skin – restoration and reinforcement of the skin’s natural lipid film as anti-pollution strategy

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According to WHO reports, air pollution continues to increase and is reaching a health alarming level in over 91% of urban population [1]. The skin as the largest organ of the body thereby offers a great contact and attack surface. Molecules such as polyacrylic aromatic hydrocarbons (PAH), localized on the surface of particulate matter, can penetrate and activate the aryl hydrocarbon receptors in skin. Decreased skin moisture, hyperpigmentation, increased inflammation tendency and ROS (reactive oxygen species) formation are some consequences. Both adhesion and penetration are efficiently prevented by the protecting properties of the natural lipid film of the epidermis. Inspired by this natural anti-pollution effect a dermally applicable formulation was developed mimicking the natural skin lipid’s physicochemical properties [2] and thus its anti-pollution effect.

smartLipids, the further development of solid lipid nanoparticles (SLN) and nanostructured lipid carriers (NLC), were made with a lipid phase composed of ceramide IIIB and ceramide VI (70%), cholesterol (15%), and 6 of the fatty acids (15%) being most present in natural skin lipids. Comparable to SLN and NLC smartLipids forms a film on skin allowing the restoration and reinforcement of skin’s natural lipid film [3] – thus where named smartLipids 2nd skin.

smartLipids 2nd skin suspensions were prepared by high pressure homogenization at 500 bar for 1-5 cycles. Surfactant screening was performed to determine the type and concentration of appropriate surfactant for stabilizing 10% of the lipid phase. Particle size was characterized by photon correlation spectroscopy (PCS), laser diffraction (LD), and light microscopy. Melting behavior of the lipid mixture was investigated by differential scanning calorimetry (DSC). According to DSC measurements the lipid mixture melts at over 50°C and therefore stays solid on skin. Due to the complexity of the matrix composed of 9 lipids, its physical stabilization was challenging. Among the 19 screened surfactants at 1.5% only 2 (Lanette E and TegoCare 450) led to liquid suspensions with PCS mean particle sizes of 187 nm (5 cycles) and 248 nm (3 cycles), respectively. All other formulations solidified or formed visible large aggregates already one day after production. Most stable physical formulation was obtained with the skin friendly and ECOCERT Lanette E. No gelation or formation of visible aggregation occurred during 2 months of storage at room temperature. Increasing or decreasing the Lanette E concentration led to poorer storage stabilities (non redispersible aggregates, viscosity increase).

smartLipids 2nd skin was successfully developed being promising in restoration and reinforcement of the skin lipid barrier in a natural way. As the formulation consists exclusively of natural ingredients, it follows the latest trend of organic cosmetics and can therefore be incorporated in a wide variety of gel products following an anti-pollution strategy. Also, smartLipids 2nd skin can be loaded with natural antioxidants, e.g. coenzyme Q10, in order to neutralize ROS already formed in the skin, enabling to create a system with dual anti-pollution effect.

References:

Development of orodispersible tablets for delivery of probiotic bacteria to the oral cavity

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Lactobacillus species can be classified as probiotics and are part of the physiological microbiome of the human oral cavity and intestinal tract. Several positive effects are described, like anti-inflammation [1], positive influence on the immune system [2], and antimicrobial activity against P. gingivalis [3]. Thus, probiotics might be an option to treat gingivitis associated inflammation of periodontal tissue. A probiotic formulation concept based on an orodispersible tablet (ODT) was developed. This convenient dosage form has outstanding benefits, because water is not needed for intake and it is appropriate for children and the elderly. The fast tablet disintegration is usually accompanied by rapid removal of the probiotic bacteria from the mouth due to saliva flow and swallowing leading to a too short residence time in the oral cavity to show the expected positive effects. The retention time of the bacteria can be extended adding mucosadhesive polymers [4]. Nevertheless, the disintegration time of the tablets should be below 30 s to still meet the acceptance level from the FDA. To support fast disintegration on the one hand and to attain intimate contact of bacteria (Lp299v) and the mucosadhesive polymer on the other hand, granulation of Lp299v and the mucosadhesive polymer with a methacrylic acid copolymer was performed first. Three mucosadhesive polymers, carboxomer, HPMC, and chitosan, were assessed. The comparison with the ungranulated formulations showed clear differences between the polymers. Granulation of carboxomer and Lp299v resulted in a distinct acceleration of tablet disintegration. This
was due to electrostatic repulsion of the negatively charged polymer and the bacteria themselves. Moreover, the very fine carborner spheres were bound to the probiotics leading to a dramatically reduced amount of free carborner. Granulation of Lp59Y and non-ionic HPFMC also led to accelerated tablet disintegration. Surprisingly, an opposite effect was found for the cationic chitosan. Here the interaction of the polycation and the polyanion during granulation prolonged the disintegration time of the tablet in comparison to the formulation that was not granulated with the methacrylic acid copolymer. The mucoadhesion of the formulation was assessed with a novel mucoadhesion test, which is related to physiological conditions in the oral cavity [5]. In the presence of the mucoadhesive polymers 20 – 30 % of the applied bacteria adhered to the mucosa, which represented a two- or threefold increase compared to 10 % when a formulation without any mucoadhesive additive was applied. Tablets consisting of a granulated mixture of bacteria and mucoadhesive polymer were proven to have good storage stability in tablet containers with a desiccant bag for 30 months (2-8 °C). For all three polymers a reduction of the mucoadhesive effect was detected after storage, but the formulation including carborner still proved superior mucoadhesion compared to HPMC and chitosan.

References:

Determination of antioxidant capacity in skin

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Oxygen Radical Absorbance Capacity assay (ORAC) is a sensitive antioxidant assay used to evaluate the antioxidant potential capacity (AOC) in food and cosmetic industries. Due to the impact of reactive oxygen/nitrogen species (ROS/RON) on aging and pathogens, ORAC is of great value in cosmeceuticals research [1, 2]. In skin aging, applying antioxidants to treat skin conditions increases the resistance towards oxidative stress and improves the signs of aging [3].

In this study, ORAC assay was validated and used to measure the AOC of two different antiaging cosmetic products, and to measure the penetration efficacy of these products into the stratum corneum layers of fresh porcine ears. Analing products were applied on porcine ears and incubated for 2 h at 30 °C. Tape stripping was then performed to remove stratum corneum and the tapes were washed using ethanol/water. Validated ORAC assay as described by Ou et al. was performed using fluorescein as a fluorescent probe, AAPH (2,2′-Azobis(2-amidinopropane) dihydrochloride) as a peroxyl radical generator, and Trolox as an internal standard [4]. The AOC results of cosmetic products and stratum corneum extracts were expressed as µM Trolox equivalents/1 mL extract.

Trolox calibration curve showed good linear relationship between net area under the curve and Trolox concentrations with a correlation coefficient (r²) ≥ 0.98. The method precision, which is expressed as %RSD was ≤ 10%, and the accuracy varied from 97% to 107%. In the results obtained, the first cosmetic serum (product A) showed much higher AOC (6.5-fold) than the second one (product B). This could be due the nature of the products, i.e. product A being classified as a natural cosmetic while the other is not. Furthermore, also the AOC that was determined from the tape strips, yielded higher AOC values for product B than for product A and not-treated skin, respectively and indicated a deeper penetration of product A into the skin. ORAC assay was successfully used to evaluate the efficacy of anti-aging serums by measuring the AOC of the products and the AOC of stratum corneum tape stripping extracts. This convenient, economical and versatile ex-vivo model can be a valuable tool to predict the potency and the penetration efficacy of cosmeceuticals.

References

Stability Studies of Proteins during Biofabrication

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PHARMACEUTICAL TECHNOLOGY AND BIOMATERIALS
POSTERS

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Biophysical characterization methods to assess the stability of formulated protein-based drugs

Introduction: The characterization of protein-based drugs within drug product development poses several challenges. There is increasing interest in early characterization and formulation optimization to accelerate drug development and thus to avoid costly late-stage failures. Early-stage development is often accompanied by tight timelines, limited API availability, limited knowledge of drug properties and the impact of formulation conditions. Due to the complex structure of proteins, they show limited stability in solution and are thus susceptible for chemical and physical degradation. Protein aggregation is known to be the most common physical instability and may occur at various points throughout the lifetime (e.g., formulation development, production, processing, storage, and packaging) and even during administration of protein-based therapeutics [1]. The formation of these larger particles depends on intermolecular interactions and may be of different quantity and quality such as size and morphology. Aggregates can considerably range in size from dimers to multimers and up to sub-visible and visible particles, endangering the safety and efficacy of the drug product due to an increased immunogenicity [2]. It is therefore important to understand the interactions and characterization of aggregates in order to control protein aggregation to enable stable and successful drug products [1]. The main goal of this study was to investigate whether analytical measures can provide useful screening tools to estimate the aggregation propensity of a model protein in liquid formulations. The analytical techniques used were based on the assessment of conformational and colloidal stability [3], including the determination of the melting temperature (Tm) as suitable indicator of protein conformational stability [4], the determination of the diffusion self-interaction parameter (kD) and the osmotic second virial coefficient (B22) as measures of colloidal stability [3,5].

Material and Methods: In this study three batches of lysozyme in liquid formulations were investigated in terms of their biophysical characteristics, and conformational and colloidal stability. Lysozyme was formulated in 20 mM acetate buffer pH 4.5 and in the absence or presence of sodium chloride. Protein concentration series ranged from 2 to 14 mg/mL. Dynamic (DLS) and static light scattering (SLS) were used to determine the diffusion self-interaction parameter (kD) and the second virial coefficient (B22), respectively. The denaturation temperature (Tm) of lysozyme in solution was investigated by intrinsic tryptophan fluorescence spectroscopy. The samples were also stored for 3 months at 5 °C and then were analyzed for visual appearance and sub-visible particles by DLS.

