Annual Meeting of the German Pharmaceutical Society – DPhG

Novel Therapies for Future Challenges

Saarbrücken, Germany
September 26 – 29, 2017
at Saarland University
Conference Book

Novel Therapies for
Future Challenges

Annual Meeting of the German
Pharmaceutical Society 2017 - DPhG
Annual Meeting of the German Pharmaceutical Society – DPhG

Conference Book

Novel Therapies for Future Challenges

Saarbrücken, Germany
September 26 – 29, 2017
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www.2017.dphg.de
Institutional Sponsors

DPhG
Deutsche Pharmazeutische Gesellschaft e.V.

The Pharmaceutical Society of Japan

UNIVERSITÄT DES SAARLANDES

Förderer der DPhG-Jahrestagung 2017

FCI
FONDS DER CHEMISCHEN INDUSTRIE

Dr. August und Dr. Anni
LESMÜLLER STIFTUNG

ACS Publications
Most Trusted. Most Cited. Most Read.
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CONFERENCE COMMITTEES

Scientific committee:
- Prof. Dr. Christian Ducho (Head)
- Prof. Dr. Thomas Efferth
- Prof. Dr. Dagmar Fischer
- Prof. Dr. Christoph Friedrich
- Prof. Dr. Peter Gmeiner
- Prof. Dr. Ulrike Holzgrabe
- Prof. Dr. Ulrich Jaehde
- Prof. Dr. Jochen Klein
- Prof. Dr. Charlotte Kloft
- Prof. Dr. Michael Lämmerhofer
- Prof. Dr. Peter Langguth
- Prof. Dr. Stefan Laufer
- Prof. Dr. Thorsten Lehr
- Prof. Dr. Andreas Link
- Prof. Dr. Irmgard Merfort
- Prof. Dr. Klaus Mohr
- Dr. Olaf Queckenberg
- Prof. Dr. Christoph Ritter
- Prof. Dr. Peter Ruth
- Prof. Dr. Achim Schmidtko
- Prof. Dr. Andrea Sinz
- Prof. Dr. Dieter Steinhilber
- Prof. Dr. Angelika Vollmar
- Prof. Dr. Hermann Wätzig
- Prof. Dr. Werner Weitschies
- Prof. Dr. Gerhard Winter

Organization committee:
- Prof. Dr. Thorsten Lehr (Chair)
- Dr. Stefan Boettcher
- Dr. Britta Diesel
- Prof. Dr. Christian Ducho
- Dr. Martin Empting
- Dr. Matthias Engel
- Dr. Martin Frotscher
- Dr. Gregor Fuhrmann
- Prof. Dr. Rolf W. Hartmann
- Prof. Dr. Anna Hirsch
- Prof. Dr. Claus Jacob
- Dr. Sonja Kessler
- Prof. Dr. Alexandra Kiemer
- Dr. Jesko Köhnke
- Prof. Dr. Claus-Michael Lehr
- Prof. Dr. Andriy Luzhetskyy
- Prof. Dr. Markus Meyer
- Prof. Dr. Rolf Müller
- Prof. Dr. Dr. h.c. Hans H. Maurer
- Prof. Dr. Marc Schneider
- Dr. Alexander Titz
WELCOME ADDRESS

Dear colleagues and friends,

The President of the German Pharmaceutical Society (DPhG), Prof. Dr. Stefan Laufer, and the Congress Chairman of this year's annual DPhG meeting, Prof. Dr. Thorsten Lehr, very cordially welcome you to Saarbrücken and the Saarland University.

The focus of this year's talks and discussions will be on novel therapies to meet future challenges. Many exciting concepts and discoveries are on their way to become next generation pharmaceuticals and we are looking forward to focused as well as interdisciplinary sessions with high level speakers and stimulating intellectual exchange.

We are also highly pleased about the high number of poster presentations by young researchers and very curious to learn about their work. This abstract book contains information on the programme as well as the abstracts to the plenary lectures, scientific lectures, poster short talks and posters, as a reference and orientation guide.

Many thanks to the scientific chairs, the organizing committee, the local helpers and to you, the participants, for your contributions.

We are glad to have you as our guests in one of the smallest federal states of Germany and we are looking forward to three days dedicated to scientific input, new ideas and time to talk. On the social side this meeting of course will serve to make new acquaintances and plans for future cooperation, to eat, drink, have fun and discover the beauty and hospitality of Saarbrücken and the Saarland.

We hope you will very much enjoy our meeting,

Prof. Dr. Stefan Laufer (DPhG-President)

Prof. Dr. Thorsten Lehr (Congress Chairman)
GENERAL INFORMATION

The Annual DPhG Meeting 2017 takes place at Saarland University, Campus E2.2 and E2.5.

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

Wednesday, September 27th, 2017: 10:00 – 18:00
Thursday, September 28th, 2017: 8:00 – 17:00
Friday, September 29th, 2017: 8:00 – 12:00

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”, poster short talks by “PST” 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 171 for direct access to specific abstracts.

POSTER SESSIONS

Topics:
Session I: Antiinfectives, Biotechnology and Biopharmaceutics, Cancer, Cardiovascular and metabolic diseases, Inflammation, Medicinal chemistry and drug desing.
Session II: Analytics, Natural Compounds, Other topics, Pharmaceutical technology and biomaterials, Clinical Pharmacy, Pharmacology, 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>Wednesday, September 27th, 2017, 14:30 – 15:00 and 19:00 – 20:30</td>
<td>Thursday, September 28th, 2017, 10:00 – 10:30 and 15:45 – 16:15 and Friday, September 29th, 2017 09:45 – 10:30</td>
</tr>
<tr>
<td>Set-up</td>
<td>Wednesday, September 27th, 2017, before 13:00</td>
<td>Thursday, September 28th, 2017, before 9:00</td>
</tr>
<tr>
<td>Dismantling</td>
<td>Wednesday, September 27th, 2017, after 20:30</td>
<td>Friday, September 29th, 2017, after 11:00</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 “Ratskeller”, Rathausplatz 1, 66111 Saarbrücken.

BADGES

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

Saarland University
The Congress will take place at Saarland University, Campus E2.2 and E2.5, 66123 Saarbrücken.

Travelling to the university by train and public transport:
High-speed ICE/TGV services are available from Frankfurt/Mannheim and Paris to Saarbrücken central station. Regional services run from Mainz, Trier and Strasbourg.

To get from Saarbrücken central station to the university campus (30 minutes), take either bus number 102 (to ‘Dudweiler-Dudoplatz’) or bus numbers 112 or 124 (to ‘Universität’). These services run every 30 minutes. There are several bus stops on campus. Bus stop ‘Universität Mensa’ is the closest bus stop for the annual meeting.
**Arriving by car:**

**From Mannheim/Karlsruhe**

Take the A6 motorway as far as the exit 'St. Ingbert West'. After leaving the motorway, follow signs to the university ('Universität').

**From Koblenz/Trier**

Take the A1 motorway as far as the Saarbrücken junction ('Autobahnkreuz Saarbrücken') where you join the A8 towards Karlsruhe. Continue on to the Friedrichsthal interchange ('Autobahndreieck Friedrichsthal') and then switch to the A623 motorway towards Saarbrücken/France ('Saarbrücken/Frankreich'). Leave the A623 at the Sulzbach exit. After leaving the motorway, join the L126 and continue on through Sulzbach. Remain on the L126 for about four kilometres, after which you will be able to follow signs to the university ('Universität').

**From France**

If driving from Paris/Metz or from Strasbourg, follow the A4 motorway as far as junction 40, where you switch to the A320/E50 towards Saarbrücken (Note: at the French/German border, the A320/E50 becomes the A6/E50). Continue on the A6 towards Mannheim as far as the exit 'St. Ingbert West'. From there you can follow signs to the university ('Universität').

**From Luxembourg**

Take the A620 motorway towards Saarbrücken. At the Saarbrücken interchange ('Autobahndreieck Saarbrücken') change onto the A6 motorway towards Mannheim. Leave the A6 at the 'St. Ingbert West' exit and from there follow signs to the university ('Universität'). While this is not the most direct route to the university, it avoids the relatively complicated route through the centre of the city.
Ratskeller (Conference Dinner):
Rathausplatz 1, 66111 Saarbrücken

Arriving from Central Station:
The bus numbers 102, 112 and 124 leading to Hauptbahnhof.
Take the Northern exit towards Kaiserstraße and keep left. After 600 m you have to turn right towards Kaltenbachstraße. After 50 m you will find Ratskeller on your right hand side.

Ratskeller

(how to get to the “Ratskeller”)
### Pre-Meeting Program

**Dienstag, 26.9.2017**

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<thead>
<tr>
<th>Zeit</th>
<th>Veranstaltung</th>
</tr>
</thead>
<tbody>
<tr>
<td>14.00-14.15</td>
<td><strong>Begrüßung</strong>, Prof. Dr. Christoph Friedrich, Marburg</td>
</tr>
<tr>
<td>14.15-15.00</td>
<td><strong>Zur Entwicklung des Hochschulfaches Pharmazie an der Universität des Saarlandes</strong>, Prof. Dr. Alexandra Kiemer, Saarbrücken / Prof. Dr. Dr. h. c. Hans H. Maurer, Homburg/ Saar</td>
</tr>
<tr>
<td>15.00-15.45</td>
<td><strong>Zur Entwicklung des Apothekenwesens in Saarbrücken</strong>, Dr. Bernhard Müller, Waldfischbach-Burgalben und Saarbrücken</td>
</tr>
<tr>
<td>15.45-16.15</td>
<td><strong>Kaffeepause</strong></td>
</tr>
<tr>
<td>16.15-17.00</td>
<td><strong>Zur Geschichte der Pharmazeutischen Industrie in Saarbrücken</strong>, Dr. Stefanie Boman-Degen, Osnabrück</td>
</tr>
<tr>
<td>17.00-18.00</td>
<td><em>’Nun lassen sie doch ihre Spielereien mit den dünnen Schichten, Herr Stahl!’ – Saarbrücken und die Chromatographie</em>, Dr. Karl Conrath, Saarbrücken</td>
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<tr>
<td>Time</td>
<td>Room</td>
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<tr>
<td>09.00 – 12.00</td>
<td>E2.5, lecture room III</td>
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<tr>
<td>13.00 - 13.45</td>
<td>E2.2, lecture room</td>
</tr>
<tr>
<td>13.45 - 14.30</td>
<td>E2.2, lecture room</td>
</tr>
<tr>
<td>14.30 - 15.00</td>
<td>E2.5, foyer</td>
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<tr>
<td>15.00 - 16.30</td>
<td>E2.2, lecture room</td>
</tr>
<tr>
<td></td>
<td>E2.5, lecture room I</td>
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<td></td>
<td>E2.5, lecture room II</td>
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<td>SL.01</td>
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<td>SL.02</td>
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<td>SL.11</td>
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<td>SL.12</td>
</tr>
<tr>
<td>16.45 - 17.30</td>
<td>E2.2, lecture room</td>
</tr>
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</table>
## Fachgruppen-Meetings

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<th>Location</th>
<th>Details</th>
</tr>
</thead>
<tbody>
<tr>
<td>17.30 – 18.45</td>
<td>E2.5, lecture room I</td>
<td>Fachgruppe Pharm./Med. Chemie, P. Gmeiner</td>
</tr>
<tr>
<td></td>
<td>E2.5, lecture room III</td>
<td>Fachgruppe Pharmakologie, J. Klein</td>
</tr>
<tr>
<td></td>
<td>E1.3, lecture room 001</td>
<td>Fachgruppe Klinische Pharmazie, C. Ritter</td>
</tr>
<tr>
<td></td>
<td>E1.3, lecture room 002</td>
<td>Fachgruppe Pharm. Biologie, A. Vollmar</td>
</tr>
<tr>
<td></td>
<td>E1.3, lecture room 003</td>
<td>Fachgruppe Pharm. Technologie, P. Langguth</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Time</th>
<th>Location</th>
<th>Details</th>
</tr>
</thead>
<tbody>
<tr>
<td>19.00 - 21.30</td>
<td>E2.5, foyer</td>
<td>Welcome reception and Poster session I</td>
</tr>
</tbody>
</table>

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### Parallel sessions II

<table>
<thead>
<tr>
<th>Time</th>
<th>Location</th>
<th>Details</th>
</tr>
</thead>
<tbody>
<tr>
<td>8.30 - 10.00</td>
<td>E2.2, lecture room</td>
<td>Antiviral therapy Chair: Christian Klein</td>
</tr>
<tr>
<td></td>
<td>E2.5, lecture room I</td>
<td>Tissue engineering Chair: Torsten Blunk</td>
</tr>
<tr>
<td></td>
<td>E2.5, lecture room II</td>
<td>Translational systems pharmacology in drug development and therapeutic use Chair: Charlotte Kloft, Meindert Danhof</td>
</tr>
<tr>
<td></td>
<td>E2.5, lecture room III</td>
<td>Arzneimittelkontrolle Fast in vitro/in vivo testing Chairs: Hermann Wätzig, Klaus Raith</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Time</th>
<th>Location</th>
<th>Details</th>
</tr>
</thead>
<tbody>
<tr>
<td>08.30</td>
<td>SL.13</td>
<td>Arnold Grünweller, A new antiviral approach: Specific inhibition of eIF4A-dependent viral mRNA translation by the natural compound Silvestrol</td>
</tr>
<tr>
<td>08.30</td>
<td>SL.16</td>
<td>Michael C. Hacker, Oligomer-cross-linked gelatinous peptides - a versatile hydrogel platform for tissue engineering and regenerative applications</td>
</tr>
<tr>
<td>08.30</td>
<td>SL.20</td>
<td>Andreas Lindauer, From Mouse to Man – Translational Modelling in Immuno-Oncology</td>
</tr>
<tr>
<td>08.30</td>
<td>SL.20</td>
<td>Dagmar Fischer, Crack the egg - Versatile applications of a shell-less hen’s egg model in pharmacy</td>
</tr>
<tr>
<td>Time</td>
<td>Session</td>
<td>Speaker(s)</td>
</tr>
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<td>----------------------------------------------------------------------------</td>
</tr>
<tr>
<td>09.00</td>
<td>SL.14</td>
<td>Stephan Ludwig, Maike Windbergs, Britta Göbel, Walter Mier, Katharina F. Wittmann, Stefanie Hennig</td>
</tr>
<tr>
<td>09.45</td>
<td>SL.19</td>
<td>Xinlai Cheng, Anya Kalayda,</td>
</tr>
<tr>
<td>10.00</td>
<td></td>
<td>Coffee break and Poster session II</td>
</tr>
<tr>
<td>10.30</td>
<td>E2.2, lecture room</td>
<td>Poster short talks (Plenary)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>10.30 Introduction to the session</td>
</tr>
<tr>
<td></td>
<td></td>
<td>10.34 Lukas Kröger PST.01</td>
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<tr>
<td></td>
<td></td>
<td>10.38 Iris Bischoff PST.02</td>
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<tr>
<td></td>
<td></td>
<td>10.42 Anke Heinrich PST.03</td>
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<tr>
<td></td>
<td></td>
<td>10.46 Marijana Jevtić PST.04</td>
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<td></td>
<td></td>
<td>10.50 Ana Sarcevic PST.05</td>
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<td></td>
<td></td>
<td>10.54 Stefanie Liening PST.06</td>
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<tr>
<td></td>
<td></td>
<td>10.58 Fabiana Troisi PST.07</td>
</tr>
<tr>
<td>11.45</td>
<td></td>
<td>Lunch break</td>
</tr>
<tr>
<td>Time</td>
<td>Room</td>
<td>Speaker/App.</td>
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</tr>
<tr>
<td>13.15</td>
<td>E2.2</td>
<td>Chris Schofield, P.03</td>
</tr>
<tr>
<td>14.15</td>
<td>E2.2</td>
<td>John Robinson, Novel Pierce</td>
</tr>
<tr>
<td>14.15</td>
<td>SL.24</td>
<td>Martin Biel</td>
</tr>
<tr>
<td>14.15</td>
<td>SL.25</td>
<td>Karel Talavera</td>
</tr>
<tr>
<td>14.40</td>
<td>SL.28</td>
<td>Armin Bauer, Lead Optimization of Griselimycins for the Treatment of Tuberculosis</td>
</tr>
<tr>
<td>14.45</td>
<td>SL.26</td>
<td>Anna Hirsch</td>
</tr>
<tr>
<td>14.50</td>
<td>SL.29</td>
<td>Anouar Belkacemi</td>
</tr>
<tr>
<td>15.05</td>
<td>SL.30</td>
<td>Marc Freichel, OCaR1</td>
</tr>
<tr>
<td>15.05</td>
<td>SL.31</td>
<td>Kristina Friedland</td>
</tr>
<tr>
<td>15.05</td>
<td>SL.32</td>
<td>Kristina Friedland</td>
</tr>
<tr>
<td>15.05</td>
<td>SL.33</td>
<td>Detlef Behrens</td>
</tr>
<tr>
<td>15.10</td>
<td>SL.34</td>
<td>Thorsten Lehr</td>
</tr>
<tr>
<td>15.30</td>
<td>SL.35</td>
<td>Joint discussion of the session topics</td>
</tr>
</tbody>
</table>

Coffee break and Poster session II
<table>
<thead>
<tr>
<th>Time</th>
<th>Session</th>
</tr>
</thead>
</table>
| 16.15 – 17.00| Molly Stevens, P.04
Bio-responsive hybrid materials for regenerative medicine and biosensing |
| 17.30 – 19.00| DPhG Hauptversammlung                                                  |
| 19.30        | Conference dinner (Ratskeller, Rathausplatz 1, 66111 Saarbrücken)      |

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### Friday, 29.9.2017

#### Parallel sessions IV

<table>
<thead>
<tr>
<th>Time</th>
<th>Session</th>
</tr>
</thead>
<tbody>
<tr>
<td>08.30</td>
<td><strong>Metabolic diseases</strong>&lt;br&gt;Chair: Oliver Werz, Eugen Proschak&lt;br&gt;Brown adipose tissue and energy homeostasis</td>
</tr>
<tr>
<td>08.30</td>
<td><strong>Nanomedicine</strong>&lt;br&gt;Chair: Claus-Michael Lehr, Marc Schneider&lt;br&gt;Bioinspired new materials - From geckos to robotics and biomedicine</td>
</tr>
<tr>
<td>08.30</td>
<td><strong>New research, new researchers I</strong>&lt;br&gt;Chair: Andreas Link&lt;br&gt;In-silico pharmacology: mechanistic models for the modulation of transmembrane proteins</td>
</tr>
<tr>
<td>09.00</td>
<td><strong>Eugen Proschak</strong>, Simultaneous treatment of hyperglycemia and hypertension in metabolic syndrome using a designed multitarget ligand</td>
</tr>
<tr>
<td>09.00</td>
<td><strong>Naoto Oku</strong>, Application of liposomal DDS for the treatment of ischemic stroke</td>
</tr>
<tr>
<td>09.45</td>
<td><strong>Simone Braig</strong>, Targeting the ER-Mitochondria Interface Sensitizes Leukemia Cells Towards Cytostatics</td>
</tr>
<tr>
<td>09.45</td>
<td><strong>Sonja M. Kessler</strong>, IMP2/IGF2BP2 expression predicts chemotherapy response in patient derived colorectal cancer xenograft models</td>
</tr>
<tr>
<td>09.45</td>
<td><strong>Anne Seidlitz</strong>, In vitro dexamethasone release from intravitreal poly(D,L-lactide-co-glycolide) model implants</td>
</tr>
<tr>
<td>09.45</td>
<td><strong>Steffen Lüdeke</strong>, The Role of Non-collagenous Protein Conformation in Biomineralization</td>
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<tr>
<td>Time</td>
<td>Location</td>
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<tr>
<td>09.30</td>
<td>SL.46</td>
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<td>09.45</td>
<td>SL.47</td>
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<td>9.45/10.00</td>
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<tr>
<td>10.15/10.30</td>
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<tr>
<td></td>
<td>E2.2, lecture room</td>
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| 13.00 – 13.45 | E2.2, lecture room | Meindert Danhof, P.05  
Future Medicines for one world: systems approaches to drug discovery, development and clinical usage |
| 13.45 – 14.30 | E2.2, lecture room | Hans Maurer, P.06  
Love potions and witch ointments in arts, literature, and opera |
| 14.30 – 15.15 | E2.2, lecture room | Award Session and Closing ceremony                                            |
| 15.30 – 17.30 | E8.1 (HIPS), Room 0.27 | Workshop "Modeling for Anyone" – BioSolve IT GmbH |
### Post-Meeting Program

**Saturday, 30.9.2017**

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| 15.00 – 16.15 | Interprofessionelle Kommunikation und Arzneimitteltherapiesicherheit – oder – Wie reden Apotheke und Ärzte bei schweren Arzneimittelinteraktionen miteinander?  
PD Dr. Guido Schmiemann  
Institut für Public Health und Pflegeforschung, Universität Bremen |
| 16.15 – 16.45 | Kaffeepause                                                                 |
| 16.45 – 18.00 | Medikationsplan – wo stehen wir?  
Dr. Hanna Seidling  
Klinische Pharmakologie & Pharmakoepidemiologie, Universitätsklinikum Heidelberg |
| 18.00 – 18.30 | Panel mit den Referenten und Publikumsbeteiligung  
18.30 Mitgliederversammlung der Fachgruppe Allgemeinpharmazie  
- Jahresbericht und Aktivitäten der Fachgruppe Allgemeinpharmazie  
  Herr Dr. Michael Hannig  
- Wahl der Fachgruppenvorsitzenden |

**Hinweis:**
Eine Anmeldung zum Tag der Offizinpharmazie ist nicht erforderlich.  
Die Teilnahme ist kostenlos.  
Die Veranstaltung wird mit 3 Punkten zertifiziert.  
www.dphg.de/apo17
1 PLENARY LECTURES
DISCOVERY AND EVALUATION OF BROMODOMAIN INHIBITORS

Georg, G. I.
College of Pharmacy and Institute for Therapeutics Discovery and Development, University of Minnesota, USA

Na,K-ATPases are transmembrane transporter enzymes that actively exchange Na+ and K+ across the plasma membrane. The α4 isoform is exclusively expressed in the sperm flagellum and is important for sperm motility and sperm hyperactivation. Both functions are essential for fertilization. Na,K-ATPase α4 knock-out animals have normal sperm counts and sperm development but their sperm have severely limited motility and are unable to fertilize an egg, as demonstrated in ex vivo studies. The natural product ouabain is a potent inhibitor of the Na,K-ATPase α4 isoform and also inhibits sperm total motility in vitro. Since ouabain is an inhibitor all four isoforms, we initiated research to discover compounds based on ouabain that are selective for the α4 isoform. Compound SS-I-54, a C17 N-benzyl-1,2,3-triazole analog was found to be a subnanomolar selective inhibitor of the α4 isoform, and an inhibitor of sperm motility in vitro and in vivo.

Bromodomains are essential protein recognition domains that bind to N-ε-acetylated lysine side chains of histones and recruit other transcription factors during posttranscriptional regulation processes. The BET (bromodomain and extra-terminal) proteins are a sub-family of bromodomain-containing proteins consisting of BRD2, 3, 4, and T. BRD4 inhibitors are currently in clinical trial for cancer treatment. BRDT, which is selectively expressed in the testis plays a crucial role in spermatogenesis. BRDT-1 knock-out mice are infertile and therefore it has been hypothesized that a BRDT-selective inhibitor could become a male contraceptive agent. Progress towards the discovery and structure-activity relationships of novel dihydropyrimidine BET inhibitors and the discovery of BRDT selective SG3-179-based bromodomain inhibitors will be discussed.
Peptides made by microbes and men

Helge B. Bode, Kenan A. J. Bozhüyük, Florian Fleischhacker, Annabell Linck, Edna Bode, Antje Heinrich, Merle Hirschmann, Peter Grün, Svenja Simony

1Merck Stiftungsprofessur für Molekulare Biotechnologie, Fachbereich Biowissenschaften and Buchmann Institute for Molecular LifeSciences (BMLS), Goethe Universität Frankfurt, 60438 Frankfurt am Main, Germany.

Microorganisms are well known for the production of biologically active natural products including clinically used antibiotics, anti-cancer or immune suppressive drugs. Although new natural products with promising activities can still be found using unusual microorganisms, the activation of previously silent biosynthesis gene clusters or other methods, there is still a great need for the rapid identification and bioactivity evaluation of further NPs.

In our group we work with the model bacteria Xenorhabdus and Photorhabdus that are able to infect and kill insects and produce a huge variety of natural products with different biological activities. We have recently identified a global regulator that allows us to shut-down all natural product biosynthesis pathways that can be combined with promoter exchange approaches of selected biosynthesis gene clusters to specifically produce only the selected natural product class and therefore allows the determination of its bioactivity directly from the crude extract.

Since it would be also desirable to increase the chemical diversity of natural products beyond the ones found in nature, we have additionally identified rules for the efficient modification of natural non-ribosomal peptide synthetases (NRPS) yielding non-natural derivatives of the original peptides. These rules can also be applied for the design and assembly of completely non-natural NRPS systems that result in the production of novel peptides (e.g. linear, cyclic, containing acyl moieties and/or L- or D-amino acids) in very good yields.
Roles of Oxygenases in Biosynthesis and the Regulation of Gene Expression

Schofield, C. J.
1Department of Chemistry, University of Oxford, Chemistry Research Laboratory, Mansfield Road, Oxford, OX1 3TA, UK

Oxygenases are involved in the biosynthesis of a range of natural products where they often catalyse synthetically impossible reactions such as occur in beta-lactam biosynthesis. Oxygenases also play important roles in the physiology of humans. In animals the response to limiting oxygen availability is mediated by the hypoxia inducible transcription factor (HIF). Both the levels and activity of HIF are regulated by its post-translational hydroxylation of conserved prolyl and asparaginyl residues. These modifications are catalysed by Fe(II) and 2-oxoglutarate dependent oxygenases which are also involved in nucleosome modifications. An introduction to the importance of the beta-lactam antibiotics will be given. The talk will then discuss the biochemical and structural features that enable 2OG oxygenase catalysis in a range of biosynthetic/biological contexts. The lecture will describe evidence that post-translational hydroxylations, including N-demethylation via hydroxylation, are much more widespread than was thought and are likely involved in the regulation of all steps in protein biosynthesis in aerobic organisms.

We thank the Wellcome Trust, BBSRC and European Union for funding our research.
Bio-responsive hybrid materials for regenerative medicine and biosensing

Prof Molly Stevens1
1Department of Materials and Department of Bioengineering, Imperial College London, SW7 2AZ, London, UK

Bio-responsive hybrid materials are of growing importance with potential applications including drug delivery, diagnostics and tissue engineering. A disagreeable side effect of longer life-spans is the failure of one part of the body – the knees, for example – before the body as a whole is ready to surrender. The search for replacement body parts has fuelled the highly interdisciplinary field of tissue engineering and regenerative medicine. This talk will describe our research on the design of new hybrid (nano)materials and nanomaterials to direct stem cell differentiation for regenerative medicine. We have also designed and developed porous silicon “nanoneedles” capable of efficiently, rapidly and safely delivering sensitive biocargoes to cells and tissues in vivo as well as interfacing with cells to inform intracellular pH and high resolution demarcation of tumorous region boundaries. This talk will also provide an overview of our recent developments in the design of materials for ultrasensitive biosensing. We are applying these nanomaterial-based approaches both in high throughput drug screening and to diagnose diseases ranging from cancer to global health applications.
FUTURE MEDICINES FOR ONE WORLD: SYSTEMS APPROACHES TO DRUG DISCOVERY, DEVELOPMENT AND CLINICAL USAGE*

M. Danhof1,2

1Leiden University, Leiden Academic Centre for Drug Research, Leiden, the Netherlands
2MDPharmacologyAdvice, Oegstgeest, the Netherlands

We are at a cross-roads in drug development. Progress in molecular biology leads to novel insights in the molecular mechanisms of disease. This opens unprecedented opportunities to develop drugs which modify the disease process rather than offering symptomatic relief. At the same time, access to medicines in many countries, especially for the poor and underprivileged, remains less than optimal. With drug treatment becoming increasingly complex and expensive, an important question is how we can ensure the access of safe and effective medicines for patients around the globe. In this presentation I will introduce the concept of systems pharmacology and address the question how it can be instrumental in the development and clinical application of novel “systems therapeutics” interventions [1,2].

Systems biology focuses on the structural and functional integration in biological systems to understand (variation in) function. A unique aspect is the multi-dimensionality of this endeavor. One of these dimensions is the analysis of biological phenomena as dynamic processes across different time scales, which is often referred to as horizontal integration. The other dimension is integration at different spatial scales (i.e. at the molecular, the cellular, the organ, and the organism level), which is referred to as the vertical integration. Systems biology applies multivariate statistical analysis to delineate the molecular pathways of the functioning of the human body in health and disease.

Systems biology constitutes a scientific basis for the development of “systems therapeutics” interventions for serious and chronic progressive disorders. Such therapeutic interventions differ in many ways from the traditional drugs. Systems therapeutics are often personalized treatments, both with regard to the selection of the drug(s) (to account for variation in the characteristics of the disease), and the dosing regimen (to account for variation in the pharmacology between patients). Moreover, these interventions are intended to be disease modifying rather than symptomatic. As a result the emphasis is on pre-emptive and preventive treatments. Finally, to overcome the plasticity of biological systems, the treatments are often complex, based on the use of multi-target drugs or rational drug combinations. Such interventions cannot be developed by trial and error.

Next to progress in systems biology, important progress has also been made in the field of mechanism-based pharmacokinetic-pharmacodynamics (PKPD) modeling. PKPD modeling aims at the characterization and prediction of the time course of drug effects in vivo. PKPD models contain mathematical expressions to characterize processes on the causal path between drug administration and response: a. the target site distribution, b. the target binding and activation, and c. the transduction and homeostatic feedback. Mechanism-based PKPD models have been found useful to describe several fundamental properties of the kinetics of drug action in vivo such as hysteresis and non-linearity. They are however unable to characterize a number of other properties, such as convergence, variability and resilience.

To overcome the limitations of classical PKPD modeling, novel systems pharmacology (SP) models consider biological networks rather than single transduction pathways as basis of drug action. Novel SP models contain expressions to characterize the functional interactions within a biological network. Such models constitute a scientific basis for the development of personalized, multi-target treatment modalities aimed at inhibition of disease progression or even cure from the disease [1].

References


* This title refers the 6th FIP Pharmaceutical Sciences World Congress which was held on May 24-27, 2017 in Stockholm
Love potions and witch ointments in arts, literature, and opera

Hans H. Maurer
Department of Experimental and Clinical Toxicology, Saarland University, Homburg (Saar), Germany

Introduction: Scopolamine and hyoscyamine (racemate called atropine) are the main alkaloids of most Solanaceae plants, which have been used within living memory as healthful, hallucinogenic, and/or eroticizing, but also deadly drugs. These effects have been described over the centuries in arts from ancient mosaics to Dali, in literature from Homer to Goethe, and in operas from Vivaldi to Martin.

Methods: Stories of dramas and music of operas were analyzed concerning action of drugs and poisons in the context of drug-facilitated sexual assaults, driving under the influence of drugs, crime scene reconstruction, and suicide assistance.

Results: In Antonio Vivaldi’s opera Orlando Furioso and Georg Friederich Handel’s Alcina, Alcina is attracted to the knight Ruggiero and she uses her magic potion to make him forget Bradamante and love her instead (similar to Siegfried in Richard Wagner’s opera Götterdämmerung, who forgot Brünnhilde and fell in love with Gutrune after consumption of her welcome potion). The imagination of flying is well described with the Hexenritt (Witch ride) in Engelbert Humperdinck’s opera Hänsel & Gretel as well in Johann Wolfgang von Goethe’s drama Faust and Charles Gounod’s opera Faust. Faust and Mephistopheles fly to the Harz Mountains to get the love potion to change his life without any fun. At the Walpurgis Night, he learned the three typical optical hallucinogenic effects of scopolamine and hyoscyamine, imagination of flying, transformation into animals, and eroticizing effects. In Ambroise Thomas’ Hamlet, Hamlet reconstructs the homicide of his father by his uncle who dropped poison into his ear. In Richard Wagner’s Tristan and Isolde, Isolde’s maid Brangäne should give Tristan and Isolde the deadly potion to atone for their fault, but she gave them the lower dosed love potion. Thus, both fell again in love although she was engaged to Tristan’s king. Finally, in Gaetano Donizetti’s L’elisir d’amore, the poor Nemorino loves the beautiful Adina, but she torments him with her indifference. When Adina is reading the book of Tristan and Isolde to her friends, Nemorino decides to buy a love potion from the travelling quack doctor Dulcamara. His name comes from the Solanaceae (Solanum Dulcamara) that contains no scopolamine and hyoscyamine...

Conclusion: The effect of poisons inspired artists, writers and composers over the centuries. With the view of a toxicologist, many effects can be related and allow funny conclusions. Some of them will be presented during the closing lecture.

References
Maurer HH (2013) Hexensalben und Liebestränke in Literatur und Oper. Pharmakon 1:450-455
2 SCIENTIFIC LECTURES
2.1 Therapeutic potential of nucleic acids
Chairs: C. Ducho, A. Kiemer

Synthetic aptamer-peptide conjugates for targeted activation of T cells

Silvana Hassel\textsuperscript{1} and Günter Mayer\textsuperscript{1}
\textsuperscript{1}Life and Medical Sciences Institute, University of Bonn and Center of Aptamer Research and Development, University of Bonn, Gerhard-Domagk Str. 1, 53121 Bonn, Germany

Immunotherapies are treatments that restore or boost the immune system’s ability to fight against infections, diseases or cancer. The development of protective long-term immunity requires activation of the effectors of the adaptive immune system, in particular T cells, by cells involved in innate immunity. Dendritic cells (DCs) represent the interface between the non-specific innate immunity and the highly specific adaptive immunity. Upon recognition of antigenic structures, DCs deliver all signals necessary for adequate activation of T cells. Hence, immunization with DC-based vaccines became of utmost importance in immunotherapy. One remarkable approach is to conjugate antigens to carrier molecules that specifically target DCs. Here, we discuss the potential applicability of aptamers as DC-targeting carrier molecules for the activation of T cells and cellular cytotoxicity.
Nucleic acid based therapeutics: synthetic delivery systems and in vivo bioimaging

Manfred Ogris1, Sebastian Gehrig1, Martina Anton2, Magdalena Billerhart1, Antonia Geyer1, Julia Maier1, Alexander Taschauer1, Haider Sami2

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2 Institute for Experimental Oncology and Therapy Research and Institute of Molecular Immunology, Klinikum rechts der Isar, Technische Universität München, Munich, Germany.

Nucleic acid based therapies offer the unique opportunity for the specific treatment of pathologic conditions whilst having the potential to avoid unwanted side effects. Nevertheless, their pharmacokinetic profile significantly differs when compared to low molecular weight drugs. Rather short circulation times in blood, enzymatic degradation and rapid clearance by the reticulo-endothelial system hinder accumulation of therapeutically active drug doses at the disease site. Hence, chemical stabilization and vectorization will improve these shortcomings and allow site-directed cellular uptake and –activation of nucleic acids.

We develop polycation-based delivery systems in combination with synthetic, cell targeting ligands, where nucleic acids are condensed by ionic interaction into nanosized particles. Biophysical and biological characterizations include receptor-ligand affinity measurements, size analysis by nanoparticle tracking analysis (NTA) and cell uptake. Pharmacokinetics and pharmacodynamics are studied in rodents with a focus on preclinical, disseminated cancer models. Tomographic, fluorescence based optical imaging allows biodistribution studies of nucleic acids labelled with near infrared fluorescent dyes, but also growth profiling of tumors genetically labelled with reporter proteins emitting light in the far red/near infrared. Bioluminescence of luciferase activity, either in transgenic animals with tissue restricted expression, in genetically labelled, implanted tumor cells or after transient gene delivery is monitored by diffuse light imaging tomography (DLIT). Optical imaging is fused with morphological data, including computed tomography (CT) [1,2] and magnetic resonance imaging (MRI), findings are corroborated by histological studies.

Figure 1: Fusion of optical tomography with morphological imaging (A) FLIT signal (red-yellow) of Alexa750-labelled siRNA polyplexes applied intratracheally fused with CT data (Geyer et al, manuscript in preparation); (B) DLIT signal of luciferase-expressing colon cancer (blue-red) fused with MRI data (Gehrig et al, manuscript in preparation).

Taken together, synthetic delivery systems for nucleic acids enable their targeted delivery. Tomographic approaches utilising multimodal molecular imaging gives detailed insights into the mechanisms of disease progression, drug distribution and therapy response.

Acknowledgments: Parts of this work have received support from the EU/EFPIA Innovative Medicines Initiative Joint Undertaking COMPACT grant n° 115363 and the uni:docs - fellowship programme for Doctoral candidates of the University of Vienna.

References:
Maintenance of tissue homeostasis by apoptotic cell death

Yoshinobu Nakanishi
Laboratory of Host Defense and Responses, Graduate School of Medical Sciences, Kanazawa University, Kanazawa, Ishikawa 920-1192, Japan

Cells that have become unwanted by the body emerge throughout our life. These cells include those that are obstacles to morphogenesis, disturb the establishment of tissue functions, have become spent and aged, have accomplished their task, or have become pathogenic. Such cells need to be selectively, rapidly, and safely removed. The removal of these cells is achieved by apoptosis-dependent phagocytosis: unwanted cells are induced to undergo apoptosis and given susceptibility to phagocytosis. Apoptotic cells are earmarked by substances, often called eat-me signals, which are bound by engulfment receptors of phagocytes. Phagocytic cells incorporate the target cells as phagosomes, which subsequently fuse with the lysosomes followed by digestion through the actions of lysosomal enzymes. This mechanism does not involve lymphocytes or antibodies, and, thus, is considered to be an innate immune response and conserved among multicellular organisms. Malfunctions in this process may lead to structural and functional defects in morphogenesis and tissue homeostasis. Therefore, molecules involved in this phenomenon may be targeted in medical treatments. The mechanisms underlying this phenomenon as well as its physiological and pathological consequences will be discussed.

Acknowledgments: This work has been supported by Kakenhi Grant form Japan Society for the Promotion of Science.

References:
A novel three-chain lysine-based cationic lipid – fast and efficient DNA transfer without co-lipids

Wölk, C.; Janich, C.; Dobner, B.; Bakowsky, U.; Langner, A.

1Institute of Pharmacy, Martin Luther University Halle-Wittenberg, Wolfgang-Langenbeck-Straße 4, 06120 Halle (Saale), Germany
2Institute of Pharmaceutical Technology and Biopharmacy, Philipps University Marburg, Robert-Koch-Straße 4, 35037 Marburg, Germany

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. Despite the enormous therapeutic potential of ATMPs only 15 licensed pharmaceutical preparations are registered by the Paul-Ehrlich-Institut (Announcement Nr. 433). Consequently, significant more research is needed.

In our research group we developed new lysine-based cationic lipids as non-viral transfection agents [1]. These lipids efficiently complex DNA and are suitable as nucleic acid carriers to get efficient DNA transfer into cells [2], a basic requirement to develop gene therapeutic medicines. The lipid DiTT4 (see Figure) belongs to this novel group of cationic lipids. The lipid has three primary amino functions and three alkyl chains. These structural characteristics cause a pH dependent changes in the molecular shape which affects the self-assembly behaviour: At acidic pH values the lipid forms rod-like micelles while at neutral and alkaline pH values liposomes and other bilayer structures are preferred (see Figure) [3]. This behaviour makes DiTT4 to an efficient DNA carrier system which achieves high gene transfer rates without neutral co-lipids. We can demonstrate efficient DNA transfer using a green fluorescent protein based in-vitro assay in different cell lines. Investigations with confocal fluorescence microscopy show very fast uptake rates of the DiTT4/pDNA lipoplexes. Furthermore, the DNA complexation was intensively characterized by agarose gel retention assay, zetapotential measurements, dynamic light scattering and ethidium bromide exclusion assay. As prior exams for planned in-vivo investigations the compatibility with biological media was examined. These investigations include haemolysis tests, effects on thrombocytes and interaction with serum proteins.

2.2 Natural compounds as tools and leads

Chairs: H. Groß

SL.05

Chemosystematics and chemical ecology: important tools for the rational search for bioactive natural products

Zidorn C¹

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Introduction – Chemosystematics is an interdisciplinary field of natural sciences investigating the distribution of natural products within all major groups of living organisms (archaea, bacteria, fungi, plants, and animals). Most forms of life share many primary metabolites and studies in chemosystematics aiming at the elucidation of relationships between taxa are therefore usually focused on secondary metabolites [1].

Chemical ecologists study the interplay of natural chemical compounds and ecological factors. Our particular field of interest is the influence of abiotic factors (e.g. season, radiation, temperature) on plant secondary metabolites. Besides being of interest to unravel ecological functions of secondary metabolites for the plants producing them, these studies provide important clues on how precisely to grow medicinal plants in order to get high yields of bioactive compounds. Such scientific data also help in optimising growing conditions for vegetables. Thus, optimal contents of desirable health beneficial secondary metabolites and low yields of secondary metabolites, which might adversely affect the taste of these vegetables, can be achieved.

Material and methods – Analytical HPLC-DAD and HPLC-MS as well as compound isolation using chromatographic techniques and subsequent structure elucidation by NMR and HR-MS are standardly used in both chemosystematics and chemical ecology.

Results and discussion – Chemosystematic combined with (macro-) molecular studies of the genus Leontodon yielded new insights into the delimitation of the genus Leontodon s.str. (i.e. excluding the genus Scorzonera but including the genus Hedypnois). Hydroxyhypocretenoids (hydroxyguaian-12,5-olides) were identified as reliable chemosystematic markers to phenetically define this group [2]. Subsequent studies proved that all investigated species assigned to this clade contained these highly bioactive sesquiterpenoids and that even the parentage of hybrid taxa could be proven based on the occurrence of these compounds [3].

Leontopodic acids, originally found in Edelweiss [Leontopodium alpinum (L.) Cass.], are excellent radical scavengers. Chemosystematic studies of the phylogenetically closely related genus Gnaphalium revealed new sources of these and related compounds [4].

In chemical ecology, altitudinal effects on secondary metabolites were investigated in Arnica montana and other taxa from the Asteraceae [5]. The ratio of ortho-dihydroxy- to other flavonoids, the total amount of caffeic acid derivatives, and the radical scavenging potential of extracts obtained from flowering heads increased with the altitude of the growing site. Initially, these results were interpreted as reactions of plants in higher altitudes to elevated UV-B radiation in these sites, but results from climate chamber experiments revealed that a decrease of the temperature caused this variation. Thus, altitudinal variation in plant phenolics is at least partially caused by lower temperatures in high altitude sites and not (exclusively) by enhanced UV-B radiation. In the course of these studies, the potent radical scavenger 1-methoxyoxaloyl-3,5-dicaffeoylquinic acid, a compound displaying a particularly pronounced altitudinal variation, was detected for the first time in flowering heads of Arnica montana.

Conclusions – Chemosystematics and chemical ecology are fascinating disciplines of basic science, but are also relevant in the search for new bioactives for medicine and agriculture. Both can give important clues for new sources of rare natural products as well as crucial information on how to grow and when to harvest medicinally used plants and vegetables containing health promoting secondary metabolites.

References:
Corallopyronin A: A Hit, a Lead – an Antibiotic?

Till F. Schäberle
Professorship for Natural Product Research with a Focus on Insect Biotechnology, Institute for Insect Biotechnology, University of Gießen

Novel drugs are needed to eliminate poverty related diseases like lymphatic filariasis and onchocerciasis. Filarial infections can be treated with antibiotics that deplete the essential Wolbachia endobacteria from their host, thereby blocking worm development and causing worm death. In a screening approach for natural products with antibiotic activity, we isolated the myxobacterial metabolite Corallopyronin A (CorA). This compound showed promising activities against Wolbachia endosymbionts [1], the mode of action against the bacterial DNA-dependent RNA polymerase (RNAP) is known and no cross-resistance occurs to RNAP inhibitors in clinical use, e.g. rifampicin.

The biosynthetic gene cluster coding for the enzymatic machinery responsible for CorA assembly was identified and analysed in detail [2-5]. In addition, in vivo activity was proven in a mouse infection model, thereby showing no toxic effects [1]. Hence, this antibiotic hit was followed up as a potential lead. The natural product depletes Wolbachia >10-fold better than the doxycycline benchmark. This allows shorter treatment times in animal filarial infections, a prerequisite to implement it as a macrofilaricidal therapeutic principle. Having demonstrated superiority of CorA over current macrofilaricides, the next steps include improvement of production and preclinical development. We have conducted non-GLP ADME and in vitro toxicity studies with technical grade CorA showing that CorA: has similar oral and intraperitoneal bioavailability, is non-toxic in vitro and in vivo, and does not inhibit host cell proliferation.