Results and Conclusion: The diffusion self-interaction parameter (kD) and the second virial coefficient (B22) indicated that repulsive interactions between lysozyme and solvent molecules are present at pH 4.5 in the absence of sodium chloride. In the presence of 400 mM sodium chloride, the DLS and SLS measurements showed attractive interactions between the molecules, indicated by negative kD and B22 values. At pH 4.5, lysozyme carries a positive net charge accompanied with a maximal number of cationic sites. The change from net repulsion (positive values) to net attraction (negative values) in presence of salt, results from the transition of charge-mediated protein-protein repulsion at low salt concentration to attraction due to short-range protein interactions (van der Waals forces, hydrophobic effects, etc.). The B22 values of lysozyme formulations in the presence of sodium chloride are within the crystallization slot, indicating a higher risk of forming precipitate, because of stronger protein-protein attractions. Lysozyme in acetate buffer pH 4.5 was therefore expected to be the most stable formulation compared to formulations in presence of sodium chloride. These findings were confirmed by the study of melting point determination. Tm of lysozyme was reduced in the presence of sodium chloride. This indicates that the addition of salt has a very unfavorable effect on the protein thermal stability at pH 4.5. The formulations were also stored at 5 °C for 3 months and were visually inspected at the end of the storage time. Formulations in the absence of salt and showing positive B22 values outside the crystallization slot were visibly clear, whereas formulations in the presence of salt and presenting negative B22 values within the crystallization slot showed precipitation. Based on the observed findings, kD, B22 and Tm values can be used to choose optimal formulation conditions to influence the protein critical solution behavior and thus to estimate colloidal and conformational stability of liquid formulations. Such studies could be performed within a few days and within a miniaturized setting, instead of real time formulation screenings.

References:
The disintegration and dissolution of enteric-coated (EC) dosage forms has been observed to be considerably delayed in vivo compared to in vitro, even with instances of associated clinical failure. 1 The reason behind this seems to be the lack of responsivity of enteric polymer dissolution to bicarbonate which is the main buffering species in human intestinal fluid, with the bicarbonate molarities present in vivo not providing sufficiently prompt enteric polymer dissolution. This is caused by the anomalous buffer action of bicarbonate in the boundary layer surrounding a dissolving ionisable solute. For, owing to the relatively slow diffusion of bicarbonate compared to the much faster dissolution of the ionisable solute, the pH buffering effects of the bicarbonate are essentially limited to the immediately surrounding boundary layer. In this case, the effective pKa of bicarbonate would be around 7.85, which is significantly higher than the pKa of the free drug, which is in the range of 4.5–4.6. This impairs the ability of bicarbonate to buffer the surface of a dissolving enteric polymer at pH values that facilitate prompt dissolution. This leads to unexpected failures of EC dosage forms to disintegrate properly in vivo and showcases the need for more in vivo-predictive dissolution testing methodologies in order to minimize the risk of such occurrences.

Acknowledgment:

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References:


POS.152

Formulation and evaluation of effervescent hydrogen generating granulates using wet (oscillating granulation) and dry (roller compactive) granulation

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The antioxidant and selectively reducing effects of hydrogen on cytotoxic radicals were first described in 2007. Since then the interest in hydrogen as a therapeutic gas is growing quickly. Most of the experiments are performed with hydrogen which is applied to animals via inhalation, injection or as hydrogen enriched drinking water (HRW). HRW can for example be prepare by electrolysis. In order to transfer hydrogen therapeutic potential to human medical use we aim to develop an effervescent hydrogen releasing tablet as a pharmaceutical dosage form to freshly prepare HRW. To achieve this hydrogen releasing effervescence granulates were manufactured by different granulation techniques (wet oscillating granulation and roller compacting). Moreover the type and amount of possible fillers were evaluated in order to explore a suitable manufacturing technology, type and amount of filler for the formulation. Granulates were characterized by measuring different parameters (angle of repose, flowing time, bulk density). The results demonstrate that the roller compacted granulates are more favourable regarding the flowing time and bulk density when compared to the wet oscillated granulates, while their angle of repose was satisfying. Concerning the angle of repose, the granulates that were prepared by oscillating granulation performed slightly better than the roller compacted ones, while the flowing time was not as good but still sufficient for a later tableting process. However, the bulk density of the wet oscillated granulates was much lower than the bulk density of the roller compacted ones.

The different amounts of the fillers compared to the hydrogen generating ingredient did not affect the results to a great extent. Granulates from each group of filler showed nearly the same results in every prepared concentration in the starting powder mixture. However, the different types of fillers showed differences in the results compared to each other. Overall the method of preparation had the biggest impact on the properties of these granulates. The type of filler that is used is also very important. The different amounts of the fillers compared to the hydrogen generating ingredients did not change their characteristics notably.

POS.153

Sustainable Nano Products: Production and characterization of PlantCrystals from the waste of black tea as a source of antioxidant phenolic compounds

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Introduction:

Black tea is one of the most common beverages consumed worldwide. It is derived by fermentation of the dried green leaves of Camellia sinensis (Theaceae). Tea is usually used due to its taste and high polyphenols' content [1]. Its residue is usually thrown away. But this wasted residue might still contain a fortune, like polyphenols. Such active constituents can therefore help to treat several oxidative stress disorders like Alzheimer’s disease. However, its poor solubility and low bioavailability lead to reduced biological activity. Recent studies showed that nanosizing of medicinal plants and their wastes is a promising, smart and easy method to release their active constituents [2]. Therefore, the waste of black tea was chosen in this study to investigate the effects of the nanosizing process on its antioxidant capacity. The characteristics of the resulted PlantCrystals from black tea waste were compared with the bulk material, including the antioxidant activity in-vitro.

Methods:

One percent of black tea was boiled in hot water for about one minute. The residue obtained was dried and grinded. The resulted residue was suspended in 1% (w/w) Plantacare2000® surfactant solution. A Small-scale bead milling method was performed on the obtained bulk-suspension [3]. The produced nanocrystals were analysed using photon correlation spectroscopy (PCS), laser diffraction (LD) and light microscopy. Total polyphenols, flavonoids and carotenoids contents were determined. The antioxidant capacity (AOC) was detected using DPPH (2,2-diphenyl-1-picylhydrazyl) assay [4]. The characteristics were conducted on the obtained PlantCrystals and compared with those of the bulk material and tea.

Results:

Nanosizing process applied to the waste of the black tea bulk material led to sizes of about 280 nm. The antioxidant capacity increased with decreasing size, i.e. the antioxidative potential increased about nine fold upon nanosization, leading to the highest AOC value from the suspensions analysed. The different contents' tests used in this study demonstrated that the produced PlantCrystals possess the highest polyphenols, flavonoids, and carotenoids contents, hence, the available content was higher due to the release occurred upon the nanosizing process. In contrast, the produced nanocrystals showed higher AOC than the bulk material. However, according to DPPH assay the prepared tea in boiled water had the best AOC followed by the obtained PlantCrystals with IC50 values of 0.013 and 0.127 mg/ml, respectively.

Conclusion:

Black tea wastes were successfully nanosized. The bulk-suspension possesses weak antioxidant activity, which was improved by the
nnpnass the best antioxidant activity. Therefore, we can conclude that plants' wastes are rich and sustainable source of phytochemicals and nanonization can further increase the health beneficial properties. The PlantCrystal technology represents a natural, environmentally friendly and cost-effective alternative source of antioxidant phenolic compounds.

References:

POS.154
Nail Patches from Bacterial Nanocellulose for the Laser Therapy of Onychomycosis

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Onychomycosis is the most common nail infection and is caused by different types of fungi, like dermatophytes, nondermatophytes and yeasts [1]. As the established treatment options, local and systemic, require a long treatment time and have a high recurrence rate, the patient’s compliance is poor [2]. Laser therapy is an improved treatment for onychomycosis with a shorter treatment time, few side effects and promises a higher patient acceptance. In the present study, the suitability of the biopolymer bacterial nanocellulose (BNC) as a softening patch for infected nails prior to laser therapy was investigated to develop an easily applicable system that increases the efficiency of laser treatment.

BNC is a biotechnologically synthesized three-dimensional network of nano-sized fibers with many desirable properties, like excellent biocompatibility, high purity, mechanical strength and a high loading capacity for hydrophilic drugs [3]. BNC was loaded with different amounts of the moisturizing glycerol and urea as a keratolytic agent. For the development of a pharmaceutical product, characteristics like form stability, adhesion, prevention of dehydration, transparency, wetting of the nail and a high loading efficacy for urea are demanded.

BNC was synthesized by the bacterial strain Komagataeibacter xylinus at 28 °C in 24-well plates for 14 days, alkaline purified and loaded by absorption with glycerol solutions at different concentrations with or without the addition of urea. Form stability was characterized by determining mass loss and compressive strain after pressurizing the loaded BNC fleeces. By comparing the absorption of the loaded fleeces relatively to the maximum opacity transparency was investigated. Horse hoof plates were used as a model to examine the wetting-capacity of the pharmaceutical formulations. To determine the loading capacity of the BNC, urea was quantified in the loading solution before and after the loading procedure.

The mechanical stability of the BNC was enhanced by the addition of > 75% glycerol, while the addition of urea did not change the compressibility slightly. The absorption measurements displayed that an increase of the glycerol concentration correlated with an increased transparency of BNC. The addition of glycerol and urea provided a sufficient wetting-capacity over more than 24 hours, which facilitates an overnight application. Investigations of the loading capacity revealed that urea solutions with concentrations up to 40%, which is comparable to commercial pharmaceutical products, could be loaded into the BNC.

The combination of BNC with urea and glycerol offers many desirable characteristics for a tailor-made pretreatment of onychomycotic nails prior to laser therapy. The easy to use application as nail patch without additional dressing material and the transparency of the BNC to evaluate the progress of softening without the need to remove the patch are beneficial for the pharmaceutical application.

Acknowledgments: We would like to thank NaNaCell GmbH for providing the K. xylinus culture.

References:

POS.155
Sterile 3D-bioprinting and mechanical characterization of hydrogels

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Biofabrication aims for biologically active constructs consisting of cells, (bio-) materials and biological molecules in hierarchical order using 3D printing technologies [1]. Fabrication yielding consistent, tailor made, and above all microbiologically safe constructs is an important task to translate from research into application [2]. Alginites are widely used hydrogels because of their good biocompatibility, gelation in contact with cations such as Ca2+ and their tunability of mechanical properties to the requirements of different cell types by variation of crosslinker, gelling conditions and molecular weight. These properties make alginate a suitable base material for bioinks [3]. Here, we compared compendial sterilization monographs with aseptic manufacturing of 3D-bioprinted alginites with a particular focus on microbiological outcome and the impact on supramolecular structures as assessed rheologically.

Compared to the untreated alginate after autoclaving for 15 min at 121 °C, alginate hydrogels showed a reduction of the number average molecular weight by 70 % and a significantly lower viscosity. In addition, after autoclaving, the alginate has transformed into a hydrogel with reduced gel characteristics. This can be seen by a significant change in the storage and loss modulus. Due to the reduced viscosity and the changed flow behaviour, the autoclaved alginate material lost its printability and therefore its suitability for 3D-bioprinting. Sterile filtration, on the other hand, had only a minor effect on the rheological behaviour and the molecular composition of the alginate hydrogel.

We conclude that aseptic production of alginate bioinks is made possible by sterile filtration without significantly influencing the rheological properties required for 3D-bioprinting.

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References:

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4.9 Pharmacology

Dextromethorphan inhibits KATP and Ca²⁺ currents in pancreatic β-cells

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Question: The NMDAR antagonist dextromethorphan (DXM) and its metabolite dextrorphan (DXO) have been recommended for treatment of type 2 diabetes mellitus because of their beneficial effects on insulin secretion [1]. It is also known that DXM and DXO, although the latter less potent, not only inhibit NMDA receptors but also voltage-gated Ca²⁺ channels [2, 3]. This study investigates the effects of DXM and DXO in islets and single β-cells on Ca²⁺ currents and KATP current, which regulate essential steps of the stimulus-secretion coupling.

Methods: Islets or β-cells were isolated from wildtype (WT) and SUR1-deficient insulins (SUR1-KO) of a C57Bl/6N background. [Ca²⁺]c was determined by fluorescence microscopy. Electrophysiology was performed with the patch-clamp-technique. Insulin release from islets was determined by radioimmunoassay.

Results: WT islets stimulated with 8 mM glucose showed a typical oscillatory pattern in [Ca²⁺]c, measurements. DXO (100 µM) was added for 10 minutes to the bath solution and slightly elevated mean Ca²⁺ of 1.27±0.04 a.u., n=14, p≤0.05). The sudden transient inhibition of Ca²⁺ oscillations in WT islets by DXM was further elucidated by measuring Ca²⁺ currents in the whole-cell configuration. DXM (100 µM) inhibited partially but significantly the Ca²⁺ current in single β-cells (control -22.2±3 pA vs. DXM -17.3±3 pA, n=5, p≤0.05).

The KATP channel current of WT β-cells was measured in the whole-cell configuration. The presence of 5 mM glucose application of 100 µM DXO acutely inhibited the KATP current (control 25±3 pA/pF vs. DXO 10±2 pA/pF, n=6, p≤0.001). Acute application of 100 µM DXM first stopped the Ca²⁺ oscillations, then after approximately 5 minutes the islets switched into a plateau pattern. Regarding the last 5 minutes of substance addition, DXM significantly increased mean Ca²⁺ (control 0.97±0.03 a.u. vs. DXM 1.27±0.04 a.u., n=14, p≤0.001). In comparison, DXM increased mean Ca²⁺ significantly more than DXO (DXO 1.05±0.04 a.u., n=17 vs. DXM 1.27±0.04 a.u., n=14, p≤0.05). The sudden transient inhibition of Ca²⁺ oscillations in WT islets by DXM was further elucidated by measuring Ca²⁺ currents in the whole-cell configuration. DXM (100 µM) inhibited partially but significantly the Ca²⁺ current in single β-cells (control -22.2±3 pA vs. DXM -17.3±3 pA, n=5, p≤0.05). The KATP channel current of WT β-cells was measured in the whole-cell configuration. In the presence of 0.5 mM glucose application of 100 µM DXM and DXO are no suitable candidates for treatment of type 2 diabetes mellitus.

Acknowledgment: We thank Prof. Dr. Gisela Drews (Institute of Pharmacy, Department of Pharmacology, University of Tübingen, Germany) for providing SUR1-KO islets.

References:

POS.155

Exotoxins from Staphylococcus aureus regulate specialized pro-resolving mediators that promote resolution of inflammation

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The knowledge about the mechanisms by the host to actively resolve bacterial infections is incomplete. A major characteristic of inflammation resolution is the biosynthesis of specialized pro-resolving mediators (SPM) by macrophages. These antiinflammatory endogenous lipid mediators (LM) lead to reprogramming of the immune response to accelerate the termination of inflammation, enhance bacterial clearance and promote tissue regeneration. Pathogenic bacteria stimulate cyclooxygenase and lipooxygenase (LOX) pathways in human macrophages to produce differential LM profiles in a phenotype-dependent manner [1]. However, the underlying factors and mechanisms that originate from bacteria to induce LM generation in these host cells remain vague. Here, we identify the pore-forming toxin α-hemolysin (Hla) from Staphylococcus aureus as selective and potent activator of 15-LOX-1 which is a key enzyme in SPM biosynthesis in human monocytes-derived M2 macrophages and in vivo in murine peritoneal infections. Hla-deficient Staphylococcus aureus mutants or depletion of Hla using a capturing Hla-antibody failed to activate 15-LOX-1 and thus related SPM biosynthesis but were still efficient to induce proinflammatory eicosanoids like arachidonic acid-derived prostaglandins (PGs) and leukotrienes (LTs). Genetic manipulation by si-RNA and pharmacological inhibition by BLX-3607 [2] of human 15-LOX-1 in M2 macrophages exclusively reduced the Hla-dependent SPM biosynthesis upon challenge with bacterial secreted toxins. Moreover, LM isolates from HLA-stimulated M2 that contain abundant SPM levels but lack proinflammatory PGs and LTs, promoted tissue regeneration in Planaria. Conclusively, our data suggest that bacterial pore-forming toxins, besides initially harming the host, may also exert beneficial functions by stimulating SPM production to resolve inflammation.


A Ruthenium(II) N-Heterocyclic Carbone (NHC) Complex with Naphthilamide Ligand Triggers Apoptosis in Colorectal Cancer Cells via Activating the ROS-p38 MAPK Pathway

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The p38 MAPK pathway is known to influence the anti-tumor effects of several chemotherapeutics, including that of organometallic drugs. Previous studies have demonstrated the important role of p38 both as a regulator and a sensor of cellular reactive oxygen species (ROS) levels. Investigating the anti-cancer properties of novel 1,8-naphthilamide derivatives containing Ru(I) and Ru(II) N-heterocyclic carbene (NHC)
ligands, we observed a profound induction of ROS by the complexes, which is most likely generated from mitochondria (mROS). Further analyses revealed a rapid and consistent activation of p38 signaling by the naphthalimide-NHC conjugates, with the Ru(II) analogue-activated MC6 showing the strongest effect. In view of this, genetic as well as pharmacological inhibition of p38α, attenuated the anti-proliferative and pro-apoptotic effects of MC6 in HCT116 colon cancer cells, highlighting the involvement of this signaling molecule in the compound's toxicity. Furthermore, the influence of MC6 on p38 signaling appeared to be dependent on ROS levels, as treatment with general- and mitochondria-targeted anti-oxidants abrogated p38 activation in response to MC6 as well as the molecule's cytotoxic- and apoptogenic response in HCT116 cells. Altogether, our results provide new insight into the molecular mechanisms of naphthalimide-metal NHC analogues via the ROS-induced activation of p38 MAPK, which may have therapeutic interest for the treatment of various cancer types.