Recent plans and progress to forward CorA into phase 1 trials with the goal to develop a novel antibiotic will be presented.

Basidiomycetes, *i.e.*, mushroom-type “higher” fungi, represent a prolific source for bioactive natural products. Some compounds have served as leads for antibiotics or fungicidal crop plant protecting agents. Others are notorious toxins whose properties, however, may still be useful tools for research purposes. This presentation highlights current research into the biosynthesis of two classes of basidiomycete compounds:

- The stereaceous mushroom BY1 produces two antilarval polyenes (18-methyl-19-oxoicosaenoic acid and 20-methyl-21-oxodocosanonaenoic acid) whose biosynthesis is initiated following injury of the mycelium, *i.e.*, the fungus employs a *de novo* synthesis strategy.[1,2] A 2736 aa polyene synthase was identified and functionally characterized that catalyzes synchronous assembly of branched-chain polyenes of different lengths.
- The so-called “magic mushrooms” of the genus *Psilocybe* produce psilocybin, a tryptamine-like natural product that features a unique 4-phosphoryloxy group. Upon oral ingestion, it becomes rapidly dephosphorylated to yield psilocin that is known for its hallucinogenic properties. The genomes of *P. cyanescens* and *P. cubensis* were sequenced and four enzymes were characterized that, together, constitute the psilocybin biosynthesis pathway. In a combined three-enzyme reaction and beginning with 4-hydroxy-L-tryptophan, enzymatic step-economic *in vitro* psilocybin synthesis was demonstrated.[3]

Acknowledgments: Support by the Deutsche Forschungsgemeinschaft and the excellence graduate school Jena School for Microbial Communication (JSMC) is gratefully acknowledged.

References:
Synthesis of a Brasilicardin A analogue featuring a simplified core

Pierre Koch

Eberhard Karls Universität Tübingen, Institute of Pharmaceutical Sciences, Department of Pharmaceutical and Medicinal Chemistry, Auf der Morgenstelle 8, 72076 Tübingen, Germany

The natural product Brasilicardin A isolated from the cultured broth of the actinomycete Norcardia brasiliensis IFM0406 is consisting of a disaccharide moiety and an amino acid side chain linked by an anti/syn/anti perhydrophenanthrene skeleton (Fig 1.). [1] Brasilicardin A was found to exhibit immunosuppressive as well as cytotoxic activity. (Pre-)Clinical evaluation of Brasilicardin A, however, is hampered by a low-yielding fermentation process as well as by an unsolved total synthetic route. Even if latter one will be completed, it is very unlikely to be able to deliver clinically useful amounts of material.

The synthesis and biological evaluation of a Brasilicardin A analogue (Brasililogue) in which the highly complex natural tricyclic skeleton is replaced with a more synthetically accessible substituted tetralin core (Fig 1.) will be presented. [2,3] Although this Brasililogue did not display immunosuppressive activity, the protecting group as well as the synthetic strategies e.g. to build up the carbohydrate side chain [2] used in this study are currently applied in semi-synthesis of this natural product.

References
2.3 Innovations in dermal delivery

Chairs: D. Fischer, P. Langguth

Innovations in dermal and transdermal controlled drug delivery

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For several dermal and transdermal applications, controlled drug release is desired. Different approaches have been proposed to achieve this goal. Intensive research on prodrugs\textsuperscript{1,2} and lipid and polymeric nanoparticles\textsuperscript{3-5} is continued. Very interesting opportunities come from the coverage of the skin by drug loaded bacterial nanocellulose\textsuperscript{6} or electrospun nanofibers mats\textsuperscript{7,8}. For intradermal and transdermal delivery, microneedles\textsuperscript{9-12} compete with needle free injection systems which might also be used for more viscous systems\textsuperscript{13,14}.

The presentation will highlight some recent achievements. In addition, the challenges of controlled release with respect to the release mechanisms, the dimensions of the delivery system and the environment will be discussed.

Stable multiple nanoemulsion (w/o/w) for dermal drug delivery – an alternative to liposomes

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Multiple water-in-oil-in water nanoemulsions (w/o/w) are complex systems within reverse micelles in an oil droplet which is surrounded by an aqueous phase. The interest in the usage of these nanoemulsions as carrier system for topical application is given by the high encapsulation efficiency of hydrophilic as well of amphiphilic and poorly soluble active ingredients for cosmetic applications and in pharmacy. In most instances for the production of such multiple emulsions a hydrophilic and a lipophilic emulsifier are combined and the vesicles are manufactured by a twostep emulsifying procedure [1].

The new multiple nanoemulsion, “Hydro-Tops”, is based on one skin friendly emulsifier (Imwitor 375) coming from the food industry and allows an easy production by mixing all components and followed by high pressure homogenization. Hydro-Tops exhibit extremely homogeneous particle sizes in the area of 100-150 nm in comparison to customary flexible liposomes. Also higher concentrations of active ingredients up to highly molecular proteins could be embedded in these vesicles in contrast to liposomes [2].

Green tea is one of the most widely consumed beverages and has gained attention due to its relevant content of polyphenols like epigallocatechin gallate (EGCG). Dermal applications of EGCG showed pharmaceutical effects like antioxidative properties, prevention of UV-induced damages and anticarcinogenic effects as well as cosmetic ones like inhibition of acne and skin aging [3,4,5]. In comparison to liposomes, the possibility to use higher concentrations of lipids to produce this nanoemulsion leads to much higher loading of actives and also improves the penetration behavior and long lasting effects.

By the encapsulation of EGCG into the multiple nanoemulsion this polyphenol penetrates into deeper skin layers which results in an enhanced scavenging of free radicals (measured by electron spin resonance spectroscopy) [6]. Even Hydro-Tops EGCG provide epidermis and dermis with an antioxidative power after dermal application in different formulations: embedded in a lamellar cream (lipid membrane structures) or loaded in bacterial nanocellulose (BNC with a portion of 99% of water) [7]. These biological effects could not be achieved with liposomes for they remain in the lamellar cream as well as in the BNC and could not be used accordingly in these very interesting formulations for cosmetics and pharmacy.

Innovations in dermal delivery

Laser assisted dermal and transdermal delivery of macromolecules

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Our largest organ, the skin, is not designed to be a primary site of drug absorption, but to protect us by preventing foreign molecules to pass through, by stimulating immune response, and by regulating body temperature and water loss. Only very few molecules possess the properties for passive diffusion from transdermal patches across its main barrier, the stratum corneum. As a result, only every second year a new transdermal patch has reached the market in the last 3 decades on average [1].

Furthermore, the classical methods to manufacture transdermal patches, such as solvent casting and hot-melt extrusion, are by no means suitable for larger biomolecules, which are in focus right now. Exposure to high temperature and inevitable production losses prohibit using these processes for most macromolecules.

To enable the dermal and transdermal delivery of e.g. therapeutic peptides, allergens and vaccines, a combination approach has been established. The fragile macromolecules are printed onto specifically designed patches, which then are applied on painlessly laser-microporated skin as outlined in fig. 1 [2].

Fig. 1: A printed patch is applied onto laser-microporated skin (pore depths ~ 59 µm)

A model peptide (ca. 1 kDa) showed a cumulative ex-vivo human skin Franz-cell permeation of ca. 320 µg/cm² over a timeframe of 24 hours in this setup, 80-times exceeding its required daily dose achieved with a patch as small as 1 cm x 1 cm. For another much larger model macromolecule (250 kDa) still up to 30 µg/cm² within 24 hours were found. First in-vivo experiments with allergens printed onto the patches placed onto the micropores demonstrated that it was possible to successfully access and activate the immune system via the dendritic cells as well. This data hints to a promising approach to extend the reach of dermal and transdermal patches to completely new classes of drugs.

References
Development of novel Imiquimod based freeze dried Nanoemulsion ImiSOL for transcutaneous immunization

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According to WHO, modern vaccination systems should no longer depend on needles and syringes. The number of annual needle-related injuries to users and patients and the transmitted diseases is increasing continuously (1.3 Million deaths and US$ 535 Million estimated for follow up treatment of Hepatitis and HIV; numbers from WHO 1999). Therefore our work focuses on transcutaneous immunization via semi-solid formulations. Imiquimod is administered as an immune-stimulant drug in combination with an antigen. By activating DCs and APCs, long-term antibody formation can be achieved. The currently available Imiquimod formulation, Aldara®, served as a comparator drug product in this study. In Aldara®, Imiquimod is dissolved, while in the newly developed ImiSOL it is present as nanoparticles of approx. 600 nm in diameter(2).

This novel semi-solid Imiquimod nanoemulsion for transcutaneous immunization was optimized with respect to a favourable lipophilic phase. Here, we tested four ointment bases (MCT, avocado oil, jojoba wax and squalene) in a Franz cell absorption model to determine the effect of the lipophilic component on permeation through ablated mouse skin. While Aldara® showed rapid permeation, jojoba wax strongly decreased imiquimod permeation across the membrane but did not outperform Aldara® in terms of activation of the immune system. MCT and squalene showed intermediate permeation properties and were further investigated for their immunization potential. Here a significant dominance of squalene was obvious when compared with all other formulations. Since squalene is susceptible to oxidative processes, tocopherol has been added as a natural antioxidant to the formulation. Furthermore, a possible influence on the formation of immunoglobulins was investigated. This resulted in a clear effect on the IgG2 population whereas IgG1 and IgM levels remain unchanged.

Further in vivo studies were carried out by vehicle administration to the shaved backs of mice (6 cm²) for determination of remaining API within and on the skin surface. 50mg preparation of the comparator and ImiSOL were applied, respectively. In addition, an antigen-basis cream DAC mixture was followed in each case to carry out the immunization. The amounts of API in the skin (1 cm²) and on the skin after three hours by HPLC were determined. Both formulations performed equally. In addition to the already observed and described CD8+ T-cell activation, an immense CD4+ T-cell response was also detected with ImiSOL, but not with Aldara. Furthermore, the expression of CD80 and CD86 on dendritic cells in the skin draining lymph nodes significantly increased following treatment with ImiSOL compared to Aldara®. Similarly, IFNγ production by T cells was determined and described as strongly enhanced. Secondly, enhanced virus protection was observed after infection with LCMV after vaccination with Imiquimod formulation performed on mice. Results showed a reduction of the virus titer with Aldara® compared to placebo. ImiSOL treated mice showed hardly any virus load, thus demonstrating its long-term protection against infections in this virus model(1).

In conclusion, the nanoparticulate solid nanoemulsion containing imiquimod demonstrated improved transcutaneous immune modulating potential compared to a commercial formulation of the same API. A clinical study is currently under way to demonstrate proof of principle in a clinical setting.

Acknowledgments
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2.4 Antiviral Therapy

Chairs: C. Klein

A new antiviral approach: Specific inhibition of eIF4A-dependent viral mRNA translation by the natural compound Silvestrol

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The dramatic outbreak of the Ebolavirus (EBOV) in West Africa and the seemingly more frequent and increased occurrence of human pathogenic viruses, like the Zikavirus, strongly demonstrate the enormous medical need for the development of vaccines and antiviral drugs against such new and emerging viruses. Particularly important is the development of drugs with broad spectrum activities because viruses can change quickly by mutations and also unknown viruses have to be addressed. Ideally, an antiviral drug should be able to inhibit not only a single virus, but a whole virus family. This is not possible at the moment because most antiviral drugs are highly specific because they inhibit defined viral structures.

We have started to analyse the natural plant compound Silvestrol, a potent and selective inhibitor of the DEAD-box RNA helicase eIF4A, for its antiviral activity. EIF4A is a promising antitumor target because its helicase activity is required for 5´-cap-dependent translation initiation of several proto-oncogenes with structured 5´UTRs. Interestingly, such capped 5´UTRs with extensive RNA secondary structures can also be found in viral mRNAs.

We observed potent antiviral effects of Silvestrol in EBOV-infected primary human macrophages at low nM concentrations [1]. The expression of EBOV proteins was strongly inhibited, whereas the levels of cellular proteins were only slightly affected by Silvestrol. This is in line with the observed very low toxicity of Silvestrol in primary cells and in mouse models. Potent antiviral activity of Silvestrol was also seen in MRC-5 cells (human embryonic lung fibroblasts) infected with Coronavirus HCoV-229E or the highly pathogenic MERS-CoV. Moreover, Polioviruses which initiate their cap-independent translation by an eIF4A-dependent IRES (Internal Ribosome Entry Site) element are also sensitive towards Silvestrol treatment, demonstrating a pan-antiviral activity of Silvestrol.

A major drawback of antiviral drugs that target the virus itself is the rapid development of resistances. Since eIF4A is a host enzyme, it is unlikely that viruses can develop resistance by escape mutations, thus making this helicase an interesting new antiviral target. Nevertheless, due to its structural complexity, the chemical synthesis of Silvestrol is difficult. Therefore we screened chemical data bases to identify molecules with similar but less complex structural features and comparable properties as Silvestrol, with the goal to develop new medically relevant antiviral lead structures.

Repurposing of Kinase Inhibitors to Fight the Flu – MEK Inhibitors Efficiently Block Influenza Virus Replication in Mice with a Prolonged Therapeutic Window

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Influenza A virus (IAV) infection results in the activation of a variety of intracellular signaling responses. IAV exploit some of these activities to support efficient replication. This dependence of IAV on cellular signaling factors provides opportunities for a novel antiviral strategy that targets essential host factors instead of viral components.

We have identified the cellular mitogenic Raf/MEK/ERK kinase cascade to be suitable for antiviral intervention. We have employed several inhibitors, which block the pathway on the level of MEK and that are under advanced clinical evaluation or even licensed for clinical use for other diseases. We have analyzed their antiviral potential on a broad range of influenza viruses in vitro and in vivo, including studies on resistance development and therapeutic treatment window, and have unraveled their antiviral mode of action.

We could demonstrate that inhibition of this pathway efficiently blocked virus replication in cells and animals. MEK inhibitors are now under advanced clinical evaluation or even licensed for clinical use for other diseases. We show that these novel signaling blockers (a) efficiently inhibit influenza virus replication in vitro and in vivo, (b) are broadly active against all influenza A and B viruses analysed so far, (c) are active against oseltamivir resistant viruses, (d) are not toxic for cells or animals in the concentration and time line used, (e) display an enhanced therapeutic window compared to standard of care, and (e) confirm the postulated mode of action: blockade of the export of viral genomes from the nucleus.

Repurposing of clinically tested MEK inhibitors is a promising approach to develop safe and efficient anti-influenza viruses with a prolonged treatment window and a high barrier towards development of resistance.
Preclinical development of Myrcludex B, a novel entry inhibitor for the treatment of HDV/ HBV infections

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The currently approved therapies for chronic Hepatitis B either block reverse transcription of the HBV-pregenomic RNA in infected hepatocytes (reverse transcriptase inhibitors) or stimulate the immune system by IFNα/pegIFNα. Both strategies show limitations as they are non-curative and in the case of nucleoside analogs the application provoke the development of drug-resistant virus strains. We have previously shown that HBV large envelope protein-derived lipopeptides efficiently block HBV entry into hepatocytes in vitro and in vivo. As these peptides address a cellular substructure they show promise to inhibit the infection by a different pathway. Myrcludex B [1,2], the lead substance, is a first-in-class HBV entry inhibitor currently studied in clinical trials [3].

For pharmacokinetic studies Myrcludex B was radioactively labeled and the organ distribution in mice was investigated. The results obtained reveal a rapid and exclusive distribution of Myrcludex B and related preS lipopeptides to the liver. Mutational analyses showed that both, myristoylation and a conserved seven amino acid sequence motif are crucial for the exclusive liver accumulation. The process is highly specific and differs from constitutive hepatic delivery via the blood, since single amino acid exchanges within the conserved motif resulted in a total loss of the specificity.

In vivo studies in rats and dogs again demonstrated the exclusive liver targeting, obviously encompassing a species-independent determinant of hepatotropism. Surprisingly, cynomolgus monkeys did not show any preference of the radiolabelled peptide for the liver. It was uniformly distributed and rapidly renally excreted. In correlation, in vitro experiments confirmed highly specific binding of Myrcludex B to mouse, rat, dog and human hepatocytes whereas cynomologous and rhesus monkey hepatocytes did not show any specificity. As human hepatocytes show a strong binding of Myrcludex B and because of the coherence of in vivo and in vitro data we assume a rapid and exclusive accumulation of Myrcludex B to the human liver both after intravenous and subcutaneous injection.

Myrcludex B is a new antiviral drug that targets and inactivates the HBV-preS1-specific receptor. Its high potency to block a HBV infection combined with its excellent pharmacokinetic properties and its low toxicity makes it a promising therapeutic option for acute and chronic HBV and HDV infections.

2.5 Tissue engineering

Chairs: T. Blunk

Oligomer-cross-linked gelatinous peptides - a versatile hydrogel platform for tissue engineering and regenerative applications

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Hydrogels, especially injectable formulations, have become the number one material choice in attempts to engineer and/or regenerate soft and, more interestingly, even hard tissues. This is attributed to the extracellular matrix-like properties and excellent cytocompatibility that can be engineering into such materials. In more detail, the materials can be made specifically cell adhesive, degradable via different mechanisms and triggers, and their mechanical properties can be broadly adjusted. Such tailored gels thus provide an excellent niche for stem cell development and tissue regeneration. The materials can also specifically stabilize, sequester and/or release cytokines, growth factors and other bioactive proteins. These properties further make hydrogels candidate materials for drug and gene delivery applications for small and macromolecular API.

Towards an economic and easy to functionalize hydrogel platform, we developed anhydride-containing oligomers and formulated two-component hydrogels with different gelatinous peptides. [1, 2] The oligomers were synthesized by free radical co-polymerization of different acrylamide, acrylate and vinyl monomers combined with defined molar fractions of maleic anhydride. Key properties of the two component gels can be controlled by oligomer chemistry and content in the formulations. The cross-linking efficacy of glutaraldehyde can be matched by the anhydride-containing oligomers. Beside cytocompatible hydrogels, the oligomeric cross-linker could be used as a replacement for glutaraldehyde in protocols to fabricate cross-linked gelatine micro- and nanoparticles as well as microribbons.

The gels presented a suitable niche for melanocyte culture. [3]

The hydrogel platform was further processed into tubular conduits that have potential for peripheral nerve regeneration. In this context, we demonstrated straightforward bio-functionalization by derivatization of a predefined fractions of anhydride groups of the oligomer component by conversion with functional amines. Such a functionalization with small molecular amines had significant effects on the adhesion of proliferation of mesenchymal stem cells, which are relevant multipotent cells for regenerative applications.

The chemistry of the oligomers also allows for convenient modifications by variation of comonomers chemistry and ratio. We have synthesized anhydride-containing oligomers bearing acrylamides with chemically accessible methyl ketone groups. [4] Gel materials with these linkers showed excellent cytocompatibility and could be conjugated with hydrazide derivatives of bioactive molecules post fabrication.

Currently, the oligomer chemistry is systematically altered to identify injectable formulations of the two component materials. [5] First candidates allow for stem cell encapsulation, spreading and proliferation. The behaviour of the cells in the hydrogel indicates that the gelatine component provides adhesion sites for the cells and facilitates gel remodelling by encapsulated cells.

The presented hydrogel platform is versatile, economic and allows for convenient bioconjugation thus presenting an interesting material platform for API delivery and regenerative applications.

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References

In vitro skin models for testing pharmaceutics – overcoming the barriers

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In vitro models of the human skin provide a versatile approach for testing novel pharmaceutics. Depending on the focus of the study, the degree of model complexity varies from simple skin cell monolayers up to complex three dimensional tissue constructs composed of different skin cell types to simulate the native hierarchical structure of human skin. Such models can additionally be converted into pathophysiological conditions like e.g. inflammation or wounded state to mimic skin diseases and test relevant pharmaceutics for their pharmacological effect.

However, unfortunately for testing the extent and kinetics of skin absorption by pharmaceutics, tissue constructs do not provide meaningful results, as the inherent barrier function of human native skin can thus far not be adequately simulated. The main absorption barrier of human skin is located in the uppermost layer of the skin, the stratum corneum, in which the composition and organization of the endogenous lipids play an integral role.

Thus, for predictive and preclinically relevant skin absorption testing excised human skin samples from operations can be used as in vitro models. Here, absorption of drug solutions as well as the effect of formulations on skin absorption can be assessed.
Bioengineered tumor-stroma models to study the contextual control of tumorigenesis

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Tumor-stroma interactions drive malignant transformation and tumor progression. However, the mechanisms by which microenvironmental cues, i.e. composition, structure and mechanics, regulate these processes remain elusive owing in part to a lack of adequate model systems. By integrating bioengineering and oncology science tools, we can gain an improved understanding of the molecular, cellular, and tissue level dynamics underlying tumorigenesis.

This talk will focus on bioengineered model systems that allow independent control of compositional, structural and mechanical cues relevant to the tumor microenvironment and emphasize the functional role of stromal components, particularly the extracellular matrix (ECM), in driving malignant cell behaviors. It will specifically highlight pathological ECM remodeling in obesity by stromal cells and how it contributes to intratumor heterogeneity and increased breast cancer aggressiveness.

These multidisciplinary studies extend our current knowledge on the relationships between contextual ECM changes and tumor cell responses and may thus inform novel therapeutic strategies for breast cancer patients, especially for the obese cohort that is confronted with a high risk for relapse and metastatic disease. Further, they enable us to gain new insights into stroma-dependent aspects of breast tumorigenesis and increase awareness for the need to critically evaluate the indiscriminate use of stromal cells and biomaterials for regenerative applications after tumor resection.

The presented work was funded by a Feodor Lynen Postdoctoral Research Fellowship of the Alexander von Humboldt Foundation to Dr. Wittmann and grants from the National Institutes of Health/ National Cancer Institute to Prof. Dr. Claudia Fischbach-Teschl, Cornell University, Ithaca, NY, USA.
Conversion of patient-specific fibroblasts to neurons on-chips with single natural product derivative and application for neurotoxicity evaluation of drugs

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Microscale engineering technology can mimic organ microenvironments through integration of multi-cellular and functional devices and thereby offers a unique niche to study physiology and pathophysiology of human tissues. Recently, several models have been developed to investigate neuroregeneration and neurodegeneration towards personalized medicine, in which patient-specific iPSCs are exploited. However, this process including generation of patient-specific iPSCs and differentiation to neurons are very complicated and time-consuming. In an effort to establish a reliable and reproducible chemical reprogramming protocol, we performed high throughput screenings based on various luciferase reporter cell lines carrying respective promoters of aspired transcription factors across a chemical library containing 250,000 compounds and identified a number of small molecules, which can activate pluripotency-associated transcription factors involved in reprogramming and transdifferentiation. Surprisingly, we found one compound is sufficient to convert fibroblasts to Tuj1+/MAP2+ neuron-like cells with comparable efficiency to virus-mediated (5%). In combination with commercially available medium, we achieved direct conversion of patient fibroblasts into neurons with more than 80% efficiency. More intriguingly, Tuj1+ neuron-like cells can be observed within days and MAP2+/NeuN+/Tau+ within one week. In cooperation with ChipShop (Jena, Germany), we applied this cocktail to a microfluidic chip-based system and reproducibly achieved conversion of fibroblasts to Tuj1+/Chat+ neuron-like cells within days. Finally, we developed a microfluid-based model in the tandem of neuron-on-a-chip with liver-on-a-chip to study the patient-specific neurotoxicity of drug metabolites, which can be potentially applied for personalized disease model and neurotoxicity study of drugs and drug metabolites via high throughput screening.

This work supported by DFG grant program (CH 1690/2-1) and the BMBF grant programs Drug-iPS (FKZ 0315398B) and SysToxChip (FKZ 031A303E).
2.6 Translational systems pharmacology in drug development and therapeutic use
Chairs: C. Kloft, M. Danhof

From Mouse to Man – Translational Modelling in Immuno-Oncology

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In recent years immunotherapy in oncology (aka: immuno-oncology) has become a game-changer in the treatment of cancer. Several therapeutic strategies to stimulate the patients’ own immune system in order to attack and eradicate malignant cells are under active development with remarkable results in the clinic. These strategies include cancer vaccines, oncolytic viruses, transfer of ex-vivo activated T-cells as well as antibodies and therapeutic proteins that block the immune check-point pathway [1]. Ipilimumab was the first immuno-oncologic antibody reaching the market in 2011, targeting the anti-cytotoxic T lymphocyte-associated protein 4 (CTLA-4). More recently, in 2014, the anti-programmed death (PD)-1-receptor antagonist pembrolizumab was approved for the treatment of advanced melanoma with remarkable response rates in general, and even achieved complete disappearance of tumor lesions in a number of patients [2]. Clearly these drugs bear an enormous potential and new antibodies targeting different immune check-points (e.g. LAG3, TIM3) as well as combinations of immuno-therapeutics are being developed [1].

A quantitative understanding of the pharmacological mode of action as well as the pharmacokinetic behaviour of these antibodies is paramount to identify the most promising candidate compound or combination and to define the most appropriate dose for patients in clinical studies. It is desirable to have an understanding of the most likely efficacious dose level already early in the development process of monoclonal antibodies. At this stage the production of an antibody is usually done at a small scale and requires careful planning of resources. More importantly, however, Phase 1 studies in oncology are usually already conducted in patients and the dose given should have a reasonable chance of being efficacious.

Translational pharmacokinetic/pharmacodynamic (PKPD) models allow the prediction of a range of likely efficacious doses early in the development. Information on drug-specific aspects (e.g. receptor binding, tumor growth inhibition in mice) from in vitro and in vivo experiments can be combined with data from the literature on system-specific parameters (e.g. blood/lymph flow in mice and man, distribution volumes) to form a mathematical description of the pharmacological system based on the current state of knowledge. Once an adequate model is available to describe pre-clinical in vivo data, model parameters are translated from animal to human making assumptions on size-dependency of parameters and the degree of conservation of pharmacology in different species.

The author had the opportunity to work in a team developing a translational PKPD model for the anti-PD1 monoclonal antibody pembrolizumab which was published recently [3]. In the talk the translational modelling approach will be illustrated using pembrolizumab as an example. Albeit in this case some clinical data (PK) was already available at the time of modelling, efficacy studies in patients with metastatic melanoma were still ongoing and decisions around the minimally efficacious dose needed to be made before the clinical results became available. The predictions with the translational model filled the gap and supported the sparse clinical data available at the time of decision-making.

While the pembrolizumab example is a success story as the predicted efficacious dose range matched very well with actual clinical results in retrospect, there are certainly pitfalls and uncertainties in the translation of a complex biological system like cancer and the immune system [4]. The limitations of the approach will be discussed as well as strategies to handle uncertainty and assumptions inherent to the model. The shortcomings will be put into perspective with the great potential that translational modelling has to improve the development of new immuno-oncologic drugs.
Acknowledgments: My former colleagues at MSD who were involved in the development of the pembrolizumab model (C.R. Valiathan, K. Mehta, V. Sriram, R. de Greef, J. Ellassais-Schaap and D.P. de Alwis).

References:
**In-silico Clinical Trials of Prandial Insulins: The FDA Accepted Type-1 Diabetes Simulator**

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**Background:** Technosphere® insulin (TI), an inhaled human insulin with a fast onset of action, provides a novel option for the control of prandial glucose. We used the University of Virginia (UVA)/Padova simulator to explore in-silico the potential benefit of different dosing regimens on postprandial glucose (PPG) control to support the design of future clinical trials. Tested dosing regimens included at-meal or postmeal dosing, or dosing before and after a meal (split dosing).

**Methods:** Various dosing regimens of TI were compared among one another and to insulin lispro in 100 virtual type-1 patients. Individual doses were identified for each regimen following different titration rules. The resulting postprandial glucose profiles were analyzed to quantify efficacy and the risk for hypoglycemic events.

**Results:** This approach allowed us to assess the benefit/risk for each TI dosing regimen and to compare results with simulations of insulin lispro. We identified a new titration rule for TI that could significantly improve the efficacy of treatment with TI.

**Conclusion:** In-silico clinical trials comparing the treatment effect of different dosing regimens with TI and of insulin lispro suggest that postmeal dosing or split dosing of TI, in combination with an appropriate titration rule, can achieve a superior postprandial glucose control while providing a lower risk for hypoglycemic events than conventional treatment with subcutaneously administered rapid-acting insulin products.

Diabetes Technol Ther. 2016 Sep 1; 18(9): 574–585.
Systems pharmacology-based TDM: better informed clinical decision and reduced burden on patients

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Therapeutic Drug Monitoring (TDM) is a multi-disciplinary clinical specialty aimed at improving patient care by individually adjusting the dose of drugs for which clinical trials have shown it improved outcome in the general or special populations. It can be based on an a priori pharmacogenetic, demographic and clinical information, and/or on the a posteriori measurement of blood concentrations of drugs (pharmacokinetic monitoring) and/or biomarkers (pharmacodynamic monitoring). [1]

Systems pharmacology and model-based TDM personalises drug doses to optimise therapy by utilising population pharmacokinetic (PK) or pharmacodynamic (PD) models. [2] While some models are complex, others are simple and may not describing all aspects of the biological systems. As quoted by Box, “all models are wrong”, [3] however some may be useful, for example to inform personalised dosing.

Traditionally TDM approaches use therapeutic ranges, reference drug/biomarker measurements or compare them against nomograms to facilitate personalised dosing. Challenges appear when total drug exposure over a dosing interval or biomarker levels over time are associated with optimal treatment outcomes, as these are often impossible to identify with one measurement. Bayesian forecasting (BF) methods use a maximum a posteriori approach utilising population models as inbuilt a priori information and current concentration observations from a patient to estimate each individual’s pharmacokinetic parameters and drug exposure. [4] BF methods then modify the initial population-derived pharmacokinetic parameters using the patient’s dosing history, demographic data and measured drug concentration to estimate an individual’s AUC0-24 and adjust treatment, if required. Although, BF programs to estimate AUC0-24 have been available for over 30 years [4], we found that widespread adoption of these programs into the clinical setting has been poor [5], despite local and international guidelines recommending computerised AUC0-24 monitoring [6]. Several freeware, freely accessible from any internet connected computer with a web-browser, and commercial programs with 24-hour support using BF methods are available to support personalised dosing for a variety of drugs, nowadays. We could show that BF methods have the potential to improve patient care by minimising drug toxicity and maximising drug efficacy whilst saving both the hospital and the individual patient time and money by reducing the number of blood samples required and providing flexibility around sample times. [7] Evidence for improved clinical decisions making and reduced burden to the patients for several drug classes has been demonstrated recently compared to traditional methods, while maintaining precision and accuracy. [8, 9] However, the rigorous evaluation and validation of BF programs compared to true measured exposure in the literature has been limited, potentially limiting their implementation in regular clinical practice. Cost-benefits analysis for commercial products and user-friendliness may be crucial factors in the next steps of the decision-making process for hospitals to adapt this approach. Furthermore, the inclusion of systems-pharmacology and biology models, which include underlying disease models together with PK and PD models are very limited to non-existent.

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Key players of cisplatin sensitivity: towards a systems pharmacology approach

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The efficacy of cisplatin-based chemotherapy is limited by the development of tumor resistance to the treatment. Cisplatin resistance is multifactorial, with several mechanisms operating simultaneously in a single cell. Therefore, the necessity to study the reaction of the whole cell to a drug and to understand the interactions between different cellular components involved is being readily acknowledged. Systems pharmacology is an emerging field focusing on the analysis of a whole cell's reaction to a drug rather than on single compound-protein interactions1.

The aim of this work was to characterize cisplatin resistance in non-small cell lung cancer (NSCLC) integrating experimental and bioinformatic methodology as a basis for a systems pharmacology approach. For this purpose we used A549 non-small cell lung cancer cell line and its cisplatin resistant derivative A549rCDDP2000.

In A549/CDDP2000 cells, cisplatin failed to induce G2/M cell cycle arrest and apoptosis was significantly reduced as compared to A549 cells, although equitoxic cisplatin concentrations resulted in comparable platinum-DNA adduct levels. These differences were accompanied by changes in the expression of proteins involved in DNA-damage response. In A549 cells, cisplatin exposure led to a significantly higher expression of genes coding for proteins, which mediate G2/M arrest and apoptosis (MDM2, XPC, SIP, p21 and GADD45a), as compared to cisplatin-resistant cells. This was underlined by significantly higher protein levels of pATM and p53 in A549 cells after cisplatin treatment compared to the untreated cells.

Additionally, a data-driven method was used to identify further key players responsible for a different reaction of the two cell lines to the platinum drug. The cellular transcriptome was screened for relevant gene candidates using a whole genome array. By combining statistical methods with available gene annotation without previously defined hypothesis, HRas, JNK3, p38, CCL2 and DOK1 were identified as genes relevant for cisplatin resistance. These genes were further analysed on transcriptome and proteome level to introduce a more systematic approach on different layers of cell signalling. The results were compiled in a model of resistance-associated signalling alterations. In conclusion, our findings suggest that acquired cisplatin resistance of NSCLC cells is a consequence of altered signalling of the identified proteins leading to reduced G2/M cell cycle arrest and apoptosis.

2.7 New antibiotics

Chairs: R. W. Hartmann

Novel Peptidomimetic Antibiotics Targeting Essential Outer Membrane Proteins in Gram-Negative Bacteria

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Infectious diseases and the emergence of multi-drug resistant bacteria are one of the major contributors to human morbidity, and it has become a pressing issue to find new antibiotic classes, which can kill bacteria via novel mechanisms of action. Although synthetic med-chem approaches have allowed the discovery of some antibiotics (e.g. sulphonamides, quinolones and oxazolidinones), most classes of antibiotics have been isolated from natural sources. The cationic antimicrobial peptides (CAMPs) are naturally occurring molecules acting in the innate immune systems of many organisms. It has been shown recently that CAMPs can provide an important source of inspiration for the discovery of new antibiotics with novel mechanisms of action.

The β-hairpin is a recurring structural motive found in naturally occurring CAMPs, and which also often mediates many protein-protein and protein-nucleic acid interactions. The design of conformationally constrained protein epitope mimetics (PEMs) based on this structural motive is now recognized as a successful approach for antimicrobial discovery [1]. A new family of β-hairpin peptidomimetic antibiotics based on the antimicrobial peptide protegrin-I was discovered recently, and several rounds of optimization gave L27-11 as a novel potent pseudomonas-specific antibiotic. A clinical lead called murepavadin (POL7080) active in the nanomolar range against Gram-negative Pseudomonas spp. is now in clinical development. These antibiotics are largely inactive against other Gram-negative and Gram-positive bacteria. Studies on the mode of action of L27-11 showed that the peptidomimetic targets the essential β-barrel outer membrane protein LptD, which functions in the lipopolysaccharide (LPS) transport pathway during outer-membrane biogenesis [2]. Based on the same approach, another interesting peptidomimetic antibiotic was discovered called JB-95, with potent antimicrobial activity against Escherichia coli. Studies on its mode of action showed that JB-95 could selectively destabilize the OM but not the inner membrane of E. coli, likely through interaction with selected β-barrel OM proteins, including BamA and LptD [3].

These discoveries demonstrate the importance of essential OM proteins in Gram-negative bacteria as targets for novel antibiotics. The ability to target essential bacterial proteins, including the LPS transport (Lpt) machinery and BAM folding machinery, may provide valuable new weapons in the fight against drug resistant Gram-negative bacterial pathogens.

Lead Optimization of Griselimycins for the Treatment of Tuberculosis

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Novel anti-tuberculosis agents with mechanisms of action distinct from current TB drugs are urgently needed due to the increasing prevalence of drug-resistant (DR) TB. As a part of our TB drug discovery strategy, we revisited under-exploited antibiotics with high anti-TB potential and re-evaluated griselimycin (GM), a natural cyclic depsipeptide that was first isolated from two strains of Streptomyces identified in the 1960s. GM was found to have antibacterial activity specifically against organisms within the Corynebacterineae suborder, notably including Mycobacterium species. Because of earlier reports of the effectiveness of GM against drug-resistant \textit{M. tuberculosis}, we re-initiated studies on this natural product lead with the ultimate goal of introducing a highly active, stable, and safe derivative of this compound class into the TB drug development pipeline.

Griselimycins are structurally unrelated to any known TB drug. Using solid phase peptide synthesis, approximately 290 griselimycin derivatives were synthesized by modification of each amino acid of the peptidic sequence. We discovered that the modification of the 8-proline unit has a pronounced effect on the compound's potency and stability. As a result, Cyclohexylgriselimycin (CGM) was identified as an outstanding candidate for further \textit{in vivo} profiling. CGM is eighteen-fold more potent than griselimycin against \textit{M. tuberculosis} \textit{in vitro} and displays enhanced metabolic stability in human liver microsomes as well as a remarkable oral bioavailability (F=89% in CD1 mice compared to F=48% for griselimycin).

Through state-of-the-art optimization of a natural product, griselimycin, known for more than 50 years, we have identified CGM, an advanced lead compound with a potential in particular against drug-resistant \textit{M. tuberculosis} strains and for clinical dosing once daily by the oral route. CGM was profiled in-depth, revealing a novel mode of action and excellent anti-TB properties.

\begin{center}
\begin{tikzpicture}
\node (GM) at (0,0) {\includegraphics[width=0.5\textwidth]{gm.png}};
\node at (GM) [above] {\textbf{R = H} Griselimycin (GM)};
\node at (GM) [below] {\textbf{R = Me} Methylgriselimycin (MGM)};
\node at (GM) [right] {\textbf{R = Cyclohexyl} Cyclohexylgriselimycin (CGM)};
\end{tikzpicture}
\end{center}

References:
Identification of potent inhibitors of the anti-infective target DXS using ligand-based virtual screening

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The enzymes of the methylerythritol phosphate (MEP) pathway are important drug targets given that pathogens such as Mycobacterium tuberculosis and Plasmodium falciparum use this pathway for the biosynthesis of the essential isoprenoid precursors isopentenyl diphosphate (IPP) and dimethylallyl diphosphate (DMAPP), while humans exclusively utilise an alternative pathway.[1] The thiamine-diphosphate-dependent enzyme 1-deoxy-D-xylulose-5-phosphate synthase (DXS) catalyses the first and rate-limiting step of the MEP pathway. To expand the structural diversity and obtain potent and selective inhibitors of DXS, we performed a ligand-based virtual screening (LBVS) campaign based on shape similarity to screen the ZINC database, starting from previously discovered DXS inhibitors as references.[2,3] Biochemical evaluation of the top-scoring compounds against M. tuberculosis DXS and further rounds of LBVS using the best hits as references afforded inhibitors in the single-digit micromolar range. In addition to the promising biochemical activity, the hits are active in cell-based assays against P. falciparum and even drug-resistant strains of M. tuberculosis. Further assays demonstrated their selectivity over mammalian thiamine-diphosphate-dependent enzymes, their lack of cytotoxicity and validated DXS as the intracellular target.[4]

References:
Two-pore cation channels (TPCs) are members of the pore-loop cation channel family that are specifically expressed in the membrane of endolysosomal organelles. Over the last couple of years our laboratory has characterized the functional properties of the two mammalian TPCs (TPC1 and TPC2) using patch-clamp approaches in intact lysosomes [1, 2]. Moreover, using genetic mouse models we have examined the roles of TPCs in a variety of physiological and pathophysiological settings. In my lecture I will give an overview on our recent work on TPC2. TPC2 is broadly expressed in the body and plays a key role in cholesterol homeostasis [3], cancer cell migration [4] and pigmentation characteristics. Moreover, this channel is involved in the intracellular trafficking of several pathological viruses including Ebola [5]. These roles make TPC2 an attractive target for future drug development.

References:
Trafficking, localization and regulation by cholesterol of the chemosensory cation channel TRPA1

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The cation channel TRPA1 serves in mammalian nociceptors as transducer of a myriad of chemical stimuli into electrical excitation and neuropeptide release, leading to pain and neurogenic inflammation. Despite their structural diversity, most TRPA1 agonists share the property of interacting with the plasma membrane. However, it is currently unknown whether these channels localize in specialized membrane micro-domains and whether cholesterol interacts with specific sites on this channel. Using total internal reflection fluorescence (TIRF) microscopy and density gradient centrifugation of Triton-X insoluble fractions we found that TRPA1 localizes preferably into cholesterol-rich domains. In functional experiments we found that depletion of cholesterol and sphingolipids decreases the chemical sensitivity of TRPA1. Finally, through molecular dynamics simulations and structure-function studies we identified four domains in transmembrane segments 2 and 4 behaving as cholesterol recognition amino acid consensus (CRAC) motifs. Our data shows that TRPA1 localizes in lipid rafts, and indicates that cholesterol influences the channel’s functionality and its trafficking and membrane localization.
New function of an old protein: Cavβ3 and intracellular signaling

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As an auxiliary subunit of voltage-gated Ca\(^{2+}\) channels Cavβ3 plays an important role in shaping the channel's biophysical properties. Bound or unbound to the pore-forming α1 subunit of voltage-gated Ca\(^{2+}\) channels, Cavβ3 can also interact with additional proteins and even may function independently of its well-established role as subunit of voltage-gated Ca\(^{2+}\) channels [1-3], especially in cells lacking this channels, like fibroblasts, and maybe, dendritic cells. Recently we could show that Cavβ3 attenuates intracellular Ca\(^{2+}\) signalling with significant effects on regeneration of skin wounds and neuroinflammation/neurodegeneration. Accordingly, targeted therapies to mitigate or to augment Cavβ3 function might be beneficial in delayed skin wound healing and neuroinflammation/neurodegeneration.

References:
OCaR1, a new player in endo-lysosomal Ca\textsuperscript{2+} signaling 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 but not with markers of the ER, the Golgi apparatus or mitochondria in non-secretory cells. 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. In my lecture I will provide evidence demonstrating that OCaR1 in lysosomes and secretory granules operates as a gatekeeper of regulated exocytosis, and that this process is governed by control of lysosomal and granular Ca\textsuperscript{2+} release mediated via NAADP and two pore channels (TPCs).
2.9 Medication safety research
Chair: M. Hug, U. Jaehde

Implementation of the national standardized medication plan: The PRIMA project
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In Germany, a common data format and an infrastructure to exchange medication data between health care professionals (HCPs) electronically are not available in primary care. Accordingly, generating and exchanging electronic medication plans (MPs) by HCPs in this setting is not yet possible. In the project PRIMA (October 2014 – December 2016), an interdisciplinary medication management (MM) service [1] is offered by general practitioners (GPs) and community pharmacies (CPs). To exchange information on patients' medication and potential drug-related problems (DRPs), a standardized MP is generated in the local software systems of both HCPs, exchanged via a central server, and subsequently printed for the patient. We aimed to evaluate (1) patients' expectations of the MM and acceptance, usage in daily routine, and potential benefit of the MP, (2) to pilot potential processes for generating and exchanging medication data between local software applications of CPs and GPs, and (3) to evaluate the acceptance of the MM process and the MP by the participating HCPs.

(1) Eleven teams (each consisting of one GP and one CP) were involved and asked to recruit approx. 10 patients each. We developed a questionnaire to evaluate patients' views. Additionally, we conducted, recorded, and transcribed qualitative face-to-face patient interviews.

103 questionnaires were available for evaluation. Ten patient interviews were conducted (30±8 min). In the survey, patients specified as reasons to participate the medication review by CPs and GPs (58.3%), the HCPs overview of their medication (50.5%), and/or the complete and checked MP (39.8%). Experienced DRPs were of minor importance (1.9%). 69.9% of the patients stated to bring the MP along when visiting a specialist, 50.5% used it regularly when preparing their medication, and 26.2% used it sometimes as a reminder. Patients considered that their main benefit from the MM service resulted from the closer cooperation between GPs and CPs (83.5%). They felt more confident in handling their medication (68.9%), and indicated an increase in knowledge on dosage and indication (64.1 and 71.8%, respectively). Only 14% of the patients believed the MP itself significantly contributed to this. The interviews emphasized that patients recognize an increase in medication safety due to the closer cooperation as the major benefit. All patients stated that every patient taking several drugs regularly should get a MP.

(2,3) A questionnaire was developed to evaluate HCP's acceptance of the MM service including the MP. Additionally, HCPs joined a workshop in September 2016 to discuss their experiences regarding collaboration, communication, and benefits of the MM service for their patients. Of the 12 teams of CPs and GPs, one dropped out due to technical problems. The remaining 11 teams recruited 196 patients. In total, 35 HCPs participated in the workshop. HCPs named improvement of medication safety as the major motivation to participate (83%, n=18). 75% (n=8) of the CPs and 60% (n=10) of the GPs agreed with the previously specified processes and responsibilities in the MM service. HCPs estimated that the service improved the implementation of drug therapy (83 %, n=24) and appropriateness of medication (79%, n=28). Furthermore, HCPs expected a reduction in overall health-related costs (82%, n=28).