POS.160

Activation of AT2 potentiates bradykinin-induced extravasation – role of ACE

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Non-allergic angioedema arises from swelling of mucosal and submucosal tissue and can be potentially life-threatening, e.g., if the swelling occurs in the larynx. Non-allergic angioedema falls into different subtypes depending on the underlying molecular mechanism. In any case, bradykinin-induced activation of bradykinin receptor type 2 (B2) has been shown to be crucially involved in animal studies [1] and in clinical trials, e.g., in ACE inhibitor induced angioedema [2]. While non-allergic angioedema induced by ACE inhibitors is most likely caused by inhibition of bradykinin degradation, the molecular mechanism of non-allergic angioedema induced by angiotensin II receptor type 1 (AT1) inhibitors (sartans) such as telmisartan is still unclear. Blockade of AT1 disrupts the negative feedback on renin secretion and increases plasma levels of angiotensin II (AT2) likely resulting in a more pronounced activation of angiotensin II receptor type 2 (AT2). Therefore, we sought to investigate whether AT2 might be involved in the molecular mechanism of sartan induced angioedema. To accomplish this, we synthesized the selective non-peptide AT2 agonist compound 21 (C21, Fig 1) and investigated its pharmacological specificity in organ bath studies using aortic rings of AT2 knockout mice. Furthermore, in two different human primary endothelial cell cultures we were able to show that C21 reduces ACE activity in cell homogenates [3]. As for an in vivo approach, we quantified dermal extravasation induced by intradermal injection of bradykinin in mice (Miles Assay). As compared to vehicle (0.1 % dimethyl sulfoxide), C21 significantly increased dermal extravasation from 3.4±0.2-fold to 4.7±0.2-fold (n=11, P<0.05). This effect was abolished by co-treatment with the AT2 antagonist PD123,319 (PD) and dependent on activation of B2. The selective B2 antagonist icatibant strongly reduced extravasation and abolished the difference between vehicle and C21. In striking contrast, extravasation induced by the synthetic B2 agonist labradimil, which cannot be hydrolyzed by ACE, showed no dependence on pretreatment with C21 (P>0.05). In another approach we compared tissue and supernatant ACE protein content by Western blot in lungs of C57BL/6J incubated in oxygenated buffer solution at 37°C for 30 min in the presence or absence of C21. C21 incubation caused a numerical decrease of ACE in tissue lysates to 0.81±0.05-fold, while ACE protein in supernatants was significantly increased to 1.33±0.17-fold (n=6, P<0.05, Tukey’s multiple comparisons test following One-Way ANOVA). The difference of ACE protein content between tissue and supernatant was significant as well (P<0.01). Likewise, lung ACE protein content as determined following in vivo treatment of mice with C21 significantly decreased to 0.5±0.1-fold (n=6, P<0.05) as compared to treatment with vehicle. A similar reduction of ACE protein to 0.4±0.1-fold (n=6, P<0.05) was observed after combined treatment with C21 and telmisartan, while
telmisartan alone showed no effect. These data suggest that activation of AT2 reduces ACE activity and that this effect is likely caused by shedding of membrane bound ACE. Circulating ACE loses its second catalytic domain and provides less catalytic capacity. This process might contribute to non-allergic angioedema occurring in patients treated with sartans.


Smad Activity: Targeting TGFβ to further explain interindividual variability in PK parameters. In MER PK parameter values between the two patient populations were largely explained by body size and CLCR CG. A covariate analysis using describe concentration-time profiles of MER in the obese and non-obese.

Conclusion: An NLME PK model was successfully developed to trigger ubiquitin-mediated depletion of non-activated R-Smads. The canonical TGFβ/BMP signaling pathway involves the activation of regulatory Smads (Smads)-Smad2/3 and Smad1/5/8 by means of receptor-mediated C-terminal phosphorylation. Activated R-Smads are then subjected to further modification at several serine/threonine residues in the linker region in response to several kinases including cyclin-dependent kinases (CDKs), glycoxy synthase kinase3β (GSK3β), and mitogen-activated protein kinases (MAPKs), creating different phosphorylated isoforms with both anti- and pro-tumorigenic functions. The differentially phosphorylated forms determine the outcome of the signaling as well as the stability of R-Smads, as they are marked for ubiquitination, which poses the molecules for proteasomal degradation.

Although the ubiquitin-proteasome pathway (UPP) plays an important role in terminating TGFβ/BMP signaling through degradation of activated R-Smads, its role in the regulation of non-activated R-Smads is less known. Here, we present an indirubin derivative, designated as E738–a known kinase inhibitor–as a modulator of TGFβ signaling through degradation of activated R-Smads. This work contributes to a comprehensive understanding of TGFβ/BMP signaling through modulation of R-Smad signaling in a panel of patient-derived cholangiocarcinoma (CCA) cells. This could lead to new therapeutic strategies, exploiting the requirement of R-Smads in cellular survival and differentiation.

References:

POS.164

Vacular (H+)-ATPase critically regulates specialized pro-resolving mediator pathways in human M2 macrophages and resolution of inflammation

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Vacular (H+)-ATPases (vATPases) are ATP-dependent proton pumps that are present within the membrane of various organelles in numerous cells and are essential to maintain pH homeostasis. Although vATPase is fundamental in cytokine trafficking and secretion in human monocytes [1] and was implicated in the M2 polarization of murine macrophages [2], its functional roles in lipid mediator (LM) biosynthesis remain elusive. Alternative (M2)-polarized macrophages possess high capacities to produce specialized pro-resolving lipid mediators (SPM) that regulate key functions in resolution of inflammation and tissue regeneration. Here, we exploited the role of vATPase using the well-established vATPase inhibitor Archazolid (ArchA) in LM biosynthetic pathways in human M2 macrophages. Monocyte-derived human macrophages were pretreated with ArchA (30 nM, 15 min), polarized towards M1 and M2 for another 48 hrs, and stimulated with pathogenic E. coli for 180 min to produce LM profiles that were analyzed by UPLC-MS/MS [3]. Blockade of vATPase during human M2 polarization abrogated 15-lipoxygenase-1 (15-LOX-1) expression and prevented the related biosynthesis of SPM. Targeting vATPase neither influenced the IL-4-triggered JAK-STAT6 pathway that is known to regulate 15-LOX-1 expression [4], nor the mTORC1 signaling cascade that promotes the M2 phenotype [2], but strongly suppressed MEK and ERK phosphorylation in M2, indicating an essential role of the MEK-ERK cascade in the regulation of 15-LOX-1 and SPM formation in M2. Targeting vATPase in vivo delayed resolution of zymosan-induced murine peritonitis accompanied by decreased SPM biosynthesis without affecting pro-inflammatory leukotrienes or prostaglandins during the resolution phase after 24 h. Together, our data propose that vATPase is required for ERK-1/2-mediated 15-lipoxygenase-1 expression and consequent SPM biosynthesis during M2 polarization, implying a crucial role for vATPase in the resolution of inflammation.


POS.165

The microtubule-targeting agent pretubulysin impairs inflammatory key features in endothelial cells in vitro and in vivo

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The process of inflammation is a physiological mechanism inevitable for providing protection against noxious stimuli. An inflammatory response is usually comprised of the recruitment of leukocytes to the site of inflammation and the elimination of the noxious stimuli, which is followed by resolution of the inflammatory state and repair of damaged tissue. However, if failed to be resolved, the mechanisms underlying inflammation can contribute to a broad range of pathological conditions, like chronic inflammatory diseases, which are characterized by constant infiltration of leukocytes, ultimately leading to tissue damage and even cancer. Microtubule-targeting agents (MTAs), which are primarily known for their use as chemotherapeutic drugs, have also been shown to have anti-inflammatory properties, with colchicine being one of the lead compounds. In this context, the aim of this study is to investigate the impact of the novel MTA pretubulysin (PT) and already established MTAs on leukocyte-endothelial cell interactions. The endothelium plays a pivotal role in the migration of leukocytes to the site of inflammation and has long been neglected in anti-inflammatory drug research.

By using intravital microscopy of postcapillary venules of the cremaster muscle in mice, we found that the treatment with PT decreases the TNF-induced firm adhesion of leukocytes onto and their transmigration through the vascular endothelium in vivo. In addition, in vitro cell adhesion assays showed that the adhesion of monocyctic cells (THP-1) onto TNF-activated endothelial cells is reduced when endothelial cells were pre-treated with the microtubule-destabilizing drugs PT, vincristine (VIN) or colchicine (COL). In contrast, the TNF-induced adhesion of THP-1 cells onto an endothelial cell monolayer was not impaired when endothelial cells were pre-treated with the microtubule-stabilizing agent paclitaxel (PAC). Based on this data, the influence of PT and other MTAs on the surface expression of the cell adhesion molecules (CAMs) ICAM-1, VCAM-1 and E-selectin (on endothelial cells) was tested by flow cytometry. While the TNF-induced surface expression of ICAM-1 and VCAM-1 is decreased by the pre-treatment of endothelial cells with PT, VIN and COL, the surface expression of ICAM-1 was not and that of VCAM-1 only slightly affected when TNFα-activated endothelial cells were pre-treated with PAC. In addition, pre-treatment with PT had no effect on the surface expression of E-selectin. It cannot be excluded that a reduction of the surface expression is not caused by effects of the used MTAs on the microtubule-mediated transport of the examined CAMs. However, western blot and RT-qPCR analysis showed that total protein content as well as mRNA levels for ICAM-1 and VCAM-1 were significantly down-regulated after treatment with PT, VIN and COL in TNF-activated endothelial cells. Surprisingly, the total protein as well as mRNA levels of E-selectin were also down-regulated upon treatment with these compounds. Based on these findings, the influence of PT, Vin, Col and Pac on the translocation of NFκB into the nucleus of TNF-activated endothelial cells was examined microscopically (immunocytochemistry) but was shown to be unimpaired by any of the tested compounds. In consequence, the influence of PT and other MTAs on the NFκB and AP-1 promoter activity was determined by a dual luciferase reporter assay. It could be shown that the pre-treatment of TNF-activated endothelial cells with PT, VIN and COL causes only a slight reduction of the promoter activity for both transcription factors, whereas this process was not affected when endothelial cells were treated with PAC.

Taken together, this study shows that the microtubule-destabilizing agents PT, VIN and COL interfere with leukocyte-endothelial cell interactions. At least in part, this influence is caused by an effect of these MTAs on the NFκB or AP-1 signaling cascades in endothelial cells. However, since these results are insufficient to explain the striking effects of these compounds on the expression of CAMs involved in leukocyte-endothelial cell interactions, further investigations into potential effects downstream of any transcription factor-induced gene regulation are required.
4.10 Poster Short Talks

PST.01

**Intracochlear PLGA based implants for dexamethasone release: Challenges and solutions**

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The effective treatment of diseases of the inner ear is still an unmet medical need [1]. Local controlled drug delivery to the cochlea is challenging due to the hidden location, small volume and high sensitivity of this organ. A local intracochlear delivery of drugs would avoid the problems of intratympanic (extracochlear) drug application, but is more invasive. The requirements for such a delivery system include a small size and appropriate flexibility. The delivery device must be rigid enough for surgical handling but also flexible to avoid traumatizing cochlear structures. We developed biodegradable dexamethasone loaded PLGA extrudates for the controlled intracochlear release.

In order to achieve the desired flexibility, Polyethylene glycol (PEG) was used as a plasticizer. In addition to the drug release, the extrudates were characterized in vitro by differential scanning calorimetry (DSC) and texture analysis. Simulation of the pharmacokinetics of the inner ear support the expectation that a constant perilymph drug level is obtained after few hours and retained over several weeks. Ex vivo implantation of the extrudates into a guinea pig cochlea indicate that PEG containing extrudates have the desired balance between mechanical strength and flexibility for direct implantation into the cochlea. The location of the implant was visualized by computer tomography.

In summary, intracochlear administration of drug releasing biodegradable implants is a new and promising approach to achieve local drug delivery to the cochlea for an extended time [2].

References

PST.02

**Stabilization of Calcium Phosphate nanoparticles (CaP-NP) as carrier system for siRNA**

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Introduction: Convection-enhanced delivery (CED) represents a promising technique to deliver therapeutics through the narrow interstitial spaces of the brain via a mild pressure gradient [1]. Application of NP by this technique allows for high local drug concentrations as well as a homogenous distribution in brain tumours [2]. One prerequisite for a successful CED application are particle sizes <100 nm [3].

Aim & Objectives: In this study we investigated the ability of a newly synthesized terpolymer to stabilize siRNA loaded CaP-NP intended for transfection of brain cancer cells. Consequently, time dependent changes in particle size distributions as well as transfection ability of the NP were investigated. The terpolymer consisted of a lipophilic anchor, polyethylene glycol (PEG) and maleic anhydride that had been hydrolyzed in order to obtain an amphiphilic anionic oligomer that interacts with Ca(II) ions and CaP.

Methods: CaP-NP were fabricated and loaded with siRNA using nanoprecipitation. Stabilisation of CaP-NP was analysed for different concentrations of terpolymer (2 µM, 5 µM, 10 µM) and siRNA (1 µg, 10 µg). For determination of particle sizes, we compared Nanoparticle tracking analysis (NTA) and Laser diffraction analysis (Mastersizer 3000: Hydro SV unit with a volume of 7 ml). In vitro transfection efficiency of stabilized siRNA-loaded CaP-NP was investigated for the rat glioblastoma cell line F98 with the WST-8 cell viability assay.

Results & Discussion: Particle size determination by NTA revealed the presence of NP whereas a microscopic analysis still showed the presence of aggregates. In comparison to NTA, Laser diffraction analysis enables particle size analysis in a wide size range from nano- to micrometer allowing for simultaneous illustration of NP and aggregates during measurement. Furthermore, Laser diffraction analysis enables for a time-dependent measurement. The small volume dispersion chamber allowed for affordable determination of particle sizes of siRNA loaded particles. Based on these findings, we chose Laser diffraction analysis as the method of choice for further particle size determinations. We analyzed the ability of the terpolymer for CaP-NP stabilization using concentrations of 2 µM, 5 µM and 10 µM. For polymer concentrations of 2 µM and 5 µM we observed the formation of aggregates, whereas 10 µM terpolymer were shown to successfully stabilize the precipitated NP at a size <100 nm. We further analysed the behavior of stabilized CaP-NP diluted in cell culture medium and in the presence of serum. Here, CaP-NP prepared with 10 µM terpolymer remained stable (particle sizes <100 nm) for at least 50 min. This stabilization remained if CaP-NP were loaded with 1 µg siRNA. Furthermore, stabilized CaP-NP showed satisfying in vitro transfection efficiencies independent of terpolymer concentration as well as low cytotoxicities.

Conclusion: Here, we present the successful use of polymer-stabilized siRNA loaded CaP-NP (<100 nm) for in vitro transfection of brain tumour cells. Using a small volume dispersion unit, Laser diffraction analysis allowed to simultaneously observe NP and aggregates formation in different media.

We like to thank the European Regional Development Fund Saxony (ERDF) and the SAB (Sächsische Aufbaubank (Saxony, Germany)) for funding this project. We further like to thank Prof. Dr. Achim Aigner & Dr. Alexander Ewe, Rothf.-Böhm-Institute of Pharmacology and Toxicology, University Leipzig for their support with the NTA.

References:
those obtained with a conventional formulation and were found to be in a therapeutic range. The developed emulsions are a promising vehicle to improve therapy of chronic skin diseases.

![Figure 1: Penetrated amount of nonivamide after 4 h from conventional formulation (HNC 0.05%) and a developed thermogelling emulsion containing 0.3% nonivamide mean ± SD, n = 3.](image)

The authors would like to thank PD Dr. Martin Schenk from the Department of Experimental Medicine at the University Hospital of Tübingen for providing pig ears and Sino-Étus Chemical Co. for the generous donation of methyl cellulose. Also thanks to Synmte AG for kindly providing avibioneze. Dominque Lunter is supported by the European Social Fund and the Ministry of Science, Research and Arts Baden-Württemberg.

References:

PST.04
Cell penetrating liposomes enable the oral delivery of peptide drugs
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Purpose:
Oral delivery of peptide drugs is limited due to their instability in the gastrointestinal tract and low mucosa penetration. A novel approach to facilitate oral bioavailability of peptide drugs such as vancomycin is the use of liposomes containing tetraether lipids (TELs) and cell penetrating peptides (CPPs).

Methods:
Tetraether lipid isolation of *S. acidocaldarius* was performed by Soxhlet extraction. The cyclic penetrating peptide R9C was synthesized by preparative HPLC and confirmed by HPLC/MS analysis. Preparation of the liposomal formulations was performed by dual asymmetric centrifugation (DAC). For in vivo studies, the oral uptake of the peptide antibiotic vancomycin was determined by radiolabelling. Therapeutic trials in a (MRSA) systemic infection model were performed according to Hertlein et al [1].

Results:
All liposomes were characterized by Zetasizer measurements. The increase in the zeta potential indicated the successful incorporation of the CPP-phospholipid-conjugates into the liposomes. TEM and cryo-TEM micrographs confirmed the appropriate liposomal size and morphology. The preparation of the liposomes by DAC enabled high encapsulation efficiencies (up to 60%) as verified by HPLC and FCS measurements [1, 2]. Microscopy studies using Caco-2 cells showed that a strongly enhanced binding of the CPP-liposomes in contrast to standard liposomes was obtained. Furthermore, a high increase in the transport of vancomycin over rat mucosal tissue in Ussing chamber studies in contrast to the free peptide was shown. Oral application in a rat model revealed that the novel liposomal formulation led to a fivefold increase in the bioavailability of vancomycin. The functional efficiency of this uptake enhancement was demonstrated by a strong increase of the antibiotic efficiency in a (MRSA) systemic infection model.

Conclusion:
The promising results clearly raise the hope that this novel liposomal formulation can be used as platform-technology for oral application of a variety of peptide drugs. Bioavailability studies in dogs are ongoing.

References:

PST.05
Identification and quantification of microdialysis variability using a dynamic in vitro microdialysis system and nonlinear mixed-effects modelling
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Objectives: In the guidelines on the use of pharmacokinetics (PK) and pharmacodynamics (PD) in the development of antinfectives, the European Medicines Agency (EMA) Committee for Medicinal Products for Human Use (CHMP) emphasises the importance of the determination of unbound drug concentrations at target sites [1] such as interstitial space fluid (ISF) to foster predictions for probability of PK/PD target attainment. This knowledge can be gathered using the minimally invasive microdialysis (µD) sampling technique [2], which is based on passive diffusion of molecules from ISF across a semipermeable membrane at the tip of a µD catheter. Unbound drug in the ISF can be determined by measuring the drug concentration in the collected dialysate and retrodialysis needs to be performed as a calibration method and this was repeated on four different µD. The objective was to investigate the impact of ISF composition on µD variability.

Methods: An in vivo ISF LIN concentration-time profile (C(t) profile) obtained from the typical patient profile of a clinical µD study [3, 6] was mimicked in the dIVMS. Samples were taken from the flask, which represents the ISF and collected from three different µD catheters (CMA 60, 20kDa cut-off) simultaneously. Retrodialysis was consecutively performed as a calibration method and this was repeated on four different

References:
occasions using the same catheters. Nonlinear mixed-effects (NLME) modelling was performed using NONMEM® (7.4.3) to characterise the LIN C(t) profile in the dIVMS and to quantify variability between catheters and occasions. An integrated ISF and microdialysis modelling approach was chosen to evaluate data from all three available sources (ISF, micro- and retrodialysis) simultaneously [7]. Afterwards, variabilities associated with relative recovery were compared to variability from in vivo clinical data. Model adequacy was assessed by goodness-of-fit plots, parameter precision and visual predictive checks.