(1) The majority of patients strongly perceives a benefit from the MM service. Patients emphasize that the service with its close cooperation between physician and pharmacist rather than the MP itself is responsible for the potential benefit.

(2,3) The electronic MP as well as the MM service were successfully implemented and accepted by the HCPs. This is an important precondition for further implementation of both the MM service and the MP in routine primary care in Germany.

Acknowledgment: PRIMA was funded by the Federal Ministry of Health (BMG), Berlin, Germany.
Reference:1. ARMIN - Arzneimittelinitiative Sachsen-Thüringen: http://www.arzneimittelinitiative.de/
Implementation of the national standardized medication plan: The metropolmediplan2016 – mmp16

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Background: A statutory entitlement to a standardized medication plan was established in October 2016 in Germany for all patients, prescribed at least three drugs.

Purpose: The main aim of the MetropolMediplan2016 – MMP16 study is evaluating acceptance and practicability of the medication plan as well as its suitability to improve medication safety.

Methods: The MMP16 consortium consists of different experts. An interdisciplinary team including clinical pharmacists and clinical pharmacologists evaluated a random sample of 300 anonymized medication plans, generated under clinical routine conditions. The aim was to investigate if the medication plans were formally complete and suitable for an assessment of medication safety.

Results: Standardized medication plans listed a median of 8 (5 to 11) drugs and were updated a median 3 (1 to 5) times by physicians and pharmacists. Only 19 (6.3%) of the medication plans met all formal requirements. However, 233 (77.7%) of the medication plans classified as providing adequate and complete data for the assessment of medication safety. In 12 (4.0%) of the medication plans we identified at least one definite medication error. Besides, 6 (2.0%) cases of most likely contraindicated drug-disease combinations could be identified by deducing the most probable diagnoses from the individual medication pattern.

Conclusion: The standardized format facilitates the identification of medication risks. Irrespective of frequent deviations from formal specifications, the majority of medication plans was found suitable for the assessment of medication safety. An additional diagnosis improved detection of drug-related risks.

We thank the BMG for funding the MMP16 consortium.
Optimizing patient information leaflets to enhance medication safety

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Patient information leaflets (PILs) are one of the most prominent sources for patients’ drug information [1]. However, the current content and design of PILs holds numerous difficulties concerning its purpose to inform the patients and to enable the patients to participate in a rational, safe and adherent pharmacotherapy [1,2]. Small writings, multiple use of technical terms and an overload of information are the most frequently mentioned problems [1,2]. Even so the issues of PILs are well recognized, very rarely studies are performed to show the impact of optimized PILs on relevant endpoints like health literacy, adherence or medication safety. This talk will summarize the results of two recent studies [3,4] addressing some of the challenges of PILs.

In study 1, the text legibility of the PILs of the 30 most prescribed drugs was analysed using the Hohenheimer Index (HI) [3]. The HI score ranges from 0 (low comprehensibility) to 20 (high comprehensibility). A score of 12 indicates for example a technical text. The median HI was 9.1 (4.7 – 13.9) for the 30 PILs analysed. Median word count was 2598 (1522-4708) and 4.8% (2.7% – 9.5%) technical terms were used per PIL. Four PILs were optimized, such as, shortened sentences and fewer technical terms. In these optimized PILs the HI increased by 2.4 points on average (1.0 – 4.4). Two PILs were studied additionally in 105 subjects. Subjects understood the content of the optimized PILs better compared to the unchanged PILs.

In study 2, a one-page summary sheet (OPSS), summarizing the most relevant drug information in a clearly structured manner and in plain language on one page, was developed as an additional document to the PIL [4]. In a study with 155 participants the benefit of OPSS over PILs was investigated for two drugs. Over 90% of the participants preferred the OPSS over the PIL. Furthermore, participants using the OPSS made significantly less mistakes when they were asked five questions about the medication and were about 50% (3.6 minutes versus 6.5 minutes) faster in their processing time.

In summary, the studies have shown that PILs can be optimized and optimized PILs lead to a significantly better understanding in the study participants. OPSS is a promising tool to support common PILs. Establishing the OPSS in a regular use could enhance health literacy significantly and could lead to further positive effects like an improved medication adherence and medication safety.

References
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4. Wojtyniak, J.-G. et al: The benefits of a one-page summary sheet (OPSS) compared to the patient information leaflet (PIL) to enhance health literacy – a randomized crossover trial, DPhG Annual Meeting 2017
2.10 Metabolic diseases

Chairs: O. Werz, E. Proschak

Brown adipose tissue and energy homeostasis

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Obesity has reached pandemic dimensions and there is a high medical need for pharmacological treatments of this metabolic disease. Obesity is characterized by an imbalance of energy intake (nutrition) and energy expenditure (e.g. physical activity). The surplus in energy is mainly stored in white adipose tissue (WAT), which is the largest energy store in the human body. The increase in WAT mass in obesity is accompanied by inflammation and increased risk of cardiovascular disease, type 2 diabetes, metabolic syndrome and certain types of cancer. In addition to WAT one can distinguish also another type of fat: Brown adipose tissue (BAT).

In contrast to WAT, BAT dissipates energy in the form of heat and BAT is essential for non-shivering thermogenesis in babies (1). Importantly, several groups showed that human adults have metabolically active BAT and that BAT activity correlates with leanness (2). Moreover, brown-like adipocytes have been detected in WAT – especially subcutaneous WAT (3). These brown-in-white (brite) cells can be induced by a broad range of hormones, pharmacological substances and cold exposure (4). Brown and brite adipocytes have received a lot of attention as a potential target for anti-obesity therapies. Our work mainly focuses on signaling pathways that activate BAT and/or regulate brown adipocyte differentiation. Recently, we identified Gq-coupled signaling as an important inhibitor of BAT differentiation using pharmacological and genetic tools (5).

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References
Simultaneous treatment of hyperglycemia and hypertension in metabolic syndrome using a designed multitarget ligand

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The cardiometabolic syndrome (MetS) is a multifactorial disease cluster consisting of dyslipidemia, cardiovascular disease, type 2 diabetes mellitus and obesity. Pharmacological intervention in the MetS is dependent on numerous drugs, thus polypharmacy is an obvious problem in the treatment of MetS patients. This study focuses on the dual target approach to accomplish a more efficient therapy for MetS. The two targets addressed by dual ligand design are the soluble epoxide hydrolase (sEH) and the peroxisome proliferator-activated receptor type γ (PPARγ). In vivo studies could demonstrate that even though an inhibitor of sEH or PPARγ agonist have benefits when used individually, the combination is more beneficial for the multidisease features in cardiometabolic syndrome. Using a split-and-combine strategy we designed a library of dual sEH/PPARγ modulators and proved that both targets can be simultaneously addressed by a merged pharmacophore. In a follow-up study, we designed lead-like merged N-benzyl benzamides which were able to modulate sEH and PPARγ. Structure activity relationship studies on both targets were performed resulting in an equipotent submicromolar (IC50 (sEH) = 0.3 μM/ EC50 (PPARγ) = 0.3 μM) propionic acid N-benzyl benzamide derivative. Evaluation in vitro and in vivo displayed good ADME properties qualifying the novel dual modulator as pharmacological tool compound for long term animal models of MetS. 8-week evaluation in spontaneously hypertensive obese rats (SHROB), a rat model of MetS, demonstrated excellent efficacy including simultaneous reduction of blood pressure, improvement of glucose tolerance, and organ protection. These results could be confirmed in an 8-week curative study in ZSF1 rat model of MetS.

References:
Metabolic diseases

A dual modulator of farnesoid X receptor and soluble epoxide hydrolase to treat non-alcoholic steatohepatitis

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Non-alcoholic steatohepatitis (NASH) arising from western diet and lifestyle evolves as serious health burden with alarming incidence.[1] Characterized by accumulation of fat in liver subsequently causing inflammation and fibrosis it is strongly associated with the metabolic syndrome.[2] Although its high prevalence elicited intense research for novel treatment options there is still no satisfying therapy.[2] Several potential targets have been identified for NASH treatment and promising clinical data has been reported for the farnesoid X receptor (FXR) agonist obeticholic acid[3]. Moreover, inhibition of soluble epoxide hydrolase (sEH)[4,5] proved effective in treating NASH in vivo. Considering the multifactorial nature of NASH involving metabolic dysbalance and inflammatory processes, modulation of multiple targets might provide a superior therapeutic effect and combining FXR activation for anti-steatotic/fibrotic activity with anti-inflammatory inhibition of sEH promises synergistic activity.

The nuclear receptor FXR acts as cellular bile acid sensor and liver protector with various beneficial metabolic effects. Most importantly for NASH treatment, its activation reduces liver fat content and fibrosis.[6] sEH, an enzyme of the arachidonic acid cascade located in the CYP pathway, degrades anti-inflammatory epoxyeicosatrienoic acids (EETs) to dihydroxyeicosatrienoic acids (DHETs) and is highly expressed in liver. sEH inhibition hindering EET degradation has anti-inflammatory properties and was found effective in animal models of NASH.[5]

To exploit the concept of dual FXR/sEH modulation for NASH treatment we developed dual agents with FXR agonistic and sEH inhibitory potency. Initially, we merged known pharmacophores[7,8] for both targets to generate a lead compound exhibiting moderate FXR agonistic and sEH inhibitory potency. Systematic exploration of the structure-activity relationship (SAR) of the compound class on both targets allowed optimization of the potency for FXR activation and sEH inhibitory potency. Systematic exploration of the structure-activity relationship (SAR) of the compound class on both targets allowed optimization of the potency for FXR activation and sEH inhibitory potency. Intensive in vitro evaluation revealed marked FXR target gene induction accompanied by robust inhibition of sEH in hepatocytes and extraordinary selectivity amongst nuclear receptors as well as the membrane bile acid receptor TGR5. Moreover, the dual modulator showed no toxic activity and a favorable metabolic profile with pharmacologically active metabolites. With this encouraging data, a pilot in vivo study was conducted which confirmed a favorable pharmacokinetic profile and engagement on both targets in vivo. Further preclinical development is ongoing to enable IND application and preliminary results are promising.

In summary, we have developed a first-in-class dual FXR/sEH inhibitor revealing high potency and favorable properties in vitro as well as a desirable kinetics and dual target engagement in vivo. Our results encourage further exploration of dual FXR/sEH modulation for NASH treatment.

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Structure-Based Optimization and Profiling of 17β-Hydroxysteroid Dehydrogenase Type 14 Inhibitors

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17β-Hydroxysteroid dehydrogenase type 14 (17β-HSD14) is a recently characterized enzyme, which is mainly located in the brain, liver and placenta. 17β-HSD14 catalyses the oxidation of estradiol and 5-androstene-3β,17β-diol into estrone and dehydroepiandrosterone, respectively using NAD⁺ as cofactor.

The specific localization of this enzyme in the brain makes it very attractive. However the physiological role of the enzyme has not been yet elucidated. Potent and selective inhibitors are useful tools to study the role of an enzyme in vivo. The goal of this study was to structurally optimize previously described inhibitors of 17β-HSD14 and evaluate their biological profiles in order to identify a good tool compound which could be used for in vivo studies.

We recently reported on the first crystal-structure of a non-steroidal inhibitor of 17β-HSD14 in complex with the enzyme. Starting from a potent inhibitor of the same structural class and using a structure-based design approach, several structure variations were performed in order to improve its solubility, its selectivity profile against other 17β-HSD enzymes, and its cytotoxicity. The behavior of the most interesting compounds toward the efflux pump Pgp was also determined as the compounds should be able to permeate the blood-brain barrier. The design strategy and the biological evaluation of the new 17β-HSD14 inhibitors will be presented.

References
2.11 Nanomedicine

Chair: C. M. Lehr, M. Schneider

Bioinspired new materials - From geckos to robotics and biomedicine

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Evolution has created a multitude of effects that inspire the development of new materials and surfaces. Anti-reflective like a moth eye, self-cleaning like a lotus leaf, slippery like a shark skin, or colorful like butterfly wings – scientifically these natural phenomena are now reasonably well understood and can be imitated or emulated in the laboratory. However, full technical exploitation of these biomimetic principles has rarely been achieved so far. At INM, we develop and investigate new dynamic surfaces for diverse functionalities: low friction, adhesion, corrosion protection, anti-reflection, electric storage, biocompatibility and combinations of these. Such surfaces either exhibit new chemistries or new topographies – and are often inspired by biological evolution.

This talk will give an overview of our successful attempts to create adhesive surfaces based on the design of a gecko toe. We were among the first to identify the governing principle of “contact splitting” [1], i.e. the gain in intermolecular adhesion due to a multitude of fine fibrillar contacts instead of one monolithic contact region. This allows optimum contact between the surfaces while establishing little elastic strain and thereby enhances the van der Waals interactions that are present between any surfaces at short distances. We have produced many different versions of artificial “gecko surfaces” in the laboratory. We have also shown that a switching action to a non-adhesive state can reproducibly achieved, for example, by inducing bending and Euler buckling in the fibrils [2]. An important element of our work is the numerical simulation of the adhesion performance as a function of materials and structure parameters, which has allowed us to rationally optimize our structures for particular applications [3]. Even surfaces with finite roughness are now accessible for our newly designed microstructures [4]. More recently, we have extended our adhesive systems to address also biological surfaces, especially skin. Appropriately designed prototype surfaces allow to exert shear stresses on tissue [5], and have been shown to exhibit cellular compatibility in combination with adhesion [6]. These properties make gecko surfaces interesting for innovative surgical procedures. Currently, these principles are being exploited to create new surface solutions for robotic pick-and-place systems, assembly machines, in space technology and biomedicine.

Acknowledgments

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References

Application of liposomal DDS for the treatment of ischemic stroke

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Ischemic stroke is one of the leading causes of death and disability especially in super aging society. Tissue plasminogen activator (t-PA) is the only one therapeutic agent for the treatment of acute ischemic stroke in worldwide. However, due to the narrow therapeutic time window and the risk of cerebral haemorrhage induced by t-PA, adaptable patients are very limited. Moreover, ischemia-reperfusion (I/R) injury often occurs as a secondary damage after recovery from cerebral ischemia. By the way, It has been known that cerebral ischemia causes the blood-brain barrier (BBB) disruption. Therefore, at first, we examined the permeability change of the cerebral blood vessels, and observed that about 100 nm liposomes were accumulated in ischemic region of the brain after I/R treatment in transient middle cerebral artery occlusion (t-MCAO) model rats. Next, to develop a DDS medicine for the treatment of ischemic stroke, we investigated the therapeutic effect of liposomal neuroprotectants, such as asialo-erythropoietin-modified liposomes and liposomes entrapped an immunosuppressant FK506. Those liposomal drugs, significantly suppressed the brain cell death after I/R treatment. Moreover, treatment with those liposomal drugs improved motor hypofunction which had been brought by the brain ischemia. Taken together, liposomal DDS would be a useful strategy for the treatment of ischemic stroke.

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Nanomedicine

SL.40

Nanoparticles for targeted brain delivery

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The blood-brain-barrier (BBB) is one of the most important barriers in the body. It protects the brain from the peripheral circulation and toxic substances and maintains the brain homeostasis. Therefore, the BBB represents an insurmountable obstacle for most drugs. However in some cases it is important to overcome this barrier, namely in case of brain located diseases. Therefore, a number of different strategies have been employed during the past years to overcome this barrier. One of them is the fast-emerging field of nanotechnology. It offers the possibility to transport drugs over the BBB by packing them into surface-modified nanoparticles (NP) [1]. Especially apolipoprotein E (ApoE) appears to play a major role in the nanoparticle-mediated drug transport across the BBB. Further studies verified a clear correlation between the ApoE adsorption and the BBB passage [2, 3]. Therefore, it was hypothesized that these NP resemble endogenously circulating lipoproteins and are taken up by a receptor-mediated pathway by the brain endothelial cells, which express the respective receptors. Concerning the transport mechanism, our studies confirmed an active receptor-mediated endocytotic uptake of nanoparticles, which had been modified by apolipoprotein E (ApoE) bound on the particle surface. The involvement of low density lipoprotein receptor family members, notably the low density lipoprotein receptor related protein 1 (LRP1), on the uptake of the ApoE-modified nanoparticles into the brain capillary endothelial cells could be shown [4].

This knowledge of the uptake mechanism of ApoE-modified nanoparticles enables future developments to rationally create very specific and effective carriers to overcome the blood-brain barrier. As an example, we focus on transporting NP loaded with an anti-Alzheimer’s disease drug to the brain: Our data suggest that embedding flurbiprofen (a drug that failed in clinical trials due to its low permeability to the brain) in poly(lactic acid) NP enables crossing of an advanced in vitro BBB model [5].

Acknowledgments

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References

In vitro dexamethasone release from intravitreal poly(D,L-lactide-co-glycolide) model implants

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Sustained intravitreal dexamethasone (DX) administration with the FDA approved Ozurdex® is indicated for the treatment of macular edema and non-infectious uveitis. Since drug release cannot be determined in vivo in humans, in vitro characterization is of great importance for such devices. The aim of this study was to provide an initial estimate on in vitro drug release from intravitreal model implants with a similar composition as Ozurdex®. Hence, 2 batches (0.4 mm in diameter and 0.9 mm in diameter) of intravitreal model implants containing 25 % DX in poly(D,L-lactide-co-glycolide) (PLGA, Resomer® RG 502 H) were produced by solvent casting and subsequent hot melt extrusion (mean extrusion temperature at the nozzle = 52-56 °C, [1]). The filaments were cut with a scalpel to model implants of a length of approximately 6 mm. Drug release was studied in the shaking incubator (Titramax 1000, Heidolph Instruments GmbH & Co KG, Germany) by placing the model implants in reagent tubes that were filled with 4 mL of ringer buffer pH 7.4 to simulate the volume of the human vitreous body. The temperature was 37 °C and shaking speed was 150 rpm. At defined times, samples of 1.0 mL were collected and replaced with 1.0 mL of fresh ringer buffer solution. Experiments were performed in triplicate. The quantification of DX was performed using high performance liquid chromatography (HPLC, Shimadzu Europe Ltd., Germany).

As expected, the fraction of released DX from the model implants with diameters of 0.4 mm was faster than from the 0.9 mm implants (fig. 1) so that after 84 d a total of 99.2 ± 3.6 % had been released from the smaller diameter implants (0.4 mm) compared to 53.4 ± 0.9 % within the same period of investigation for the implants with the larger diameter (0.9 mm). The uniform surface of the cylindrical implants turned rougher during the drug release study. Changes in shape were recognizable within the first week of release testing and swelling as well as a surface enlargement of the model implants were observed after three weeks (fig. 2). After four weeks, the model implants disintegrated into several fragments. Such fragmentation has also been reported in clinical cases [2].

The clinical relevance of fragmented intravitreal implants and the potential of retinal toxicity are currently controversial discussed and need to be investigated in more detail. Moreover, the influence of different in vitro test parameters such as the release medium, the release volume and the type of movement patterns should be evaluated in further studies in order to gain a better understanding of drug release from intravitreal implants.

Fig. 1: Mean relative DX release of PLGA model implants (0.4 mm and 0.9 mm) in ringer buffer pH 7.4 (37 °C, shaking incubator at 150 rpm), means of n = 3 ± SD.

Fig. 2: Microscopic images of the DX-loaded PLGA model implants before release testing and after 3 weeks. Magnification factor of 40 (upper series) and 200 (bottom series).

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New research, new researchers I

Chairs: A. Link

In-silico pharmacology: mechanistic models for the modulation of transmembrane proteins

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The transmission of chemically encoded information across the cell membrane represents a crucial prerequisite for every living cell. The communication of cells with their environment is mainly shaped by transmembrane proteins that intermit the barrier function of the membrane and thereby provide highly complex and effective signaling machinery. Despite the essential role of transmembrane proteins for biological processes, surprisingly little is known about the underlying mechanisms. This includes the binding of ligands, the conformational changes and the subsequently triggered biological response.

Driven by the hypothesis **form follows function** we aim at a characterization of the large conformational ensembles of transmembrane proteins. Over the last decades, specific protein-ligand complexes were determined by crystallography providing an indispensable structural view on transmembrane proteins including G protein-coupled receptors (GPCR), ion channels, toll-like receptors (TLR) and pore-forming toxins among others. Since these crystal structures represent single static conformations, we need functional models that consider transmembrane proteins as dynamic entities.

Here, we demonstrate in three examples how a combination of *in silico* approaches and experimental methods can draw a dynamic and mechanistic view on the functionality of transmembrane proteins [1,2]

First, we will focus on GPCR functionality and explain ligand-dependent effects taking muscarinic acetylcholine receptors as classical model systems. We link conformational characteristics triggered by certain ligands to distinct receptor functions including subtype selectivity, partial agonism and functional selectivity [3,4,5]. Second, we show how TLRs recognize a broad variety of pathogen-associated molecular patterns, which is essential for the activation of innate immune response [6]. And third, we look at bacterial toxins that enter the host cell membrane upon cholesterol recognition, oligomerize and finally form a pore, which destroys the barrier function of the membrane leading to cell death.

Based on the shown examples we will point out that a mechanistic understanding of transmembrane proteins, their highly complex functionality and the possibility to modulate them specifically is key for the rational design of a majority of novel therapeutics.

Targeting the ER-mitochondria interface sensitizes leukemia cells towards cytostatics

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Combination chemotherapy has proved to be a favorable strategy to treat acute leukemia. However, the introduction of novel compounds remains challenging and is hindered by a lack of understanding their mechanistic interaction with established drugs.

In the present study, we demonstrate a highly increased response of various acute leukemia cell lines, drug resistant cells and patient derived xenograft (PDX) cells by combining the recently introduced protein disulfide isomerase (PDI) inhibitor PS89 with cytostatics. In leukemic cells, a proteomics based target fishing approach disclosed that PS89 impacts a whole network of ER homeostasis proteins. We elucidate that the strong apoptosis induction in combination with cytostatics is orchestrated by the PS89 target B-cell receptor-associated protein 31 (BAP31), which transduces apoptosis signals at the ER-mitochondria interface. Activation of caspase-8 and cleavage of BAP31 stimulate a pro-apoptotic crosstalk including ER calcium release and increased ROS levels resulting in amplification of mitochondrial apoptosis.

This study promotes PS89 as a novel chemosensitizing agent for acute leukemia treatment and uncovers that targeting the ER-mitochondria ‘social network of cell death’ is a promising approach in combination therapy.
IMP2/IGF2BP2 expression predicts chemotherapy response in patient derived colorectal cancer xenograft models

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Overexpression of the oncogene insulin-like growth factor 2 (IGF2) and its mRNA-binding proteins IGF2 mRNA binding protein 1-3 (IMP1-3/IGF2BP1-3) has been observed in different cancer entities including colorectal carcinoma. Although members of the IGF2/IMP axis have been suggested as diagnostic and prognostic biomarkers, attempts towards an individualized therapy of IMP overexpressing tumors have yet to be reported. The aim of this study was to investigate the effect of IGF2 and IMP1-3 expression with respect to the responsiveness towards different cancer therapeutics in vivo.

Samples from n=72 patients were analysed by RNA sequencing. IGF2 as well as IMP1, -2, and -3 were significantly overexpressed in primary tumor tissue compared to normal colon mucosa. In addition, metastatic tissues showed a significant increase of IGF2, IMP1, -2, and -3 levels compared to normal tissue adjacent to the metastatic site. Expression of downstream targets of IGF2 was changed accordingly: IGF1 receptor (IGF1R) levels were increased, whereas expression of the tumor suppressor Phosphatase And Tensin Homolog (PTEN) was decreased. In order to test the responsiveness of these tumors towards different therapeutics, patient-derived xenografts were established. Tumors were treated with oxaliplatin, irinotecan, 5-fluorouracil, sapitinib (AZD8931), selumetinib, afatinib, avastin, regorafenib, nintendanib, the mTOR Inhibitor BI860586, the IGF1/2-antibody BI836842, TANK inhibitor AZ1, and cetuximab for four weeks or until tumor volume exceeded 1 cm³. Tumors, which highly expressed IGF2, IMP1, or IMP2, showed an improved response to anti-EGFR therapy with Cetuximab and Sapitinib. High IMP3 levels correlated with lower tumor volumes after Selumetinib treatment. Interestingly, the monoclonal antibody against IGF1/2 BI836842 failed to improve therapy of IGF2/IMP overexpressing tumors. The response towards Oxaliplatin and 5-Fluorouracil, two chemothterapeutics, which are part of the standard regimen FOLFOX, did not depend on IGF2 or IMP expression levels.

In conclusion, IGF2 and IMP1-3 are frequently overexpressed in colorectal cancer. Patients with IGF2, IMP1, or IMP2 overexpression might profit from anti-EGFR therapy, whereas patients showing IMP3 overexpression are expected to benefit from an anti-MEK therapy with Selumetinib.

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The Role of Non-collagenous Protein Conformation in Biomineralization

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Non-collagenous phosphoproteins organize the defined formation of nano-platelets of hydroxyl apatite in bone or enamel. However, the contribution of protein secondary structure to this process is still under debate.1 While some studies suggest a particular role of β-sheet conformation,2 others emphasize that most proteins being associated with biomineralization are intrinsically disordered.3 A protein being putatively involved in skeletal development and being capable of adopting both β-sheet (at low pH) and disordered structure (at high pH) is the hen egg yolk protein Phosvitin.4

By the use of circular dichroism (CD) spectroscopy we monitored the pH-dependence of phosvitin secondary structure and identified the involved conformational species and the pK-values for the corresponding transitions through matrix least-squares global fitting (Figure 1).5 In addition to the expected β-sheet at low pH, we found two disordered structures, one with low (PII−) and one with high content of PII helix (PII+).5 To elucidate the role of the different conformations in hydroxyl apatite formation we generated different conformers at different pH and entrapped these conformations through immobilization on a polycationic polymer surface. Using the phosvitin/polymer scaffold as a biomimetic platform for hydroxyl apatite formation (pH 8), we found a clear correlation of the amount of bound protein, calcium binding, and of crystallite formation with the entrapment conditions, particularly those that support PII− disordered structure.5 By the CD analysis of DMP1, a protein involved in enamel formation, we also identified PII− structure at the entrapment conditions supporting efficient crystallite formation. This suggests a general role for disordered structure with low PII helix content in biomineralization.5

Figure 1. CD spectra of phosvitin measured at different pH. Matrix-least-squares global fitting shows that every spectrum is a linear combination of only three spectra that are mixed at different proportions at different pH.

References
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].

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Total Synthesis, Target Elucidation and Structure–Activity Studies on Mycolactones and their Analogs

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Mycolactones are a group of complex macrolides, which exhibit cytotoxic, immunosuppressive and analgesic properties. As the exotoxins of the human pathogen Mycobacterium ulcerans, mycolactones are central to the pathogenesis of the neglected disease Buruli ulcer, a severe and chronic medical condition characterized by necrotic skin ulcers. Mycolactone A/B, the most pathogenic member of the mycolactone family of polyketides naturally occurs as a 2:3 mixture between the cis-∆4',5' and the trans-∆4',5' isomer of the lower pentaenoate side chain. However, despite extensive research in several academic laboratories, it is not yet clear whether the cis-∆4',5' or the respective trans-form represents the major contributor to bioactivity. Moreover, the molecular mechanisms of action of mycolactones are still heavily debated and several targets including the Wiskott–Aldrich syndrome protein (WASP), the Sec61 translocon and the type 2 angiotensin II receptor (AT2R) have been proposed within the last years [1].

Driven by the desire to understand the action of mycolactones on a molecular level, we prepared a variety of mycolactone analogs by means of total synthesis. These compounds featuring modifications and tags at both, the lower (southern) side chain (R2) and the upper (northern) core extension (R1), were used for structure–activity relationship and target deconvolution studies [2,3]. By employing two distinct biotinylated mycolactone-derived probes in conjunction with real-time PCR, RNA interference and other techniques, we recently identified the mechanistic Target of Rapamycin (mTOR) signaling pathway as the key-driver of mycolactone-promoted apoptosis [4]. By interacting with the intracellular 12 kDa FK506-binding protein (FKBP12), mycolactone A/B inhibits the assembly of the mTORC1 and mTORC2 multiprotein complexes, which are crucially involved in the regulation of various cellular processes. We found that mycolactone-promoted inhibition of mTORC2 assembly prevents the phosphorylation and activation of the serine/threonine protein kinase Akt. As a consequence, dephosphorylation of the Akt-targeted transcription factor Forkhead box O3 (FoxO3) triggers the expression of the pro-apoptotic Bcl-2-like protein 11 (Bim), which finally drives cells into apoptosis. Intriguingly, Bim knockout prevented the typical Buruli ulcer phenotype in M. ulcerans-infected mice thus confirming our results in vivo.

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Steroid hormones are essential for many physiological processes. Acting similarly quorum sensing molecules regulate the cell-to-cell (c2c) communication in bacteria and thereby control important processes like virulence and biofilm formation. As steroid hormones are also associated with severe diseases, selective inhibition of hormone biosynthesis has turned out to be an effective therapeutic strategy. Conversely, interference with the bacterial c2c communication has recently emerged as an alternative approach toward anti-infective treatment. In this talk, examples from our lab in both fields are presented.

First attempts to obtain selective inhibitors of estrogen and androgen biosynthesis started decades ago. Selective inhibitors of corticoid biosynthesis only recently came into the focus of research efforts because the homology between aldosterone synthase (CYP11B2) and cortisol synthase (CYP11B1) is very high and it was considered impossible to obtain selective inhibitors. Nevertheless, we could develop highly active and selective CYP11B2 inhibitors as candidates for the treatment of several cardiovascular diseases as well as potent inhibitors of CYP11B1 for treating Cushing’s syndrome and the promotion of chronic wound healing. Research efforts for treating hormone-dependent diseases are not only focused on steroid biosynthesis in the endocrine glands. A more targeted approach addresses the activation or deactivation of the steroid in the target cell. For the modulation of estrogen activity 17ß-hydroxysteroid dehydrogenases are responsible. As one example of our work in this field, the design and optimization of highly active and selective 17ßHSD2 inhibitors for treating osteoporosis and bone fracture healing is described.

The blockade of bacterial virulence without affecting cell viability is a new paradigm for the treatment of bacterial infections. In contrast to conventional antibiotics such compounds should not instigate selection pressure and treated bacteria ought to be less prone to resistance development. We developed the first compounds which interfere with the PQS c2c communication of P. aeruginosa. Inhibitors of PqsD, an enzyme involved in the biosynthesis of the QS molecule PQS, and antagonists of its receptor PqsR were designed and synthesized and shown to block biofilm and virulence factor formation, and importantly, were active in an in vivo infection model.

In all examples shown in this presentation we were the first to start drug discovery research. Meanwhile some of the targets are in the focus of the pharmaceutical industry.
Neuroallianz: A New Academia-Industry Partnership Model for Promoting Innovation in Drug Discovery

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Drug discovery and development in the post-genomic era have so far been less successful than anticipated. Despite high and continuously increasing investments by the pharmaceutical industry the number of new drugs approved by the authorities has remained low [1]. In 2016 FDA drug approvals dropped to the lowest number in almost 20 years, and in Germany the situation was not much better (see Fig. 1). The attrition rate in clinical trials has remained high, the main reasons being lacking efficacy or toxicity of the new drugs [2,3].

Collaboration of academic institutions and industry provides new opportunities to accelerate the translation of experimental research discoveries to novel therapies. The government-funded BioPharma consortium Neuroallianz is a successful example of private-public partnerships in drug development. The programme started in 2009 and is now in the last funding period. Several cooperation projects that are already no longer funded by the BMBF (Federal Ministry of Education and Research) are successfully continued without public support. The purpose of BioPharma, namely initiating academic-industrial partnerships to foster drug development, has been fully achieved. In conclusion, Neuroallianz represents a successful concept to bridge the gap between basic academic research and industrial drug discovery and development.

Common criticisms of the drug discovery and development process are that it is too inefficient, promising early candidates often failing in the clinic and academic discoveries with commercial potential, particularly in Europe, frequently not being developed further [1, 2]. The latter is predominantly due to lack of understanding or interest among academics in the detailed requirements for drug development. The Project Group Translational Medicine and Pharmacology TMP of the Fraunhofer Institute for Molecular Biology and Applied Ecology IME was established in 2012, with support from the LOEWE program of the State of Hessen, to act as a bridge between academia and industry and to promote the effective translation of novel ideas and projects into clinical studies. This is facilitated by teams of Goethe University and Fraunhofer researchers - many with pharmaceutical industry backgrounds - collaborating with other external institutions, on drug discovery and formulation, compound validation, analytics and predictive human models and clinical research, for the indications pain, autoimmune and neurodegenerative disease, sepsis and resolution of inflammation. This collaborative team approach is promoted by interdisciplinary laboratory sharing by graduate students and has proved effective in generating a “can-do” attitude to project development and a consistently high standard of science (mean IF of >5). Active project portfolio management and standardised documentation maintain quality and optimize resources. Drawing on innovative academic research, emphasis has been laid on novel disease targets and biomarkers, use of novel functional readouts such as optical imaging and both repurposing of known drugs, as well as structure optimization and novel formulation.

Among the successes have been the development to clinical trials of repurposed drugs. TMP-001, was found to be an effective inhibitor in murine experimental autoimmune encephalomyelitis [3], and after passing through phase I, is in phase II trials for multiple sclerosis. TMP-10010 was identified in a high-throughput screen for CYP2J2 inhibitors and found to be effective in reducing paclitaxel-induced neuropathic pain in mice [4]. A phase II trial is in preparation. New targets for pain-relieving drugs have also been identified, including distinct K⁺ channels for neuropathic pain and BLT2 receptors for inflammatory pain [5, 6] and are currently being validated with newly synthesised compounds. Medicinal chemical studies have also identified selective antagonists of PPARγ for use in inflammatory disorders [7].

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Our research in RNAi- and Crispr/Cas9 based functional genomics especially focuses on the identification of new cancer genes and therapeutic targets in therapy-resistant solid tumors. For such studies, clinically relevant mouse tumor models, which closely resemble the human disease, were available. Specifically, we are combining so-called mosaic mouse models with stable RNAi technology to dissect tumor suppressor networks in gastrointestinal tumors and to identify and validate new therapeutic target genes. Together with a limited number of other laboratories worldwide, we have the expertise to conduct RNAi screens for new cancer genes directly in orthotopic and immunocompetent cancer mouse models in vivo.

To best translate data from our unique RNAi platform into new cancer therapies, we recently systematically connected our RNAi expertise with the research areas virtual screening/modelling and medicinal chemistry to build an academic drug discovery unit, designated TuCAD2 (Tübingen Centre for Academic Drug Discovery). Our unit was recently approved as a member of the worldwide acting Academic Drug Discovery Consortium (ADDC, http://addconsortium.org/drug-discovery-factsheet.php?ddc_id=DC1000196). TuCAD2 represents an interfaculty and interdisciplinary endeavor and was founded by the Dept. of Pharmaceutical/Medicinal Chemistry (Stefan Laufer) and the Dept. of Internal Medicine VIII (Lars Zender).

In our talk we will discuss the pivotal role of academic drug discovery infrastructures for rapidly translating validated therapeutic target structures into clinical applications and will give an example of a novel and promising drug for the treatment of liver cancer which entered the phase of clinical testing only 13 month after completion of pivotal preclinical proof of concept.
Using targeted oxylipin metabolomics to understand a pharmacological modulation of the arachidonic acid cascade

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Several oxidized polyunsaturated fatty acids (PUFA) are potent endogenous mediators. These eicosanoids and other oxylipins are formed in the arachidonic acid (20:4 n6, ARA) cascade in three enzymatic pathways as well as during autoxidation: Conversion of ARA by cyclooxygenase (COX) leads to highly potent prostanoids such as PGE$_2$, a mediator of pain, fever and inflammation. Lipoygenase action gives rise to hydroperoxy-PUFA which are further transformed, e.g. in case of 5-HpETE to leukotrienes. Research of the past decade also demonstrated that via cytochrome P450 formed epoxy-PUFA and hydroxy-PUFA also play key roles maintaining homeostasis, e.g. in the regulation of inflammation and blood pressure. Combined action of the enzymes of the ARA cascade leads to further oxylipins, such as inflammation terminating multiple hydroxylated PUFA namely lipoxins or resolvins. Autoxidation of PUFA also leads to a vast number of oxidized lipids, ranging from simple hydro(peroxy)-PUFA to prostanoid like isoprostanes of which at least some show a distinct biological activity.

The ARA cascade, particularly the COX pathway is still one of the today’s major drug targets (NSAID, COX-2-inhibitors and aspirin). However, biological effects may not only result from the decrease in single prostanoids but from a shift in the whole oxylipin pattern caused by the drug. In the aforementioned complex network of enzymatic as well as autoxidative reactions almost all PUFA are substrates for the enzymes of the ARA cascade leading to a pleiotrop of oxylipins.

A “targeted metabolomics” approach, monitoring as many oxylipins as possible, is the most promising strategy to comprehensively analyze the modulation of the ARA cascade by pharmaceuticals/drugs. For this purpose liquid chromatography mass spectrometry (LC-MS) is the best suited analytical method. Low (sub-nanomolar) concentrations and the large number of structurally similar analytes, including regioisomers, require high chromatographic resolution and selective as well as sensitive MS analysis. Currently, reversed phase LC in combination with detection on sensitive triple quadrupole instruments, operating in selected reaction monitoring (SRM) mode, is dominantly used for quantitative oxylipin analysis.

In the talk the challenges for the quantitative analysis of oxylipins are summarized and an application of the targeted metabolomics approach is presented showing the effects of aspirin on the oxylipins pattern in human gut tissue.

Overcoming resistance mechanisms against cytoskeleton-targeting drugs by modulating the membrane phospholipid composition

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The serine-threonine kinase Akt (protein kinase B) mediates drug resistance of cancer cells. Using functional lipidomics [1], we recently identified phospholipids with polyunsaturated fatty acids (PUFA-PC) as negative regulators of Akt membrane translocation and thus activation [2]. Moreover, we reported a role of the phospholipid-dependent regulation of Akt for cell cycle progression [2], apoptosis and long-term vitamin A signalling [3]. Whether targeting the cellular phospholipid composition succeeds in overcoming Akt-dependent resistance is unknown. An in-house lipidomic screening campaign revealed synergistic effects of PUFA-PC and cytoskeleton-targeting agents on cellular PUFA-PC ratios and Akt signalling, with a derivative of the myxobacterial actin-targeting miuraenamide A as promising lead compound. PUFA-PC and miuraenamide derivatives were efficiently taken up by cells, substantially induced apoptosis and reduced viable cell numbers as polydisperse liposomal formulation. The potent inhibition of Akt activation by liposomal miuraenamides depends on the reprogramming of cells towards an accumulation of a broad PUFA-PC spectrum in line with the emerging link between actin dynamics, lipid remodelling and Akt signalling. Cytoskeleton-targeting agents were markedly less effective when combined with saturated phospholipids, used in marketed liposomal chemotherapeutics. Among 17 cancer and immortalized cell lines, human breast adenocarcinoma cells (MDA-MB-231) were most susceptible towards miuraenamide derivatives and PUFA-PC. The viability of primary human monocytes and macrophages - non-cancer cells from myeloid origin - was instead not decreased. Conclusively, our study fosters dietary phospholipids as potential chemopreventive agents and opens the door for the development of innovative drug carrier systems, which exploit phospholipids as auxiliaries to overcome Akt-dependent resistance.

References:
Lipidomics approaches are nowadays widely adopted to have a comprehensive view on lipid profiles in biological samples. Alterations in lipid profiles may well serve as informative biomarkers in various diseases for diagnostic and prognostic purposes. We are primarily interested in using lipidomics for correlating lipid profiles with disease severity in coronary artery disease and address other questions of clinical relevance. For this purpose we investigate the performance and advantages of UHPLC-QTOF-MS/MS with data independent acquisition (DIA) workflows using SWATH (sequential window acquisition of all theoretical fragment ion mass spectra) both for untargeted and targeted analysis of lipids. In SWATH, precursor ions for simultaneous fragmentation are selected with intermediate Q1 mass window width (e.g. typically 10-30 u) and the generated fragments readout by TOF analysis creating composite MS/MS spectra for each SWATH window. This approach provided better analyte coverage compared to data-dependent acquisition (DDA) and superior spectra quality compared to MS/MS in which precursors of the entire mass range are fragmented simultaneously at two distinct collision energies. Our understanding is that DIA can provide untargeted comprehensive MS/MS data over the entire chromatographic space from which by appropriate software tools and MS/MS spectral databases lipids can be identified in an automated manner and MS/MS chromatograms can be extracted for profiling workflows and quantitative analysis, respectively. If precursor and fragment ions are reassigned by deconvolution software assay specificity should be improved resulting in robust analysis. Possibilities, advantages and challenges of DIA with SWATH in untargeted and targeted lipidomics workflows will be discussed on clinical examples. In one study, lipid extracts of platelets of patients with stable angina pectoris and acute coronary syndrome have been profiled against controls and revealed significant alterations in lipid profiles of platelets which correlated with disease severity to some extent [1]. In another study, the advantages of SWATH acquisition for targeted analysis of steroid hormones in plasma samples of patients treated with steroid hormone patches will be discussed.

Reference:
Pharmacological profile of diflapolin, the first dual inhibitor of 5-lipoxygenase-activating protein and soluble epoxide hydrolase

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Arachidonic acid (AA) is metabolized by different enzymatic cascades to diverse bioactive lipid mediators. While leukotrienes (LT) are formed via the 5-lipoxygenase (5-LOX) pathway facilitated by the 5-LOX-activating protein (FLAP), the soluble epoxide hydrolase (sEH) converts anti-inflammatory epoxyeicosatrienoic acids (EETs) to pathological relevant dihydroxyeicosatrienoic acids (DiHETE). Therefore, inhibition of FLAP and sEH might be advantageous over single-interference, and may represent a promising pharmacological approach for intervention with complex diseases as inflammation.

Here, we present the in vivo pharmacological profile and efficiency of N-[4-(benzothiazol-2-ylmethoxy)-2-methylphenyl]-N’-(3,4-dichlorophenyl)urea (diflapolin) that targets both, FLAP and sEH [1]. In intact human leukocytes, diflapolin inhibits FLAP and the corresponding 5-LOX product formation with an IC50 of 30 and 170 nM for monocytes and neutrophils, respectively. Additionally, in a cell-free assay, diflapolin potently suppressed sEH with an IC50 of 20 nM. In order to confirm FLAP inhibition, distinct required characteristics were demonstrated: diflapolin (I) only inhibited 5-LOX product formation in intact leukocytes and failed to block isolated 5-LOX, (II) lost potency when exogenous AA was added, (III) potently suppressed 5-LOX product formation only in HEK-5-LOX cells that co-express FLAP, and (IV) hampered 5-LOX / FLAP complex assembly at the nuclear membrane in leukocytes. Diflapolin did not interfere with other enzymes of the AA-cascade (i.e., COX1/2, 12-LOX, 15-LOX, LTC4S, mPGES1, and cPLA2) and thus showed strong target specificity. Beside in vitro efficiency, diflapolin evoked anti-inflammatory properties in the zymosan-induced mouse peritonitis model as it inhibited the formation of cysteinyl-LTs and LTB4, impaired vascular permeability, and reduced neutrophil infiltration. Together, we present diflapolin as a novel potent dual FLAP/sEH inhibitor in vitro and in vivo with strong target specificity suitable for treatment of inflammation-related diseases. Additionally, diflapolin serves as a valuable chemical tool to study the biology of FLAP and sEH.

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References:
Manipulating cancer cell lipid metabolism by interfering with lysosomal function

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The lysosome has long been simply considered as the cells' trash can, however, we evolve our understanding of the various important lysosomal functions within the cell besides degradation processes and their therapeutic potential. Proper lysosomal function is crucial for instance for endocytotic trafficking, autophagy and regulation of cellular metabolism, which are often deregulated in cancer cells. In the search for novel therapeutic approaches, targeting aberrant lipid and cholesterol metabolism, which favour proliferation and survival of cancer cells, has come into focus recently, yet remains quite demanding. Our work provides a novel approach to meet this challenge showing that interference with lysosomal function in cancer cells leads to alterations in cholesterol and lipid metabolism. A crucial regulator of lysosomal function is the enzyme vacuolar-type H+ ATPase (V-ATPase), which provides the acidic pH necessary for lysosomal enzymes, regulates endocytotic trafficking and is a central part of the nutrient sensing machinery. Our study shows that inhibiting this important enzyme with the potent natural compound archazolid affects cancer lipid metabolism on multiple levels. Archazolid treatment leads to drastic changes in cholesterol distribution, by inducing lysosomal trapping and thereby restricting cellular access to free cholesterol. As a consequence, cholesterol-dependent activation of Ras signalling at the plasma membrane is reduced, leading to impaired proliferation in vitro and in vivo. Furthermore we have evidence that, V-ATPase inhibition induces a cross-talk between the lysosome and other organelles, such as mitochondria and the lipid storage organelles, lipid droplets. We found that upon archazolid treatment, lipid droplets are changed in number and size, along with altered cytosolic free fatty acid levels and changes in the triglyceride and cholesteryl composition. Additionally, mitochondrial function is influenced by changes in PPARα signalling and enzymes implicated in beta-oxidation. Manipulating lysosomal function using the potent V-ATPase inhibitor archazolid therefore represents a novel approach to address deregulated lipid metabolism and functional consequences in cancer cells, providing the basis for attractive and innovative anti-cancer strategies (Fig 1).