**Results:** The LIN C(t) profile in the dIVMS was successfully described using a one compartmental model with linear clearance describing LIN elimination from the flask and three µD measurement compartments. The predictive performance of the PK model was adequate and typical parameter estimates (95% CI) were in line with the experimental settings. The model-predicted and experimentally derived relative recovery were comparable. Recovery variability was split up in intr- and intercatheter variability which were lower in vitro compared to 27.2%CV (21.8 – 32.0%CV) and 26.1%CV (16.7 – 33.8%CV) in vivo.

**Conclusion:** The established dIVMS and model-based analysis provide quantitative and qualitative insights into the µD sampling technique. Changing the target site fluid from Ringer’s solution to artificial ISF increased intra- and intercatheter variability which were still lower than in vivo. This suggests that the current in vivo assessment of sources of µD variability might be confounded by further factors such as variability in the sampled target site or location of the µD catheter. An integrated approach considering additional in vitro and in vivo data might identify these confounding factors and thus aid in further elucidating the high variability in clinical µD studies.

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**References**


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**PST.06**

Pharmacology meets (clinical) pharmacy – all virtual patients for pharmacy students

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**Background**
Clinical pharmacy is the ‘youngest’ of the five main subjects taught in the German pharmacy curriculum being introduced in the year 2001. It is the only clinical subject amongst subjects related to natural sciences like chemistry. One particular challenge of clinical sciences is that they need to be taught in a practical and clinical context, i.e. with patients present. In order to close the gap between theory – the lecture – and practice – the patient, a blended learning project was introduced to the pharmacology curriculum in 2018.

**Aim**
Closing the gap between theory and clinical practice in pharmacology and clinical pharmacy through the introduction of a blended learning concept using e-learning cases and tutorials in addition to the traditional lecture.

**Methods**

The blended learning concept consists of the lecture in pharmacology, which is being held over the course of four semesters, e-learning cases, and tutorials. With each introduction of a new subject in the pharmacology lecture, a correspondent e-learning case is accessible for students, providing an opportunity to review/rehash the pharmacological subject, i.e. pharmacokinetics, within a clinical setting. After completing the e-learning case, students can additionally attend a tutorial with the same subject, led by a medical student, in order to discuss particular problems even further. Any unresolved questions from the tutorials are fed back to the main lecturer, who can discuss these within the lecture if needed. The whole course is organised via the learning platform Moodle®.

**Preliminary Results**
We have started the third term of P3, attendance is increasing over time, for the e-learning cases as well as the tutorials. We have trained three instructors (all medical students), designed 14 e-learning cases which were retrieved 300 times; and held 18 tutorials (some themes were offered twice due to high demand) with 144 participants altogether. Evaluation shows that students appreciate the peer-to-peer situation and relaxed atmosphere during the tutorials as well as the overall structure of the e-learning cases with different questions types and detailed explanations. Students also choose particular subjects rather than attempting all subjects.

**Discussion**
Evaluation of participants shows that students appreciate to have protected opportunities to apply theoretical knowledge in a more clinical and practice-based setting. Participation rates in particular for the e-learning cases however are extremely low compared to the number of students enrolled in the pharmacology lecture. Further qualitative evaluation will be needed to explore reasons for non-participating in e-learning as well as the tutorials. We hope to provide P3 in an interprofessional context in near future by inviting medical students to take part in both, e-learning and tutorials.

We like to thank Lahn@LMU for the financial support of the project.

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**PST.07**

Addressing targets relevant in neurodegenerative disorders: synthesis and biological activity of multifunctional heterobivalent carboline derivatives

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In an ageing society, therapy of neurodegenerative disorders such as Alzheimer’s disease or Parkinson’s disease is becoming increasingly important. Due to the complex nature of these diseases, therapeutics that combine several pharmacophores into one molecule and act as multitarget drugs may improve the effectiveness of therapy. Recently, it was reported that compounds consisting in a tetrahydro-gamma-carboline (2,3,4,5-tetrahydro-1H-pyrido[4,3-b]indole) scaffold are potent inhibitors of NMDAR in cell-based assays with IC50 values similar to the marketed drug memantine, yet they similarly inhibit both cholinesterases in the nanomolar range [2]. In the present study we produced novel heterobivalent compounds based on a lead structure presented by Malhaveta et al. [1] and performed structure-activity relationship to evaluate whether the unique combination
of a carbolinium scaffold with phenothiazine produces improved NMDAR-inhibitory activity while retaining strong selectivity for inhibition of BCHE. We found that, in contrast to homobivalent compounds, N-methylation of the pyridine to obtain permanently charged carbolinium moieties was dispensable in heterobivalent carbolinium-phenothiazine compounds to act as NMDAR blockers. The new compounds retained their high selectivity as cholinesterase inhibitors, as they blocked BCHE with IC50 in the low nanomolar range while they were inactive at AChE. Selected compounds were furthermore characterized in vitro with regard to their physicochemical properties and potential mutagenic potentials.

References:

PST.08

“Gender Medicine” in the 18th century?! Pharmacotherapy in the hessian hospital Merxhausen.

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Since the founding of the „Hohen Hospitäler“ in the middle of the 16th century, a few charity institutions for the rural population in Hesse existed. The hospital in Merxhausen was a sanctuary for old, infirm or disabled women as well as for those with mental-health diseases. Once a week, a surgeon visited the hospital to take care of the women in cases of acute sickness or injury. Basically, the surgeon prepared the medicine himself and was requested to issue a calculation of the expended drugs at the end of the year.

We analyzed these medical calculations from the 18th century as an origin for practical pharmacotherapy of the past. Therefore, these hand-written manuscripts including various alchemical signs had to be transcribed; we identified the drugs, arranged them accordingly to the dosage form and described the commonness.

We exemplary present the analyze based on the year 1755. The surgeon treated 523 patients during that year and included 660 drugs in his calculation. It’s promising to compare the outcome with the analyze for another hessian hospital in Haina, which only treated men.1 In fact, there are differences in the most commonly used dosage forms and in the medical range. In summary we considered some indications for gender specific pharmacotherapy in the past.

PST.09

Platelets induce a chemoresistance of tumor cells by upregulating drug efflux transporters

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Background: Lately, the crosstalk between tumour cells and blood platelets has been the objective of multiple investigations in oncological research. It was shown that direct contact with certain types of tumour cells activates platelets leading to a release of soluble factors, which in turn can bind to surface receptors on the cancer cell. While most of the work focused on the metastatic behaviour of these circulating cancer cells, the impact of platelets on the mechanisms of chemoresistance remains unclear. An increased resistance to chemotherapeutic drugs is often associated with the upregulation of ATP-binding cassette transporters (ABC-transporters) and the concomitant drug efflux. ABCB1, ABCG1 and ABCG2 are particularly well studied because of their responsibility for multi-drug-resistance in cancer therapy. Nevertheless, a functional axis of platelet activation and transporter upregulation of tumour cells has not been described before.

Aim: The project aims on elucidating the influence of platelets on the chemoresistance of different tumor cells in vitro and the underlying molecular mechanisms, e.g. a probable involvement of ABC-transporters. The elucidation of a platelet / tumour cell resistance activation axis should provide novel targets for a pharmacological sensitization strategy.

Methods: AsPC1 human pancreatic cancer cells and MDA-MB231 human breast cancer cells were co-incubated with human platelets. The survival in presence of the chemotherapeutic drugs doxorubicin and mitoxantrone was detected by MTT assays, ABC-transporter activities were followed by the conduction of fluorescence assays using transporter substrates and inhibitors. The expression of ABCB1, ABCG1 and ABCG2 was visualized via Western blot. In order to verify the role of EGFR / MAPK signalling for cell activation and resistance, the cancer cells were co-incubated with inhibitors of key molecules of the pathway in addition to the treatment with platelets.

Results: MDA-MB231 and AsPC1 tumour cells displayed a higher resistance against doxorubicin and mitoxantrone induced cytotoxicity when cells have been treated with platelets. The chemoresistance could be associated with the involvement of the transporters ABCB1 and ABCG2, confirmed by higher activity of these transporters in the fluorescence transporter assays. Western blot data confirm the connection between platelets and transporters by showing increased expression of aforementioned transporter proteins. When treated with an inhibitor of CREB – a transcription factor and important signal transductor in both EGF- and P13K-signalling – the effects of the platelet / tumour cell interaction is partly reversed referring to the critical role of the EGF-pathway in these terms.

Conclusion: It can be assumed that platelets influence circulating cancer cells, beside the known effects on attenuating the attacks of the immune cells, also with respect to induce a higher resistance against a chemotherapeutic treatment, which is closely related to the clinical prognosis. Therefore, the interference with platelet / tumour cell communication and especially the EGF-pathway appears as a promising therapeutic perspective for sensitization strategies.

PST.10

Glycolytic Flux and p53 status influence Growth Inhibition in response to the G6PD Inhibitor DHEA in Colorectal Cancer Cells

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Pyruvate kinase and glucose-6-phosphate dehydrogenase contribute to cancer aberrant metabolism. The low pyruvate kinase activity of PKM2 leads to accumulation of upstream glycolytic metabolites to be used in different anabolic pathways supporting the cancerous elevated proliferation rate. In this study, we investigated the effects of DHEA, a G6PD inhibitor, on colon cancer (CRC) cells with different p53 activity (p53 status). DHEA triggered increased G6PD expression in a ROS dependent mechanism. DHEA also reduced PKM2 activity in the presence and absence of wild-type p53 causing a "Warburg-like" phenotype that restored glucose dependency and enhanced lactate production. PKM2 activation synergizes with DHEA treatment further
Comprehensive understanding of the complex interplay between antibiotic, patient and pathway and applying this by optimising dosing is crucial to prevent further emergence and spread of antimicrobial resistance. Escherichia coli (E. coli) is an important cause of life-threatening diseases, such as sepsis and pneumonia, and fluoroquinolones are critically important antibiotics with a challenging resistance situation [1]. The aim of these investigations was to elucidate bacterial adaptation and resistance mechanisms and pharmacokinetic/pharmacodynamic (PK/PD) relationships exposing bacteria to human target-site concentration-time profiles (C(t)) profiles in a dynamic in vitro infection model (di/VIM) [2]. In addition to sequencing of fluoroquinolone resistance determining regions (QRDR) of the bacterial genome, PK/PD modelling of time-kill curve data was applied to unveil phenotypic adaptation.