Figure 1. Graphical abstract. The central role of the V-ATPase is acidification of the endolysosome. The low luminal pH is crucial for the generation of free cholesterol, which is subsequently released into the cytosol and used as building block and for the integration into membranes. The membrane-bound small GTPase Ras is mainly localized in cholesterol-enriched membrane microdomains, where it can be activated. Ras in turn activates downstream signalling pathways necessary for proliferation and survival. Furthermore, the V-ATPase is implicated in the induction of transcription factors, inducing lysosome-to-nucleus signalling leading to the transcription of lipid regulating genes such as PGC1α and PPARα. Hence, V-ATPase activity influences lipid metabolism and mitochondrial function thus being an innovative target in cancer biology.

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References:
Best before – lyophilisation as novel storage alternative for extracellular vesicles

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Extracellular vesicles (EVs) are cell-derived lipid membrane particles decorated with surface and membrane proteins [1]. EVs are nature’s way to deliver information as they transfer protein and nucleic acid based cargoes selectively to their target cell. They are increasingly studied for biosignalling, pathogenesis and biomedical applications [1,2]. However, little is known about the optimal conditions for their transfer and storage. Currently, the international consensus supports to keep them at -80°C [3] but whether this affects EVs’ biological functionality remains unclear. Lyophilisation (freeze-dry) of EVs would allow easy handling at room temperature (RT) and thus significantly boost their expanded investigation but has not been evaluated to date. We present the first comprehensive assessment of different EVs during various storage conditions including freeze-drying and -80°C. Moreover, we evaluated for the first time EVs’ stability and bioactivity upon model enzyme loading, and we studied the effect of different cryoprotecting agents to preserve EV functionality.

Figure 1. (a) Size evolution of EVs stored at 4°C and in PBS. (b) Size and (c) normalised number of EVs and liposomes analysed by nanoparticle tracking analysis and upon storage at -80°C or after lyophilisation without cryoprotecting additives (Mean ± SD, *p<0.05, vs. storage at -80°C, ANOVA, Tukey post-hoc test).

EVs were isolated from 48 h conditioned culture medium by ultracentrifugation (120,000 x g, 2 h), loaded with glucuronidase via saponin treatment [4] and purified by gel filtration (Sepharose CL-2B). EVs were stored at RT, 4°C or -80°C, and lyophilised with/without addition of cryoprotectants (mannitol, trehalose, PEG), and analysed by nanoparticle tracking analysis and electron microscopy (TEM, phosphotungstic acid stain). Residual enzyme activity was assessed with fluorescein glucuronide (37°C) and compared to liposomes (phosphocholine/cholesterol 60/40 mol%). EVs from MSCs were stable at 4°C while size of HUVEC EVs increased during 14 d of storage (Fig. 1a). EVs from MSC stem cells or HUVEC endothelial cells, and liposomes showed average sizes of ~190 nm for all storage conditions (Fig. 1b). A 30% decrease in particle number was observed for HUVEC EV during freeze-drying compared to storage at -80°C but was less pronounced for MSC EVs and liposomes (Fig. 1c). Addition of 1wt% mannitol caused cryoprotection and inverted this effect, with EV morphology not altered as imaged by TEM. The glucuronidase activity of loaded MSC EVs was lost after 14 d of storage but addition of 1-4wt% trehalose induced recovery of enzymatic cleavage comparable to activity levels of liposomes, indicating that low sugar concentrations preserve the EV functionality.

We provide first evidence that EV functionality is not impaired during freeze-drying, further optimised by the addition of cryoprotecting sugars. Our findings provide new insight for exploring lyophilisation as novel storage modality and we create a fundamental basis for standardised EV applications in biomedical research.

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References:
Exploring food-drug interactions: solubility screening of ziprasidone-HCl in biorelevant media

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The increase in bioavailability after the oral administration of poorly water soluble drugs together with food is often associated with enhanced solubility in gastrointestinal fluids.1 However, the mechanisms behind these effects are still not fully understood. Therefore, it was the aim of this study to identify parameters that potentially affect the luminal concentration of ziprasidone (BCS II) in the human GI tract after drug administration together with food.2 For this purpose, the apparent solubility of ziprasidone-HCl (ZIP) was measured in various simple and complex biorelevant media with the aid of the shake-flask method and HPLC. Owing to its weakly basic character, the solubility of ZIP was clearly depending on pH with better solubility at low pH values. Interestingly, at highly acidic pH values the use of physiologically relevant HCl had a detrimental effect due to the common ion effect. In pure lipids, the solubility was also low, but partitioning experiments revealed that this effect was limited to the salt. By using 1:1 mixtures of aqueous media of different pH and long- or medium-chain lipids, we could demonstrate partitioning of the free base into the lipid, especially at neutral pH values. Further solubility experiments with different biorelevant media suggested that the process of drug solubilization might be the most critical aspect in case of ziprasidone. In full-fat milk as well as media containing mixed micelles formed by bile salts, higher ZIP concentrations could be measured. However, in milk the concentration showed a sharp drop after diluting the milk with 0.1 M HCl in a biorelevant manner due to the loss of emulsion stability. In order to simulate the effects of gastric lipolysis, a fungal lipase (AMANO A12) was used. These experiments revealed that the generation of medium- and long chain fatty acids clearly enhanced drug solubilization in simulated gastrointestinal fluids. The observations made in this pilot study imply that in case of ziprasidone the food-induced stimulation of bile secretion in combination with lipolysis products are a likely explanation for the occurrence of the positive food effect on oral drug bioavailability that was observed in vivo. In future experiments, the protocol of this solubility screening shall be applied to other drugs with positive food effects to elucidate the mechanisms behind food-drug interactions.

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References
Ex vivo skin penetration of antipruritic nonivamide from film-forming formulations and in vivo skin tolerability

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Chronic pruritus is a common symptom accompanying various chronic skin diseases. Conventionally, it is treated with antihistamines and local anesthetics. However, these drugs often cannot provide sufficient relief. As an alternative, capsaicinoids can be used. Their long-lasting antipruritic effect is caused by continuous stimulation of TRPV1 at the epidermal pain conducting fibers. To achieve this, currently available formulations need to be applied 4-6 times a day. This is inconvenient and results in poor patient compliance.

The aim of our study was to develop a film-forming formulation (FFF) with sustained release for dermal use making it easy to treat large areas of affected skin over a long period. Nonivamide (synthetic capsaicin) was used as active. FFFs were prepared by loading a solution of nonivamide in refined castor oil into mesoporous silica. This was subsequently incorporated into a plasticized film-forming polymer dispersion.

Film forming capacity of the FFFs was investigated by confocal Raman microscopy. Color coded images show that the oil is bound to the silica and immobilized in a polymeric matrix. The inclusion of the nonivamide containing oil was regarded as a prerequisite to achieve sustained penetration into the skin.

Ex vivo permeation experiments were carried out to parametrically compare permeation of nonivamide from FFFs to a standard formulation (Hydrophilic Nonivamide Cream; HNC; prepared according to "Hydrophile Capsaicinoid Creme" in: Neues Rezeptur Formularium; monograph #11.125). It was found that permeation rate from a FFF with 0.9 % nonivamide was comparable to that from HNC containing 0.05 % nonivamide. The permeation rate from the FFF falls thus into a therapeutically suitable range [1].

As the site of action of capsaicinoids is located within the viable epidermis, ex vivo penetration experiments were performed to compare nonivamide penetration from FFF and HNC into excised skin. It was found that the FFF was capable of delivering a similar amount of nonivamide to the skin as the HNC. Nonivamide levels in the viable epidermis decreased rapidly if it was applied in HNC but were kept constant over a period of 24 hours if it penetrated from FFF. The capability of FFF to sustain penetration was thus shown.

Furthermore, skin irritation potential of FFF was tested against the vehicle and the control formulation HNC in an in vivo experiment in human volunteers (signed written consent obtained, approved by local ethics committee and in accordance with the declaration of Helsinki). Transepidermal water loss, skin hydration and erythema index were assessed. It was found that FFF did not alter any of the measured parameters within the application time. This shows the excellent skin tolerability of the FFF [2].

Our investigation clearly shows that FFFs exhibit the desired sustained penetration profile while being well tolerated. As a result, dosing intervals can be prolonged and patient compliance to the treatment can be improved.

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New chemical tools for probing the NAD⁺-dependent lysine deacylase sirtuin 2

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Sirtuins are a highly conserved class of NAD⁺-dependent lysine deacylases. The human isotype sirtuin 2 (Sirt2) has been implicated in the pathogenesis of cancer, inflammation and neurodegenerative diseases, which makes a modulation of Sirt2 activity a promising strategy for pharmaceutical intervention [1]. Recently, we discovered a highly potent new class of Sirt2-selective inhibitors. These inhibitors enabled us to obtain co-crystal structures, which revealed their unique binding mode to the active site of Sirt2. Upon binding, they occupy the extended C-site and induce a major rearrangement of the active site. Therefore, these inhibitors were termed "Sirtuin Rearranging Ligands" (SirReals) [2]. By structure-guided optimization and extensive structure–activity relationship (SAR) studies, we were able to discover SirReal analogues with enhanced potency and cellular efficacy [3,4]. A triazole-based SirReal (1), which was shown to feature an extended binding mode by forming a hydrogen bond with Arg97, was identified as an excellent template for the design of different SirReal-based probes. Using a Cu(I)-catalyzed cycloaddition we were able to link an alkynylated SirReal analogue with divers azido-conjugated functional labels. A rhodamine-labelled SirReal (2) was used for the development of an in vitro fluorescence polarization assay and as an intracellular fluorescent reporter for Sirt2. Conjugation with a thalidomide moiety allowed the development of a proteolysis targeting chimera (PROTAC, 3) that chemically induced the proteasomal degradation of Sirt2 [5]. A biotinylated SirReal (4) was shown to be suitable for pull-down experiments as well as biolayer interferometry [4]. All these unprecedented tools for Sirt2 feature the high potency and unique isotype selectivity of the parental SirReal (1) and open up new avenues to dissect the role of Sirt2 in biology and medicine.

[References]
Expanding the chemical space of genetically encoded small molecule screening libraries through a dedicated encoding strategy

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The identification of small bioactive compounds is a crucial step towards drug development. Genetically tagged libraries of small molecules (DNA-encoded libraries, DELs) have found widespread use in drug research as screening technology.[1,2] Tagging compounds with genetic information allows for pooling them to large compound mixtures. Bioactive compounds can be efficiently identified from these compound mixtures by a generic selection assay, followed by sequencing of the DNA-tag. DELs are synthesized through cycles of alternated organic synthesis and encoding steps. Thus, DNA-compatibility of any organic preparative reactions is prerequisite for library synthesis. Essential reactions utilized in the synthesis of drug-like compounds do not meet this requirement. Transition metal catalysts, and acid organocatalysts enable access to diverse drug-like heterocycles from simple starting materials, but interact or even react with purine bases eventually causing depurination of the DNA tag. To circumvent this impediment to methods development for DELs, we utilize a solid phase-bound hexathymidine sequence “hexT” as an adapter oligonucleotide in the initial step of DEL synthesis (Figure 1).[3,4] The hexT DNA oligonucleotide tolerated a broad spectrum of reaction conditions, reagents, and catalysts for target molecule synthesis. Testimony of the stability of the hexT was the synthesis of hexT-pyrazole conjugates through a Au(I)-mediated three-component annulation reaction in glacial acetic acid.[3,4,5] The hexT-heterocycle conjugates were readily ligated to coding DNA sequences with a hexa-adenosine overhang.

Figure 1: Access to DNA-encoded libraries based on the chemoresistant hexathymidine sequence “hexT”. The solid phase-bound hexathymidine sequence “hexT” tolerated harsh reaction conditions, among them transition metal ions, and strong Brønsted acids, to synthesize substituted heterocycles from simple starting materials. The hexT-heterocycle chimeras were readily ligated to coding DNA sequences.

References:
Sex differences in eicosanoid biology and consequences for the development and progression of acute inflammation and related pharmacotherapy

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Eicosanoids such as leukotrienes (LTs) and prostaglandins (PGs) are lipid mediators implicated in inflammatory and autoimmune diseases, often characterized by a sex-bias (e.g., asthma). Here, we report on sex differences in eicosanoid biology as key variable for the acute inflammatory process and related pharmacotherapy. We have previously shown sex-dependent biosynthesis of LT during acute inflammation where testosterone impaired the subcellular trafficking of the key enzyme 5-lipoxygenase (5-LO) and consequently suppressed LT formation in human innate immune cells (e.g. isolated neutrophils or monocytes), in mouse peritoneal macrophages (PM), and in vivo in zymosan-induced murine peritonitis [1]. Additionally, we have recently identified a sex bias in PG production in neutrophils during acute inflammation (higher in male) under conditions where LT production is elevated in females at the sites of injury [2]. Here, we report on a sex bias in the efficiency of clinically relevant inhibitors of LT biosynthesis, i.e., various 5-LO-activating protein (FLAP) inhibitors and “novel-type” 5-LO inhibitors that are superior in females. We provide evidence that androgens cause these sex differences in vivo and in vitro, and we show that androgens impede the agonist-induced, tight assembly of the 5-LO/FLAP complex at the nuclear membrane of human and murine leukocytes. Our findings show that this testosterone-mediated sex bias impacts the efficiency of clinically relevant LT biosynthesis inhibitors, strongly suggesting that therapy with LT-modifiers should be evaluated with respect to sex [3].

3 POSTER SHORT TALKS
Development of protein-protein interaction inhibitors to affect the heterotetrameric architecture of protein kinase CK2

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During the last years, protein kinases have emerged as interesting targets for the development of new drugs. Kinase inhibitors can be used to treat many different diseases such as Alzheimer’s Disease, Parkinson’s Disease and cancer.[6]

The kinase CK2 (former name casein kinase 2) is a heterotetrameric serine/threonine kinase consisting of two catalytically active α- and two regulatory β-subunits.[2] CK2 plays a prominent role in the control of the cell cycle and apoptosis. Interestingly the CK2α subunit shows constitutive activity even in its monomeric form. In the holoenzyme complex the CK2β subunits regulate the substrate specificity and catalytic activity of CK2α.[3] Overexpression and enhanced activity of the enzyme were observed in different types of cancer.[4]

Whereas most of the known CK2 inhibitors are targeting the ATP binding site of the enzyme or showing allosteric modulation, inhibition of holoenzyme assembly has been shown to be a promising strategy to affect the enzyme’s selectivity and catalytic activity.[5]

Podophyllotoxin derived tetrahydrocarbazole analogues are known for their inhibitory effect on both, the subunit interaction and catalytic activity of monomeric CK2α.[6] Based on these findings, several new compounds have been synthesized and tested for their ability to inhibit interaction of CK2’s subunits and catalytic activity of the holoenzyme.

Three component synthesis of tetracyclic compounds 4 is described.[7] The synthetic method allows broad modification. Relationships between structure, phosphorylation activity and inhibition of CK2 protein-protein interaction are presented.

![Figure 1. Synthesis of CK2 protein-protein interaction inhibitors](image-url)

Chronic inflammatory diseases, such as psoriasis or rheumatoid arthritis, are characterized by constant leukocyte infiltration and ongoing angiogenesis in the inflamed tissue. As current anti-inflammatory pharmacotherapy is not always satisfying, there is a great need for the discovery of new drug leads and targets. The synthetic carbazole derivative C81 acts as a kinase inhibitor. Results of a thermal shift assay revealed that C81 shows by far the highest binding affinity to the BMP-2-inducible kinase (BMP2K/BIKE) and the adaptor-associated kinase 1 (AAK1). Both kinases belong to the Numb-associated kinase (NAK) family, which has been linked to various biological functions, such as osteoblast differentiation or receptor-mediated endocytosis. Since the vascular endothelium crucially regulates inflammatory processes, we hypothesized that these kinases might play a pathophysiological role in the inflammation-activated endothelium. The functional role of both kinases has not been characterized in the endothelium so far. Therefore, we aimed to analyze the pharmacological potential of C81 and to investigate the role of BMP2K in angiogenic and inflammatory processes in the vascular endothelium.

Initial experiments show that only high concentrations of C81 affected the viability of human umbilical vein endothelial cells (HUVECs) after 24 hours of treatment (IC$_{50}$: 171 μM). Longer incubation periods (72 h) reduced the proliferation of a human microvascular endothelial cell line (HMEC-1) with an IC$_{50}$ of 7 μM. C81 treatment (10 μM) resulted in a reduced migratory capacity of HMEC-1. A tube formation assay on Matrigel $^\text{TM}$ demonstrated that C81 significantly impaired the formation of capillary-like structures in a concentration-dependent manner. Interestingly, C81 (3 μM) also reduced the formation of VEGF-induced but not bFGF-induced sprouts in HUVECs. Western blot analysis indicated that the serum-induced activation of signaling molecules that play a crucial role in cell proliferation and angiogenesis (e.g. ERK, Akt) was not reduced neither by C81 treatment nor by knock-down of BMP2K (RNAi).

In regard to inflammatory processes, C81 treatment decreased the adhesion of THP-1 cells (monocytic cell line), peripheral blood monocytes and primary lymphocytes onto the activated endothelial cells in vitro. BMP2K silencing of HUVECs also resulted in a significantly decreased adhesion of THP-1 cells. In vivo results of intravital microscopy in the murine cremaster muscle demonstrated that the adhesion of leukocytes was significantly reduced after C81 treatment. As the interaction of leukocytes and the endothelium is mainly mediated by cell adhesion molecules (CAMs), the effect of C81 or BMP2K silencing on their expression was analyzed (flow cytometry, qPCR) in HUVECs. While the expression of CAMs was strongly decreased after C81 treatment, the knock-down of BMP2K did not markedly affect their expression. Furthermore, neither C81 treatment nor BMP2K silencing led to the reduction of TNF-α-induced iκBα degradation (Western blot) or p65 translocation into the nucleus (microscopy). Silencing of BMP2K resulted in a marked decrease of TNFα-induced COX-2 expression, while C81 treatment strongly induced its expression (Western blot). Interestingly, C81 treatment also resulted in a reduced prostaglandin release (LS-MS/MS), although COX2 was upregulated. The analysis of the MAPK pathway revealed that TNF-induced activation of JNK was inhibited, while the expression of the MAPK inhibitor DUSP-1 was strongly upregulated (Western blot).

Our study provides first insights into the anti-inflammatory and anti-angiogenic potential of the carbazole derivative C81 in vitro and in vivo. Since the inhibition of BMP2K seems to be responsible only for some actions of C81, we will investigate the role of AAK1 in these processes. The precise role of BMP2K/AAK1 in angiogenic and inflammatory endothelial processes as well as the involved pathways during BMP2K/AAK1 silencing and C81 treatment will be further elucidated.
Skin model for testing autoinjectors administering highly viscous formulations

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Biopharmaceuticals have reached an important role in the drug market. Two-thirds of all drugs in development are biopharmaceuticals [1]. Because of their high molecular mass they cannot easily permeate through biological barriers like skin, mucous membranes, or cell membranes. In addition, proteins are not stable in the gastrointestinal tract. The only way to administer biologicals is the parenteral route [2]. Several biologicals have to be self-administered by the patients into subcutaneous tissue. Compared to intravenous application the patient can self-administer the doses by a special device, like an autoinjector and can therefore reach a higher level of self-management [3].

We present a tool-box which is intended to optimize the drug delivery task for new drug-device combinations. The box consists of the following set of model building blocks intended for bio-relevant testing at the bench.

- Research grade auto autoinjector that is designed for multiple use treatment and that can deliver different viscous formulations, with different volumes and injection speeds in one device.
  - Technical data of the autoinjector
    - Volume: 1ml, 2ml
    - Viscosity: 1cP, 40cP
    - Flow rate: 1ml/s, 0,1ml/s, regulated by spring forces (up to 90N possible)
- Animal skin as a bridge to in-vivo conditions
- Planning of a multi-factorial placebo study in human volunteers to evaluate the influence of viscosity, volume and injection speed on the pain perception and the liquid distribution documented by ultrasound measurements.

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References:
The importance of dermal fibroblasts for adequate skin differentiation and homeostasis is widely discussed and believed to play significant roles in stimulation of keratinocyte proliferation and re-epithelialization. Current research indicates that the expressions of type IV collagen and laminin-5, the latter of which is synthesized by keratinocytes alone, are delayed and reduced in the absence of fibroblasts. Indeed the formation of a histologically typical epidermis is dependent on the presence of fibroblasts; skin equivalents generated from keratinocytes alone develop only three of the four viable cell layers and form a thinner than normal stratum corneum. Recent work indicates that expression of filaggrin in epidermal keratinocytes is regulated by dermal fibroblasts. However, little is known regarding the effects of fibroblast–keratinocyte interactions on the skin barrier formation.

Here, we generate skin equivalents with and without fibroblasts to gain a more fundamental understanding of the role fibroblasts play in adequate skin differentiation, skin homeostasis and the skin barrier function. Normal human keratinocytes and fibroblasts were isolated from juvenile foreskin, cultivated in vitro, and used for the construction of the skin equivalents generated according to a previously published procedure. Additionally, keratinocytes were seeded on top of the acellular dermal equivalent to generate equivalents lacking fibroblasts. Equivalents were cultivated over two weeks before they were processed for further analyses.

Skin equivalents generated without fibroblasts showed a reduction in the thickness of the dermis as compared to controls. Protein and mRNA expression levels of the essential cornified envelope proteins filaggrin and involucrin, plus the tight junction proteins occludin and claudin-1, were increased in the absence of fibroblasts. This might suggest that an upregulation of barrier proteins occurs to compensate for other barrier dysfunctions, or that fibroblasts play a direct role in their downregulation in epidermal keratinocytes. On-going work aims to resolve the precise mechanisms by which fibroblasts influence the formation of the stratum corneum with regard to lipid composition and organisation. Furthermore, investigations into signalling pathways involved with the here identified keratinocyte–fibroblast interactions will be conducted.

By comparison of normal skin equivalents and skin equivalents without fibroblasts, we examined a significant influence of fibroblasts on the proliferation and differentiation of keratinocytes. Our data indicate that for the correct formation of the cornified envelope of corneocytes and the tight junctions between them, interactions between epidermal keratinocytes and fibroblasts play an essential role. These findings could help us to understand the physiology and pathophysiology, and may lead to the identification of new signalling pathways that could be targeted in several skin diseases demonstrating deficient barrier formation.

References:
Application of the Patient-Reported Outcomes version of the Common Terminology Criteria for Adverse Events (PRO-CTCAE™) within a pharmaceutical care service on the oncology ward

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Background and Objective: Pharmaceutical care is a valuable service to optimise individual drug therapy and to ensure medication safety. Including patient's perspective has become an increasingly important component of adverse event reporting. The National Cancer Institute has developed a Patient-Reported Outcomes version of the Common Terminology Criteria for Adverse Events (PRO-CTCAE™) to consider patient's perspective more systematically. We aimed to assess its contribution to a pharmaceutical service on the oncology ward.

Setting and Method: A research pharmacist and an oncology pharmacist (pharmaceutical team) provided the pharmaceutical care service for four months on the oncology ward at the University Hospital RWTH Aachen, Germany. The service included conducting an extended medication history, medication reconciliation and medication safety review, and providing pharmaceutical recommendations to the medical team and patients upon detection of DRPs. The most important tools used were the German PRO-CTCAE item questionnaire, the Adverse-Drug-Reaction Risk Score, the APS-Doc classification system for DRPs in the hospital setting and the Doku-PIK documentation system for pharmaceutical interventions in the hospital setting. Statistical analysis was performed using IBM SPSS Statistics 24.

Main outcome measures were type, frequency and occurrence of DRPs leading to an intervention and the proportion of pharmaceutical recommendations influenced by PRO-CTCAE.

Results: 101 patients were included in the study. Median time on the ward was seven days. The most frequent cancer diagnosis were malignant neoplasm of bronchus or lung (23.8 %), diffuse large B-cell lymphoma (15.8 %), and multiple myeloma (13.9 %). 65.3 % of patients had a palliative therapy regimen and 59.4 % of patients were in the first or second chemotherapy cycle. On average each patient had two DRPs leading to an intervention. The most common DRPs according to APS-Doc were related to potential drug-drug interactions (53.4 %), to drug dosage (13.6 %), to drug prescription/monitoring (8.4 %), to an indication (7.3 %), and to a contraindication (5.2 %). 89 % of DRPs occurred on the ward, 8.4 % before admission and 2.6 % on admission.

According to Doku-PIK, the pharmaceutical team provided the recommendation mostly on symptom surveillance (32.8 %). Attending physicians and nurses received additional information in 20.5 % of pharmaceutical recommendations. Pharmaceutical team initiated additional diagnostic tests in 14.9 % of recommendations. Further, in 12.8 % of the recommendations there was a need to discontinue or interrupt drug treatment, in 12.3 % to change the drug.

For 13 patients (12.9 %) recommendations were based on the PRO-CTCAE items. Implementation rate of pharmaceutical recommendations was 93.2 %. In the multivariate Poisson regression model, number of drugs, comorbidity and previous adverse drug reactions were shown to be significant factors predicting the DRP pattern.

Conclusion: Our results show that the PRO-CTCAE questionnaire may support pharmaceutical care for cancer patients. Furthermore, pharmaceutical care is a feasible approach to identify and reduce DRPs on the oncology ward. The high acceptance of pharmaceutical recommendations indicates the need for oncology pharmacists on the ward.
Influence of electrospray ionization on mass spectrometry analysis of cysteinyl leukotrienes and eoxins

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Pro-inflammatory glutathione (GSH)-conjugates comprise the cysteinyl-leukotrienes (cys-LTs) and eoxins (EX), both formed in immunocompetent cells. Cys-LTs are produced by 5-lipoxygenase (5-LOX) from arachidonic acid (AA) yielding the unstable epoxide LTA₄ that is subsequently conjugated with GSH by LTC₄ Synthase (LTC₄S). LTC₄ is processed in the extracellular space by sequential cleavage of the tripeptide side chain to yield LTD₄ and LTE₄. For EXs formation, AA is metabolized by 15-LOX-1 to the hydroperoxide 15-HPETE, that is converted by LTC₄S yielding EXC₄ (=14,15-LTC₄). EXC₄ was also shown to be processed to EXD₄ and EXE₄.

Previously, GSH-conjugates were analyzed by HPLC-MS/MS with negative electrospray ionization used to be advantageous for eicosanoids. However, the revealed product ion scans for GSH-conjugates were devoid of significant structural information and further investigations were required to distinguish between LTC₄ and EXC₄.

The main purpose of our work was to establish an UPLC-MS method for detection of pro-inflammatory GSH-conjugates. Both conjugates, LTC₄ and EXC₄, could be separated by gradient elution with acetonitrile and water as mobile phases, but mass spectrometric analysis carried out in negative ionization mode revealed undistinguishable MS/MS spectra. However, positive ionization [1] gave characteristic fragmentation and structural information for LTC₄ and EXC₄.

Taken together, we established a UPLC-MS/MS method carried out in negative and positive electrospray ionization to detect pro-inflammatory GSH-conjugates. Analysis in negative ionization mode is useful for samples containing GSH-conjugates together with other eicosanoids, whereas positive ionization mode is indispensable for structural distinction of GSH-conjugates.

Sex bias in lipid mediator biosynthesis during acute inflammation and resolution

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Acute inflammation is generally a self-limited process in which lipid mediators (LM) play a pivotal role. LM, derived from the omega-6 polyunsaturated fatty acids (PUFA) like leukotrienes (LT) and prostaglandins (PG), are potent enhancers of innate and adaptive immunity during the acute phase of the inflammatory process [2]. These pro-inflammatory LM are mainly produced by the 5-lipoxygenase (5-LO) and cyclooxygenase-2 (COX-2) enzymes. However, the omega-3 PUFA-derived specialized pro-resolving mediators (SPM) such as resolvins, maresins, and protectins are LM that regulate the resolution process and are mainly products of the 12/15-lipoxygenases (12-LO, 15-LO) [1].

Due to the disparity in the incidence of immune disorders between males and females, several studies were focused on the role of sex in inflammation. We have recently demonstrated superior LT biosynthesis in human neutrophils, monocytes and in mouse macrophages from females and we confirmed these sex differences in vivo in a model of acute inflammation such as mouse zymosan-induced peritonitis [3].

In this study, we report sex differences in the production of a broad range of pro-inflammatory and pro-resolving LM during the acute and the resolving phases of inflammation. We performed a time-dependent mouse zymosan-induced peritonitis up to 24 hours to evaluate the differences in LM production in the two sexes. Significant higher amounts of LM produced by 5-LO, 12-LO, 15-LO and COX-2 were found in the peritoneal cavity of male compared to female animals under healthy conditions. Interestingly, the expression of the main enzymes involved in LM biosynthesis did not differ between the two sexes. Four hours after zymosan administration, a significantly higher production of 5-LO metabolites was found in exudates from males, whereas more 12-LO metabolites were produced in females. Note that a higher cell afflux in female peritoneal cavity was evident as compared to males after 4 hours [3]. No differences were evident at 24 hours for the LO metabolites, whereas greater activity of COX-2 was found in males, with PG formation being significantly higher compared to females. Accordingly, at this time point, the number of cells in the peritoneum of females was significantly lower as compared to males.

Conclusively, our data clearly demonstrate that sex is an important variable in the biosynthesis of LM with consequences for the resolution and possible implication for therapies.

Characterisation of novel phospholipid-based injectable depot formulations for controlled drug delivery

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Besides commonly employed methods for drug administration, such as oral, dermal or pulmonary routes, parenteral injections are often chosen to administer drugs with low absorption or bioavailability issues. Long-acting injectable formulations, such as parenteral depots, offer additional advantages in comparison with conventional formulations of the same active principle, such as a better patient compliance and a predictable drug-release profile upon injection. Furthermore, the fluctuations in drug blood level can be smoothed and thereby side effects could be remarkably reduced [1]. In the last years several depot formulations enabling release kinetics from days to months were developed. Some technologies on the market or in clinical studies are based on phospholipids, like multivesicular vesicles (DepoFoam®) [2], a lipid-based in situ forming depot (FluidCrystal®) [3] or a proliposomal oil [4]. An alternative approach to induce depot formation may rely on the use of large unilamellar liposomes formulated with negatively charged phospholipids to prompt a controlled aggregation with polyvalent cations. Although the aggregation behaviour of this class of phospholipids in presence of cations such as calcium or magnesium ions was already described [5, 6], so far its use as depot formulation for controlled drug delivery has not been investigated in a detailed manner.

The aim of this study is to develop an alternative technology for lipid-based depot injectables in contrast to the existing manufacturing processes. Different negatively charged phospholipids were screened with respect to their ability to form depots. The physico-chemical properties of the aggregates were characterized in order to evaluate the influence of the different phospholipids on the aggregation behaviour and the aggregate properties.

A series of negatively charged phospholipids with glycerol-, serine- or phosphatic acid head groups were mixed in different compositions with the zwitterionic phospholipid L-α-phosphatidylcholine. Liposomes were prepared by film hydration method and subjected to freeze-thaw-cycles. Upon extrusion, homogenous large unilamellar vesicles with a mean diameter of 150 nm were obtained, as measured by dynamic light scattering. As expected, with increasing amounts of negatively charged phospholipids a decrease in the zeta potential values of the formulations was observed. The extent of aggregation in presence of calcium or magnesium, assessed by turbidity measurement (OD_{400}), suggested that the aggregation profile of the tested formulations is dependent on the nature of the phospholipid head group. Particularly, distearoyl phosphatidyl glycerol and dipalmitoyl phosphatidic acid showed a noteworthy aggregation tendency with both cations. A morphological characterization via cryogenic transmission emission microscopy (cryo-TEM) revealed a tendency of the formulations to aggregate in presence of cations without fusing, as also confirmed by a terbium/dipicolinic acid fusion assay.

In conclusion, the two negatively charged phospholipids with the most pronounced aggregation behaviour could be possible candidates for a depot formulation. Further investigations, such as active and passive drug loading and release studies based on these liposome formulations, are currently ongoing.

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References
Distinct cardiac metabolic patterns in two mouse models of diseased heart

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Cardiac hypertrophy is associated with changes in cardiac metabolism. However, the cause of these changes is not completely understood. The cAMP regulated transcriptional coactivators (CRTC) are known to regulate metabolism [1]. Our previous data show that CRTC1 is regulated by β-adrenergic signaling in cardiomyocytes.

In the present study, the influence of CRTCs on cardiac metabolism was investigated in Crtc1-deficient (KO) mice and in Mybpc3-targeted knock-in (Mut) mice. Mice globally deficient in Crtc1 develop cardiac hypertrophy and dysfunction. Mybpc3-targeted knock-in mice are a well-accepted model for HCM, overexpressing Crtc1.

RNA expression in the hearts of 5-6 week old mice was analyzed by RT-qPCR. Analysis showed a reduced mRNA content of PGC-1α (peroxisome proliferator activated receptor γ coactivator 1-α) and PPARα (peroxisome proliferator activated receptor α) by 31±6% and 46±5% (n=7) respectively, in Mut mice compared to KO mice. PPARα and PGC-1α contribute to the expression of key enzymes necessary for β-oxidation. Nevertheless, the mRNA expression of carnitine palmitoyltransferase 1B (CPT1b), necessary for fatty acid uptake into the mitochondrion, was increased in Mut mice by 42±6% (n=7). Furthermore, PGC1α mediates mitochondrial biogenesis. Consistent with PGC1α mRNA reduction, the mt-Nd1 (mitochondrial NADH dehydrogenase 1) mRNA expression was reduced by 52±7% (n=7) in Mut mice compared to WT. Examining pathways of glucose metabolism, decreased mRNA expression of the regulatory subunit of the pyruvate dehydrogenase phosphatase (PDPγ) by 31±4% (n=7) was found in Mut mice. Comparison between WT and Mut mice showed reduced expression of the insulin dependent glucose transporter GLUT4 in Mut mice. Together, these findings point at an increased fatty acid metabolism and reduced glucose metabolism in the Mut mice. No differences in mRNA expression of GLUT1, AMPK and Tfam were found. As a marker of AMPK activity, phosphorylation of acetyl-CoA carboxylase (ACC), as substrate for AMPK, was analyzed by immunoblot analysis. No difference in ACC phosphorylation was detected. There were no differences in the mRNA expression concerning genes involved in metabolism in the KO mice. Crtc3 mRNA was increased in both the Mut and KO mice compared to WT mice. The mRNA content of Crtc2 was unchanged in the Mut mice but increased by 46±9% (n=6-7) in the KO mice. The Crtc1 deficiency might be compensated by a higher Crtc2 expression in the KO mice. This could contribute to an attenuation of the changes in cardiac metabolism.

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Medication Reviews for Patients in Long-Term Care Facilities

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**Background**
Drug-related problems (DRP) are common in the elderly due to polypharmacy [1]. Community pharmacies supplying drugs to long-term care (LTC) facilities may play an important role in detecting and solving DRPs in LTC residents.

**Objectives**
This study aimed to evaluate the results and the quality of medication reviews (MRs) provided by community pharmacists for residents of LTC facilities and to derive recommendations for the implementation of MR services into routine healthcare practice.

**Methods**
Patients insured by AOK Rheinland/Hamburg (AOK), aged at least 65 years and taking regularly five or more drugs per day were invited to participate in the study. Pharmacists supplying LTC facilities in North Rhine Westphalia could apply for participation and obtained a special training focusing on DRPs in the elderly. Based on the medication history, they performed a ‘simple MR’ and documented all identified DRPs and interventions. Their report was sent to the AOK and remunerated once per patient. ‘Documented DRPs’ were compared to ‘Reference DRPs’ obtained by MRs performed by two experienced clinical pharmacists retrospectively. The agreement rate between reference DRPs and documented DRPs served as a surrogate to assess the quality of the MR service. To evaluate the feasibility, an acceptance analysis was conducted.

**Results**
A MR was performed for 94 patients by one of 12 community pharmacies. The residents’ mean age was 84 years, 66% were female. On average, they took 13 different drugs per day, self-medication included. 35% had at least one regularly scheduled drug considered as potentially inappropriate in the elderly (PIM) according to the PRISCUS list. The pharmacists documented 154 DRPs (mean 1.6, SD 1.5) of which the most common were drug-drug interactions (40%), followed by PIM (16%) and inappropriate dosages (14%). The mean number of DRPs differed between the pharmacies and ranged from 0.5 to 3.5 per patient. Documented interventions (n=129) mainly concerned monitoring issues (25%), followed by recommendations to change a dosage regimen (19%) or to stop a certain drug therapy (18%). The physicians’ acceptance rate of recommended medication changes varied between pharmacies ranging between 0-86%. Out of 235 reference DRPs, 36% were concurrent with the documented DRPs, the highest agreement rate concerned potential serious drug-drug interactions. Regarding the agreement rate, a high variability was found between the pharmacies (15-100%). Of all documented DRPs, the clinical pharmacists considered only five (3%) to be incorrect. Although limited time resources were claimed by 7 of 11 pharmacists, MRs were mainly regarded as feasible.

**Conclusions**
The results indicate that many DRPs can be detected in LTC residents even by a MR based solely on medication history. Most of the documented DRPs were drug-drug interactions. For the detection of other types of DRPs more training may be needed. Besides, the relatively low physicians’ acceptance rate of interventions suggests that pharmaceutical MR services may benefit from a closer cooperation between pharmacists and physicians.

**Acknowledgments**
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**References**
Development of a peptide macrocycle FXIIa inhibitor for safe anticoagulation therapy

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Inhibiting coagulation factor XII (FXII) has been shown to reduce thrombosis in various animal models without increasing the risk for bleeding [1], a major problem of current anti-coagulants. A range of natural protein inhibitors of FXIIa as well as monoclonal antibodies have been used in animal models of thrombosis [1,2,3], but no high affinity small molecule inhibitor has been reported until recently. In our laboratory, we have evolved by phage display a potent and highly selective FXIIa inhibitor based on a macrocyclic peptide format (MW < 2000 Da) [4]. Furthermore, we have improved the potency and plasma stability of the inhibitor using unnatural amino acid incorporation [5,6]. The final peptide shows inhibitory affinity in the picomolar range while retaining the high selectivity over physiologically relevant homologous proteases, and we achieved a high stability in plasma by replacing preferable cleaved positions. The final inhibitor prolongs the intrinsic coagulation measured by the activated partial thromboplastin time (aPTT) in human, mouse and rabbit plasma (EC50 human = 1 µM). In a FeCl3-induced thrombosis model in mice, the peptide could reduce thrombosis substantially while it showed no abnormal bleeding. Our results suggest that FXIIa inhibition by a peptide macrocycle can potentially offer a safe anticoagulation therapy. Pharmacokinetic studies in mice and rabbits showed a short plasma half-life, most likely due to fast glomerular filtration. Currently we are working on several strategies to prolong the circulation half-life of the inhibitor by conjugation to an albumin-binding tag developed in our lab [7] or through PEGylation.

2-Substituted indole-3-carbonitriles as new DYRK1A inhibitors

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The dual-specificity tyrosine phosphorylation regulated kinase 1A (DYRK1A) is highly expressed in the brain. It plays an important role for neuronal development, neurogenesis and normal brain function. The DYRK1A gene is located on chromosome 21 in the Down syndrome critical region [1]. The overexpression of DYRK1A leads to hyperphosphorylated tau protein, which forms neurofibrillary tangles, and formation of neurotoxic β-amyloid plaques. DYRK1A is an interesting target for the development of new drugs because of its role in Down syndrome and Alzheimer pathomechanisms [2].

In our group 10-iodo-11H-indolo[3,2-c]quinoline-6-carboxylic acid (1) was developed as a potent inhibitor of DYRK1A (IC50 = 6 nM) with considerable selectivity compared to closely related kinases of the CLK and DYRK families. Unfortunately, the inhibitory activity against DYRK1A in cell-based assays was significantly lower, probably because of poor membrane permeability [3].

Starting from 7-chloro-1H-indole-3-carbonitrile (2) as a small analogue of the 10-iodo-11H-indolo[3,2-c]quinoline-6-carboxylic acid, a fragment-based design was performed to develop new potent DYRK1A inhibitors with improved water solubility and reduced molecular weight. In the presentation, the synthesis and kinase inhibitory activity of 2-substituted indole-3-carbonitriles and also the results of docking experiments will be presented.

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Tables made from paper – a simple method for improved drug delivery

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Introduction: Today, most of the new chemical entities (NCE) possess poor solubility. Many strategies were developed in the past to overcome this issue which employ the use of cyclodextrines, micelles, liposomes or drug nanocrystals. Recently a new method to improve the solubility of poorly soluble actives was introduced. The so-called smartFilm®-technology uses commercially available paper. The paper is loaded with active by adding a solution of active in a solvent to the paper. Upon drying of the paper, the active remains in the pores of the paper in amorphous state leading to an increase in dissolution velocity and kinetic solubility. However sheets of paper are not convenient for oral administration, therefore it was aimed at transferring the paper into an easy to swallow solid oral dosage form by compressing paper into tablets.

Materials and Methods: Different sorts of paper were obtained from local supermarkets, were cut into small pieces and compressed by a single punch tablet press (EK0, Korsch, Germany). Resulting paper tablets were tested according to the requirements of the European Pharmacopeia 8.0, e.g. resistance to crushing, friability, disintegration. In a second step drug loaded tablets were produced. Caffeine (model drug) was dissolved in water and this solution was added dropwise onto the paper. After drying, the paper was processed as described above. In addition to the tests performed previously, disintegration and drug release were determined. Furthermore, scanning electron microscopy (SEM) (Hitachi S-510, Hitachi High-Technologies Europe GmbH, Germany) and differential scanning calorimetry (DSC) (DSC 7, PerkinElmer, USA) were performed to evaluate the drug localisation within the paper and the crystallinity of caffeine, respectively.

Results and Discussion: From all sorts of paper tablets were obtained by simple compression without further excipients. Tablets appeared glossy and with a smooth surface [Fig. 1]. Loaded and unloaded tablets fulfilled the requirements of the European Pharmacopeia. However, drug release was found to be slightly influenced by the type of paper. SEM images confirmed the assumption, that the localisation of the active within the paper is different, because each type of paper possesses different properties, i.e. hydrophilicity, pore size and pore density [Fig. 2A/B] and thus leads to a different localisation of the active. Fig. 3 shows the localisation of caffeine in disposable washing cloth [Fig. 3A] and in envelope [Fig. 3B]. The envelope possessed low hydrophilicity, a small pore size and relatively low pore density. In contrast, higher pore density and larger pores were seen in case of disposable washing cloth. Consequently, only very small amount of caffeine could be loaded into the pores of the envelope. Hence, most of the caffeine was located on top of the paper and re-crystallised as large needles [Fig. 3B]. In case of the disposable washing cloth, caffeine was located within the paper and could not form large crystalline needles [Fig. 3A]. DSC measurements confirmed the absence of a large crystalline melting peak. Therefore, it can be concluded that caffeine was – at least partly – incorporated in amorphous state into the disposable washing cloth, but not in the envelope.