Methods:
Two fluoroquinolone resistant E. coli isolates were investigated. Firstly, mutations in the QRDR of gyrA and parC and the presence of the plasmids qnrA, qnrB and qnrS were assessed by polymerase chain reaction (PCR) and electrophoresis. PCR products of gyrA and parC were analysed by Sanger sequencing. Secondly, the isolates were exposed to C(t) profiles of a 750 mg, 90 min i.v. levofloxacin (LEV) infusion in septic patients (n=4 per isolate), which were mimicked in a di/VIM based on a previously published population PK model [3]. Bacterial and LEV concentrations over time were quantified using a plate counting assay and a fluorescence assay, respectively. PK/PD analysis was performed for replicates with clinically relevant LEV exposure, which was determined over time as cumulative area under the LEV C(t) profile (cumAUC(t)). The PD effect was assessed as the cumulative area between the growth control (GC) and the bacterial killing and regrowth curve over time (cumABC(t)) [4] and normalised to the growth of unexposed bacteria, determined as cumulative area under the GC curve. Nonlinear regression was performed in R Studio™ to estimate in a sigmoidal maximum effect (Emax) model the cumulative exposure causing 50% of Emax (cumAUC50) and the steepness of the exposure-effect curves (Hill factor: 0.994).

Results:
Both investigated isolates harboured the gyrA mutation S83L, but no mutations in the QRDR of parC were present. Qnr plasmids were only detectable in strain 2. In the di/VIM, initial reduction of bacterial load and regrowth within 24 h was observed for both isolates. The extent of reduction and regrowth was different for the two strains, with a ≤ 2 log10-fold reduction for strain 1 and a reduction to the lower limit of quantification of the applied assay for strain 2. While strain 1 showed more pronounced regrowth up to the GC level, bacterial concentration of strain 2 did not reach the inoculum concentration again within 24 h. Using cumulative areas as dynamic exposure and effect metrics enabled describing the PK/PD relationship with a sigmoidal Emax model. The exposure-effect curve was steeper for strain 2 (Hill factor: 0.994) compared to strain 1 (Hill factor: 0.886). The higher cumAUC50 value for strain 1 compared to strain 2 (326 mg-h/L, and 39.6 mg-h/L, respectively) was in accordance with the observed growth and kill behaviour and the minimum inhibitory concentrations (MIC), indicating a higher susceptibility of strain 2 (MIC: 2 µg/mL) compared to strain 1 (MIC: 8 µg/mL). For strain 1, cumAUC50 was not reached with the investigated dosing regimen.

Conclusion:
Detected target-site mutations alone were not capable to fully explain the observed differences in the time-dependent growth-kill behaviour of the investigated clinical isolates. PK/PD modelling strongly indicated additional adaptation and LEV resistance mechanisms underlying the different PD parameter values of the strains. The mimicked LEV dosing regimen was not capable to eradicate bacteria in vitro, but might have triggered expression of adaptation mechanisms, such as SOS response. Further investigations will focus on phenotypic adaptation, applying nonlinear mixed-effects PK/PD modelling.

References:
Computational treatment simulations to assess the risk for non-efficacy in tamoxifen treatment for breast cancer patients of different ethnicities

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Background: The antihormonal drug tamoxifen (TAM) is used worldwide for the treatment of breast cancer (BC). TAM is extensively metabolised to its main active metabolite endoxifen (ENDX) via two metabolic pathways, both including the cytochrome P450 (CYP) isoenzyme 2D6. CYP2D6 is a highly polymorphic enzyme and isoforms with reduced or impaired function have been shown to result in ENDX minimum concentrations at steady-state (C_{min,ss} ENDX) below the proposed therapeutic threshold of 5.97 ng/mL. As the frequency of CYP2D6 functional variants and thus the extent of ENDX formation differs between ethnicities, this study, by applying pharmacometric simulation, aimed to assess which populations might be at highest risk for subtherapeutic C_{min,ss} ENDX under TAM standard dosing and therefore benefit the most from therapeutic target concentration interventions.

Methods: CYP2D6 diploidy frequencies in 8 ethnic populations (Africans, African-Americans, Americans (Latin-Americans and Canadians), Middle-Easterns, Caucasians (Europeans and North Americans), Oceanians, Central Asians and East Asians) were extracted from tables of the Clinical Pharmacogenetics Implementation consortium (CPIC). Reported CYP2D6 diploidy frequencies were transformed into CYP2D6 activity scores (AS), which were translated into genotype-predicted phenotypes (gPM; AS=0: poor metaboliser (gPM), AS=0-0.5: intermediate metaboliser (gIM) and AS≥1.5: normal metaboliser (gNM)) according to the most recent CPIC guideline. As previously proposed, the CYP2D6 wildtype (WT; AS=2) was assigned to patients for who no allele-defining sequence variations could be identified. Based on the extracted ethnic-specific AS frequencies, 8 large virtual populations were generated. Using an earlier developed nonlinear mixed effects parent-metabolite PK model of TAM and ENDX, each population was simulated to receive 20 mg TAM once daily (q.d.) for reaching steady-state, the respective number of patients not reaching therapeutic target concentration interventions. Results for all ethnicities were compared and ranked according to their respective risk for therapeutic target-non attainment. Simulations were performed in NONMEM (v. 7.4).

Results: Even though gNM was found to be the most prominent genotype-predicted phenotype in all ethnicities, the frequency of gNM differed up to 2-fold between ethnicities (highest in Oceanians (67.1%) and lowest in Africans (43.3%)). While highest proportions of gIM were observed in Africans (37.1%) and African-Americans (36.5%), Caucasians and African-Americans showed the highest proportion of gPM (4.83% and 2.16%, respectively). These findings had direct implications on the proportion of patients at risk for subtherapeutic C_{min,ss} ENDX under TAM standard dosing and therefore benefit the most from therapeutic target concentration interventions.

Conclusions: By collecting rich CYP2D6 genotype information and subsequently applying this information in treatment simulations for 8 different ethnicities, we determined Africans, African-Americans and Caucasians as ethnicities with the highest number of patients at risk for TAM treatment failure. Within these populations, more than 1 out of 5 patients will not reach the therapeutic ENDX threshold under TAM standard dosing. Thus, we strongly recommend CYP2D6-guided and model supported patient-tailored dosing to ensure treatment success. Efforts to increase population-specific genotyping data along with ethnicity-specific imputing of missing CYP2D6 diploidy information could further improve the predictive power of treatment simulations.

References:

Triple-color reporter system to follow up directed differentiation of iPSC towards the three germ layers

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Ectopic expression of transcription factors, such as OCT4, SOX2, KLF4 and MYC converts terminally differentiated somatic cells into induced pluripotent stem cells (iPSCs), which are comparable to embryonic stem cells in terms of self-renewal and differentiation potential to all three germ layers. iPSC technology provides a promising approach for tissue regeneration, autologous transplantation and disease modelling in the field of regenerative medicine. The clinical application of iPSCs is currently limited by the lack of sufficient differentiation protocols leading to the desired functional cells. Directed differentiation of human iPSCs is a field widely studied and developed in recent years, which, however, is often a complicated, time-consuming, and cell-line dependent process. To better understand cellular events occurring during the differentiation, we generated a reporter hiPSC line, which contains a triple-color reporter system, namely GFP, mCherry and mRFP regulated by endoderm-related genes (Sox17, Gata4, and, HNF4), mesoderm-related genes (brachyury, Hand1, and Eomes) and ectoderm-related genes Pax6 and Sox1, respectively. Applying this system, we successfully recorded landscapes of cellular differentiation stimulated by distinct differentiation protocols. Recently, we performed cell-based high throughput screening and identified a number of small molecules able to modulate pluripotency associated transcription factors. Our next aim is to optimize current differentiation protocols by screening our chemical library for the generation of fully functional differentiated cells.

Acknowledgments: Thank others for any contributions. Funding: This work supported by the DFG grant program (CH 1690/2-1) and the BMBF grant programs Drop-iPS (FKZ 0315398A-FKZ 0315398B) and SynTurChip (FKZ 0314304A-FKZ 0314304B).

References:

Ketamine promotes early changes in dendritic morphology in the hippocampus of a genetic rat model displaying depressive-like behaviour

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Background: The antipsychotic drug ketamine is used worldwide for the treatment of depression. However, ketamine-induced behavioral changes might be accompanied by early changes in dendritic morphology in the hippocampus of a genetic rat model displaying depressive-like behaviour.
Psychiatric disorders constitute a major burden for society in terms of productivity and years lost to disability. Recently it was shown that ketamine (KET), a non-competitive NMDA receptor antagonist, induces a rapid and sustained antidepressant effect in treatment-resistant patients. However, the mechanism by which KET ameliorates depressive symptoms is still unclear.

Here we used the Flinders Sensitive Line (FSL) rat, and its control strain the Flinders Resistant Line (FRL) rat to investigate morphological and molecular changes in the hippocampus that may be involved in the rapid antidepressant-like effect of KET.