Conclusion: Tablets made from paper - unloaded and drug loaded - can be easily obtained from various sorts of paper without further excipients and possess good pharmaceutical quality. In paper with large pore size, due to high hydrophilicity and high pore density the active remained in the pores of the paper in amorphous state and led to an increase in dissolution velocity. In fact, tablets made from paper are a promising method for improved drug delivery, especially for poorly soluble actives.
Differential regulation of the c-fos gene promoter by dual leucine zipper kinase (DLK)

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In our aging society diseases such as diabetes mellitus type 2, neurodegenerative, and cardiovascular diseases will gain more importance and the need for improving existing therapies or finding new targets will increase. Although our understanding of neurodegenerative conditions such as Alzheimer's disease has improved, the underlying pathomechanisms are still incompletely understood. The dual leucine zipper kinase (DLK) is expressed predominantly in brain, neurons and also in the β-cells of the endocrine pancreas. Upon stimulation this mitogen activated protein triple kinase (MAP3K) leads to seemingly contradictory responses in the nervous system, ranging from apoptosis to axon regeneration after injury [1]. The importance of DLK for the developing brain is highlighted by the finding that DLK knockout mice die perinatally [2]. However when the DLK is deleted in adult mice no gross abnormalities are observed. Our previous work showed that the DLK is involved in the pathogenesis of diabetes type 2 [3]. Studies found that patients with diabetes type 2 have an increased risk for developing Alzheimer's disease [4, 5]. Recent findings indicated that the activation of DLK contributes to the pathogenesis of Alzheimer's disease through a new non-canonical pathway leading to increased phosphorylation of c-Fos protein [6] which is a transcription factor and is used as marker for neuronal activity [7]. In the present study the effect of the DLK on the transcriptional activity of the c-fos promoter was investigated. To this aim transient transfections into the electrically excitable cell line HIT and a luciferase reporter gene assays were performed. DLKwt, an empty vector and a kinase dead mutant of DLK, were co-transfected with a luciferase reporter gene under control of the human c-fos promoter (-711/+51). DLKwt but not the kinase dead mutant of DLK, increased c-fos transcriptional activity 2.6 fold. When cells were treated with KCl and forskolin c-fos promoter activity increased 7.9 fold; no further enhancement in promoter activity by DLKwt was detected. To identify the DLK responsive elements 5' and 3' deletions were studied. There are 5 well described binding elements in the c-fos promoter, the cis inducible element SIE, the serum response element SRE containing the binding sites Ets and CArG, the AP-1 binding site, and the c-AMP response element CRE [8]. After transfection into HIT cells the activity of the c-fos promoter in full length compared to the deletions was investigated. 5' deletion had no influence on basal promoter activity. Deletion of the proximal promoter elements enhanced promoter activity whereas further 3' deletion profoundly reduced it. The stimulatory effect of DLK on c-fos gene promoter activity was lost after 5' deletion of the SIE element. 3' deletion of the proximal promoter elements diminished the DLK responsiveness. The present data show that DLK enhanced c-fos gene promoter activity and indicate that SIE, known to bind STAT family members, and a proximal promoter element confer the stimulatory effect of DLK.

3. Oetjen E.: Archiv der Pharmazie 2016; 349(6) :410 -413
Physiologically-based pharmacokinetics/pharmacodynamics (PBPK/PD) systems pharmacology model of glucose homeostasis in human

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Introduction: The complex pathophysiology of type 2 diabetes mellitus (T2DM) raises demands for individualized treatments. A systems pharmacology approach combined with physiologically-based (PB) pharmacokinetics/pharmacodynamics (PK/PD) modeling can help to better understand the underlying processes and aid the development of new drugs and treatments at various disease stages. Our goal is to develop a PBPK/PD model of glucose homeostasis in human that captures the most relevant processes to assess personalized disease progression and treatment outcomes.

Methods: PB Glucose Homeostasis Model (GHM) was developed with PK-Sim® and MoBi® as part of the Open Systems Pharmacology Suite (OSPS), version 7.1 [1]. The model includes the relevant compounds glucose, insulin, glucagon, GLP-1, and GIP. Literature knowledge about human physiology and glucose metabolism and regulation was used for model development. Values of parameters describing PK of modeled compounds and PD effects were identified by fitting the model to concentration-time profiles and production rates from various perturbation experiments involving exogenous administrations of the modeled substances.

Results: The developed model combines 9 PBPK sub-models (endogenous and exogenous glucose and insulin, c-peptide, GLP-1, GIP, glucagon, and dapagliflozin) and contains 1834 ordinary differential equations (ODE). Published experimental data from 72 sources were used for identifying the parameters describing PD of the system. Due to the high complexity of the model, different processes were parametrized sub-sequentially.

The final model describes regulation of glucose production and uptake in healthy individuals at various mechanistic levels. In the liver, glucose is produced via gluconeogenesis (GNG) and glycogenolysis (GIL). Simultaneously, glucose is taken up from the bloodstream and stored as glycogen; the balance between hepatic glucose uptake and production is given by the effects of glucose, insulin, and glucagon on the respective processes. In the kidneys, a mechanistic representation of glomerular glucose filtration and reabsorption, facilitated by the transporters SGLT1/2, is implemented [2]. Additionally, glucose is produced via GNG in the cortex and metabolized by the cells in the medulla. In the peripheral tissues fat and muscle, glucose is taken up through insulin-dependent transporter GLUT4.

A mechanistic model of insulin receptor is implemented in insulin-sensitive tissues for receptor-mediated insulin degradation and PD of the hormone. Effects of insulin on GNG, GIL, glycogen synthesis, and GLUT4 trafficking depend on the phosphorylation level of insulin receptor substrate 1 (IRS1).

Further implemented processes are insulin secretion, production and degradation of glucagon, and PBPK models of the incretin hormones GLP-1 and GIP, including their secretion in intestinal mucosa and PD effects on insulin and glucagon secretion.

The GHM is able to reproduce concentration-time profiles of glucose, insulin, and glucagon, observed in various perturbation experiments, including intravenous (iv) glucose tolerance tests (IVGTT), iv insulin tolerance tests (IVITT), continuous insulin infusion tests, and intraduodenal glucose infusion protocols as well as oral glucose tolerance tests (OGTT).

Conclusion: The developed GHM describes regulation of glucose balance at high level of detail, while maintaining agreement with observed data from different sources. The implemented processes provide interfaces for modeling the pathology of T2DM and modes of action of anti-diabetic medications. Provided a comprehensive individual dataset of perturbation experiments, the model could be further trained to create a T2DM population and predict individual disease progression.

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Presented at: PAGE 2017, Budapest, Hungary
4 POSTERS
4.1 Analytics

**POS.1**

**Determination of pre-existing anti-PEG antibodies in pediatric patients with ALL and the effect on PEG-Asparaginase activity.**

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Introduction: Polyethylene glycol (PEG) is often attached to therapeutic proteins to improve their pharmacoconomic properties [1]. Anti-PEG Antibodies (ABs) have been detected at ranges from 0.2% to 22-44% in healthy individuals [2-5] and were able to compromise the safety and effectiveness of PEGylated proteins [3,6,8]. Objective: In pediatric patients with acute lymphoblastic leukemia treated according to the AIEOP-BFM ALL 2009 protocol, we evaluated the prevalence of anti-PEG ABs prior to first PEG-asparaginase (PEG-ASNase) administration and their influence on PEG-ASNase activity after first PEG-ASNase administration. Patients and Methods: Anti-PEG AB samples were collected from 680 patients (m/f, 404/276, age: range 1-18, median 5.5 years) and determined as reported by Armstrong et al. with slight modifications (3). PEG-ASNase activities were analysed on day 7±1 (n=567) and on day 14±1 (n=579). Cut offs for IgG, IgM anti-PEG ABs were determined with samples from 58 infants aged ≤1 year (Anti-PEG IgG Optical density value (OD)≥8, Anti-PEG IgM Od≥2). Results: Prior to first PEG-ASNase administration a prevalence of 10.9% for both ABs (IgG, IgM) was detected. On day 7±1 as well as on day 14±1 PEG-ASNase activities were significantly lower in samples with anti-PEG AB OD values above the cut-offs, compared to samples with OD values below the respective cut-offs (Wilcoxon rank-sum test, day 7±1: median 51, 156 U/L – 1681 U/L vs. 883 U/L (486, 0 U/L - 2959 U/L), p=0.03; day 14±1: 430 U/L (80, 42 U/L - 961 U/L) vs. 536 U/L (519, 0 U – 1809 U/L), p<0.001). The trend to lower PEG-ASNase activity was not associated with complete loss of activity. Conclusion: 10.9% of pediatric patients with ALL showed anti-PEG ABs over the cut off prior to first PEG-ASNase exposure, which seemed to influence the PEG-ASNase activity. Though no complete loss of PEG-ASNase efficacy was observed, the clinical relevance of the anti-PEG ABs for the ASNase therapy needs further investigation.

References


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**POS.2**

**Different in vitro and in vivo tools for elucidating the human metabolism of α-cathine derived drugs of abuse – A comparison study for developing toxicological screening procedures**

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A stability-study of expired ampoules manufactured more than 40 years ago and use of stability assurance analytical methods

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Pharmaceutical manufacturers have to study the stability of drug products before marketing according to the ICH guideline Q1A(R2); data of those investigations aim to set expiry dates of finished products. The expiry date on the container of a remedy assures the physician and the patient the stability of the drug formulation i.e. within a specification of 95% to 105% until expiry [1]. Only a few studies show that shelf-lives of pharmaceutical products are often longer than expiration dates shown on the container [2-4]. Investigating drug stability of finished products includes storage conditions like temperature and humidity. The objective of the study presented here was the determination of the content of nine expired ampoules manufactured in the 20th century and the identification of the impurity profile by means of HPLC-UV and HPLC-MS, respectively. The ampoules are part of a collection of long expired finished pharmaceutical products at IBMP, Nürnberg-Heroldtschild (Germany), and consists among others of epinephrine and Adrenaline in Oil, ephrine (Effortil®), synephrine (Sympatil®), caffeine and procaine (Impeltil®), caffeine and sodium salicylate (Caffeinum Salicylicum), dipyrindamole (Persantin®), furosemide (Lasix®), and metamizole (Impletol®). For determination of the active ingredient and drug products methods of the European Pharmacopoeia were used. For determination of method the contents were validated for linearity, precision, and accuracy. The results were compared to current reference ampoules. Five of nine ampoules were still within the limits of specification of content. In Suprarenin and Adrenalin in Oil, both containing epinephrine, Impeltil (procaine), and Persantin® (dipyrindamole) contents were decreased to 70%, 74%, 79%, and 86%, respectively, and therefore out of specification. In the present contribution, the analytical results will be presented.

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POS.3

Optimization of microwave-assisted acidic hydrolysis of peptides and proteins by GC-MS

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Proteins and peptides have characteristic amino acid compositions. Certain fields such as the food industry, clinical diagnostics and pharmaceutical quality control of therapeutic proteins and peptides require a reliable and accurate compositional analysis. Liberated amino acids can be qualitatively and quantitatively analysed by liquid or gas chromatographic methods [1]. To perform an accurate amino acid compositional analysis, the conventional approach includes protein hydrolysis by treatment with 6 M hydrochloric acid at 110°C for 24 h [2]. However, the main disadvantage of this classical approach is the long hydrolysis time. Furthermore, degradation of certain amino acids was reported [3].

To overcome these obstacles, a microwave assisted acidic hydrolysis procedure was tested and crucial parameters such as temperature, time and microwave power were optimized. The major advantage of a microwave-assisted hydrolysis compared to the classical heat block approach is an enormous reduction of sample preparation time. The best results were obtained applying a temperature of 150°C, 300 W, and a hold time of 15 min.

Method optimization was performed with bovine serum albumin (BSA) as a test protein. The final procedure was applied to three therapeutic peptides which partially consist of uncommon and isobaric amino acids. The performance of the developed procedure was validated by GC-MS and UHPLC-QTOF-ESI-MS using norvaline as an internal standard for quantification. Separation of isobaric amino acids was achieved by GC-MS using a chiral Chirasil-L-Val column.

References:

POS.4

Development of an amidoxime-reductase assay

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The mitochondrial amidoxime reducing component (mARC) is the fourth human molybdenum containing enzyme and identified recently in our lab. The three component system consisting of mARC, cytochrome b5 and t NADH depending cytochrome b5 reductase is able to reduce endogenous and xenogenic N-hydroxylated compounds [1].

Due to the recent discovery the enzymology has not been investigated in greater detail so far. Until now the detection of possible substrates is performed by HPLC after incubations and work-up procedures. However, the development of a suitable HPLC method for each substrate is very time consuming. Also a variety of interesting potential substrates are not measurable via standard HPLC detectors. A more convenient way of detection is the photometric decrease of NADH. If such an assay is positive a more detailed analysis could follow.

For the validation of this method biotransformation assays with recombinant human proteins were performed and compared with the turn over rate of the model compound benzamidoxime (see figure 1). Rates of benzamidoxime reduction were determined by HPLC and also based on the molar extinction coefficient of NADH. As similar results for benzamidoxime were obtained other known substrates of mARC like guanoxanbenz or benzhydroxamic acid were tested. Again the HPLC results could be confirmed with the new assay. Taking this in account we investigated possible substrates that had not been verified yet. By this method new substrates were detected and the corresponding transformations are now under investigation.

In summary a fast and reproducible method for the detection of new substrates of mARC was developed leading already to so far unknown transformations.

References:
Towards the development of a capillary electrophoresis based Asparaginase assay

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Acute lymphoblastic leukaemia is the most common haematologic disorder in children and various treatment protocols were developed and are still being developed. L-Asparaginase, an amidohydrolase most common in microbes which generates ammonia and aspartic acid out of asparagine, is a core agent used in the majority of these protocols for several decades [1]. It hypothetically acts by selectively impairing protein biosynthesis in leukemic blasts and is thus complementary to conventional chemotherapeutics [2].

Currently, there are three different drugs approved for use in the EU. Native Asparaginase derived from Escherichia coli (ASNase), Asparaginase derived from Erwinia chrysanthemi and a polyethylene glycol-conjugated E. coli Asparaginase (PEG-ASNase). Hypersensitivity reactions can occur occasionally leading to therapy failure. The development of PEG-ASNase improved utility as the coupling of polyethylene glycol prevents the enzyme of being cleared by the mononuclear phagocyte system and also results in significantly less antibody formation as well as a reduced clearance leading to less frequent administration [3].

While anaphylactic reactions towards the enzyme are identified by clinical symptoms of the patient, detecting so called silent inactivation without any sign of an immunological reaction still remains a challenge. Several methods are currently in use or discussed in research, ranging from activity assays and detection of anti-ASNase or anti-PEG antibodies to ammonia levels. They all have a major disadvantage as they only indirectly determine asparaginase by measuring surrogate parameters. Using activity assays, might pose a problem as there is baseline asparaginase activity found in several species – among them also humans (e.g. by asparaginase-like protein 1) [4].

In our lab, we developed a direct assay to simultaneously determine ASNase and PEG-ASNase from a ready-to-use solution using a BeckmanCoulter PAR80plus capillary electrophoresis. Samples were prepared from commercially available and approved drugs and serially diluted. Analysis was carried out by using a 40.2 cm bare-fused capillary (30.0 cm effective length) and 75 µm inner diameter. Buffers with a pH of 7.5 were prepared with Trometamol (120 mM) and triethylentetramine (12 mM), as a dynamic coating to prevent absorption of proteins onto the capillary wall. Perchloric acid was used to adjust the pH to the predefined level. Samples were applied to the capillary by electrokinetic injection with 300 kV*s in reverse polarity mode after introducing a plug of a buffer solution with a lower concentration (10-51 µM). Therefore, we suggested αS1-casein as an inhibitor of the extracellular binding site of TL4/CD14. These findings were supported by binding experiments using microscale thermophoresis (MST).

The human α-casein bound to the purified extracellular TL4/MD2-complex with a KD of 2.2 µM in comparison to LPS binding TLR4/MD2 with a KD of 8.7 µM. Furthermore, we suggested α-casein as an inhibitor of the extracellular binding site of TL4/CD14. These findings were supported by binding experiments using microscale thermophoresis. The human α-casein was recently reported to induce proinflammatory cytokines via Toll-like receptor 4 (TLR4) and by binding experiments using microscale thermophoresis (MST).

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Acknowledgments:

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Characterizing the TLR4 binding-site of αS1-casein by selective inhibitors

Psychoactive Substances (NPS). In the last few years, they were involved in several intoxication cases. Recently, a new group of NBOMe-derived compounds were found on the drug market. Westphal et al. [1] described the analytical properties of four amphetamine-based NBOMes discovered by German custom authorities in a seizure from China. The aims of the presented studies were to elucidate the phase I and II metabolism and the detectability in standard urine screening approaches (SUSA) of 25B-, 25C- and 25I-NBOMe as well as 4-EA-NBOMe using GC-MS, LC-MS^n, and LC-HR-MS/MS.

After application of the compounds to male Wistar rats for toxicological diagnostic reasons (10 and 0.1 mg/kg BW for metabolism and toxicological detection studies, respectively), urine was collected over 24h. In addition, for 25B- and 25I-NBOMe, human urine samples, submitted for toxicology analysis to the author’s laboratory, were analyzed. The phase I and II metabolites were extracted and analyzed directly or after enzymatic cleavage by SPE (HCX) or simple urine precipitation with acetonitrile followed by GC-MS (TF ISQ) after acetylation and LC-HR-MS/MS (TF Q-Exactive Plus) according to Caspar et al. [2-4]. Furthermore, incubations with pooled human liver microsomes (pHLM) or pooled human S9 fractions (pS9) were performed to compare in vivo rat or human with in vitro human metabolism [2-4]. For the detectability studies, our standard urine screening approaches (SUSA) by GC-MS (TF ISQ), LC-MS^n (TF LXQ), and LC-HR-MS/MS (TF Q-Exactive) were applied to rat urine and authentic human urine samples. Finally, initial CYP activity screenings were performed to identify CYP isozymes involved in the major metabolic steps.

The four NBOMes were extensively metabolized, mainly by O-demethylation, N-dealkylation, aryl-hydroxylation, and combinations of them as well as by glucuronidation of the main phase I metabolites in both species. Furthermore, for 4-EA-NBOME oxidation of the ethyl sidechain to benzoic or phenylacetic acid could be found. Intake of the compounds was detectable via its metabolites by both LC-MS SUSAs and for 25B-NBOMe and 4-EA-NBOMe in addition by the GC-MS SUSA. With exception of the oxidation to benzoic acid for 4-EA-NBOMe, all main metabolic reactions could be confirmed in the incubations with pHLM or pS9. Initial CYP activity screening revealed the general involvement of CYP1A2, CYP2B6, CYP2C19, and CYP3A4 in the metabolism of the investigated compounds.

The presented studies demonstrated that all NBOMe derivatives were extensively metabolized in rats as well as in humans and that an intake could be detected by both LC-MS screening approaches and in case of acute poisoning also by the GC-MS SUSA. Since several CYPs were involved in initial metabolic steps, interactions might not be expected.

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References:
4.2 Antiinfectives

Synthesis and biological activity of phenylethylene glycol-derived LpxC inhibitors

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Over the last decades, many bacterial strains have developed multidrug resistance to the common antibiotics as a result of the overuse and misuse of these drugs. The successful treatment of bacterial infections is posing an increasing challenge due to the decreasing development and approval of new antibacterial drugs.1 To avoid a post-antibiotic era, there is an urgent need for the development of new antibiotics with a novel mode of action to circumvent the established mechanisms of resistance. A new promising target for the treatment of bacterial infections caused by Gram-negative bacteria (GNB) is the Zn2+-dependent enzyme LpxC present only in these bacteria. This enzyme catalyzes the deacetylation of UDP-3-[3-hydroxymyristoyl]-N-acetyl-glucosamine, the first committed step in the biosynthesis of lipid A. Lipid A forms the membrane anchor of lipopolysaccharides, which are part of the outer membrane of GNB. The inhibition of lipid A biosynthesis leads to a destabilization of the outer membrane and thus affects the growth and viability of GNB.2 The aim of this project was to synthesize LpxC inhibitors based on the structure of compound 1, which has promising antibacterial and inhibitory activity against the enzyme LpxC of E. coli. To improve the biological activity, the structure-activity relationships of new phenylethylene glycol derivatives should be investigated. To achieve this goal, the Zn2+-chelating hydroxamate moiety, the hydrophobic side chain, which imitates the fatty acid side chain of the enzyme’s natural substrate,3 and the hydrogen bond donor moiety were varied. The antibacterial and LpxC inhibitory activity of these synthesized compounds were tested in a disc diffusion test and an enzyme assay.

![Zinc binding group](image)

![Hydrophobic side chain](image)

![Hydrogen bond donor](image)


References:


POS.9

PBPK based Dose Recommendation of Cefuroxime for a Perioperative Antibiotic Prophylaxis, with a special Focus on Tissue Concentrations.

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Optimization of the perioperative antibiotic prophylaxis (PAP) is still a matter of debate in human medicine and pharmacy. Despite existing guidelines and recommendation, there are important issues of uncertainty regarding the timing and dose before incision and the intraoperative follow-up administrations. Another issue is the tissue penetration of the antibiotics used for PAP. The most important antibiotics for PAP are the beta-lactam antibiotics ceftazolin and cefuroxime.

To achieve a maximal bactericidal effect, the free drug concentration of beta-lactams must exceed the pathogens minimal inhibitory concentration (MIC) for 60-70% of the time during the dosing interval in the targeted tissue. To reach this target, it is advocated that the blood concentrations should also exceed the MIC by a factor of 4 to 6 [1].

Literature data concerning tissue concentrations of cefuroxime, especially during surgery, is limited. To address this lack of information we collected plasma and lung tissue samples from 25 thoracical surgery patients (18 to 77 years). After induction of anesthesia, a dose of 1.5 g Cefuroxime was administered intravenously. Another 1.5 g cefuroxime was given every 2.5 h thereafter.

We set up a physiologically-based pharmacokinetic (PBPK) model, using PK-Sim®/MoBi® [2]. Relevant changes during surgery which could influence the kinetic, like the blood loss, administered fluid volume, protein shift from plasma to interstitial and the effect of anesthetic drugs, were included into the final model.

The adjustment of the model according to the physiological changes during surgery improved the model performance (MPE = 1.4% and MAPE = 29.0%), with 84.5% of all predicted plasma concentration being within 50% of the observed. The lung tissue concentration could also be described adequately (MPE = 0.4%, MAPE = 34.5%). The results indicate that a prediction of changes in the PK triggered by surgery as well as a prediction of cefuroxime concentrations in lung tissue is possible.

We used our final model for population simulations and a scale-up from the fitted tissue concentrations to interstitial concentrations. To be able to give clear dose recommendations in the field of perioperative antibiotic prophylaxis using cefuroxime, we simulated alternative dosing regimens in varying populations (see below). We tested the difference between long-term infusions and standard short infusions. The simulated populations, which were in line with our study population, show different BMI levels, kidney functions, age and gender.

For Staphylococcus aureus, one of the most relevant pathogen in surgical site infections, adequate plasma and interstitial concentration were reached. In addition, we are able to give clear dose recommendations to optimize perioperative management of PAP. Whereas when looking at other bacteria (especially Escherichia coli) the given or simulated dose regimes do not lead to adequate interstitial and plasma concentrations in most populations.

Our results show that the use of cefuroxime for perioperative prophylaxis to prevent Staphylococcal surgical site infections, seems to be reasonable and recommendable. According to our data the use of cefuroxime for perioperative prophylaxis in abdominal surgeries seems to be at least questionable.

In today’s clinical practice, monoclonal antibodies have become a well-established therapy option for a range of indications, such as cancer and autoimmune diseases [1]. To develop various specific antibodies, huge antibody libraries have to be screened. For this purpose phage display has been used with great success in the last 25 years. Nevertheless, this method is associated with some drawbacks as the possible discrimination of the most potent binders during the biopanning process, the incompatibility with flow cytometry or the size limitation of the protein displayed on the surface [2]. To circumvent these disadvantages, we developed a screening tool using E. coli cells presenting a full-length antibody on their surface. These antibodies and in particular their libraries enables the screening for new variants against pre-given epitopes using flow-cytometry without losing the highly potent binders.

In this work, the autodisplay technique [3,4] was utilized to present a functional full-length antibody on the surface. As a proof of principle, the display of the antibody T84.66 which is directed against carcinoembryonic antigen (CEA) was investigated. Based on this antibody a library was generated. Therefore, restriction sides were introduced in front of and behind the complementarily determining region 3 (CDR3). This enables the exchange of the CDR3 fragment through a randomized fragment. After ligation, this construct was used to transform E. coli strain UT6500 (DE3) via electroporation. The resulting library consists of up to 10^10 clones which can be analyzed and sorted via flow cytometry after incubation with a fluorescently labelled target protein. To examine the optimal conditions for the screening, two different autotransporters in combination with two promoters were investigated: the AIDA-1 autotransporter [3] under control of a T7-promoter and the EhaA-autotransporter [4] controlled by an araBAD promoter. Experiments with the T84.66 antibody as a passenger revealed that the EhaA-autotransporter under araBAD control suited better with regard to surface presentation and cell survival after sorting via flow cytometry. These results indicate that it is possible to generate a full-length antibody library on the surface of E. coli which afterwards can be screened with the advantageous high-throughput screening system of flow cytometry. Further investigations should be performed to identify an antibody variant out of the constructed library which binds a pre-given epitope of therapeutically interest.

References:
In today's pharmaceutical research protein-protein interactions (PPI) play an important role for the characterization of drug targets and for the identification of new therapeutics. Most applications of PPI are based on fluorescence detection requiring a fluorophore labelled target protein. Compared to other common used labelling strategies, an incorporation of an unnatural amino acid followed by a click chemistry based bioorthogonal reaction with a fluorophore is distinguished by high site-specificity. Autoradiography is a surface display technology based on the secretion mechanism of autotransporter proteins in gram-negative bacteria and enables the presentation of target proteins on their outer membrane [1].

In this study click chemistry and Autoradiography were combined to fluorescently modify CK2α as a model protein on the surface of Escherichia coli. CK2α, the regulatory subunit of the human protein kinase CK2, functions as a modulator of CK2 activity and stability as well as a docking platform for CK2 substrates. We report the successful incorporation of the unnatural amino acid para azidophenylalanine (pAFL) [2] into the purified and the surface displayed CK2α. Therefore a suitable position in the sequence of CK2α was chosen and mutated to the amber stop codon, TAG. Afterwards pAFL was incorporated at this position using an orthogonal amber suppressor RNA. Performing the SPAAC click reaction [3,4] with dibenzycyclooctyne-fluorophores the purified as well as the surface displayed CK2α was site-specific labelled and analyzed by SDS-PAGE. We were able to verify the fluorescence modification of surface displayed CK2α by flow cytometry. With this innovative procedure of protein labelling on the surface of bacterial cells, screening assay and protein interaction studies with whole cells were enabled. Moreover this method offers further investigations on the interaction of CK2α and its substrates for example by fluorescence activated cell sorting, Fluorescence resonance energy transfer or microscale thermophoresis.

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References:
with a mean hydrodynamic size of ~200 nm, PDI of ~0.2 and ζ-potential of ~28 mV.
mRNA encoding for a model fluorescent protein (mCherry) was incorporated at different ratio to the NCs followed by an evaluation of their physicochemical properties. Subsequently we used the NCs to perform transfection studies on both cell lines revealing a higher transfection rate in phagocytic cells over keratinocytes, while the starch-chitosan polyplexes indicated a better transfection efficiency (~10%) over CS-PLGA nanocarriers (~2%) (Figure 1).

Our next objective is to incorporate a messenger RNA encoding for a vaccine-relevant antigenic fragment of the influenza virus into the nanocarriers to investigate the ability of the NCs to stimulate a protective immune response for transcutaneous vaccination.

Acknowledgments:
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Helmholtz Centre for Infection Research. Kai Schulze for discussions on vaccination strategies.

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Patient-derived organotypic models of head and neck cancer emulate tumor grading in vitro
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3 Charité Comprehensive Cancer Center, Berlin, Germany

Head and neck cancer is the sixth most common cancer type with about 650,000 new cases and 350,000 cancer deaths annually worldwide [1]. The main risk factors are tobacco and alcohol consumption and 90% of the head and neck cancers are of squamous origin [2,3]. The main therapy options are currently surgery, chemotherapy, and radiotherapy or most likely a combination of them [4]. Despite considerable efforts, survival rates have not improved for the last decades. To improve drug evaluation, we developed and comprehensively characterized patient-derived, organotypic models of oral squamous cell carcinoma (HNSCC models). Thus extending our modelling of squamous carcinoma of human skin [5].

For HNSCC models patient-derived cancer cells [6] were mixed with normal human oral keratinocytes and seeded onto lamina propria equivalents with primary human fibroblasts. Models with the SCC-25 [7] cancer cell line were built for reference. Whereas normal models (normal keratinocytes and fibroblasts only) showed a well structured squamous epithelium with basal and spinous layers, HNSCC models in contrast presented an increased suprabasal layer with rounded swollen cells. These novel HNSCC models combine the advantages of the murine patient-derived-xenograft (PDX) models, with their high availability of human tumor cells and the human cell-based organotypic mucosa models, showing polarity, in vivo like tumor grading and interactions between the tissue layers. We will use these patient-derived head and neck cancer models for the evaluation of topically-applied anti-cancer drugs. This approach fosters also the reduction, replacement, and refinement of animal testing.

Acknowledgments: Financial support of the Berlin-Brandenburg research platform on the 3R (BBFRP)

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Immunofluorescence staining of HNMSC models well reflected the differences in the laminin-5, Ki-67 and cytokeratin-13 expression, seen in the patient.

PO16
Patient-derived organotypic models of head and neck cancer emulate tumor grading in vitro
Leonie Gronbach1, Christopher Wolff1, Konrad Klinghammer2, Christian Zoschke1, Ulrich Keilholz3, Monika Schäfer-Korting1
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4.4 Cancer

The IGF2 mRNA binding protein p62 induces the appearance of liver progenitor cells and promotes cirrhosis

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Suggesting carcinogenic changes. Human cirrhotic (n=41) and HCC remained positive for the IGF2 mRNA binding protein p62 (immunostaining p62+ cells: fetal=1.032, adult=0.122) [1]. Hepatocytes in transgenic mice (E14.5)=2.056, adult (8wk)=0.118; RPKM (IGF2BP2) human liver: 1.032, fetal=0.122, adult=0.118; RPKM (IGF2) human liver: 1.032, fetal=0.122, adult=0.118; RPKM (IGF2BP2) murine liver: fetal=2.056, adult (8wk)=0.118; RPKM (IGF2) murine liver: fetal=2.056, adult (8wk)=0.118. When fed a methionine- choline deficient (MCD) diet in order to induce tumor progression, the MCD diet had a significantly reduced survival compared to their controls, and mice presented increased levels of a number of LPC markers, such as an increased expression of BEX1, CDH1, CD44, EPCAM, BEX1, CDH1, CD44, EPCAM, CD44, EPCAM, BEX1, CDH1, CD44, and several LPC markers (PROM1, CDH1, CD44, EPCAM, PROM1, CDH1, CD44, EPCAM, PROM1, CDH1, CD44, EPCAM, PROM1, CDH1, CD44, EPCAM). Livers of transgenic mice (E14.5=2.056, adult (8wk)=0.118, and Huh7 cells showed an increased expression of NEAT1_2. Last, immunocytochemistry paracrine staining (PSPC1) was performed in the hepatocellular carcinoma chemoresistant cells, and PSPC1-positive signals were detected in all hepatocellular carcinoma cells, whereas no signal could be detected in the control cells.

Our data for the first time support a role of NEAT1 in HCC chemoresistance. We could further show a significant overexpression of both NEAT1 isoforms in human HCC patient samples. Therefore, NEAT1 should be considered as a promising target for the development of novel HCC therapies.

Acknowledgments: Kevan Hosseini 1, Sonja M. Kessler 2, Alexandra K. Kiemer 2

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References:

Hepatocellular carcinoma (HCC) represents the second most common cause of cancer-related death worldwide [1], not least to its high chemoresistance [2]. The long non-coding RNA nuclear paraspeckle assembly transcript 1 (NEAT1), localised in nuclear paraspeckles [3], has been shown to enhance chemoresistance in several cancer cells [4]. Since data on NEAT1 in HCC chemosensitivity are completely lacking, we aimed to study NEAT1 expression in human HCC and in HCC chemoresistance.

First, expression of total NEAT1 and the isoforms NEAT1_1 and NEAT1_2 was analysed in the TCGA data set of liver tissues [5]. Total NEAT1 and both of its isoforms were increased in tumour tissues compared to non-tumour liver tissues, and NEAT1_1 expression was distinctly higher than NEAT1_2 expression. Next, total NEAT1 and NEAT1_2 expression was determined in sorafenib and doxorubicin resistant HepG2, Plc/prf/5, and Huh7 cells by qPCR. Total NEAT1 was overexpressed in all three sorafenib and doxorubicin resistant cell lines compared to their chemosensitive counterparts. In addition, sorafenib resistant HepG2 and Huh7 cells as well as doxorubicin resistant Plc/prf/5 and Huh7 cells showed an increased expression of NEAT1_2. Last, immunocytochemistry paracrine staining (PSPC1) was performed in the hepatocellular carcinoma chemoresistant cells, and PSPC1-positive signals were detected in all hepatocellular carcinoma cells, whereas no signal could be detected in the control cells.

Our data for the first time support a role of NEAT1 in HCC chemoresistance. We could further show a significant overexpression of both NEAT1 isoforms in human HCC patient samples. Therefore, NEAT1 should be considered as a promising target for the development of novel HCC therapies.

Characterization of a new in vitro model for tumor-associated macrophages and comparison with patient-derived macrophages from lung tumor tissue

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Lung cancer represents the most common cause of cancer-related death worldwide. Macrophages have been shown to play a complex role in tumor progression. Depending on the microenvironment, macrophages can either be polarized to resemble a classical pro-inflammatory (M1 macrophages) or an alternatively-activated, anti-inflammatory subtype (M2 macrophages). Tumor-associated macrophages (TAM) are predominantly of the M2 subtype and therefore tumor promoting, which correlates with a poor clinical prognosis [1]. Tissue resident macrophages in the lung, such as alveolar macrophages (AM), are of central importance in the immune response against infections and tumors.

POS.17

Paraspeckle formation and induction of IncRNA NEAT1 in hepatocellular carcinoma chemoresistance

Kevan Hosseini, Sonja M. Kessler, Christina S. Schultehöfel, Markus List, Marci Schulz, Alexa K. Kiemer, Stephan Llagai

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POS.18
Understanding the process of macrophage polarization in the tumor microenvironment might contribute to therapeutic advances. Therefore, we aimed to characterize two established and a novel in vitro model of human macrophage polarization and compare them to patient-derived TAM from lung tumors.

Due to the limited availability of primary in vivo polarized human TAM, we used a general and well established in vitro model for M1 and M2 polarization and compared it to a novel "TAM-like" model. Primary human monocytes were first differentiated into macrophages with M-CSF and subsequently polarized with either LPS/IFNγ towards an M1-like phenotype or with IL10 toward an M2-like phenotype. In order to mimic a tumor microenvironment, macrophages were alternatively exposed to A549 lung tumor cell supernatant to obtain the "TAM-like" phenotype. As indicated by FACS analysis with common M1 (CD68, HLA-DR) and M2 markers (CD14, CD163) and qRT-PCR experiments, the "TAM-like" cells in our model seemed to adopt an M2-like phenotype. In cell migration assays, which allow testing for metastasis and invasive potential of tumor cells, we could show that migration of A549 cells was enhanced with M2-as well as TAM-like-conditioned medium compared to M1-conditioned medium.

In order to compare the properties of the new and established cell models with in vivo differentiated macrophages, we isolated primary TAM and autologous AM from patient tissue. The cell preparations were >95% pure, as indicated by flow cytometric analysis of CD68 expression [2]. We compared both macrophage types from three adenocarcinoma patients according to age and cancer stage using paired-end mRNA-Seq. As expected, we found previously described markers of M2-polarization, such as matrix metalloproteinases and angiogenesis related genes to be upregulated in TAM. Surprisingly, a major regulation of lipid metabolism-associated genes was significantly altered. First qRT-PCR data suggest that our newly established TAM-like cells are a suitable model for primary in vivo differentiated TAM, since similar genes were differentially expressed in both.

By establishing this in vitro model of TAM-like macrophages, we generated a cheap and easy to set up tool to study tumor associated macrophage polarization processes. This model might help to develop therapeutic advances, because TAM may be re-educated to a tumor-suppressive phenotype and elicit a potent anti-tumor immune response.

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POS.20

Systems Medicine Modelling in Hematopoietic Stem Cell Transplantation

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Background and objectives: Hematopoietic stem cell transplantation (HSCT) is a complex and high-risk treatment for patients with certain malignancies like leukemia or multiple myeloma. Potential life-threatening complications of HSCT are viral and bacterial infections. Furthermore, transplant rejection, relapse and death can be unforeseen complications. Predictions of the course of HSCT cannot be made as the individual course has not yet been fully clarified. Modelling seems to be an adequate instrument for pursuing the underlying processes. Many influencing factors must be taken into account, as for example chemotherapy, immunosuppression, but also individual risk factors like patient characteristics (age, sex, medication, graft type, immune status).

The overall objective of this project is to develop a systems medicine model covering thrombopoiesis and leukopoiesis, as well as the reconstitution of CD4+ (helper) and CD8+ (killer) T-cells after HSCT.

Methods: Model building process was performed stepwise by developing four basic models independently for thrombopoiesis, leukopoiesis, CD4+ and CD8+ T-cells. Various structural models were tested, for instance turnover models and literature models, e.g. myelosuppression [1] and T-cell dynamics [2]. Each of the submodels account for the effect of medication during HSCT. Finally, all four submodels were linked to a joint model that was fitted simultaneously to clinical data as well. Data analysis and simulations were performed using non-linear-mixed-effects modelling implemented in the software NONMEM® (version 7.3.0) [3]. Statistical evaluation and graphics were created within the software R (version 3.4.1).

Results: The dataset consists of 49 patients (42.9% female), who underwent HSCT at Saarland university hospital between March 2014 and September 2015. All patients received allografts with a majority (53.1%) for acute myeloid leukemia (AML). Thirty-six patients (73.5%) received hematopoietic stem cells (HSC) from unrelated donors. Median age at HSCT was 54 years with a range of 22-72 years. Twelve patients (24.5%) died at median day +60 (range day 4 to +404) after transplantation. Thrombopoiesis and leukopoiesis were best described by a compartmental model of myelosuppression, whereas for the dynamics of CD4+ and CD8+ T-cells a simple turnover model described the clinical data best. Simultaneous estimation of the model parameters in our joint model showed moderate to strong correlation between several model parameters.

Conclusion: Development of a joint model of hematopoiesis and immune reconstitution is a first step in establishing a comprehensive systems medicine model of HSCT. The model shows a good agreement with the clinical data. In a following step, we will incorporate the dynamics of natural killer cells and viral infections, especially human polyomavirus BK [4].

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POS.21

Do ABC-transporters contribute to development of environment mediated drug resistance in breast cancer cell lines MCF-7 and MDA-MB-231?

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Background: Therapy of malignancies is often restricted by the development of cellular resistance against chemotherapeutics. Interaction of tumor cells with extracellular matrix (ECM) components in their microenvironment is known to induce a lower sensitivity for cytostatic drugs in different malignancies. This phenomenon, called "environment mediated drug resistance" (EM-DR) covers versatile resistance mechanisms e.g. downregulation of apoptosis signals. EM-DR can ultimately lead to acquired drug resistance by initially protecting the cells and allowing them to mutate. Adhesion receptors such as integrins and proteoglycans are considered to be essential for this kind of de novo resistance [1]. ABC (ATP-binding cassette) transporters like ABCB1 (P-gp), ABCG2 (BCRP) and ABC11 (MRP1) are important key players in multidrug resistance fostering a cellular efflux of cytostatic drugs. Consequently, resistance against prominent cytostatic drugs, such as doxorubicin (substrate of MRP1 and P-gp) and mitoxantrone (substrate of BCRP and P-gp) is often associated with higher expression level of the indicated exporters. [2]

Aim/objectives: The aim of this study was to investigate the contribution of ABC-transporters to EM-DR phenomenon in breast cancer cell lines MCF-7 and MDA-MB-231.

Methods: First, sensitivity against cytostatic drugs (i.e. doxorubicin and mitoxantrone) after 72 h was measured by MTT viability assay. The IC50...
values obtained from the dose-effect curves were used as a parameter for cell sensitivity. The impact of EM-DR related factors on chemoresistance, such as providing the ECM components collagen or fibronectin was illustrated by comparing the IC50 to untreated IC50 values. Intracellular accumulation assays of doxorubicin and mitoxantrone were performed by flow cytometry analysis. Additionally, differences between expressions of surface epitopes under cytostatic influence were analyzed by proteome profiler array. In order to elucidate ABC transporters, different assays were performed using fluorescent transporter substrates such as phenothiazine A (BCRP), calcein-AM (MPP-UP-gp), rhodamine 123 (P-gp) or Hoechst 33342 (BCRP/P gp) and analyzed by flow cytometry or fluorescent plate reader.

Results: Interaction with ECM components (collagen or fibronectin) increases resistance against doxorubicin and mitoxantrone in both, MCF-7 and MDA-MB-231 cells. Furthermore, the interference with the adhesion-mediated signalling pathways, e.g. by inhibition of the integrin associated focal adhesion kinase (FAK) declines the resistance indicating a clear role of EM-DR in these terms. The cultivation of cells on collagen also resulted in a decreased intracellular amount of doxorubicin and mitoxantrone. This is functionally reflected by an increased activity or expression of the efflux transporters, especially in case of P-gp. Doxorubicin and mitoxantrone incubation also induces changes in surface adhesion receptors, referring to a highly probable interlinkage between EM-DR and ABC transporter functionality in resistance.

Conclusions: These results support both the role of ECM components and adhesion receptors (e.g. integrins) in EM-DR in both breast cancer cell lines and a possible connection to ABC-transporters.

References:

Comparison of ovarian carcinoma cell lines regarding to the mechanisms of cisplatin resistance

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Ovarian carcinoma is one of the most lethal gynaecological malignancies. Ovarian cancers are comprised of a variety of tumor types with different histopathological features and biological behavior. 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.

Cell lines of ovarian cancer are helpful tools to obtain insight into the underlying mechanisms of cisplatin resistance. The molecular mechanisms of chemoresistance are manifold, but in case of cisplatin resistance efflux transporters appear to be less important. Cell surface molecules, such as integrins and glycosaminoglycans, as well as their downstream pathways, have often been associated to resistance [1]. We have recently reported that the cisplatin resistant A2780cis cell line is, in terms of resistance strongly triggered by the vnt-signaling pathway [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, which presents different resistance features, such as collagen overexpression [3]. The aim of this study was to compare the two ovarian carcinoma cell lines with regard to their cisplatin resistance mechanisms. Cytotoxicity (MTT-assay) and protein expression (SDS-PAGE/WB) were investigated in A2780 and W1 cells and the cisplatin resistant cell lines A2780cis and W1CR. FH535 was used as a -pathway inhibitor. In order to investigate whether integrin activation could have an impact on sensitivity to cisplatin, cells were grown on collagen type-I coated surfaces. Furthermore, E-cadherin and vimentin were detected at the protein level as indicators of epithelial to mesenchymal transition (EMT).

MTT data confirm that the cisplatin resistant sublines of A2780 and W1 ovarian cancer cells display 3- to 10 fold higher EC50 values of cisplatin cytotoxicity. FH535 displayed a distinct toxicity in A2780 and A2780cis cells, which reveals the critical role of this pathway in cell survival. Interestingly, FH535 is more toxic to the resistant cell line, which points to the fact that the resistance in A2780cis cells is triggered by the eck-signaling pathway. Accordingly, pre-treatment with FH535 in sub-toxic concentration sensitized A2780cis cells to cisplatin cytotoxicity. In contrast, FH535 displayed only low toxicity in the W1 and W1CR cells, which illustrates that this pathway in obviously not decisive in their cell survival. Otherwise, if subjected to integrin activating treatment (collagen I), W1 and W1CR cells showed a significant increase in resistance to cisplatin, referring to a certain impact of adhesion mechanisms on resistance. Furthermore, inhibition of integrin-linked kinase (ILK) or focal adhesion kinase (FAK) leads to a higher gain in sensitivity, especially in cells grown on collagen. These data support the hypothesis of integrin-triggered cisplatin resistance of the W1 cell lines, which explains the collagen overexpression present in the resistant cells [3]. This effect was not observed for the A2780 and A2780cis cells. Since no changes in E-cadherin and vimentin were noted, EMT involvement in this scenario appears unlikely for both cell lines.

This work provides further insight into the peculiarities of cisplatin resistance and illustrates that despite the same tumor entity the respective ovarian carcinomas differ strongly in their chemoresistance mechanisms. W1 cells were introduced as promising novel cell models for further studies on the heterogeneity of the ovarian carcinoma.

References:

Heparin affects the tumor cell induced VEGF and chemokine release from platelets both in a contact and coagulation dependent manner

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Metastasis is still the major cause for cancer associated fatalities. Tumor cells, that undergo the hematogenous metastasis, detach from the primary tumor, migrate through tissues and invade blood vessels. Inside the blood circulation tumor cells immediately interact with platelets. This contributes to platelet activation, which in turn augments cancer cell survival and proliferation in several ways. Amongst others, tumor cells are protected by platelets from blood shear forces and apoptosis induced by natural killer cells. Furthermore, activated platelets induce tumor cell arrest at the vascular wall and subsequent extravasation into the tissue. Platelet secreted chemokines CXCL5 and CXCL7 recruit granulocytes to tumor cells, which foster the formation of the early metastatic niche [1].

Angiogenesis in the metastatic nodule is facilitated by platelet-derived growth factors like Vascular Endothelial Growth Factor (VEGF). We characterized MV3 melanoma and MCF7 breast cancer cells in terms of their ability to induce platelets activation with subsequent granule release and the impact of unfractionated heparin (UFH) on the interaction between tumor cells and platelets in several in vitro approaches. In a plasma-based assay, we were able to distinguish between platelet activation either resulting from the direct contact between tumor cells and platelets, or triggered by the coagulation cascade. The ability of the tumor cells to induce a thrombin formation and subsequent platelet activation was assessed using a thrombinogenic thrombin generation assay. The granule release resulting from the activation was quantified by a human enzyme-linked immunosorbent assay (ELISA) focusing on the release of VEGF, CXCL5 and CXCL7.

It is shown that a direct interaction between platelets and MV3 or MCF7 cells induces platelet activation and a VEGF release in isolated plasma that cannot be further elevated by the coagulation cascade and generated thrombin. In contrast, the release of platelet-derived CXCL5 is further increased by thrombin. This makes heparin an interesting candidate for a therapeutic approach to cancer metastasis.

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and CXCL7 depends on thrombin-mediated platelet activation and direct interaction between tumor cells and platelets. Preincubation of platelets with therapeutically relevant concentrations of UFH reduces the tumor cell initiated VEGF release from platelets. In contrast, tumor cell induced CXCL5 and CXCL7 release from platelets was not impacted by heparin pretreatment in citrated plasma. On the contrary, in defibrinated, re-calculated plasma heparin is able to reduce CXCL5 and CXCL7 release from platelets by thrombin inhibition.

These results indicate that different mediators are located in different granules of platelets that were released in a tightly regulated process by various trigger mechanisms. Our data display for the first time that heparin is able to reduce the mediator release induced by different tumor cells both in a contact and coagulation dependent manner.

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The mRNA-binding protein TTP inhibits tumor progression in liver cancer

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Hepatocellular carcinoma (HCC), the predominant form of liver cancer, represents the second most common cause of cancer-related death worldwide and typically develops in an inflammatory environment [1]. The initiation and progression of cancer are impaired by dysregulated expression of proteins controlling diverse cellular phenotypes [2]. The cytoplasmatic stability of many oncogenic and proinflammatory mRNAs is controlled by AU-rich elements (ARE) in their 3'-UTRs [3], which can be targeted by ARE-binding proteins like tristetraprolin (TTP, ZFP36), a mediator of mRNA decay [4]. Dysregulated TTP seems to play an important role in the pathophysiology of cancer since it has been reported to be downregulated in several non-hepatic human malignancies [5]. The aim of this study was to determine the role of TTP in liver cancer. In order to study a potential dysregulation of TTP in human HCC, two publicly available GEO data sets (GSE52097 and GSE14520) comprising a total of almost 500 HCC tissues in comparison to a comparable number of non-tumour liver tissues was analysed. The data revealed strongly decreased levels of TTP in HCC tissue. Previous data suggested that TTP expression is suppressed in hepatoma cells due to promoter methylation. Neither experiments employing the methyl transferase inhibitors azacytidine or decitabine in the three hepatoma cell lines HepG2, HuH7, and Pclprf5 nor DNA methylation profiles of 50 non-tumour liver tissues supported this mechanism. Although the mechanism of TTP-downregulation remains unclear, we decided to study the role of TTP in cancer initiation and progression. In order to reveal the effects of TTP on tumour initiation, in vivo experiments were performed using hepatocyte-specific TTP KO and wildtype (WT) mice. Animals were treated with either 100 mg / kg body weight diethylstilbestrol (DEN) at the age of nine weeks for 48 h to induce acute inflammation, or 5 mg / kg body weight DEN at the age of 2 weeks to induce tumour formation for six months. However, no difference between TTP KO and WT animals was observed. Studies on tumour progression involved investigations on cancer cell migration, which represents an important step in tumour expansion. A wound-healing assay in three different TTP-overexpressing hepatoma cell lines, i.e. HepG2, HuH7, and Pclprf5, showed a significantly decreased migration ability in HuH7 and Pclprf5 cells. Ki-67-FACS staining in the same cell lines revealed that proliferation was strongly inhibited in all of the TTP-overexpressing cells. Furthermore, the expression of several oncogenes, which had been shown to be TTP targets in non-hepatic tissues, was analysed. Oncogene RNA expressions were significantly downregulated or showed a strong tendency to be decreased in TTP-overexpressing hepatoma cells. Chemosensitivity towards doxorubicin and sorafenib was rather unchanged in TTP-overexpressing cells.

Our data show that TTP inhibits the migration ability, proliferation, and oncogene expression of hepatoma cells. In conclusion, TTP plays no substantial role in hepatic tumour initiation and inflammation, whereas its role in hepatic tumour progression seems to be important.

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References:

Comparison of chromosomal aberrations of mouse models for hepatocellular carcinoma with human data by aCGH analysis

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Chromosomal aberrations are frequently observed in a wide range of diverse malignancies including hepatocellular carcinoma (HCC). HBV- and HCV-infections are the major causes for HCC development. Due to the increasing incidence of HCC and its high mortality there is a special need for successful animal models reflecting the human pathogenesis of HCC for both basic as well as for translational studies in HCC research. However, the variety of different risk factors and the heterogeneity of HCC itself make it impossible to generate an ideal model for all purposes. Each animal model recapitulates only some aspects of hepatocarcinogenesis. Aim of this study was to compare aCGH data of different HCC mouse models with human HCC data to find common genetic events. Publicly available datasets of aCGH array analyses were analysed and compared regarding gains and losses. A 0.25 threshold was applied to find gains and losses. A gain or loss of a gene was considered if there was a gain or loss in at least 75% of the samples. The human dataset (GSE14322) contained an HBV-associated HCC cohort showing 447 genes with 24 gains and 291 losses. In murine hepatocellular carcinomas of AlbLtah transgenic mice (GSE14467) 115 genes were affected by gains and 535 genes by losses. The latter two datasets had only 12 common genes with losses. Mice with an inducible telomere dysfunction on a telomerase-deficient background (GSE36813) had 5278 gains, 4059 losses, and 101 genes as losses in common with the human samples. Interestingly, p53 knock-out mice (GSE63100), in which 110 genes had gains and 490 genes had losses, 134/490 losses were also observed in the mice with induced telomerase dysfunction. Concordantly, 356 genes out of 1,571 genes with losses were shared with the telomerase-deficient mice.

Pathway analysis revealed that the mostly affected pathways were metabolic and cancer-associated pathways. In conclusion, chromosomal aberrations are a common feature of human HCC and all mouse models investigated. These models share about 25% of genetic aberrations with each other, but only a minor part with the human dataset suggesting that also for studies on chromosomal events one has to choose the ideal model the respective research hypothesis.
**IMP2/IGF2BP2 expression predicts poor outcome in patients and high tumor growth rate in xenograft models of gallbladder cancer**

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Overexpression of oncofetal insulin-like growth factor 2 mRNA-binding proteins (IMPs/IGF2BPs) has been described in different cancer types. Gallbladder carcinoma (GBC) is a rare but highly aggressive cancer entity with a typically late clinical detection and poor prognosis. Aim of this study was to investigate the role of IMPs in human GBC. Tissue microarrays (TMAs) of an international multi-center GBC sample collection from n=483 patients were analyzed by immunohistochemistry. IMP2 immunoreactivity was found in 74.3% of the tumor samples on TMA, of which 14.0% showed strong and 56.0% low staining intensity. 72.4% of the tumor samples were IMP1 positive, but IMP1 showed lower expression in tumor tissue compared to control tissues. IMP3 immunoreactivity was observed in 92.7% of all tumors, of which 53.6% revealed strong IMP3 expression. Kaplan-Meier analysis linked high IMP2 immunoreactivity to shorter survival time (p<0.033), whereas neither IMP1 nor IMP3 expression was linked to a decreased survival time. Eight different human biliary tract cancer cell lines were evaluated for tumor growth kinetics in mouse xenografts. Cell lines with high IMP2 expression levels showed the fastest increase in tumor volumes in murine xenografts. IMP1 or IMP3 expression did not correlate with tumor growth rates.

More advanced tumors are often characterized by increased DNA damage and chromosomal instability, which can be induced by reactive oxygen species (ROS). Interestingly, IMP2 expression was associated with a higher ROS production after stimulation with phorbol 12-myristate 13-acetate (PMA) in biliary tract cancer cell lines. NADPH oxidase represents an ROS-generating enzyme complex that is activated by the small GTPase RAC1, which has previously been shown to be activated in IMP2 overexpressing hepatoma cells. Interestingly, RAC1 mRNA also highly correlated with IMP2 expression in the human biliary tract cancer cell lines.

In conclusion, IMP2 is frequently overexpressed in GBC and significantly associated with poor prognosis and growth rates in vivo. This might be at least in part due to increased ROS generation by RAC1 induction. IMP2 might therefore represent a new target for treatment of advanced GBC.

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**Stearoyl-CoA desaturase-1 derived phosphatidylinositolosols regulate the activation of p38 MAPK in apoptosis**

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As central enzyme in the biosynthesis of monounsaturated fatty acids (MUFAs), stearoyl-CoA desaturase-1 (SCD-1) introduces a cis-9 double bond into saturated fatty acids (SFAs). SCD-1 has been proposed as a promising target for the treatment of cancer, skin disorders and metabolic diseases. While the regulation and biological implications of SCD-1 have been extensively investigated, the molecular mechanisms through which SCD-1 mediates cellular responses remain a mystery. We have shown that changes in SCD-1 activity specifically counter-regulate the stress-activated p38 mitogen-activated protein kinase (MAPK) [1]. Both the SCD-1 inhibitor CAY10566 and the knockdown of SCD-1 significantly enhanced the phosphorylation of p38 MAPK. Since the cellular ratio of MUFAs-containing phosphatidylinositols (MUFAs-PI) is strongly regulated in response to changes in SCD-1 activity, we hypothesized that MUFAs-PIs might represent lipid mediators, which specifically act as bioactive signaling factors upstream of p38 MAPK. Here, we compensated the depletion of MUFAs-PI upon treatment with CAY10566 by selectively enriching NIH-3T3 fibroblasts with MUFAs-PI species or control phospholipids that were applied in form of liposomes. The MUFAs-PI species PI(18:1/18:1) was efficiently taken up by fibroblasts within 48 h as determined by ultraperformance liquid chromatography ESI tandem mass spectrometry (UPLC-MS/MS), fully preventing p38 MAPK phosphorylation and partially restored proliferation and cell morphology. Cellular levels of free MUFAs were not increased upon supplementation with PI(18:1/18:1) which points towards the phospholipid rather than released fatty acids as signaling molecule. Due to the central role of p38 MAPK for survival, we investigated the role of MUFAs-PI during early apoptosis. MUFAs-PI-containing phospholipids were found to be co-regulated independent of the mechanism by which apoptosis was induced. The apoptotic depletion of MUFAs-PI is associated with a decrease of SCD-1 expression and inversely correlates with an activation of p38 MAPK. Together, we here identified PI(18:1/18:1) as novel signaling molecule that regulates p38 MAPK with apparent relevance for stress-signaling in apoptosis.

4.5 Cardiovascular and metabolic diseases

POS.28

Angiotensin-converting enzyme activity is attenuated by selective stimulation of angiotensin II type two receptor

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Angiotensin-converting enzyme (ACE) is known for its catalytic activity in the degradation of vasopeptides that are amongst others angiotensin I and bradykinin (BK). ACE inhibitors (ACEi) are used as gold standard in the treatment of hypertension and heart failure with reduced ejection fraction. As an adverse side effect of ACEi non-allergic angioedema can cause potentially life-threatening obstructive swellings in the upper respiratory tract, most likely favoured by increased BK activity [1]. However, angiotensin II (Ang II) receptor (AT) type 1 (AT1) blockers (ARB) can also induce angioedema, yet with a lower incidence. As ARB such as telsimartan (Tel) raise Ang II plasma levels and shift activity towards AT type 2 (AT2) we considered its contribution to the generation of ARB induced angioedema. We used the selective non-peptide AT2 agonist compound 21 (C21) which we synthesized previously according to published synthetic route [2]. In organ bath studies, we specified C21’s agonistic selectivity towards AT2 in endothelium-intact aortic rings of C57BL/6, FVB/N and AT2 knockouts backcrossed to FVB/N (0.001 µM – 10 µM for C21). C21 induced relaxation of pre-constricted aortic rings with half-maximal vasodilator concentrations (pD2) of 6.2±0.21 in C57BL/6 and 6.7±0.16 in FVB/N, while in AT2 knockouts there was no effect (P<0.001). Two-wayANOVA, n=6 each). These findings confirm the specificity for C21. Further studies in homogenates of human umbilical vein endothelial cells (HUVEC) and human dermal microvascular endothelial cells (HDMEC) showed the impact of AT2 on ACE activity. HUVEC incubated with Ang II/Tel (0.1 mM each) and HDMEC incubated with C21 (0.1 mM) showed a significantly reduced ACE activity compared to vehicle (mean±SEM; 45±8 % with P<0.05 for AngII/Tel in HUVEC and 58±13 % with P<0.001 for C21 in HDMEC (Tukey test following One-Way ANOVA, n=5 each). These effects were abolished by the selective AT2 antagonist PD123319 (10 µM). To translate these finding into an in-vivo model, we performed in vivo assays in C57BL/6 that were intravenously pre-treated with either vehicle, C21 (0.05 mg/kg BW) or C21 combined with Tel (10 mg/kg BW). Each mouse was intra-dermally injected into the dorsal skin with 2 mmoles BK. The extravasation induced by BK was expressed as fold increase compared to saline (mean±SEM). Treating mice with C21 (5.2±3.08) and C21/Tel (5.19±0.10) resulted in significantly higher extravasations than vehicle (4.5±2.017, P<0.001, n=6 each), while no difference was found between the treatment groups (P>0.05). These findings show that activation of AT2 results in a quenching effect on ACE activity leading to reduced degradation of BK which might contribute to ARB induced angioedema.


POS.29

Mathematical model of the oral glucose tolerance test including glucose, insulin and c-peptide levels: an IMI DIRECT study

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Objectives: Development of a mechanistic model of blood glucose, insulin and c-peptide levels during an oral glucose tolerance test (OGTT) in pre-diabetic subjects at high risk of developing type 2 diabetes mellitus (T2DM).

Methods: Data from the Diabetes Research on Patient Stratification (DIRECT) study was used [1]. The subjects in the study were non-diabetic, which was defined by the inclusion criteria of HbA1c <5.5% and fasting plasma glucose <7 mM with anti-diabetic treatment, and an elevated risk of developing T2DM according to the DIRECT-DETECT risk algorithm [2]. All subjects underwent a 75 g OGTT. Glucose, insulin and c-peptide concentrations were measured before and 0, 15, 30, 45, 60, 90 and 120 min after oral glucose intake. Modelling and simulation was performed using non-linear mixed-effects methods implemented in the software NONMEM (version 7.3.0). Stochastic simulations were performed for model evaluation.

Results: The dataset included 2237 subjects and 46830 data points. Glucose, insulin and c-peptide concentrations were modelled simultaneously using one compartment turn-over models. Oral glucose uptake was described using a transit model with a first-order absorption rate constant and one transit compartment. Glucose utilization followed a second-order process. Endogenous glucose release was calculated as a zero-order process based on glucose baseline and degradation rate to assure steady-state conditions in absence of glucose intake. C-peptide and insulin releases were described using a hill function. Insulin release was calculated by the c-peptide release times a bioavailability factor accounting for pre systemic hepatic clearing of insulin after release by the pancreas. Upon glucose digestion, a group of hormones called incretins is released to enhance insulin secretion. Their effect was implemented on the hill function. C-peptide elimination followed a first-order process. Insulin degradation was described by a saturable process. The precision of all parameter estimates was excellent (relative standard error <4%).

Conclusion: An OGTT model simultaneously describing the changes in glucose, insulin and c-peptide levels was successfully developed for pre-diabetic subjects. The inclusion of both c-peptide and insulin release enabled the distinction between changes in beta-cell function and first pass clearance of insulin, which improved the characterization of the individual glycemic condition. The study participants have further OGTTs scheduled after 18 and 48 months. Hence in future application this model is planned to be used to quantify disease progression of T2DM.


POS.30

Regenerative potential of adipocytes – Potential treatment for hypertrophic scars?

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Hypertrophic scars can result from surgery (e.g. mastectomy) or burn wounds depending on the wound depth. Patients are not only affected by cosmetic problems because of the elevated and red appearance of the scar tissue, but also suffer from pain, pruritus and contractures. Following autologous fat grafting in plastic surgery, significant improvements in morphology and function of hypertrophic scar tissue have been observed repeatedly [1]. This phenomenon indicates crosstalk between the injected adipose tissue and the connective tissue [2]. However, the underlying mechanisms are largely unknown. Therefore, this project aims to unravel the interactions between adipose tissue and connective tissue during wound healing and to identify the cell types involved.
After isolation of adipose-derived stem cells (ASC) from adipose tissue, cells were differentiated into adipocytes over 14 days. Concurrently, primary human fibroblasts were stimulated with transforming growth factor β1 (TGF-β1) for 72 h to generate myofibroblasts. Additionally, fibroblasts were isolated from hypertrophic scar tissue. (Myo)fibroblasts were then incubated with conditioned media from ASCs or adipocytes. Following 24 h, exposure to conditioned medium from adipocytes but not ASCs induced a significant downregulation of the myofibroblast marker α-sma on gene and protein level. Notably, this effect was even more pronounced in fibroblasts derived from hypertrophic scar tissue. A similar downregulation of α-sma could be induced indicating an involvement of PPARγ interferes with TGF-β-signalling, the contribution of PPARγ was tested.

After 24 h, exposure to conditioned medium from adipocytes but not ASCs induced a significant downregulation of the myofibroblast marker α-sma on gene and protein level. Notably, this effect was even more pronounced in fibroblasts derived from hypertrophic scar tissue. A similar effect was observed for collagen I and III expression. When myofibroblasts were pre-treated with GW9662 (1 μM) followed by 24 h incubation with adipocyte-conditioned medium. Since nuclear receptor PPARγ interferes with TGF-β-signalling, the contribution of PPARγ was tested.

Downregulation of α-sma and ECM proteins may indicate a modification of the myofibroblast differentiation state. A re- or dedifferentiation of myofibroblasts would be a possible explanation for the regeneration of hypertrophic scars. This aspect is currently under investigation.

References:
7. POS.31 Effect of food intake and different formulations on the pharmacokinetic of metformin: a mathematical modelling approach

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Background and Objective: Metformin is a widely used biguanide glucose lowering agent used as the first-line treatment for type 2 diabetes mellitus [1]. However, the detailed mechanism of action is still unknown and the available pharmacokinetic (PK) data of metformin indicates a high interindividual variability. We aimed to develop a mathematical model describing the PK of metformin after single and multiple dose(s) of different formulations with and without food intake and therewith to explain parts of the variability.

Methods: Metformin plasma concentration-time profiles and corresponding urinary data were used from four clinical phase I trials in healthy adults performed by Boehringer Ingelheim. Volunteers received immediate (IR) or extended release (ER) formulations between 850 mg and 1500 mg as single or multiple dose administration under fasted or fed conditions. The model was developed stepwise: first, the structural and the stochastic model was established. Second, a covariate analysis was performed using the forward inclusion and backward elimination procedure, with significance levels of 5% and 0.1%, respectively. Parameter estimation and simulations were performed using non-linear-mixed-effects methods implemented in the software NONMEM® (version 7.3.0).

Results: The dataset included 5644 plasma and 316 urinary concentrations of metformin from 175 healthy subjects. 32 volunteers received multiple doses of IR and 143 of ER formulation. The PK profile of metformin was best described by a two-compartment disposition model following flip-flop kinetics, which is already described in literature [3] (first-order absorption rate constant 0.308 h-1; first-order elimination rate constant 0.908 h-1). To describe the PK of the ER formulation, a zero-order infusion into the absorption compartment was implemented with a duration of 1.95 h in fasted volunteers. It was increased to 4.08 h under fed conditions. The bioavailability was estimated at 25.2% using the total amount recovered in the urine. As metformin is not metabolized and fully cleared by the kidneys [4] this amount reflects the fraction absorbed and therewith the absolute bioavailability. Goodness-of-fit plots confirm that the final model successfully describes the plasma and the urine data of metformin. For model evaluation, simulation-based model diagnostics for different formulations under fasted and fed conditions were performed. Visual predictive checks demonstrate a good descriptive performance with neither under- nor overprediction. Including relevant covariates decreases the interindividual variability from 55.9% to 36.9% for the bioavailability and 35.2% to 20.5% for the absorption rate. To investigate the food effect of metformin, simulations for 1000 mg metformin were performed under fasted and fed conditions and for IR and ER formulations. Interindividual variability and residual variability were considered. The simulations confirm a 1.73-fold and 1.70-fold higher AUC under fed conditions compared to fasting conditions for IR and ER formulation, respectively.

Conclusion: To conclude, metformin blood concentrations as well as urinary concentrations were accurately quantified by a two-compartment model. Food-intake and different formulations were successfully implemented as covariates. Therewith, the variability in the PK of metformin can partly be explained. Hence, the model can be used to individualize dosing regimens regarding food-intake or drug formulations.

POS.32

Physiologically-based pharmacokinetic (PBPK) modeling of drug-drug interactions: the rifampicin - alfentanil interaction via CYP3A4

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Objectives: PBPK modeling is a powerful tool to explore and quantitatively predict the magnitude of drug-drug interactions (DDIs) and may even offer an alternative to dedicated clinical studies. Alfentanil is a sensitive CYP3A4 substrate (≥ 10-fold increase of AUC with strong inhibitors) and recommended by the FDA for the assessment of the DDI potential of investigational new drugs [1]. Our objective was to establish a full PBPK model of alfentanil and to demonstrate its ability to predict the rifampicin-alfentanil DDI.

Methods: PBPK models of alfentanil and rifampicin were built in PK-Sim® modeling software (Version 7.0.0) as part of the Open Systems Pharmacology Suite [2,3]. Alfentanil drug-dependent parameters as well as plasma and urine concentration-time profiles of various clinical studies (dosing range 15-50 μg/kg as intravenous and 60-75 μg/kg oral application) were obtained from literature and used to establish a model accurately describing and predicting observed clinical data. The alfentanil model was then coupled to a previously established rifampicin model [4], clinical DDI studies were predicted and the results were compared to published observed data.

Results: Model development was accomplished with data of 3 clinical studies as an internal dataset; model evaluation was performed with an external dataset of 4 different trials. The newly developed alfentanil model applies metabolism by CYP3A4. The passive glomerular filtration rate was reduced to a fraction of 0.06 to recover the low urinary excretion of approximately 0.3% as unchanged drug [5]. Although in clinical use alfentanil is administered solely in intravenous form, some DDI studies published plasma concentrations of alfentanil after oral application. Colonic absorption was disabled in our model, as late absorption was not consistent with the reported concentration-time profiles after oral administration. Simulation of 12 different DDI scenarios with the coupled models generates alfentanil plasma concentration-time profiles during rifampicin treatment that are in very good agreement with observed data. Predicted AUC ratios (AUC with rifampicin /AUC without) show a low fold bias of 1.19 (geometric mean fold absolute deviation, range 1.01-1.84, n=12).

Conclusion: We provide a full body PBPK model of alfentanil as a tool for the drug development process for dynamic evaluation of the DDI potential of investigational drugs that are CYP3A4 inducers or inhibitors.

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POS.33

The benefits of a one-page summary sheet (OPSS) compared to the patient information leaflet (PIL) to enhance health literacy – a randomized crossover trial

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Introduction and objectives

In Germany – as well as in many other countries – patient information leaflets (PILs) are one of the most prominent sources for patients’ drug information [1]. However, the current content and design of PILs holds numerous difficulties concerning its purpose to inform the patients and to enable the patients to participate in a rational pharmacotherapy [2]. Small writings, multiple use of technical terms and an overload of information are the most frequently mentioned problems [1]. To overcome these issues a one-page summary sheet (OPSS) which can be added to the PIL was developed. The OPSS summarises on one page the most relevant information regarding to the patient needs in a clearly structured manner and in plain language. Thus, to investigate the benefits of the newly developed OPSS compared to the PIL, a cross evaluation was aspirated.

Methods

To elucidate whether patients informed by OPSS or PIL have a better pharmacotherapeutical understanding, a randomized crossover study was conducted. For doxycycline and metoclopramide OPSS were generated. Afterwards, the study was performed following the protocol depicted in figure 1. Subjects were randomized and had to read either the OPSS or the PIL for doxycycline or metoclopramide as the first drug. Afterwards they had to answer five pharmacotherapy relevant questions, for example how to dose the drug correctly. Subsequently, study participants had to read the contrary version for the second drug. To measure the pharmacotherapeutical understanding the processing time and the rate of mistakes during the interrogation phase was tracked.

Results

A total of 155 study participants were enrolled. Median age was 52 years (range: 18 to 84 years). Sixty-two percent were females and 46% of the study participants had a long-term medication. Besides, 43% stated that they read PILs of their medication regularly. Over 90% of the study participants preferred the OPSS regarding content and structure. Furthermore, in the OPSS group study participants made about 27% (138 versus 191) less mistakes and were on average about 50% (3.6 minutes versus 6.5 minutes) faster in executing the interrogation phase. Both, processing time and the rate of mistakes were significantly superior compared to the PIL.

Conclusion

Our study provides high evidence that the OPSS is a reasonable tool supporting the common PIL as one of the most important sources for patient’s drug information. Establishing the OPSS could enhance health literacy significantly. The OPSS is a supplemental information to the PIL, which could lead to further positive effects like increased medication adherence and reduced adverse drug events.

References:

POS.34

Physiologically-based pharmacokinetic (PBPK) modeling of dronedarone and its main metabolite N-debutyldronedarone

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Objectives: Dronedarone is described as the most potent P-gp (P-glycoprotein) inhibitor. The FDA (US food and drug administration) recommends dronedarone as a model perpetrator drug to evaluate the impact of P-gp inhibition on P-gp substrates (victim drugs) during co-administration [1]. Our objective was to establish a whole-body PBPK model of dronedarone and its main metabolite N-debutyldronedarone.
model of dronedarone including its main metabolite N-debutyl-dronedarone to explore and predict plasma concentrations of dronedarone and its metabolite and to use this model for drug-drug interaction prediction.

**Methods:** The parent-metabolite PBPK model was built in PK-Sim® (version 7.1.0) and MoBi® (version 7.1.0). Drug-dependent parameters (e.g. logP and solubility) as well as plasma concentration-time profiles (dosing range 10-80 mg as intravenous and 100-1600 mg as oral administration) and population data (e.g. age, weight) from clinical studies with dronedarone and its metabolite were obtained from literature. Model parameters, that could not be inferred from literature, were optimized to accurately describe an internal dataset of plasma concentration-time profiles. Model evaluation was carried out by the comparison of predicted versus observed plasma concentration-time profiles, AUC (area under the curve) and Cmax (peak plasma concentration) values.

**Results:** Dronedarone and its metabolite both exhibit non-linear pharmacokinetics and their AUC and Cmax values vary greatly between different clinical studies with the same administered doses of dronedarone. The developed whole-body PBPK parent-metabolite model includes metabolism by CYP3A4 (cytochrome P 450) to describe the building rate of the main metabolite. The metabolite is eliminated through metabolism by MAC-A (monoamino-oxidase A) [2]. Furthermore, a mechanism-based inhibition (MBI) of CYP3A4 by dronedarone and its metabolite was implemented [3]. This MBI is extremely relevant for the description of the PK after oral administration because it leads to almost complete inhibition of CYP3A4 in the duodenal mucosa already at low doses of dronedarone. In contrast, the CYP3A4 MBI in the liver shows time- and dose-dependency.

**Conclusion:** The newly developed whole-body parent-metabolite PBPK model of dronedarone precisely describes plasma concentrations of dronedarone and its metabolite after intravenous and oral administration of dronedarone and is a valuable tool to predict the maximum impact of P-gp inhibition on the PK of P-gp victim drugs.


**References**

Calculated model parameters for the target-mediated drug disposition (TMDD) model of the three endothelin receptor antagonists (ERAs) with different binding affinities of the ERAs to the two receptor subtypes, ETA and ETB [2], resulted in highly significant differences in the PK profiles of the three ERAs, as presented in Table 1. The PK model development was performed using data from first-in-human studies after i.v. administration of single ascending doses of the three ERAs. Bosentan was administered over 5 min with doses ranging from 5 to 750 mg [1]. Clazosentan was given as.i.v. infusion of 3, 10, 30, or 60 mg/h over 3 h, 60 mg/h over 6 h, and 30 mg over 12 h [3]. Tezosentan doses ranged from 5 to 600 mg and were infused over 1 h [4]. Population modeling was performed sequentially evaluating several structural models with NONMEM 7.3. Model selection was performed with SAS 9.4 based on statistical and graphical procedures.

**Results**

Overall, 706 bosentan, 472 clazosentan, and 539 tezosentan plasma concentration-time data from 54, 48, and 56, respectively, healthy subjects were analysed. The PK of all three ERAs were best described by two-compartment TMDD models. Variations in internalisation of the drug-receptor complexes could be observed; bosentan-target complexes are internalised by a first-order process with one rate constant, clazosentan is not internalised at all and tezosentan-target complexes are internalised with two rate constants differentiating two populations. Interindividual variability observed was ≤ 57%. A diurnal variation of the receptor synthesis rate improved the models for all three ERAs significantly (p<0.001).

**Conclusion**

PK of all three ERAs were well described by two-compartment TMDD models with variation in drug-target complex internalisation processes. Further investigations are needed to evaluate if this might be related to different binding affinities of the ERAs to the two receptor subtypes, ETA and ETB, which represent the target. Model predictions were improved by incorporation of a cosine function on receptor synthesis [1] with diurnal fluctuation. The results presented indicate a class effect in PK behaviour of ERAs in general and could serve as basis for developing dosing strategies at multiple-dose administration.

**Method**

PK model development was performed using data from first-in-human studies after i.v. administration of single ascending doses of the three ERAs. Bosentan was administered over 5 min with doses ranging from 5 to 750 mg [1]. Clazosentan was given as i.v. infusion of 3, 10, 30, or 60 mg/h over 3 h, 60 mg/h over 6 h, and 30 mg over 12 h [3]. Tezosentan doses ranged from 5 to 600 mg and were infused over 1 h [4]. Population modeling was performed sequentially evaluating several structural models with NONMEM 7.3. Model selection was performed with SAS 9.4 based on statistical and graphical procedures.

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**Conclusion**

PK of all three ERAs were well described by two-compartment TMDD models with variation in drug-target complex internalisation processes. Further investigations are needed to evaluate if this might be related to different binding affinities of the ERAs to the two receptor subtypes, ETA and ETB, which represent the target. Model predictions were improved by incorporation of a cosine function on receptor synthesis [1] with diurnal fluctuation. The results presented indicate a class effect in PK behaviour of ERAs in general and could serve as basis for developing dosing strategies at multiple-dose administration.

**References**

uptake through OATP1B1 into hepatocytes with additional renal plasma clearance.

For the PopPK analysis, NONMEM 7.3 was used. Possible covariates included demographics including age, weight and sex. Plasma albumin, creatinine clearance, and concomitant drugs were also documented. Subsequently, experimental twice-weekly dosing regimens for prophylactic treatment in outpatient care with applications every 72 h and 96 h were simulated with both models. Simulated doses were escalated from 70 mg/m² up to 210 mg/m².

Results:
The transfer of the adult PBPK model to a pediatric study population was achieved successfully. Nearly all observed PK plasma profiles were within the 2-fold error range for the simulations. Including individualized fraction unbound improved the model fit in the distribution phase. Inclusion of the hepatic influx transporter OATP1B1 sufficiently defined the hepatic clearance processes.

The PopPK model that best described the data consisted of two compartments with linear elimination. Inter-individual variability was found for both volumes of distribution and clearance, including a full covariance matrix. As paediatric data was used, the allometric approach for scaling parameters based on bodyweight was included. No other covariate was found to be significant on a 5% level of significance.

Depending on the dose applied, simulated twice-weekly applications resulted in plasma concentrations below the target minimum inhibitory concentration (MIC90) of 0.25 μg/mL for the most common Candida species (3) within median of 55 h to 71 h.

Conclusion:
The simulation revealed that Cmax was predicted more precisely in the PopPK approach; similarly, undefined variabilities throughout the population are taken better into account. For outpatient care, a twice-weekly regimen with 210 mg/m² resulted in plasma concentrations above the MIC90. As no maximum tolerated dose has been reported for echinocandines, this option might be feasible for outpatient care. Nevertheless, appropriate clinical trials with PK, safety and efficacy endpoints are needed to further define this regimen’s clinical usefulness.

References:

POS.37
Smartphone compliance apps in praxis: How reliably can a medication plan be entered?
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Background and Objectives:
Non-adherence to medical protocols is one major reason for ineffective drug therapy[1–3]. The reasons for non-adherence are highly diverse [4]. Apart from therapy-related or social-economic factors, patients often simply forget to take their medication, especially if there is a lack of self-perceived need for treatment [4]. A device reminding the patients to take the right tablet at the right time could be one option to counter that problem. Due to the widespread usage of smartphones, compliance apps could be one solution to improve medication adherence.

Previous studies have already shown an improvement of adherence to medication plans if patients used reminder systems like smartphone apps or SMS reminders[5–8]. In our study, we aimed to evaluate the correctness of the entry of a medication plan in one selected compliance app by patients.

Methods:
To assess the correctness of a medication plan, the app MediSafe® was selected as one of the most commonly used compliance apps available for Android and iPhone based smartphones. Volunteers were asked to enter two drug regimens (ibuprofen, 400 mg, 1-1-1; comment: administration with food; levothyroxine, 75 µg, 1-0-0; comment: 30 min in front of food intake) into the app. For evaluation, a scoring system to assess the correctness was established. For each drug, the maximal score was four points that correspond to 100% correctness: name of the drug, dosage, dosing regimen and comments. Further, the time required for entering the medication plan into the system was measured. In addition, the volunteers were asked to answer a questionnaire regarding demographics and smartphone use and knowledge.

Results:
Overall, 100 subjects entered the study, 65% of whom were women. The average age of the test population was 31 years (range from 14 to 64 years). 95% of the subjects owned a smartphone and 21% already used at least one app focusing on healthcare or lifestyle. More than 50% stated they would use the MediSafe® app if they have to take medication regularly. Moreover, 75% would appreciate a recommendation of a compliance app by their physician or pharmacist. The average time for entering the medication plan was 6.37 min and the average correctness achieved was 66% (5.29 of 8 points). Only 18 subjects achieved the maximum score of 8 points. The most frequent errors were related to the dose or comment. Participants complained, that the user interface of the app was not intuitive. Most of the participants did not find the button to enter the dose or the additional comment regarding food intake.

Conclusion:
Previous studies have already shown that compliance apps are an easy-access-tool to enhance medication adherence. However, information about the individual medication plan has to be entered and individually adapted in a correct way. As our study shows, entering an exemplary, simple medication plan seems to be more difficult than foreseen. As a consequence, either the usability of the app has to see major improvements or the assistance by healthcare workers is needed in order to make compliance apps truly helpful. Thus, support provided by healthcare workers for setting up the app could be an opportunity to improve the medication adherence of patients and thereby medical care and public health.

POS.38
Package information leaflets (PILs) fail to inform patients – a study on the readability of PILs
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Background and objectives: In order to support patients with using medications, package information leaflets (PILs) contain important descriptions of medicinal products which include medical conditions, dosage, and side effects. Unfortunately, several studies have shown patients find PILs unintelligible and in need of literacy, and structural enhancements [1]. This project’s first objective was analyzing PILs readability for the 30 most prescribed drugs in Germany, Second, was determining how PILs can be optimized for greater readability.

Methods: PILs analyzed during this study belonged to the 30 most prescribed drugs in Germany, as was determined in literature [2]. TextLab, a software application, was used for its ability to implement text analysis that measures readability according to the Hohenthal Index. The Hohenthal Index is a scale that measures text legibility with a score from 0 (low comprehensibility) to 20 (high comprehensibility). A score of 12 indicates a technical text.

PILs belonging to simvastatin, prednisolone, salbutamol, and ramipril received linguistic optimizations, such as, shortened sentences and fewer technical terms. Furthermore, readability of optimized PILs for ramipril and prednisolone were investigated in a randomized crossover online study. Each of our subjects received and read a pair of PIL
excerpts, one from each drug, of which one was from an optimized PIL and the other not. After reading both, subjects answered questions concerning their understanding of the content. Processing time was tracked.

Results: Median Hohenheimer Index for the 30 PILs was 9.1 with a range of 4.7 – 13.9. Median number of words was 2598 with a range of 1522-4708 and median percentage of technical terms to nontechnical was 4.8% with a range of 2.7% – 9.5%. A total of 26 from the 30 PILs (86.7%) holds a Hohenheimer Index below 12. Median Hohenheimer Index for optimized PILs was 12.7 with a range of 10.9 – 14.2, whereas the median Hohenheimer Index for all four marketed PILs was 10.3 with a range of 6.5 – 13.1. Marketed and optimized PILs have a Hohenheimer Index median difference of 2.4 with a range of 1.0 – 4.4. Overall, 105 subjects (64.6% female) participated in the study, 77 (73.3%) of which were members of the pharmaceutical profession. Median age was 28 years with a range from 19 to 77 years old. The most impressive results came from PIL optimization of prednisolone (p-value < 0.05).

Conclusion: Results of our study indicate that marketed PILs are unintelligible to the patients. Moreover, readability of the PILs for the 30 most prescribed drugs is highly variable. This study was a first step to show that PILs can be significantly optimized by making simple improvements through shortening sentences and using terminology that is friendly for layman. A next step will be the improvement of the remaining 26 of the 30 most prescribed drug substances to confirm previous findings.

References:
patients with cSSSI and intraabdominal infections [3] and healthy volunteers [4].

Results: A two compartment model best described the data and was extended by a dialysis model based on both plasma flow rate and dialysate flow rate for the CVVHDF method and based on dialysate and ultra-filtrate flow rate for the CVVHD method. Dialysis clearance was 1.47 l/h (Inter-individual variability (IV): 42.4%) for CVVH patients and 2.57 l/h (no IV supported) for CVVHD patients, while the intrinsic clearance was much higher (4.6 l/h, IV: 54.5%). A mass balance analysis revealed a proportion of 9.51% (SD 4.78%) of the tigecycline dose to be eliminated by haemodiafiltration. This is comparable to renal elimination for patients without renal impairment. Simulations indicated a comparable 12-h area under the concentration-time curve (AUC_{0-12h}) for patients undergoing renal replacement therapy, healthy subjects and critically ill (median: 3.16, 3.12, 2.83; 10th percentile: 1.72, 2.63, 1.73; 90th percentile: 5.91, 4.16, 4.89; mg·h/l respectively).

Conclusion: A population pharmacokinetic model was successfully developed which included two dialysis methods. The mass balance analysis results suggest that continuous haemodialysis is sufficient to replace the renal function regarding the tigecycline clearance. AUCs of patients undergoing RRT were similar to AUCs of the comparison groups.

Acknowledgement: The authors thank Prof. Dr. Frieder Kees, University of Regensburg, for his analytical support.


Results: PAO1 displayed MIC values of 1 and 32 mg/L for colistin and streptomycin, respectively. Streptomycin alone decreased bacterial concentrations from 10.3 (growth control) to 2.9 log10 CFU/mL at 2x MIC. Colistin alone displayed full eradication if concentrations were ≥ 1xMIC. Bliss Independence indicated synergistic interactions in 8 of the 16 combination scenarios and no antagonism. Loewe Additivity indicated synergy in 4 of the 16 scenarios and antagonism in 4 of the 16 scenarios. The criteria agreed in only 50% of the combination scenarios.

Conclusions: The determined pharmacodynamic interaction agreed in synergism for subinhibitory concentrations of colistin and inhibitory concentrations of streptomycin on the selected additivity criterion and differed for subinhibitory concentrations of colistin and streptomycin, where Loewe Additivity indicated antagonism. Regardless of the additivity criterion and the interaction classification, streptomycin augmented the effect of colistin when compared to the single drug effect and decreased the concentration threshold for bactericidal activity from 1 to 0.25 mg/L for colistin. Further studies in static and dynamic time-kill curve settings with pharmacometric modelling are warranted to assess the clinical significance of the pharmacodynamic interactions between colistin and streptomycin.

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Variability of voriconazole exposure after approved sequence dosing in humans

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Background and objectives: Voriconazole (VRC), a broad-spectrum antifungal drug used to treat invasive fungal infections, shows complex pharmacokinetics (PK) and is primarily metabolised by the CYP isoenzyme 2C19 [1]. Apart from timely initiation of VRC therapy, effective and safe concentrations in all individuals, identified as minimal total plasma concentration (C_{min,total}) range of 1.5 mg/L – 4.5 mg/L, are crucial for therapy success [2]. The aim of the current work was to investigate the PK of VRC after the approved standard dosing in healthy volunteers in plasma using nonlinear mixed-effects modelling, focussing on PK variability and attainment of the PK target range.

Methods: A prospective, open-labelled, uncontrolled study was conducted in collaboration with the Medical University of Vienna. 10 healthy male individuals (age: 21-46 years, weight: 65-83 kg) received the standard dosing regimen for VRC of initially short-term i.v. infusions and subsequently p.o. administrations every 12 hours (2x6 mg/kg i.v., 2x4 mg/kg i.v., 3x200 mg p.o.). Intensive plasma sampling was carried out over 84 h and the unbound VRC concentrations were determined after ultrafiltration (UF) by high-performance liquid chromatography [3]. Total plasma concentrations were measured for three individuals over a wide concentration range (n samples=22) to quantify VRC plasma protein binding (PPB). Data analysis, as well as modelling and simulation activities were performed using R (3.3.2) and NONMEM (7.3.0, first-order conditional estimation method with interaction option).

Results: High interindividual variability in the VRC concentrations, especially for minimal concentrations (observed C_{min,total}) was detected and increased from the first (50.7 CV%) to the last dosing interval (63.6 CV%). The determined mean PPB of 47.4% (± 5.6%) was...
lower than previously described in the literature (58%), confirming current investigations on VRC PPB [4]. A two-compartment PK model with zero-order input (i) and first-order absorption (p) was suitable to describe the PK of unbound VRC in healthy volunteers. VRC unbound clearance (CL) was estimated to be 14.4 L/h, unbound central volume of distribution (Vc) 161 L, intercompartmental clearance (Q) 71.9 L/h, unbound peripheral volume of distribution (Vp) 603 L and absorption rate constant (ka) 2.21 h⁻¹. Interindividual variability implemented on CL, Vc, Vp, Q and ka using an exponential model was highest for CL (84.4% CV%). The developed PK model was used for stochastic simulations of 10,000 concentration-time profiles over 84 h. After converting predicted minimal unbound plasma concentrations (predicted Cmin,unbound) to predicted minimal total plasma concentrations (predicted Cmin,total) using the determined PPB, the attainment of the desired Cmin,oral target range was evaluated. Overall, only 41.8% of predicted Cmin,oral of healthy volunteers were within the PK target range, increasing from 3.9% at the end of the first dosing interval (12 h after first VRC administration) to 51.3% after the initial i.v. phase (48 h after first VRC administration).

Conclusions: The developed model adequately described the PK of unbound VRC in plasma and identified substantial interindividual variability, despite standard dosing in healthy volunteers. Next, a covariate analysis will be performed to identify factors (e.g. CYP2C19 genotype) explaining the high PK variability and in addition time- and/or concentration- and/or formulation-dependent influences on the PK will be further investigated. Ultimately, based on the final PK model alternative dosing regimens for different subgroups will be proposed.

References:

POS.43
Pharmacokinetic-pharmacodynamic, disease- and patient-related considerations for the optimization of infliximab therapy in inflammatory bowel disease

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Background and objectives: Infliximab (IFX) is an anti-tumour necrosis factor α (TNFα) monoclonal antibody used in the treatment of inflammatory bowel disease (IBD). Due to a high fraction (up to 60%) of treated patients that lose response to IFX over time and the fact that IFX exposure was found to be predictive of the loss of response, therapeutic drug monitoring (TDM) plays a crucial role for successful IFX therapy [1]. Hence, a quantitative IFX exposure-response model that enables prediction of a patient’s response based on measured IFX serum concentrations would substantially support the therapeutic decision making. The aim of the current study is to identify patient subpopulations at risk of therapy failure and contribute to rational use of IFX in IBD patients by using a pharmacometric approach to individualise therapy.