Methods

To validate the antidepressant-like effects of KET at 1 h post injection, we exposed the rats to the forced swim test. For morphological analysis, one hemisphere per animal was processed for the Golgi-cox staining. Molecular studies were performed on the controlateral hemisphere (2).

Results

We found that FSL rats exhibited higher immobility times (p<0.001) while KET treatment reduced immobility times (p<0.0001). Moreover, the swimming behaviour was lower in FSL rats compared to FRL (p<0.01) and it was higher in FSL rats treated with KET compared to FSL vehicle (p<0.05). These data demonstrate an antidepressant-like effect of KET only 1 h after injection.

Regarding the morphological study, we found a significant increase in the number and density of spines in the apical dendrites (p<0.01) in FSL rats treated with KET. We also found an overall decrease in the basal dendritic length in the FSL rats (p<0.05) and an effect of KET treatment on spine number in FSL rats treated with KET (p<0.05).

At synaptic level, KET decreased the phosphorylation of cofilin and the NMDAR2A subunit level while it increased the HOMER 3 level.

Conclusion

These data suggest that morphological and synaptic reorganization of both apical and basal dendrites may be involved in the fast antidepressant-like effect of KET (2).

References


PST.16

Sex dimorphism in rat platelet aggregation

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Platelets are key players in maintaining the homeostasis controlling the blood loss after vessel injury. However, a not-controlled platelet activation can lead to the formation of pathologic blood clots connected to several diseases (i.e. myocardial infarction or stroke). Clinical observation show a sex-biased prevalence and severity of vascular thrombosis and/or mortality, with men having a worse prognosis. Nonetheless, in the last decades there are conflicting reports on the effects of sex on platelet aggregation, prompting for a deeper investigation on the mechanisms behind. The purinergic system is involved in platelet aggregation, in fact, endothelial cell ecto-nucleoside triphosphate diphosphohydrolase 1, E-NTPDase1 (CD39) promotes vascular homeostasis, but how this is modulated by sex and sex hormones is still not elucidated. Here we show that the basal expression of CD39 in rat platelets is higher in female rats than in males. Notably, adenosine-diphosphate (ADP, 1 µM)-induced aggregation was stronger in platelets from female rats as compared with females. Important to note is that the efficiency of the CD39 inhibitor ARL was stronger in platelets derived from females than males.

Another metabolic pathway with pivotal roles in platelet activation is the arachidonic acid (AA) cascade mediated by the activity of 12-lipoxygenase (12-LOX) and cyclooxygenase (COX)-1. While a more pronounced expression of 12-LOX was found in platelets from male rats than in females, no sex differences were evident in the expression of COX-1. Of interest, when platelet aggregation was induced by the addition of AA (20 µM), platelets from female animals aggregated more efficiently than those from males.

Together, here we show for the first time a sex-difference in the basal expression of key enzymes in the regulation of platelet activation in rats together with a sex-biased aggregation dependent on the stimulus utilized.

Further experiments will be carried out to understand the molecular mechanisms underlying these sex differences.

Acknowledgment

Funding was provided for G.T. by a NARSAD Young Investigator Grant from the Brain & Behavior Research Foundation and a postdoctoral research grant from the Danish Council for Independent Research (DFF-7003-00103)

PST.17

Thiocyanates as a new class of selective Sirt1 inhibitors

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Sirtuins are class III histone deacetylases (HDAc) that, unlike other HDAcs, are not Zinc-dependent but use NAD+ as a cofactor to cleave off various different acyl groups from the ε-amino residues of lysines. Through their great range of substrates they influence various cellular processes like metabolism, stress response, DNA repair, cell survival or apoptosis. Therefore sirtuins are associated with the pathogenesis of various diseases, like cancer, metabolic or neurodegenerative diseases. Of the seven human sirtuin isoforms Sirt1 is the most extensively studied one. It is linked to aging in general and specifically to age related diseases, like for example Alzheimer’s or Huntington’s Disease (HD). The first Sirt1 inhibitor to pass phase I and II clinical studies, Selisistat is currently examined in a phase III trial for treatment of HD. More modulators of sirt1 are needed to exploit and further characterize its therapeutic use.

We tested a small library of commercially available compounds proposed by docking studies against Sirt1, 2 and 3. OSSK 221646 was found to selectively inhibit Sirt1 with an IC50 of 13.0±0.6 µM. Analouges showed that the thioacrylate structure of the compound was key to the selectivity and high affinity towards Sirt1. To enable cellular studies new analogues of the thioacrylates with higher solubility were identified through docking studies and tested. Compounds that showed micromolar IC50 values in vitro were further studied in cells. Therefore levels of hYH2AX, which are lower in Sirt1 KO cells than in WT, were examined. We obtained similar protein levels of hYH2AX for Sirt1 KO cells as well as cells treated with Selisistat or the thioacrylates. These results show that thioacrylates are a promising new class of selective Sirt1 inhibitors.

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References


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Small molecules as MKK4 inhibitors for the regeneration of hepatocytes

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About 1 million deaths per year are affiliated with chronic or acute liver failure, having viral infections (hepatitis B/C), non-alcoholic fatty liver disease (NAFLD) or metabolic syndrome as main causes. As no medical treatment for liver failure is available, the finding of a molecular target is pursued. Using RNA experiments, the mitogen activated protein kinase kinase 4 (MKK4) was found by Wuestefeld et al. to play a key role in liver regeneration. Silencing MKK4 expression by shRNA resulted in an increase in robustness and regenerative potential of the liver via amplified hepatocyte proliferation. MKK4 silencing is suspected to lead to a higher activation of mitogen activated protein kinase 7 (MKK7) and thus to a higher phosphorylation and activation of the downstream c-Jun-N-terminal protein kinase (JNK1) [1,2].

For the finding of a small molecule inhibitor of MKK4 a virtual screening with MKK4 crystal structure (PDB: 3ALO) using Glide and SurflexDock methods on the Taito: HP Apollo 6000 XL230aSL230s Supercluster (taito.csc.fi) Finland was pursued. Docking was carried out using an incremental approach. In the first screening, fast approximated scoring/pose scanning was used and later with more robust/time consuming settings. The combined docking lead to 180 compounds, which were purchased for in vitro testing with DiscoveryX KINOMEScan assay. A POC (percent of control) of 5 @ 10µM was determined for MKK4 and a good selectivity profile for the compound was determined, which lacked hydrolytic stability due to an imino group. For the chemically stable assay, a POC of 23 @ 10µM was determined for MKK4. Further scoring/pose scanning was used and later with more robust/time incremental approach. In the first screening, fast approximated scoring/pose scanning was used and later with more robust/time consuming settings. The combined docking lead to 180 compounds, which were purchased for in vitro testing with DiscoveryX KINOMEScan assay. A POC (percent of control) of 5 @ 10µM was determined for MKK4 and a good selectivity profile for the compound was determined, which lacked hydrolytic stability due to an imino group. For the chemically stable assay, a POC of 23 @ 10µM was determined for MKK4. Further derivatisation and optimization resulted in a compound with a POC of 5 @ 10µM and 51 @ 1µM.

For the replacement of the imino group to a hydrostably configured E configured C-C double bond, HWE-reactions were carried out for the first compounds. In the synthesis of the further derivatives, HWE-reaction was replaced by Heck-reaction.


The final compounds were evaluated for their inhibitory activity on GSK-3β in an ADP-Glo kinase assay. The biological data of this compound series revealed a crucial role of the nitro group and highlighted its importance for the compound activity. While the original piperidine moiety proved optimal in terms of the ring size of the aliphatic ring, a successful rigidization approach resulted in an increased compound activity in the low triple-digit nanomolar range. The most potent compounds were further characterized regarding their metabolic stability in human liver microsomes and inhibitory potency on the likely off-target JAK3. Furthermore, we conducted 1 µs molecular dynamics simulations to examine the putative binding modes of these compounds within the ATP binding site of the kinase.

Reference:

Bioisosteres of the Natural Product Taxifolin and their Impact on Amyloid-β 42 Aggregation and Intracellular Oxidative Stress

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Several polyphenolic compounds have shown neuroprotective properties and anti-aggregative effects towards fibrilization of amyloid-β 42 (Aβ42), a key protein in the pathogenesis of Alzheimer disease. [1] Especially the flavonol (+)-taxifolin is well known for its inhibitory effect on Aβ aggregation.[2] The chemical structure shows a catechol moiety on the B-ring, three hydroxy groups at position 2, 5 and 7 and a ketone at position 3. (Fig. 1)

The structure-activity relationship studies could show the importance of the hydroxy groups of the catechol unit for the inhibition of Aβ42 fibril formation.[3] Also computational studies suggest that the catechol plays a crucial role for aggregation inhibition of β-amidloid aggregation via oxidative formation of an o-quinone. Ginex et al. postulated the aza-Michael addition as binding mechanism of taxifolin to β-amidloid fibrils.[4]
Based on the structure of (+)-taxifolin and supported by computational studies we designed six new bioisosteric compounds with an azobenzene scaffold carrying the catechol moiety. (Fig. 1) All compounds show high aggregation inhibition in transmission electron microscopy (TEM) experiments. Additionally, the compounds show protection against glutamate-induced intracellular oxidative stress in HT22 mouse hippocampal neuronal cells at low concentrations. Taken together, we synthesized six bioisosteric compounds which have shown higher activity than the parent compound (+)-taxifolin for inhibition of Aβ42 fibril formation and exhibit neuroprotection on HT22 cells.

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