Methods: As the first step, an extensive literature research was performed in order to (1) summarise (a) all relevant pharmacokinetic (PK) and pharmacodynamic (PD) pathways of IFX, (b) disease characteristics and (c) underlying immune events and (2) understand the sequence of processes induced by an IFX administration. An exploratory graphical and PK pharmacometric analysis was performed on data (n=722) collected as a part of an investigator initiated study at the Medical University of Vienna. The IBD patients received 2× IFX infusions of absolute doses between 100 and 1300 mg. The serum samples (n=388, response markers and patient characteristics were collected approximately at the middle and at the end of dosing interval. Impact of patient and disease characteristics on IFX exposure was investigated and a population PK model was developed using the Bayesian (frequentist prior) approach. The analysis was conducted using R, NONMEM, PSn and Pirana software packages.

Results: The literature research identified the sequence of processes which might be responsible for IFX effects on the disease, as well as factors that could potentially impact PK of IFX (disease activity through inflammatory burden and target abundance, anti-IFX antibody presence, nonspecific clearance extent, faecal loss). The factors influencing IFX PK were further evaluated using nonlinear mixed-effects modelling approach. A PK model that approximates the body to two compartments, representing vascular space and all other organs, described the data best. Estimated parameters (drug clearance CL, volumes of the two compartments and intercompartmental drug exchange) were in the range of previously reported values [2-6]. Interestingly, target-mediated CL was found to be negligible, indicating that linear elimination pathways were predominant. Covariates significantly influencing CL were identified to be: (1) anti-IFX antibody status, (2) protein turnover (serum albumin concentration), (3) disease activity index, (4) concomitant therapy with immunomodulators and (5) body size. After inclusion of the covariates in the PK model, unexplained between-subject variability in CL was estimated to be ~40%. IFX exposure and response (captured by C-reactive protein concentration, serum albumin concentration and Harvey- Bradshaw disease activity index) were found to be related, enabling further development of a PKPD model.

Conclusions: A population PK model on outpatient data was successfully developed based on prior knowledge from the literature. Based on the model, covariates influencing CL of IFX were identified, contributing to the identification of subpopulations at risk. As a next step, the model will be linked to PD data in order to establish a PKPD disease model that can be used to support therapeutic decision making in a clinical setting.

References:
patients. STW 5 is very well suitable also in self-medication, and, as an European product, also not raising questions re. biopiracy.

References

PO.45
Marshmallow (Althea officinalis L.) as an established therapy in dry cough associated with pharyngeal irritation

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Background
According to a monograph of the European regulatory authority EMA, herbal preparations from Althaea officinalis L., radix (marshmallow root) are used as a demulcent preparation for the symptomatic treatment of oral or pharyngeal irritation and associated dry cough in Europe since centuries, and therefore are not subject to questions re. biopiracy (1).

Study design/Hypothesis/Purpose
The two prospective, non-interventional studies reported here aimed on creating a better documentation of the users’ impression of the effectiveness and tolerability and their satisfaction.

Methods
In two independently performed surveys with altogether 822 consumers buying either lozenges or syrup of the aqueous Marshmallow root extract STW 42 for treatment of their dry cough were recruited in pharmacies. They were asked to fill in a questionnaire covering a treatment duration of seven days for the documentation of the course of symptoms, and the global assessments of effectiveness, tolerability and satisfaction.

Results
The users stated that both preparations showed a good effect with respect to the symptomatic treatment of oral or pharyngeal irritation and associated dry cough with a very rapid onset of effects, in the majority of cases within 10 minutes. The tolerability was very good (3 minor adverse events for the syrup).

Conclusions
The results of the surveys support the long-standing use of Marshmallow root syrup and lozenges for symptomatic treatment of dry cough.

References
1. European Union herbal monograph on Althaea officinalis L., radix, EMA/HP/436679/2015
4.7 Inflammation

**POS.46**

**Glucocorticoid induced leucin zipper (GILZ) downregulation promotes anti-bacterial host defense**

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Macrophages are important elements of the innate immune system and form the first line of defense against host invaders. Their activation via Toll-like receptors (TLRs) leads to the recruitment of adapter proteins like myeloid differentiation factor 88 (MyD88) or TIR domain-containing adapter inducing IFN-β (TRIF), resulting in inflammatory actions, e.g. the liberation of cytokines, chemokines, reactive oxygen and nitrogen species [L 2]. In parallel, GILZ (glucocorticoid-induced leucine zipper, TSC2D3), an important mediator responsible for anti-inflammatory and immunosuppressive actions of glucocorticoids, is downregulated. This downregulation takes place on mRNA and protein level for all TLR-agonists except for TLR3 activation whereas the mRNA level remains constant [2].

Aim of this study was to elucidate the potential, adapter-molecule-dependent mechanisms of GILZ downregulation and its functional relevance in anti-bacterial host defense. Therefore we treated primary human macrophages with lipopolysaccharide (LPS), TLR4 agonist, Pam3CSK4 (TLR1/2 agonist) or Poly(I:C) (TLR3 agonist) to activate the cells through MyD88 and/or TRIF pathways. Employing IkB kinase (IKK) or proteasome inhibitors for quantitative reverse transcription PCR and western blot analysis, we show their involvement in the process of GILZ downregulation.

We characterized different types of murine GILZ wildtype and knockout macrophages, primary bone-marrow-derived macrophages (BMM) and a non-transformed murine macrophage cell line (MPI, Max-Planck-Institute macrophages), for the functional consequences of GILZ downregulation [3]. In GILZ knockout macrophages, tumor necrosis factor-α (TNF-α) secretion was increased after TLR1/2 (MyD88) as well as TLR3 (TRIF) activation. After LPS or Pam3CSK4 treatment, there was an increased NO production in GILZ knockout cells. These results underline once more the functional importance of GILZ as an anti-inflammatory modulator.

In conclusion, we propose a model of potential, IKK and proteasome dependent mechanisms of GILZ downregulation. First data show the functional significance of GILZ knockdown leading to an increased production of TNF-α and reactive nitrogen species. In present investigations we are testing the relevance of GILZ downregulation in bacterial infection.

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**References:**

**POS.47**

**Urate Transporter Inhibitor Lesinurad (Zurampic®) is a Non-Adipogenic PPARγ Modulator**

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With lesinurad (zurampic®), a first-in-class urate transporter (URAT1, SLC22A12) inhibitor has recently gained approval by both FDA (December 2015) and EMA (February 2016). It is used for the treatment of hyperuricemia associated with gout in combination therapy with conventional xanthine oxidase inhibitors (XOI). Lesinurad efficiently blocks the URAT1-mediated reuptake of uric acid into proximal tubular epithelial cells with an IC50 value of 7.3 μM causing lower serum uric acid levels and enhances urate elimination.[1]

Due to its Y-shaped structure resembling fatty acid mimetics,[2] we tested lesinurad for nuclear receptor modulation in vitro and identified a remarkable off-target activity on the peroxisome proliferator-activated receptor (PPAR) γ. Activated by endogenous fatty acids and eicosanoids, PPARs are involved in the regulation of lipid and glucose homeostasis as well as inflammation.[3–6] In contrast to both other subtypes PPARα and PPARδ, PPARγ plays a key role in lipid storage in adipocytes, where it is abundantly expressed, and in adipocyte differentiation.[6]

We confirmed direct interaction and activation of PPARγ by lesinurad under cell-free and cellular conditions by co-factor recruitment, isothermal titration calorimetry and hybrid transactivation assays. Furthermore, we studied PPAR-mediated effects on mRNA expression and the ability to differentiate fibroblasts into adipocytes. Therein, lesinurad revealed target-gene specific modulation of PPARγ and in contrast to rosiglitazone did not cause fat accumulation in adipocytes. Our findings support the notion that PPARγ activation has to be considered for the drug safety of zurampic®. However, this side-activity does not necessarily lead to adverse effects but could even contribute to the desired therapeutic goal with its anti-inflammatory properties because PPARα agonists e.g. have been shown to suppress the production of inflammatory cytokines.[7] Further experiments will be necessary to assess the PPARα modulatory potency of lesinurad.

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**References:**

**POS.48**

**Macroph-aging: Role of glucocorticoid metabolism**

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The aging process is characterized by a gradual deterioration of the immune function that leads to a chronic, low-grade inflammatory state, termed “inflamm-aging.” It has been suggested that macrophage activation plays a key role in the induction and maintenance of this state. In the present study, we aimed to evaluate the aging-associated changes in the myeloid compartment of mice in order to characterize the process of “macroph-aging”. Aged mice showed a pro-inflammatory phenotype characterized by decreased basal serum glucocorticoid levels, higher basal TNF-α, and LPS-induced IL-6 and TNF-α serum levels in comparison to young mice. In peritoneal macrophages and bone marrow macrophages (BMMs) isolated from aged animals higher levels of H2O2, TNF-α, and LPS-induced ERK1/2 phosphorylation were found. These changes were accompanied by a downregulation of the glucocorticoid-
receptor target genes GILZ (glucocorticoid-induced leucine zipper) and MKP-1 (map kinase phosphatase) in peritoneal macrophages and peripheral blood monocytes. Given that GILZ plays a central role in macrophage activation [1,2,3], we hypothesized that the loss of GILZ contributed to the process of macrophage-aging. Indeed, we observed that the phenotype of macroages from aged mice was mimicked in macrophages from young GILZ knockout mice. In addition to the lower serum glucocorticoid levels, the decreased GILZ levels in cells from aged mice were associated with lower 11β-HSD1 expression in peritoneal macrophages and peripheral blood monocytes, but not to changes in expression of glucocorticoid receptor. The above data provide insight into the role of glucocorticoid metabolism and GILZ regulation during aging.

Acknowledgments: this project was partly funded by the Deutsche Forschungsgemeinschaft (DFG KI70/3).

References

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<th>R</th>
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</table>

Table 1: Vorinostat analogues and their in vitro IC50 values

Acknowledgments: We thank Deutsche Forschungsgemeinschaft (DFG ZU295/13-1) for funding.

References

4.8 Medicinal chemistry and drug design

POS.49

Soft drug analogues of the histone deacetylase (HDAC) inhibitor Vorinostat

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Posttranslational modifications (PTM) of amino acids like acetylation, methylation or ubiquitination are important mechanisms to regulate gene transcription and cell regulation [1]. Acetylation on the Nε of lysine plays a crucial role in physiological processes like apoptosis, brain function and cell differentiation but are likewise involved in many diseases, like neurodegenerative diseases and cancer [2]. Histone deacetylases (HDACs), also known as lysine deacetylases (KDACs), catalyse the deacetylation on Nε of lysine on histones but also other proteins e.g. the tumour suppressor p53. Overexpression of this enzyme class is involved in inactive and non-toxic metabolites. Soft drugs (e.g. Fluocortinbutyl) are usually short acting which undergo a predictable metabolism. Therefore we utilized the soft drug principle. Soft drug analogues of the histone deacetylase (HDAC) inhibitor Vorinostat, Figure 1, the first HDAC inhibitor for the treatment of cutaneous T-cell lymphoma [3].

Figure 1: SAHA, Vorinostat

For drugs not only pharmacodynamics but also pharmacokinetics is essential. The aim of this project is to synthesise Vorinostat derivates, which undergo a predictable metabolisation. Therefore we utilized the soft drug principle. Soft drugs (e.g. Fluocortinbutyl) are usually short acting drugs with a metabolic labile group, which is converted into predictable, inactive and non-toxic metabolites [4].

We established a synthetic route for different derivates of Vorinostat, including as well esters and α-ketoesters as the corresponding free acids (Table 1). Contrary to the esters α-ketoesters the free acids are not cell permeable and hereby have no intracellular HDAC-inhibition effect anymore. Rapid hydrolysis of the ester structures should enable a topical delivery of HDAC-inhibitors with high GluN2B affinity and selectivity over related receptors [2]. In this project the electron rich methoxybenzene ring of 1 should be replaced bioisosterically by the thiophene ring. Moreover, the resulting [7]annuleno[b]thiophen-6-amines should be metabolically more stable than the methoxybenzene derivatives. Currently we determine the cellular activity of the esters in comparison to the free acids. Thereby our work represents a promising starting point to affect the pharmacokinetics preferably for local application and leading to less side effects.

<table>
<thead>
<tr>
<th>IC50 KDAC1±S D [nM]</th>
<th>IC50KDAC6±S D [nM]</th>
</tr>
</thead>
<tbody>
<tr>
<td>R = CH3</td>
<td>51±19</td>
</tr>
<tr>
<td>R = H</td>
<td>820±32</td>
</tr>
</tbody>
</table>

Table 1: Development of [7]annuleno[b]thiophen-6-amines from Ro 25-6981 and 1

Compound Ro 25-6981 is a highly potent but non selective GluN2B subunit containing NMDA receptor antagonist with [7]annuleno[b]thiophen-6-amine scaffold.

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The excitatory neurotransmitter (S)-glutamate plays a key role in the progression of neurodegenerative diseases. It interacts with various glutamate receptors and one of these subtypes, the NMDA receptor is in the focus of this project. Its activation leads to increased Ca2+ influx into the neuronal cell, resulting in some unique features such as long term potentiation, which is supposed to be crucial for brain functions such as learning and memory.

On the other hand pathological overstimulation of the NMDA receptor is often found in neurodegenerative diseases, resulting in neuronal cell death induced by high concentrations of Ca2+ ions. Thus NMDA receptor inhibition is a promising therapeutic approach. Since an overall inhibition would lead to undesired side effects, one of its subtypes, containing the GluN2B subunit should be addressed.

Figure 1. Development of [7]annuleno[b]thiophen-6-amines from Ro 25-6981 and 1

Compound Ro 25-6981 is a highly potent but non selective GluN2B antagonist [1]. Formal ring closure and further structure affinity relationship investigations, led to compounds 1 with high GluN2B affinity and selectivity over related receptors [2]. In this project the electron rich methoxybenzene ring of 1 should be replaced bioisosterically by the thiophene ring. Moreover, the resulting [7]annuleno[b]thiophen-6-amines 2 should be metabolically more stable than the methoxybenzene derivatives.

References
Flupirtine (Katalon®) is a drug currently used in Germany for pain management, especially when patients show contraindications to opioids or non-steroidal anti-inflammatory drugs (NSAIDs). In contrast to opioids and NSAIDs, the analgesic activity of flupirtine as well as its muscle relaxant effects are a result of the opening of voltage-gated potassium channels (Kᵥ) in the central nervous system. However, the occurrence of relaxant effects are a result of the opening of voltage-gated potassium channels.

In vitro studies show both flupirtine and retigabine to be potent openers of the Kv 7.2/3 channels, with retigabine possessing greater potency and intrinsic activity compared to flupirtine. On the other hand, flupirtine is more toxic in vitro. To better understand the cellular causes of flupirtine hepatotoxicity in the hopes of designing better, less toxic analogues, the potential for hepatotoxicity in vitro was assessed by using both transgenic mouse hepatocytes (TAMH) and human hepatocarcinoma cells (HEP-G2). Various cell-based assays such as MTT reduction, intracellular ATP levels and LDH release were used to gain information about cell viability and toxicity after treating cells for 24 and 48 h with increasing concentrations of flupirtine. For comparison, a structural analogue of flupirtine, retigabine, which is used in the treatment of epilepsy but has not been reported to be hepatotoxic, was also studied. To better understand the mechanisms of death in liver cells, specific markers of necrosis, apoptosis and autophagy were investigated. In addition, the Kᵥ 7.2/3 (KCNQ2/3) opening activity of the two compounds was measured by a thallium flux-based functional assay by using transfected HEK-293 cells that overexpress human KCNQ2/3 channels. The results of these studies show both flupirtine and retigabine to be potent openers of the Kᵥ 7.2/3 channels, with retigabine possessing greater potency and intrinsic activity compared to flupirtine. On the other hand, flupirtine is more toxic to liver cells in vitro.

3D-cell culture with spheroids of HEP-G2 and TAMH were also investigated as possible model systems to better study flupirtine hepatotoxicity. Interestingly, spheroids exposed to flupirtine and retigabine take up strong fluorescence properties that cannot be directly caused by the parent compounds since the compounds only very weakly fluoresce. We hypothesize that this fluorescence may be a result of oxidative metabolism of the compounds within the spheroids, which may be related to their toxicity.

During the last decades, computational approaches have become indispensable for drug design campaigns but also as auxiliary tool for structural biology. In particular for the research on G protein-coupled receptors (GPCR), the combination of in-silico methods and pharmacological experiments represent a strong alliance for functional investigations. Over the last decade, specific GPCR-ligand complexes were determined by crystallography providing an indispensable structural view on this protein class, although these crystal structures represent snapshots of a highly dynamic conformational ensemble. Taking muscarinic acetylcholine receptors as model systems, we combine computational simulations and 3D-pharmacophore analysis to provide a more dynamic view on GPCR function [1,2].

Starting from carefully developed homology models of MACHRs, extensive binding mode analyses were performed by means of receptor-ligand docking and 3D pharmacophore modeling. In order to sample the flexibility and the dynamic properties of the receptor-ligand complexes we carried out all-atom molecular dynamics simulations. This combination led to mechanistic GPCR models that comprise both inactive and active-like receptor states [3]. After characterization of the orthosteric binding pocket, we focused on dualistic ligand binding. Dualistic (bitopic) ligands simultaneously bind to the orthosteric and the allosteric binding site and combine the high affinity of orthosteric ligands with the high specificity of the allosteric binding site leading to subtype selective modulators like at-6-naph or iper-6-pht [3,4]. Furthermore, we developed mechanistic models to show that partial agonists for the muscarinic M₁ receptor stabilize distinct fractions of inactive agonist-bound receptors [5]. Using bitopic ligands as pharmacological tools, we clearly identified a dualistic and a purely
allostERIC binding mode stabilizing active and inactive receptor states, respectively. Modulation of the resulting ligand binding ensembles by various means reveals that agonists’ preference for inactive receptor complexes decreases its overall efficacy. Our findings suggest a more general role of ligand binding ensembles in determining agonist efficacy. Since GPCRs can activate multiple signaling roles, we investigated the structural basis for functional selectivity (biased signaling). We demonstrate on a molecular level a ligand-specific restriction of the extracellular domain [6]. Our data indicate a link between this conformational restriction and a shift of the physiologically imprinted signaling preference towards G\textsubscript{i}, representing a rational explanation for functional selectivity.

Taking together, a deep mechanistic knowledge of GPCR functionality might pave the way towards the rational design of tailor-made GPCR modulators with reduced side effects.


Boron Neutron Capture Therapy
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Boron in an emerging element in drug development. Boronic acids and recently benzoxaborole therapeutics have already reached the market.[1] In contrast to most other elements in organic drug molecules, boron offers additional properties for therapeutic applications, such as Boron Neutron Capture Therapy (BNCT). BNCT is a feasible alternative for the treatment of challenging tumors. The high cross section of the stable and non-radioactive \(^{10}\) B isotope allows the capture of slow neutrons inducing a nuclear fission reaction (Fig. 1). Importantly, this nuclear event destroys surrounding cells only.[2]

![Figure 1. Boron Neutron Capture.](image)

Crucial for the success of BNCT is the selective enrichment of about 30 \(\mu g\) of \(^{10}\) B of tumor. Therefore, two strategies are applied. First, \(^{10}\) B is integrated into a tumor-seeking molecule. Second, boron clusters are used as boron carriers to increase the boron load. Our research addresses the chemistry and therapeutic application of 12-vertex dicarba-closo-dodecaborane (short carbonarate, Fig. 2) clusters carrying ten boron atoms pericosahedron.[3] Here, novel chemical methods to modify the clusters at selected positions, the formation of carbonaryl building blocks and conjugation protocols to connect the \(^{10}\) B-carrier-cluster into tumor-seeking molecules are presented.[4,5]

![Figure 2. Carborane Isomers.](image)

**References**

POS.55
Computer-assisted discovery and optimization of bioactive compounds from natural sources

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Natural products from plants, animals, marine life, fungi, bacteria and other organisms are an important resource for modern drug discovery. Their biological relevance and structural diversity make natural products good starting points for drug design [1-3]. Natural-product-based drug discovery can benefit greatly from computational approaches, which are a valuable precursor or supplementary method to in vitro testing [4]. Computational approaches can be used, for example, to predict binding affinities to a particular target, to predict ADME (absorption, distribution, metabolism, excretion) and toxicological properties, and to elucidate the biological significance of an observed effect, such as that of an herbal remedy.

This contribution will give a succinct, critical overview of the scope and limitations of current computational methods in natural products research, including methods for the discovery of bioactive natural products and the optimization of natural product derivatives. Today, the high chemical structures of more than 250,000 natural products are available from about 30 public databases. We have overlaid this collection of natural products with data on about 7 million readily available and about 300 million on-demand compounds to determine the purchasable natural product space. In total, we found that more than 10% of known natural products are readily purchasable from about 200 different vendors. By allowing small deviations in molecular structure, about 60,000 readily purchasable natural products and derivatives were identified. These are particularly interesting for computer-assisted drug discovery, as they allow the effective identification of promising natural products at a low cost. Many of the readily purchasable natural products are of small size and hence also of relevance to fragment-based drug discovery. There are also an increasing number of macrocyclic natural products and derivatives becoming available for screening.

In the last part of this contribution, we will present new computational models for the prediction of metabolically labile atom positions in natural products as well as a robust, shape-based model for target prediction. The latter is employed in our lab for predicting the activity profile of natural products on 3,000 different protein targets, and for identifying new targets for known natural products. The model can also be applied to assess the target space coverage of whole natural product libraries. This is useful for determining whether readily purchasable natural products cover a specific target of interest or whether extraction of natural products from selected plant material is necessary.

**References**

POS.56
Molecular pharmacology studies of PqsR modulators

Inorg. Chem.
Antibiotic resistance is rapidly spreading among clinically relevant pathogens. The medical need for alternative anti-infective treatments is tremendously high today. A novel paradigm in combating infectious diseases is the antivirulence concept. Pathoblockers are compounds which disarm bacteria of their arsenal of virulence factors instead of directly killing them. As such, interference with bacterial cell-to-cell communication globally regulating the formation of various virulence factors and biofilm is a very promising approach. PqsR (MvfR) is a transcriptional regulator controlling communication among bacteria and production of such factors in Pseudomonas aeruginosa, most prominently pyocyanin. The discovery of such antipathogenic compounds necessitates more understanding of the underlying pharmacology and molecular regulation mechanisms of its respective targets. As an example, we herein discuss a series of tool compounds based on the structure of PqsR natural ligand 2-heptyl-4-quinolone (HHQ) that were used for probing the structure-functionality relationship. Four pharmacological profiles were identified namely agonists, antagonists, inverse agonists and biphasic modulators. Molecular docking studies revealed that each class of the PqsR modulators showed distinctive interactions in the PqsR binding domain. Interestingly, it was found that subtle modifications at one single position of the HHQ moiety (C-3) act as a switch between the different profiles, according to their ability to donate or accept a hydrogen bond, or forming a hydrophobic interaction. Finally, it was shown that PqsR – a bacterial transcriptional regulator – behaves in a similar fashion to human receptors i.e. GPCRs and an inverse agonistic mode of action is required to induce an impactful biological response via inhibiting pyocyanin. As a general conclusion, we would like to emphasize that in drug discovery it may not be sufficient to find potent compounds, instead, their pharmacological profile should be determined as it might be more important than the absolute IC50 value.

Although TLR2 has been implicated in protective immunity against infection, excessive TLR2 signaling may contribute to inflammatory and metabolic disease such as sepsis, rheumatoid arthritis, and diabetes type II [1]. Thus, TLR2 represents an important pharmacological target for the control of inflammatory conditions. In the present study, we experimentally characterized and validated TLR2 ligands which have been identified by computational modelling. A total of 18 compounds were tested for TLR2 antagonism and compared with CU-CPT22 using human embryonic kidney TLR2 overexpressing cells [2-4]. CU-CPT22 and three compounds reduced TLR2-mediated responses at 25 µM by more than 60% without showing cellular toxicity. However, there was poor selectivity towards TLR2/1 or TLR2/6 heterodimers for all inhibitors. Compound 16 proved to be a more potent TLR2/1 inhibitor than CU-CPT22 as determined by IC50 values in the low µM range. In addition, TNF and IL-8 levels were reduced in TLR2-stimulated THP-1 macrophages and human peripheral blood mononuclear cells. CU-CPT-22 and 16 had no effect on TLR4-induced cytokine release. In conclusion, one out of 18 virtually screened small molecules was experimentally confirmed as a selective and potent TLR2 inhibitor with low cytotoxicity.

Acknowledgments: This work was supported by grants from the Deutsche Forschungsgemeinschaft (DFG) to Wolber, G. and Weindl, G.

References:

POS.57
Experimental validation of Toll-like receptor 2 inhibitors identified by computational modelling
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Toll-like receptor 2 (TLR2) is a type 1 transmembrane receptor and heterodimerizes with either TLR1 or TLR6 to induce early inflammatory responses to pathogen and damage-associated molecular patterns.

POS.58
Synthesis and characterization of trisubstituted pyridine derivatives as modulators of the potassium channel K+7.2/3
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Flupirtine (1) and retigabine (2) are effective drugs for the treatment of analgesia and epilepsy, respectively. Even though retigabine was withdrawn from the worldwide market entirely in June 2017, flupirtine is still used in numerous countries including Germany. Flupirtine’s wider usage is strictly limited due to hepatotoxicity. The mechanism of analgesic action of flupirtine, which has been sold as Katadolon since 1984, is still a subject of debate; modulation of voltage gated potassium channels and resulting hyperpolarisation of neurons is most likely responsible for its central analgesic effect [1]. Consequently, without any further knowledge about the binding-site or structure activity relationships (SARs) it has been a challenge to develop the next generation derivatives of flupirtine and retigabine with reduced side effects. Previous findings of our group demonstrated that trisubstituted pyridine derivatives show a correlation between their oxidation potentials and K+7.2/3-channel opening ability but not their toxicity to liver cells [2]. To investigate this hypothesis further, we synthesized an expanded series of derivatives with modifications in position X, Y, R1 and R2 that follow closely the structure of flupirtine. We report here the characterization of their oxidation potentials by cyclic voltammetry, their cytotoxicity to liver cells growing in culture as well as their activity for opening K+7.2/3-channels in a cell-based assay.

Acknowledgements: We thank our colleagues Christine Mauermann, Martin Empey and Benjamin Kirch who provided insight and expertise that greatly assisted the research. We thank Simone Amann for her assistance with the biological assays and Lorence Siebenbürger for the purification of a few compounds.


DPhG Annual Meeting 2017 Conference Book -121-
Studies on Backbone-Modified Oligonucleotides as Prodrugs

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Oligonucleotides (ON) are promising therapeutic agents, e.g. by binding the mRNA that encodes proteins which are essential for cellular functions (antisense mechanism) [1]. However, ON are challenging drug candidates as they usually have poor chemical stability along with high polarity that hinders their penetration of cell membranes. Therefore, chemical modifications of the backbone structure are required [1]. A novel approach in this field is the preparation of prodrugs of antisense ON (AON), which are designed to efficiently cross biological barriers such as cell membranes and to be enzymatically deprotected inside the cell (see figure). This approach has already been successful for siRNA [2].

Our overall goal is the synthesis of electroneutral AON prodrugs, composed of about 20 nucleotides with backbone-masking groups, which will allow for an esterase-mediated intracellular release of the active form. The first step has been the synthesis of model compounds, such as TxT dimers and TxTxT trimers (x = modified internucleotide linkage), to test the stability of different masking groups in several media. First results from these studies will be presented.

References:

Metabolic Stability Determination in vitro of Tetra-Substituted Pyridinylimidazoles

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Huntington’s disease (HD) is an autosomal dominant genetic disease, which leads to brain cell degeneration in the striatum [1, 2]. For HD-treatment, the dual inhibition of c-Jun N-terminal kinase (JNK) 3 and p38 mitogen-activated protein (MAP) kinase might be a promising strategy [3]. Therefore, tetra-substituted imidazoles were designed as dual JNK3/p38α inhibitors. The metabolic stability of substance 1 and 2 was determined to further profile these best-balanced and therefore most favourable dual inhibitors. Phase 1 biotransformation was investigated in vitro in male human liver microsomes (HLM). Both tested dual inhibitors seem to undergo metabolic degradation, which leads most likely to the oxidation of the imidazole-C2 sulfur. Nevertheless, neither 1 nor 2 reached its half-life during the longest incubation time of 240 min and their concentrations declined about 31% and 44% (table 1) relating to the starting concentrations, respectively. Therefore, they can be considered sufficiently metabolically stable and suitable for in-vivo probes.

References:

Table 1: Metabolic Stability Data for Tetra-Substituted Pyridinylimidazoles 1 and 2

<table>
<thead>
<tr>
<th>compound</th>
<th>R1</th>
<th>R2</th>
<th>OxMet1</th>
</tr>
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<tbody>
<tr>
<td>F</td>
<td>Na</td>
<td>Na</td>
<td>CYP-mediated biotransformation</td>
</tr>
</tbody>
</table>
A dual topoisomerases inhibitor with markedly superior
antitumor activity in ovarian cancer relative to standard
therapeutics

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Ovarian cancer is one of the most frequent malignant tumor diseases in
gynecology with the highest rate of mortality [1,2]. Insufficient therapeutic
options are responsible for this frightening increase of cancer incidence
and cancer deaths. Hence, research on substances such as the aza-
derived tetrasubstituted imidazoles as Dual Inhibitors of Phosphatidylinositol-3-

The results indicated a significant higher apoptosis rate by treating
cancer cells with P8D6 compared to standard therapeutic drugs. This
difference was very clear for the 48h treated cells. A considerably
increased death rate after treating with P8D6 could also be clarified on
the microscopic pictures. The incubation study evidenced the best effect
for P8D6 at 48h. Besides, the flow cytometry experiments show very
promising results for P8D6 in rate of apoptosis. P8D6 is a further cytostatic agent in the development phase, which can
counteract a resistance development by its nonselective inhibition of both
topoisomerases. Our studies demonstrate its promising antitumoral
effect.

Persons with Huntington’s Disease: The PREDICT-HD Study. Movement Disord. 2009, 24 (12),
1763-1772.
disease and its relationship to markers of disease progression: evidence of early lack of
Tofacitinib was one of the first small molecule JAK inhibitors. It shows a potent and selective JAK3 inhibitors with a covalent-reversible binding within the JAK-family [4]. Recently we reported the development of highly

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Figure 1: Schematic representation of the postulated mode of action

POS.65

New opportunities for the treatment of NASH

POS.66

Non-alcoholic fatty liver disease (NAFLD) is considered as hepatic manifestation of the metabolic syndrome and can progress to non-alcoholic steatohepatitis (NASH). NAFLD has a high global prevalence and the amount of circulating triglycerides accompanied by improved bile acid receptor FXR. Activation of FXR decreases gluconeogenesis and the amount of circulating triglycerides accompanied by improved lipid metabolism and a decrease in hepatic steatosis by increasing mitochondrial \( \beta \)-oxidation. In obese patients, FXR activation leads to additional anti-inflammatory effects [1,4]. The GOLPh program indicates that NASH was resolved without fibrosis and during the next decade, NASH is expected to replace hepatitis C virus infection as leading cause of liver transplantation in the US. Until now there is no FDA-approved therapy available. Currently, the farnesoid X receptor (FXR) agonist obeticholic acid and the dual peroxisome proliferator-activator receptor \( \alpha/\delta \) (PPAR\( \alpha/\delta \)) agonist elafibranor suggest that a combination of FXR and PPAR\( \alpha/\delta \) activation could have synergistic effects in NASH treatment [3, 5].


Structure-based design and synthesis of novel hydroxamates as dual target inhibitors

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In the present study we designed histone deacetylase (HDAC) inhibitors coupled to different bromodomain-inhibiting scaffolds. Although this approach was previously reported [3-5], we were the first to use aromatic hydroxamic acids as specific HDAC8 inhibitor. HDAC8 is an interesting epigenetic target not only because it was related to different types of cancer, but also as it is over expressed in all life stages of the major human parasite Schistosoma mansoni [10-11]. General structures of these dual inhibitors (1-2) are shown in the figure below.

In addition, we synthesized further compounds with expected dual inhibitory activity against HDACs and dihydrofolate reductase (DHFR) enzymes. We hypothesized that incorporating a hydroxamic acid moiety, to a reported nanomolar DHFR inhibitor [12] can potentiate its activity against Plasmodium falciparum, the causative pathogen of human malaria. Encouraged by docking studies, some hydroxamic acid analogues were synthesized. Biological evaluation of the synthesized compounds is in progress. Most of the compounds tested so far showed good in vitro HDAC inhibitory activity. However, the activity against bromodomains was not optimal and some modifications are to be done to overcome this problem.

References:

Crystallographic Validation of Amide Bonds as Bioisosteres of Acylhydrazones in the Modell Protease Endothiapepsin

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Acylhydrazone-based DCC is a powerful strategy for the rapid identification of novel hits in drug discovery [1]. The further progression of acylhydrazones in development may, however, be hampered owing to major stability and toxicity issues under physiological conditions. It is therefore important to identify stable replacements for acylhydrazone linkers of initial hits discovered by DCC. Here, we present the first report on the design and synthesis of stable bioisosteres of acylhydrazone-based inhibitors of the aspartic protease endothiapepsin [2, 3]. The most successful bioisostere bears an amide bond and its binding mode was validated by X-ray crystallography [4]. Having some validated bioisosteres of acylhydrazones readily available with similar binding modes might accelerate the hit-to-lead optimization for future acylhydrazone-based DCC projects.

Figure 1: a) Superimposition of the acylhydrazone inhibitor 1 (green) and the amide bioisostere 2 (light blue). Shown as black lines are the hydrogen bonds that participate in the binding of both compounds. (color code: protein backbone: C: gray, O: red, N: blue, 1: C: green and 2: C: light blue) b) Structures of the inhibitors with corresponding IC50 values.

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Design, Synthesis and Biological Testing of novel EGFR Inhibitors with Low Nanomolar Activity against the Osimertinib-Resistant L858R/T790M/C797S Mutant

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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 (W2402, 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 dependence on this particular interaction means that additional mutation of Cys797 results in poor inhibitory activity, which leads to tumour relapse in initially responding patients [2, 3].
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 IC50-values in an enzyme assay and down to 14 nM EC50 in a cellular activity assay against p38, led us to pick this compound as a lead structure for further improvements in terms of mutant EGFR inhibition. 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 IC50-values in an enzyme assay and down to 14 nM EC50 in a cellular activity assay against p38. Therefore, it was necessary to check the EZERK1 morphoe of the created salts by using XRPD and DSC. The kinetic and intrinsic solubility were determined with titration measurements and the water solubility was checked by conducting shake flask experiments. In the present contribution, the analytical results of the newly created salts will be presented.

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References:

POS.70

Development of Pharmacophore Model for Indeno[1,2-b]indoles as Human Protein Kinase CK2 Inhibitors and Database Mining

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Protein kinase CK2, initially designated as casein kinase 2, is an ubiquitously expressed serine/threonine kinase. This enzyme, implicated in many cellular processes, is highly expressed and active in many tumor cells. A large number of compounds has been developed as inhibitors comprising different backbones. Besides others, structures with an indeno[1,2-b]indole scaffold turned out to be potent new leads. With the aim of developing new inhibitors of human protein kinase CK2, we report here on the generation of common feature pharmacophore model to further explain the binding requirements for human CK2 inhibitors. Nine

POS.69

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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The implementation of new reliable product development programmes for active Pharmaceutical Ingredients (API) is very interesting for the pharmaceutical industry, because very few of the evaluated drugs in clinical tests actually make it to the market, decreasing the accessibility of more efficient therapies. Although solids are easy to handle and offer a high thermal stability, the lack of water solubility often minimize their clinical application. Therefore, the design and synthesis of new solid forms offers a way to improve their efficiency by enhancing their solubility, dissolution, thermal stability, bioavailability and/or pharmacokinetics.1 Since large counterions are able to prevent strong lattice forces within the API crystals, it was aimed to use those large natural acids within a huge screening program for salt formation of basic, poorly water-soluble APIs, in order to enhance their solubility.2,3 The dried milky sap from Papaver somniferum, known as opium, was chosen as model system, because it consists of alkaloids of different backbones. Beside others, structures with an indeno[1,2-b]indole scaffold turned out to be potent new leads. With the aim of developing new inhibitors of human protein kinase CK2, we report here on the generation of common feature pharmacophore model to further explain the binding requirements for human CK2 inhibitors. Nine
common chemical features of indeno[1,2-b]indole-type CK2 inhibitors were determined using MOE software. This pharmacophore model was used for database mining with the aim to identify novel scaffolds for developing new potential and selective CK2 inhibitors. Using this strategy several structures were selected by searching inside the ZINC compound database. One of the selected compounds was bikaverin (6,11-dihydroxy-3,8-dimethoxy-1-methylbenzo[b]xanthene-7,10,12-trione), a natural compound which is produced by several kinds of fungi. This compound was tested on human recombinant CK2 and turned out to be an active inhibitor with an IC50 value of 1.24 µM [1].

The chemical structure of bikaverin (6,11-dihydroxy-3,8-dimethoxy-1-methylbenzo[b]xanthene-7,10,12-trione), first isolated from the culture of *Fusarium vasinfectum*

References

CYP121 and CYP125 as Targets for Novel Antimycobacterials

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Tuberculosis, caused by *Mycobacterium tuberculosis* (Mt), is still the most deadly bacterial infection worldwide. In 2014, the WHO registered about 1.5 million cases of death related to Mt infections.[2] Despite a broad arsenal of available antimycobacterial agents, treatment becomes ineffective due to the increased abundance of multiresistant mutants.[3] These circumstances underline the urgency for new therapeutic strategies with novel modes of action. The antimycobacterial P450 enzymes CYP121 and CYP125 are such a promising new drug targets for prospective antitubercular agents. CYP121 was shown to be essential for Mt growth in *vitro*.[4] and CYP125 plays an important role in the essential cholesterol metabolism of Mt.[5] Previous studies revealed azole antifungals (e.g. econazole)[6] to be affine binders of CYP121 and CYP125 with effectiveness against Mt in *vitro*.[7] For the rational discovery of new CYP121 and CYP125 inhibitors a systematic screening of a focused P450-inhibitor library was preformed based on biophysical and microbiological methods. With a combined screening approach consisting of surface plasmon resonance spectroscopy (SPR) and a heme coordination assay new CYP121 and CYP125 binders could be identified. For CYP121 14 heme type II binders with low micromolar Kd's were discovered. The initial screening procedure was followed by the evaluation of the antimycobacterial activity which resulted in single digit µg/ml MIC50's against M. bovis BCG in *vitro*. The frontrunner compound showed also significant growth inhibition against the human pathogen Mt with a MIC90 of 0.3 µg/mL and inhibition of the enzyme reaction in *vitro*. For CYP125 we could identify first hits with low nanomolar affinity and preferable ligand efficiencies (LE). Furthermore, the frontrunner compounds showed selectivity towards other CYP enzymes specific to Mt. Due to a low molecular weight and suitable physicochemical properties, our compounds are intriguing starting points for the development of novel drugs that might be able to overcome *M. tuberculosis* resistance.


Development of 3-Amino-benzhydroxamates as selective co-inhibitors of the human HDAC8 and 10 for the treatment of neuroblastoma

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Besides the genome and proteome itself, their modifications that are described as epigenome plays a major role in the cell cycle e.g. maturation or differentiation and the up or down regulation of genes or enzymes and became an interesting target for the treatment of various diseases. In a previous study [1] we found 3-amino-benzhydroxamates as potent inhibitors of the human HDAC8 that are now under investigation in childhood cancer. For neuroblastoma it was shown that high HDAC8 expression correlates with advanced stage disease and poor overall survival [2] and high HDAC10 expression is associated with exceptionally poor outcomes in advanced stage patients [3]. Here, we report that the novel compounds combine characteristics of HDAC8 and HDAC10 inhibition, resulting in strongly impaired colony growth of neuroblastoma cells. Since such compounds show promising effects on neuroblastoma cells we started with the synthesis of a series of inhibitors targeting human HDAC8 and 10. We wanted to develop selective HDAC8/10 inhibitors since inhibition of other HDACs leads to a dose limiting toxicity that causes side-effects like leukopenia, weight loss and fatigue syndrome.

During the last ten years, in Europe the mortality caused by diseases of the central nervous system in Europe increased. [1] Overactivation of the N-methyl-D-aspartate receptor (NMDA receptor) is associated with the pathogenesis of neurodegenerative diseases. Therefore the NMDA receptor represents a promising target to develop new therapeutic drugs and diagnostic tool compounds. [2] Ifenprodil was found to be a potent antagonist at the NMDA receptor containing the GluN2B subunit. Although it shows a high GluN2B affinity (Kᵢ = 10 nM), its selectivity towards other receptors is rather low. [3] To overcome this problem sterically restricted analogues of ifenprodil with a benzazepine structure such as 1 (Kᵢ = 14 nM) have been synthesized. [3] The aim of this project is the further increase of GluN2B affinity and GluN2B receptor inhibiting activity by replacing the phenol of 1 by another electron rich aromatic system such as thiophene 2. Moreover, the particular reactivity of thiophene will allow the regioselective derivatization in α- and β-position.

![Chemical structure of compounds 1 and 2](image)


POS.74


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Innovation is cost and time expensive. Thus, the pharmaceutical industry must be able to provide the financial resources. Whereas the industry of the 70’s and 80’s of the past century had enough financial resources, a turnaround appears. Less successful research [1], higher investments [2] and more regulatory pressure [1, 3] force the industry to invest profitably and to maximize the return of investment. Hence, the entire life of a medicinal product (MP) must be managed successfully to gain enough for further research.

We characterize the life cycle (LC) of a MP and scrutinize the corresponding management possibilities by literature research. It is evident, that three core questions have to be answered throughout the entire life of a MP:

1) What is reasonable - to stop or continue the LC?
2) How can the time-to-market be maximally reduced?
3) How can the company expand the LC and therefore delay the market withdrawal?

The first question is an individual one that cannot be answered within this work. Nevertheless it is crucial: only 0.01-0.02% of the initially synthesized lead structures make it to the market [4]. At some point during Research and Development (R&D), the companies must decide to abandon a lead structure because of failure or to prioritize other structures due to financial aspects.

The answer for the second question must be found in the first part of a LC. R&D of a medicinal product reduces the patent protection from 20 years [3] to seven or eight years [4]. Each shortening of the R&D time generates valuable time with patent protection and higher earnings. The attempts of the pharmaceutical industry are: optimization of the internal structures [2], open source drug discovery [2], a growing interest in rare diseases [1] and computer-based tools [5]. Additionally, new trends in process design shall ease the development of production facilities and thus the approval from regulatory bodies [6].

The last question is addressed throughout the entire LC. Directly from the beginning, the pharmaceutical company must start further R&D activities in order to receive secondary patents [3]. Besides differentiation [7], own generics [3] and patent settlements [8], a switch of the prescription status [1, 3] is a common strategy.

Findings suggest that the pharmaceutical industry is seeking solution statements for parts of life cycle management. Yet, only few companies started to seek solutions in restructuring their business models [9]. This contribution sets the basics for further research in which product and process design will evolve through computer simulations.

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References:
is the introduction of polar or ionic groups to increase the hydrophilicity. As an alternative option, the introduction of a fluoro substituent in ortho-position of an aromatic ring system was recently suggested [3,4]. In order to explore the general applicability of this concept, we compared the melting temperature as surrogate parameter of solubility of ortho-fluorinated compounds and their unsubstituted congeners. It turned out that in many cases ortho-fluoro substituents indeed have significant impact on melting temperature and solubility. However, the effect cannot be generalized and depends on the respective compound class. In the presentation we report two series of molecules, namely benzamidines and 2-arylbenzimidazoles, in which ortho-fluorination consistently decreases the melting temperature. The kinetic and thermodynamic solubility of ortho-fluoro substituted and non-substituted examples from both compound classes will be reported and compared with calculated solubility values.

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Antimycobacterial wollamide analogues: Synthesis, PK data, and activity

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Antimycobacterial peptides have great potential to be the next anti TB drugs owing to their rapid bacterial action, high selectivity for the prokaryotic cell envelope, unique mode of action that differs from conventional antibiotics and their proven record of use as a host first line defense without developing notable bacterial resistance [1-3]. Wollamide B is a cationic antimycobacterial cyclic hexapeptide that exhibits activity against Mycobacterium bovis (M. bovis) (IC50 of 3.1 µM) [4]. Aiming to study its structure activity relationship (SAR) and optimize potency and pharmacokinetic properties, libraries of analogues were synthesized and tested against Mycobacterium tuberculosis (Mtb) H37Rv. Parallely, the in vitro pharmacokinetic (ADME) profile was synthesized and tested against Mtb H37Rv. The in vitro pharmacokinetic (ADME) profile was examined to evaluate their drug likeness.

Seven of the new wollamides had potent in vitro antimycobacterial activity (MIC = 0.60 – 3.1 µM) and were devoid of toxicity against human HepG2 cells. The MICs were corroborated by their excellent intracellular activities (IC50 = 0.16 – 2.0 µM) against Mtb H37Rv infected human macrophages. The in vitro ADME profiling proved the remarkable plasma stability and very good aqueous solubility of the class while the metabolic stability was found to be moderate to low. For two of the wollamides that showed a good balance of activity vs pharmacokinetic properties, an in vivo proof of concept (PoC) study was also performed using an acute mice model of TB.


Characterization of and Interference with Protein-Nucleic Acid Interactions Guided by Fluorescence Polarization Assays

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Recognition and binding of specific sites on DNA or RNA by proteins plays a central role for many essential cellular functions such as transcription, replication, and recombination. This holds also true for processes driving and/or maintaining bacterial and viral infections providing fascinating, yet challenging, opportunities in drug discovery [1]. Hence, finding new approaches for the modulation of such macromolecule-macromolecule interactions has become of increasing interest. We use fluorescence polarization (FP) as a rapid and quantitative method for the identification of small-molecular hits, which interfere with these molecular recognition machineries. We study two different target proteins, the bacterial carbon storage regulator A (CsrA) and the viral Latency-Associated Nuclear Antigen (LANA), which interact with RNA or DNA, respectively. The bacterial regulator CsrA mediates global effects on translation by binding to GGA-motifs in mRNAs and is involved in regulation of pathogenicity traits [2]. The viral LANA protein is required for the latent viral replication and persistence of KSHV (Kaposi Sarcoma Herpesvirus) via tethering the virus episome to the host chromatin [3]. Using a variety of biophysical approaches we discovered the first hits for both targets and evaluated these molecular scaffolds in a FP competition assay regarding their ability to disturb the protein-nucleic acid interaction. The gained structure-activity relationship (SAR) insights will be exploited for future fragment-growing efforts in hit-to-lead optimization campaigns.


Structure-activity-relationship of lipid-lowering agent pirinixic acid on PPAR and RXR

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Pirinixic acid is a dual peroxisome proliferator activated receptor (PPAR) alpha and gamma agonist and has shown good lipid-lowering effects in rodent models [1]. Since its discovery in the mid-1970s it was extensively used as a tool compound to investigate PPAR mediated effects resulting in the discovery of a variety of beneficial effects in various diseases [2]. But although its lipid lowering efficacy is similar to that of fibrates, pirinixic acid has never reached clinical trials, which might be explained by multiple side-effects [3]. We have discovered that pirinixic acid also activates all three subtypes of retinoid X receptors (RXR), which could be an explanation for the observed adverse effects and open new possibilities for pirinixic acid as lead compound for selective PPAR and RXR agonist development.

Fig. 1: Structure of pirinixic acid
To study the structure-activity relationship (SAR) of prinixin acid for agonistic activity on PPAR and RXR we systematically varied the scaffold and its substituents and characterized the derivatives in specific cell-based hybrid reporter gene assays for all PPAR and RXR subtypes. We observed a different SAR for both receptor types. Especially the 2,3-xylen moeity turned out crucial for activity on RXR whereas alky chain substituents in α position were favoured by PPAR but diminished RXR agonistic activity. Furthermore, the chlorine atom of the central pyrimidine ring has a major impact on RXR activity while PPAR activation is less affected by different substituents in this position. Our findings on one hand give an explanation for the diverse side-effects of prinixin acid and on the other hand implicate an interesting SAR profile with specific differences to either activate PPAR and RXR. Thus, prinixin acid may also serve as valuable lead compound for selective RXR modulator development.

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Structure-Based Design of Novel P2Y14 Receptor Antagonists

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P2Y14 receptor (P2Y14R) is a G-protein-coupled receptor (GPCR)[1], that modulates cell functions related to inflammation[2], diabetes[3], and asthma[4]. Only a limited set of P2Y14R antagonists are currently known. PPTN, one of the most potent (IC50 = 6 nM) and highly selective P2Y14R antagonists, suffers from poor physicochemical properties due to its unwieldy naphthalene ring. A computational pipeline was set up to suggest alternatives to the naphthalene ring of PPTN (unwieldy naphthalene ring). A computational pipeline was set up to suggest alternatives to the naphthalene ring of PPTN (unwieldy naphthalene ring). A computational pipeline was set up to suggest alternatives to the naphthalene ring of PPTN (unwieldy naphthalene ring). A computational pipeline was set up to suggest alternatives to the naphthalene ring of PPTN (unwieldy naphthalene ring). A computational pipeline was set up to suggest alternatives to the naphthalene ring of PPTN (unwieldy naphthalene ring). A computational pipeline was set up to suggest alternatives to the naphthalene ring of PPTN (unwieldy naphthalene ring).

In silico improved physicochemical properties. Thus, guided by in silico studies, a library of triazole-based derivatives was designed leading to a highly potent P2Y14R antagonists.

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Development of Inhibitors Targeting Elastase (LasB) from Pseudomonas aeruginosa and Collagenase H (ColH) from Clostridium histolyticum

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The increasing appearance of bacteria that are resistant to commonly used antibiotics poses a threat to public health and makes the development of novel antibiotics an urgent necessity. 1 Targeting bacterial virulence factors is a new approach in the field of antibacterial drug development. Virulence factors are known to play a pivotal role during the infection process of pathogenic bacteria. The zinc metalloproteases elastase (LasB) from the gram-negative lung pathogen Pseudomonas aeruginosa and collagenase H from the gram-positive mycochromocytic Clostridium histolyticum are such extracellular virulence factors. These proteases enable the bacteria to colonize a niche in the host, to evade the host immune response and to obtain nutrition from infected cells, thereby playing a pivotal role in the infection process.1,4 Considering the difficult challenge of crossing the bacterial cell wall1, targeting these extracellular enzymes becomes conceptually attractive.

Hence, LasB and ColH represent prime targets for novel inhibitors that attenuate the aforementioned virulence mechanisms. In order to identify novel active and selective inhibitors, we employed biophysical and functional screening of a focused protease inhibitor library. This led to the discovery of a mercaptoacetamide-based compound class as broad-spectrum bacterial collagenase inhibitors, which are highly selective towards human matrix-metalloproteases (MMPs). X-ray co-crystal structures of our hits enabled us to identify the binding mode of the mercaptoacetamides and serves as a starting point for further optimization.


POS.80

Screening a fragment library for potential ligands of the β-ketoacyl-(ACP)-synthase II (FabF) of the fatty acid synthesis pathway of multi-resistant bacteria

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Fragment Based Drug Discovery (FBDD) was established within the last 20 years as a useful method to find lead structures as starting point for the development of new drug candidates. 651 compounds of the Solubility Fragment Library of OTAVA chemicals were screened using Bio-Layer-Interferometry (BLI) for potential ligands of FabF. The protein is part of the fatty acid synthesis pathway of bacteria and a potential target for the treatment of multiresistant bacteria. The screening was performed with FabF wildtype having a blocked binding site and FabFC164Q having an open binding site. This allowed us to identify hits specific for the co-substrate binding pocket. The screening had an initial hit rate of 10.3% (67 compounds). Finally 16 compounds (2.5%) were validated as hits and selected for crystallisation experiments.

References:

Using a pool of small hairpin RNAs (shRNAs) MKK4 (mitogen-activated protein kinase kinase 4) was identified to be a major regulator of liver regeneration. Silencing of MKK4 increased the robustness and regenerative capacity of hepatocytes during acute and chronic liver failure in a mouse model experiment. Mechanistically this leads to a higher phosphorylation of MKK7 and JNK1 and an enhanced activation of the transcription factors ATF2 and ELK1, causing a faster replication and less apoptosis and fibrosis of hepatocytes. [1,2]

Our approach developing small molecules as inhibitors for MMK4 is based on a virtual screening. PubChem-3ALO structure was selected as a target for molecular docking. The compound libraries used were general hit-identification libraries and we specifically decided not to use any kinase-targeting libraries. Since no X-ray structure of phosphorylated MKK4 exists the models are only valid for type-2 inhibitors but this do not rule out also type-1 inhibitors. The used docking methods were Glide and SurfexDock and in both cases the docking site was based on the ATP-binding region of MKK4. As the Mg2+ ion was included in the X-ray structure (3ALO) it was included also in the docking experiment. In both methods docking was carried out using incremental approach, so at first screening with fast approximated scoring/pose scanning and at later stage with more robust/true refining settings. The combined docking resulted 180 compounds for purchase and in vitro testing. In our in-house in vitro assay approx. 56% of the compounds showed detectable MKK4 inhibition. To validate the hits we also used DiscoverX® in vitro assay for a selected inhibitors resulting POC (percent of control) of 5 @ 10µM at a screening at and a good selectivity profile, but lacking chemical stability. The chemically stable derivative was determined with a POC of 23 @ 10µM. Further derivatisation and optimization resulted in a compound with a POC of 39 @ 1µM.

During testing, divergent results for the binding affinity and inhibitory activity against MKK4 were obtained for one and the same structure by different assay-suppliers. These results raised the question about the binding-mode of these new inhibitors. Therefore, a cascade assay was commissioned at ProQinase® to identify the compounds as type-I or type-II binders. The kinase assay is based on a radiometric system employing [3H]-yATP. This includes MEKK2, as the activating kinase upstream of MKK4, inactive MKK4 (MKK4*) and mutated JNK1 (K55R/K56R) as the substrate. Additionally, active MKK4 (MKK4*) was tested to investigate the activity for the active kinase conformation. To exclude false negative results due to inhibition of MEKK2, a third run with active MEKK2 and cascin as substrate was conducted. Based on the previous findings the substance class of these novel MKK4 inhibitors incorporates both types of kinase binders, type-I as well as type-II.

References:

![Image](POS.82)

**The Visual Affinities Concept: an easy guidance for drug designers**


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Design of new drugs is - surprisingly often – done with paper and pencil, compounds synthesized are based on the chemist's intuition. But, how would the efficiency of the LO change, if the chemists would be able to a) Look at a binding site and a starting structure (fragment, compound) in 3D and b) Edit it interactively, playing out all ideas right there, in the binding site and c) See the implication the edits have on: affinity, logP, conformation, hERG, BBB, LE, LLE etc.? We introduce a new way to do that, which we call visual affinities. The computed affinity (an estimate, of course) is visually displayed for all atoms of a molecule [1], the change of the affinity compared to the previous one(s) can be monitored, while having other important ADME properties (Optibrium's ModelRunner integrated [2]) under control. We will highlight the science behind the affinity calculation, and present a few application scenarios for further illustration of SeeSAR [3].

References:

![Image](POS.84)

**Novel analogues of arylamino alcohols with halo-substituted phenanthrene scaffold cure Plasmodium berghei-infected mice after peroral administration**


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We introduce a new way to do that, which we call visual affinities. The computed affinity (an estimate, of course) is visually displayed for all atoms of a molecule [1], the change of the affinity compared to the previous one(s) can be monitored, while having other important ADME properties (Optibrium’s ModelRunner integrated [2]) under control. We will highlight the science behind the affinity calculation, and present a few application scenarios for further illustration of SeeSAR [3].

References:
The latest World Malaria Report (2016) estimated 212 million new malaria cases in 2015 and 429 000 malaria deaths were recorded worldwide [1]. The WHO’s recommended therapy is an artesininsin-based combination therapy (ACT), which consists of an endoperoxide and a co-drug (e.g. arteether and lumefantrine). Due to the plasticity of the mosquito Anopheles and the parasite Plasmodium, increasing resistances against standard drugs were registered. This circumstance emphasizes the urgent need for new antimalarial compounds. In the past, we reported analogs of aryl amino alcohols, so-called 3-hydroxy-N-arylidenepropanehydrazonamides with high activity against Plasmodium falciparum [2]. Structural optimization provided new derivatives with nanomolar to subnanomolar antiplasmodial activity against asexual blood stages of P3D7 and PDX2 and excellent parasite selectivity. Moreover, derivatives exhibiting a halogen-substituted phenanthrene scaffold showed potent in vivo properties and some demonstrated cutative activity in the Plasmodium berghei mouse model after peroral administration [3].

References:

Identification of new BRD4 inhibitors using a tailor-made scoring function.

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Understanding interactions in protein ligand complexes is a key factor in structure based drug design. Here, we present a novel approach for representing and comparing protein ligand interactions that allows generating a protein specific scoring function. This approach is well suited for assessing docking results and was successfully employed for the identification of novel bromodomain-containing protein 4 (BRD4) inhibitors with new scaffolds. BRD4 is an important epigenetic regulator that recognizes and binds acetylated lysine residues [1]. A Python based workflow was developed to improve the scoring of docking poses by comparing their interaction patterns to those of experimental complex structures of the target protein with help of Protein Atom Derived Interaction Fingerprint (PADIFs). These PADIFs are generated from the binding pocket per atom scores of the GOLD scoring functions [2], which can be easily extracted from the respective solutions or rescoring files. The PADIF score of each pose is calculated by an empirically developed, protein-specific scoring scheme which takes into account the similarity to a reference PADIF representing the interactions in already known complexes of that protein. As only the interactions of binding pocket residues are considered, the method is independent of the ligand structure and thus a promising tool for scaffold hopping.

Besides extensive validation on benchmark datasets, we applied this approach for the identification of new BRD4 inhibitors. A reference PADIF was created based on more than 100 available BRD4-ligand complex structures. This reference PADIF was used to score the docking poses of more than 150000 compounds of a screening library. Overall, 65 compounds were tested that showed a highly similar interaction pattern based on the PADIF comparison. Eight showed a reduced residual activity of less than 50% at a compound concentration of 20 µM with two of them showing IC50 values of 2.2 ± 0.5 µM and 14.5 ± 1.7 µM. The most important point is that all these identified hits showed new scaffolds, although the information about existing inhibitors was used.

All in all, tailor-made PADIF scoring is a promising approach for finding novel ligands with new scaffolds by making use of already acquired structural knowledge.

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A Novel Synthetic Route to (N)-Methanocarba Ribose Analogues – A Crucial Scaffold for Purinergic Receptor Ligands

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Ribose pucks between north (N) and south (S) conformation. Purinergic receptors, as well as nucleoside and nucleotide degrading enzymes, are known to differentiate between the possible conformations. Biosososteric replacement of the ribose moiety by the bicyclo[3.1.0]hexane ring scaffold, often referred to as methanocarba, allows to rigidify the conformation in either north (N) or south (S) form, thus leading to increased receptor affinity and selectivity, e.g. purinergic P2Y1 receptors prefer ligands bearing (N)-methanocarba scaffold, whereas P2Y2 receptors display higher affinity for (S)-methanocarba-UDP than for the native ligand [1,2,3]. We are highly interested in purinergic receptor ligands bearing (N)-methanocarba scaffold.

Several methods for the synthesis of the analogue fixed in northern (N) conformation have been reported. However, they feature either enzymatic kinetic resolution of enantiomers, which leads to low yields [4] or the need for expensive catalysts or highly sensitive reagents [5,6]. Herein we present a novel and convenient chiral pool approach for the synthesis of the (N)-methanocarba scaffold.

Structure-Activity Relationship (SAR) Studies on Muraymycin Nucleoside-Peptide Antibiotics and their Analogues

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Bacterial infections are a rising threat in health care. Due to emerging resistances against antibiotics in clinical use, infections of bacterial strains that once have been well treatable became a serious menace again. To fight back these infections new antibiotics with new modes of action and new targets are strongly needed. Even so there are many antibiotics targeting bacterial cell wall biosynthesis available, early intracellular and membrane bound steps of this essential process offer several still unexploited targets. Translocase I (MraY), an enzyme that catalyses the anchorage of Park’s nucleotide 1 in the cellular membrane and thus a promising tool for scaffold hopping.

References:
the nucleobase scaffold will allow us to explore the role of the nitrogen atoms for P2Y1R binding, to expand the structure-activity-affinity-relationships at the P2Y1Rs and to generate receptor ligands with improved metabolic stability. Therefore, herein the synthesis of 1- and 3-deazapurine-based novel P2Y1R ligands with general structure B is presented.

Acknowledgements: The support with catalyst by Umicore AG & Co. KG is gratefully appreciated.

POS.89
Synthesis of Muraymycin Analogues for Structure-Activity Relationship Studies: Influence of the Peptide Moiety

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The increasing occurrence of bacterial resistance demands current research to identify new potential drugs and explore novel modes of action. Nucleoside antibiotics qualify as promising candidates as they address bacterial membrane protein MraY, thus interfering with an early, intracellular step of bacterial cell wall assembly. In this context, we are particularly interested in the subclass of muraymycins, as some representatives of these Streptomyces-produced natural products have shown promising antibacterial activity (e.g. muraymycin A1 and D2, cf. Figure 1) [1,2].

Figure 1. Structures of naturally occurring muraymycins A1 and D2.

A co-crystal structure of the MraY enzyme and muraymycin D2 provided some deeper insights into the binding mode of muraymycins to their target [3]. Due to the pronounced conformational plasticity of the enzyme, possible target interactions of the muraymycin peptide moiety cannot be unambiguously derived from this structure though. We therefore intended to evaluate the influences of specific parts of the peptide chain in a detailed structure-activity relationship (SAR) study. In order to reduce synthetic effort, we decided to perform these studies on 5'-deoxy analogues, as we found reasonable activities for such compounds. Thus, we synthesised a 5'-deoxy analogue of muraymycin D2 employing our established tripartite approach for the synthesis (Figure 2) [4]. The synthesis involves urea dipeptide 1, aldehyde 2 and nucleoside 3 as suitably protected building blocks.

Figure 2. Building Blocks 1-3 for synthesis of 5'-deoxy analogue 4.

With analogue 4 as reference compound at hand, we further planned to investigate structural variations, i.e. simplifications and truncations within

POS.88
Development of bicyclo[3.1.0]hexane-based P2Y1 receptor ligands with novel nucleobase scaffold

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The P2Y1 receptor (P2Y1R) is arising as novel promising target for the treatment of atherosclerosis [1,2] and prostate cancer [3]. Several potent P2Y1R ligands with general structure A have been reported [4]. Surprisingly, in the recently published X-ray crystal structure of P2Y1R in the complex with its high affinity antagonist MRS2500, no interactions between the pyrimidine nitrogen atoms of the purine nucleobase and the amino acid residues of the P2Y1R could be observed [5]. Bioisosteric replacement of the pyrimidine ring (red) by a pyridine ring (blue) within

Figure 1. Reaction catalysed by MraY: Park’s nucleotide 1 is linked to undecaprenylphosphate 2 under the loss of uridinemonophosphate, thus yielding lipid 3. Dansylated substrate analogue 4 enables a fluorescence based in vitro activity assay, yielding the more fluorescent dansyl-lipid 5.

Muraymycins [1], a class of nucleoside natural products isolated from Streptomyces, are inhibitors of MraY and might guide the development of new antimicrobial agents [2,3]. Figure 2 shows the muraymycin core structure with arrows indicating positions where we introduced structural modifications. Activity of synthesised muraymycin analogues was evaluated using a fluorescence based in vitro MraY assay. We therefore synthesised a dansylated analogue of Park’s nucleotide 4 [4] and cloned and expresses MraY from S. aureus. Furthermore, antimicrobial growth inhibitors of MraY and might guide the development

Figure 2. Muraymycin scaffold. Arrows indicate positions where modifications have been introduced.

Our SAR study indicates contribution of several structural motifs of the muraymycins to MraY inhibition. This enables future structural optimisation, aiming at parameters such as cellular uptake or pharmacokinetic properties. Results and discussion of the SAR study will be presented.

the peptide chain. Current results of these synthetic studies will be presented. Biological evaluation of these analogues will contribute to the understanding of the relevance of the peptide moiety and help designing new lead structures for the development of potent inhibitors.

Acknowledgments: Deutsche Forschungsgemeinschaft (DFG, SFB 803 "Functionality controlled by organization in and between membranes" and grant DU 1095/5-1) and the Fonds der Chemischen Industrie (FCI, Sachkostenzuschuss) for financial support.

References:

POS.90

Synthetic Studies Towards Novel Analogues of Caprazamycin Nucleoside Antibiotics

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Continuing emergence of drug resistance to various antibacterial agents poses a major problem in current healthcare. Therefore, studies on antibacterial natural products with novel modes of action such as nucleoside antibiotics are of great importance. The Streptomyces-produced caprazamycins, e.g. caprazamycin B (Figure 1), inhibit the bacterial membrane protein MraY, a key enzyme in the early, endocellular stages of peptidoglycan biosynthesis. They show very good antibacterial activity against drug-susceptible and multidrug-resistant Mycobacterium tuberculosis as well as against mithicillin-resistant S. aureus (MRSA) and vancomycin-resistant Enterococci (VRE) [1].

Our overall goal is the synthesis of structurally simplified, chemically more tractable bioactive caprazamycin analogues. Some structure-activity relationship (SAR) studies on caprazamycins have already been performed [2]. Ichikawa et al. found that acylcaprazols still showed good antibacterial activity [3]. However, their work was mainly focussed on variations of the diazepanone motif, whereas variations of the nucleoside-derived GlyU core structure were not studied. Our results on S-defunctionalised muraymycins [4], another uridine-derived nucleoside antibiotic, encouraged us to apply this approach also on the caprazamycin scaffold. We aim to develop an efficient and highly modular synthetic approach in which modifications can be introduced at a late synthetic stage.

Figure 2 shows the retrosynthesis of the envisioned S-deoxy acylcaprazol target structures. The desired products are obtained by global acidic deprotection. Prior to that the fatty acid is introduced by esterification and the diazepanone ring is built up by peptide coupling and reductive amination.

Acknowledgments: Deutsche Forschungsgemeinschaft (DFG, SFB 803 "Functionality controlled by organization in and between membranes" and grant DU 1095/5-1) and the Fonds der Chemischen Industrie (FCI, Sachkostenzuschuss) for financial support.

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POS.91

Design and diversity-oriented synthesis of peptoid-based HDAC inhibitors with dual-stage antiplasmodial activity

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Malaria continues to be a life threatening disease, especially in underdeveloped countries of the world, and still causes over 400,000 deaths each year. Due to their weak immune system, children and pregnant women are at the highest risk of dying. Among the several millions of deaths each year. Taking all this into account, HDACi are now considered as potential malaria intervention strategies. However, one major drawback is the often high toxicity of HDACi, which hampers the application of these compounds as antimalarial drugs.

In this study we present the synthesis and biological evaluation of a mini library of peptoid-based HDACi. To synthesize this library, we applied different diversity-oriented approaches like the Ugi four component reaction (Ugi 4CR) and submonomer pathways. A set of 20 novel HDACi were obtained by these diversity-oriented syntheses and subsequently tested for in vitro activity against axenial stage P. falciparum parasites as well as for toxicity against mammalian cells. The biological evaluation disclosed that some compounds showed excellent activity against the 3D7 strain of P. falciparum with IC50 values in the single digit nanomolar concentration range (IC50(PSB7) ~ 5-9 nM) and moderate toxicity (IC50(HepG2) ~ 4.6-13.1 µM), while other compounds revealed slightly lower potency (IC50(PBS7) ~ 71-120 nM) with low toxicity (IC50(HepG2) > 50 µM). Although only moderate activity was observed against gametocytes, several compounds showed potent submicromolar activity against P. bergheri exo-erythrocytic stages. Thus, this series of compounds represents a valuable starting point for the development of novel antimalarial drug leads with potent dual-stage antiplasmodial activity and low toxicity.
Oligonucleotides represent attractive pharmaceutical agents due to their antisense, antiguene, and RNA interference potential. A problem lies within their poor pharmacokinetics, mainly related to rapid degradation by endogenous nucleases and a defective cell permeability caused by their polyanionic phosphodiester backbone. Thus, we focused on the establishment of a novel backbone modification - a combination of already known amide internucleotide linkages [1] and the core structure of muraymycin nucleoside antibiotics [2] - that allows the introduction of positively charged residues [3] into the otherwise negative oligonucleotide backbone (Fig. 1).

Utilizing this newly created nucleosyl amino acid modification (NAA-modification) as backbone linkage, several partially zwitterionic oligonucleotides have been synthesized using dimer phosphoramidites as building blocks for automated DNA synthesis under standard conditions [4,5]. The resulting oligonucleotides have the potential to evade decomposition through nucleases and to increase cellular uptake by reducing the overall negative charge. In preceding experiments we showed that typical chemical properties of nucleic acids, including recognition of base-pairing mismatches and the capability to form stable helical duplexes with only moderate destabilization, are retained in NAA-modified DNA oligonucleotides [4]. Recent research focused on further properties of the NAA-oligonucleotides especially regarding their biological and thermal stability. The latest results will be presented.

Acknowledgments: We thank the Deutsche Forschungsgemeinschaft (DFG, grant DU 1095/2-1), the Fonds der Chemischen Industrie (FCI, Sachkostenzuschuss) and the Studienstiftung des deutschen Volkes (doctoral fellowship for B. S.) for financial support.

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POS.94
Properties of DNA Oligonucleotides with a Partially Zwitterionic Backbone Structure

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Over the past decade the β-emitter 22Na has emerged as a promising radioisotope for position-emission tomography (PET). Due to its facility production and beneficial decay properties (t1/2 = 2.6 h, Eβmax = 901 keV) it is a perfect match for antibody- and small protein-coupled radiopharmaceuticals that have biological half-life times of several days [1]. Despite these advantages the usage of zirconium is accompanied by several challenges. Zirconium forms highly charged Zwitterionic Backbone Structure

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ions (Zr⁴⁺) with a small ion radius (59-89 pm) making hard donor chelators with coordination numbers of 4-9 a necessity [2]. One of the most extensively studied chelators for zirconium is desferrioxamine B (DFO), a hydroxamate based siderophore found in the bacterium Streptomyces pilosus [3]. However, several studies showed a limited complex stability of Zr⁴⁺-DFO conjugates in vivo resulting in reduced S/N-ratios and accumulation of the radiometal in bone tissue [4]. Because of these shortcomings, tremendous effort has been put into the development of new zirconium chelators. Recent studies have proven that an octadentate coordination scheme found in novel DFO-derivatives such as DFO* and DFO-squaramide esters is superior to the hexadentate nature of DFO [5,6].

We herein report the synthesis of a modular peptidic DFO-analogue bearing four hydroxamate functionalities and its zirconium complexes. The ligand is synthesized via facile peptide coupling from a monomeric building block derived from L-lysine or L-ornithine coupled to δ-alanine. Additionally the modular approach allows for the introduction of additional side-chains bearing azides, enabling for orthogonal modification of the ligand via copper-catalysed “click-chemistry”. These may be used to incorporate zwitterionic moieties, which have proven to reduce unspecific binding to serum proteins in vivo and thus improving the pharmacokinetic properties [7]. Variation of the monomer unit allows for a flexible synthesis of different derivatives to evaluate their complex stability in vitro and in vivo.

**POSTERS**

Molecular recognition of agonists and antagonists by the nucleotide-activated G protein-coupled P2Y2 receptor

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The P2Y2 receptor (P2Y2R) is a nucleotide-activated G protein-coupled receptor (GPCR). Although it has great potential as a drug target, the development of ligands has met with limited success so far. Here we report on a homology model of the P2Y2R based on the recently reported X-ray structures of the related P2Y1 and P2Y12 receptors. Computational docking studies were performed with selected ligands, and receptor mutants were created to probe the identified binding interactions. Mutation of residues that were predicted to form direct interactions with the ribose moieties (Arg110) and the phosphate groups of the agonists UTP and dideoxinosine tetraphosphate (Ap4A), Arg265 and Arg292, or that contribute indirectly to ligand binding (Tyr268), abolished activity. The Y114F, R124A, and F261A mutations led to complete inactivity of Ap4A and to a reduced response of UTP. A significant reduction in potency of UTP and Ap4A was observed for all of the other receptor mutants (Phe111, His184, Ser193, Phe261, Tyr268, Tyr269) predicted to be involved in agonist recognition. According to the model, anionic lock between Asp185 and Arg202, which is thought to play a role in receptor activation, interacts with the phosphate groups of the agonists. The uracil-derived P2Y2R antagonist AR-C118925 and antheraquinoine-based antagonists likely also bind to the orthosteric site.

The optimized homology model will be useful for virtual screening to discover new potential hit structures, and for the rational design of superior ligands and drug candidates.

Due to increasing resistances against established antibiotics, the need for novel drugs which address unexplored targets in prokaryotic cells is urgent. Nucleoside antibiotics e.g. muraymycins and mureidomycins (see Figure 1) represent such a new class, which inhibit peptidoglycan formation on an early intracellular step [1,2,3].

**Studies on the Selectivity of Nucleoside Antibiotics**

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Due to increasing resistances against established antibiotics, the need for novel drugs which address unexplored targets in prokaryotic cells is urgent. Nucleoside antibiotics e.g. muraymycins and mureidomycins (see figure 1) represent such a new class, which inhibit peptidoglycan formation on an early intracellular step [1,2,3].

**Figure 1. Structures of muraymycin A1 and mureidomycin A.**

So far, the reason for different antimicrobial activity and selectivity of the nucleoside antibiotics is poorly understood. Muraymycins are mainly active against Gram-positive bacteria (e.g. Staphylococci MIC 2-16 µg/mL) but also against Gram-negative E.coli with increased membrane permeability (imp mutant MIC: up to 0.03 µg/mL), while there is a loss of activity against wild-type E.coli (MIC > 128 µg/mL) which might be a result of poor cellular uptake. Mureidomycins on the other hand are mainly active against Gram-negative P. aeruginosa (MIC 0.1-3 µg/mL) but inactive against most Gram-positive bacteria. In 2014 Ichikawa and Matsuda reported structurally modified muraymycin analogues which gained antimicrobial activity against Gram-negative P. aeruginosa [3] (see Figure 2).
Our main goal is the synthesis of simplified analogues of nucleoside antibiotic in a detailed structure-activity relationship study to gain more insights into the differences in selectivity and activity. Current results of these synthetic studies as well as first biological data will be presented. These data will provide an insight on which molecular features improve cellular uptake, resulting in antimicrobial activity against different bacteria.

Figure 2. Structure of the most potent P. aeruginosa inhibitors synthesized by Ichikawa and Matsuda [3].

References:

Folic acid derivatives for prostate cancer imaging

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Folates are essential cofactors in the de novo biosynthesis of pyridine and pyrimidines [1]. Moreover, antifolates are key components in cancer therapy [2]. Targeting tumor specific cell surface epitopes, so called 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]. Phosphinic acids like GPI, for example, 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 like dyes without altering the binding properties [6]. However, GPI has suboptimal binding properties and needs to be improved [3]. Although the mitochondrial amidoxime reducing component (mARC) in mammals is encoded for two mARC proteins and that both of its endogenous substrates are largely unknown. However, the fact that the mitochondrial genome encodes for two mARC proteins and that both of them have been found highly conserved in all so far examined mammals substantiates the physiological importance of these enzymes [4,5]. To further investigate the physiological function of mARC, a mARC2 knockout mouse model was established enabling us to perform both in vitro and in vivo studies. For the in vitro investigations, murine liver, lung, and kidney tissues of wildtype and knockout mice were incubated with the established model substrate benzimidazole (BAO). The protein expression was examined via SDS-PAGE and western blot analysis using specific antibodies for all components of the N-reductive enzyme system, namely mARC1 and mARC2, Cytb5 and Cyt5R. Additionally, four other selected substrates of mARC were incubated with the murine knockout liver tissues. We could show that the mARC2 knockout was successful and had no influence on the expression of the other proteins of the N-reductive enzyme system. The N-reductive activity, evaluated by BAO-reduction, was significantly decreased in knockout mice tissues. Among the other selected substrates, the N-hydroxylated nucleoside N-hydroxyacetophenone and the N-hydroxymethylquinazoline guanosine also undergo a significantly reduced N-reduction in the murine knockout liver tissues. Interestingly, these findings could not be confirmed for the other two tested substrates, benzhydroxamic acid and trimethylamine N-oxide (TMAO), which goes in concert with studies of the recombinant human enzyme system.

Medicinal chemistry and drug design

Murine knockout-studies confirm the involvement of the mitochondrial amidoxime reducing component (mARC) in N-reductive metabolism

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The mitochondrial amidoxime reducing component (mARC) is a recently discovered molybdenum-containing enzyme in mammals. In the presence of NADH and in conjunction with the two electron transport proteins cytochrome b5 type B (Cytb5) and NADH cytochrome b5 reductase (Cytb5R), it catalyzes the reduction of various N-hydroxylated compounds [1,2,3]. Although the N-reductive metabolism has been studied extensively, the physiological function of mARC and its endogenous substrates are largely unknown. However, the fact that the mammalian genome encodes for two mARC proteins and that both of them have been found highly conserved in all so far examined mammals substantiates the physiological importance of these enzymes [4,5]. To further investigate the physiological function of mARC, a mARC2 knockout mouse model was established enabling us to perform both in vitro and in vivo studies. 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For the in vivo investigations, the mice were treated with BAO i.v. The murine serum was analyzed to determine the extent of BAO-reduction. Underlining the results of the in vitro experiments, we found a significantly decreased N-reduction in the mARC2 knockout mice. On the one hand, our results clearly show that murine mARC2 is mainly responsible for the N-reduction of BAO. On the other hand, the mARC2 knockout mice were still able to reduce BAO to a small amount. The in vitro experiments also showed a remaining N-reductive activity of the knockout tissues. We hold mARC2 responsible for these findings, indicating that one mARC protein can function as a backup enzyme if the dominant protein, which in mice is mARC2, should be inactive. Thus, we assume that mARC fulfills an essential physiological function which still needs to be further characterized.

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Lipid mediators derived from phospholipid-bound fatty acids play key roles in inflammatory conditions, such as asthma or cardiovascular diseases. Upon stimulation of immune cells, the unsaturated fatty acid amidoxime reducing components hmARC-1 and hmARC-2 suggests the existence of a new molybdenum enzyme family in eukaryotes.

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References

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First Dual Inhibitors of Steroid Sulfatase and 17β-Hydroxysteroid Dehydrogenase Type 1: Designed Multiple Ligands as Novel Potential Therapeutics for Estrogen-Dependent Diseases

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The etiology of estrogen-dependent diseases (EDDs) such as endometriosis and a high percentage of breast cancers are strongly coupled to elevated local estrogen levels in the diseased tissues. This elevation is a result of the overexpression of the involved enzymes in situ e.g. Steroid Sulfatase (STS) and 17β-Hydroxysteroid Dehydrogenase Type 1 (17β-HSD1). These enzymes catalyze the final biosynthesis steps of the highly estrogenic 17β-estradiol (E2) from the precursors estrone sulfate (E1S, not estrogenic) and estrone (E1, weakly estrogenic). Consequently, inhibition of STS and 17β-HSD1 is an attractive novel concept for the treatment of EDDs.[3,4] The simultaneous inhibition of both enzymes appears more promising than blockade of either protein alone. We describe a designed multiple ligand approach based on known selective inhibitors of STS and 17β-HSD1,[5,6] resulting in highly potent dual inhibitors. The most interesting dual inhibitor showed nanomolar IC50-values for both proteins, good membrane permeability and no interference with estrogen receptors. It efficiently reversed E1S- and E1-induced T47D cell proliferation.[7]
The issue of obesity resulting from unhealthy lifestyle is an ever-growing problem, particularly in Western countries and metabolic diseases such as type 2 diabetes mellitus, dyslipidemia and liver steatosis occur more frequently. As future targets to new drugs facing this problem, nuclear receptors, especially the farnesoid X receptor (FXR), are arising. FXR activation enhanced glucose-dependent insulin secretion, reduced liver steatosis and fibrosis and might even have weight-lowering activity according to the clinical development of the first-in-class FXR agonist obeticholic acid (OCA)\(^{[5,6,7]}\). Recently, OCA was approved for the treatment of primary biliary cholangitis and further indications such as non-alcoholic steatohepatitis are expected to follow soon\(^{[8,9]}\). However, the steroidal scaffold of OCA and its limited selectivity are unfavorable and novel FXR agonists are required.

An efficient strategy to establish new lead structures is the approach of selective optimization of side-activities (SOSA)\(^{[1,2]}\). In this concept, a side-activity of a validated drug on the target of interest is increased by classical medicinal chemistry compound optimization.

We have identified the leukotriene receptor antagonist pranlukast as a moderately potent FXR agonist (EC\(_{50}\) = 14.9 ± 3.0 µM; 27 ± 1% maximum relative activation) suitable as lead structure for FXR targeting SOSA. We systematically characterized the SAR of pranlukast but initial compounds revealed a rather flat SAR. A considerable potency improvement was then achieved by opening of the chromenone ring system to a benzoic acid residue.\(^{[10]}\) Further variations on this optimized lead compound revealed a much deeper SAR and resulted in remarkable improvements in FXR agonistic activity. Particularly shortening of the aliphatic ether chain was strongly favored by FXR. Altogether, the structural variations produced a 570-fold improvement over the lead compound pranlukast (EC\(_{50}\) = 0.0010 ± 0.0003 µM; 35 ± 1% max.). Systematic introduction of nitrogen atoms in the compound’s aromatic rings revealed positions tolerating more polarity thereby increasing solubility while retaining high FXR activating potency. Key compounds of the SAR study were intensively characterized in vitro and revealed high selectivity, low toxicity and favorable metabolic stability. The successful optimization of pranlukast to highly potent FXR agonists confirms that the SOSA concept is particularly promising amongst fatty acid mimetic drugs.

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**Figure 1:** Structure of pranlukast

**References:**

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**Development of novel non-steroidal Farnesoid X Receptor (FXR) Antagonists**

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The nuclear farnesoid X receptor (FXR) is a ligand-activated transcription factor, which acts as cellular sensor for bile acids and is primarily expressed in liver, kidney and intestine [1-4]. It takes part in the self-regulation of bile acid homeostasis with the result that bile acid synthesis is blocked and their metabolism is enhanced when high levels of toxic bile acids occur. Furthermore, FXR is involved in many other metabolic systems such as glucose and lipid homeostasis and seems to have anti-inflammatory effects as well [4]. Since both, FXR activation with obeticholic acid (OCA) and whole-body FXR knockout mouse models showed metabolic improvements in FXR holds promise in the treatment of obesity and metabolic syndrome. However, the site of FXR modulation appears to be crucial for these effects [5-7]. According to recent studies, inhibition of intestinal FXR activity through glycine-β-muri nic acid (Gly-β-MCA) improved obesity, non-alcoholic fatty liver disease (NAPLD) and insulin resistance [5]. In obese mouse models, oral treatment with Gly-β-MCA prevented weight gain and also reduced absolute fat mass. In addition, blood glucose levels were reduced and insulin sensitivity improved. To prove the site of action, intestine specific FXR-knockout mice were equally treated and did not benefit from Gly-β-MCA [5-6]. Altogether, the study suggests significant therapeutic value of intestine specific FXR antagonism. However, intestinal stability and selectivity of Gly-β-MCA are questionable. Thus potent and selective non-steroidal FXR antagonists are required to confirm beneficial effects of (intestinal) FXR antagonism [8-10].

In an in-house library screening, we discovered a benzamidophenylacetic acid derivative as lead compound for the development of non-steroidal FXR antagonists. It already offers respectable FXR antagonistic potency with a submicromolar IC$_{50}$ value. Its structure can be divided into two building blocks allowing a systematic, antagonistic structure-activity relationship (SAR) compilation for each block. Our first investigations focused on the position and size of the acidic group while the benzamido moiety was unaltered. By variation of the substitution pattern, we could improve potency to a two-digit nanomolar IC$_{50}$ value. Since spatial extension is promising to enhance antagonistic potency, we then systematically methylated all free positions of the scaffold to discover new compounds for a more specific evaluation of FXR antagonism as therapeutic concept.

References:

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POS.105 Development of pharmacological tools for the orphan G protein-coupled receptor GPR18

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GPR18 is a rhodopsin-like class A orphan G protein-coupled receptor (GPCR) activated by cannabinoids such as Δ9-tetrahydrocannabinol (THC). It was reported to promote migration of immune cells including M1-macrophages, T-lymphocytes, and microglial cells [1]. The endogenous lipid N-arachidonylglycerine has been proposed as a physiological agonist of GPR18 [2] but several research groups, including ours, could not confirm the reported results [3,4]. Recently, Chiang et al. proposed the arachidonic acid metabolite resolin D$_1$ as an endogenous agonist but its confirmation has yet to be awaited [5]. To further elucidate the (patho)physiological roles of GPR18 and to investigate its potential as a drug target, potent and selective ligands - agonists and antagonists - are required.

In a screening campaign, we identified bicyclic imidazolo[4,1-b]quinoline-2 derivatives synthesized by the group of K. Kiec-Kononowicz (Cracow, Poland) [6] as a novel class of GPR18 antagonists. We subsequently investigated the compounds’ structure-activity relationships at GPR18 and evaluated their selectivity versus related GPCRs. PSB-BC-5 was developed as a potent and selective GPR18 antagonist with an IC$_{50}$ of 0.279 µM. It represents the most potent and selective GPR18 antagonist known to date [3]. Moreover, a synthetic agonist for GPR18 was identified (PSB-KK-107, EC$_{50}$ 0.556 µM), which represents the first potent, non-lipid-derived small molecule GPR18 agonist. We subsequently investigated the structure-activity relationships of this new compound class and developed PSB-KK-1415 with an EC$_{50}$ value of 0.0191 µM, the most potent GPR18 agonist to date. Preliminary results indicate that the lipid-like agonists may occupy a different binding site than the newly discovered class of heterocyclic agonists. Both, novel GPR18 agonists and antagonists, are currently further optimized. They will serve as useful tools to investigate GPR18 functions.

References:

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POS.106 Lead-like PqsR Antagonists as Pathoblockers for the Adjutivative Treatment of Chronic Pseudomonas aeruginosa Infections

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Pseudomonas aeruginosa (PA) can cause severe chronic lung infections in patients suffering from cystic fibrosis or bronchiectasis. This opportunistic, ubiquitously distributed bacterium is able to switch to the biofilm mode of life, which serves as a physical barrier to survive antibiotic treatment and host immune defense. In addition, PA develops a high resistance towards antibiotics resulting in maintaining of chronic infections and high mortality of infected patients. The Pseudomonas Quinolone Signal (PQS) quorum sensing (QS) system is essential for bacterial virulence and biofilm formation making it a suitable drug target to block PA’s pathogenicity [1].
To this end, we aim at the design, synthesis, and optimization of QS inhibitors targeting the innate transcriptional regulator PqsR. A first compound series inspired by the natural PQS auto-inducer molecule HHQ showed activities in the nanomolar range. [2] In a study on another compound class, we could demonstrate that inhibitors PQS QS enhance the efficacy of antibiotic treatment in a biofilm setting. [3]

Currently, new structure-divergent lead series of PqsR antagonists derived from a fragment-based approach are being developed through means of structure-guided medicinal chemistry. The results demonstrate even more favorable properties compared with the first compound series. Our PqsR antagonists will preferably be administered by inhalation and will be developed as preemptive or adjunctive treatment in PA eradication and suppression therapy for cystic fibrosis and bronchiectasis.

Wagner et al. [3] established an assay platform for the screening of Spindlin1 inhibitors. This was used to test analogues of the lead structure, resulting in inhibitors showing IC50 values in the low nanomolar range on both human 17ß-HSD2 enzyme and its murine orthologous leading to the development of a SAR of the compound class. Furthermore, the compounds show good in vitro ADME/Tox profiles as well as promising PK results, making them suitable candidates for further evaluation in an osteoporosis disease model in mice.

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References:

POS.108
Development of novel 17ß-Hydroxysteroid Dehydrogenase Type 2 Inhibitors for the Treatment of Osteoporosis

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Estradiol (E2), as the most active estrogen in humans, plays an important role in various diseases. Depletion of Estradiol, as it is the case in women after menopause, is associated with loss of bone mineral density resulting in osteoporosis. [1] The inactivation of E2 into its less active form, Estrone (E1), is catalyzed by 17ß-Hydroxysteroid dehydrogenase Type 2 (17ß-HSD2). Inhibition of this enzyme therefore is an attractive target for the treatment of osteoporosis. [2] Following a ligand based approach, we describe the use of combinatorial chemistry to synthesize a series of potent and substituted thiophenyl hydroxyphenylmethanones. The resulting inhibitors show IC50 values in the low nanomolar range on both human 17ß-HSD2 enzyme and its murine orthologous leading to the development of a SAR of the compound class. Furthermore, the compounds show good in vitro ADME/Tox profiles as well as promising PK results, making them suitable candidates for further evaluation in an osteoporosis disease model in mice.

References:

POS.109
Protein-based nanoparticles for the delivery of hydrophobic drugs

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Modern drug delivery vehicles are artificially manufactured nanosystems that are similar in size and shape to biological nanostructures, such as exosomes and viruses. [1] Biopolymers, like polysaccharides and proteins, are promising macromolecules as starting materials for nanoparticles. They are readily accessible, can be obtained in high quantity, are structurally well defined and trigger in most cases only a low reaction of the immune system. [2,3] Especially proteins are attractive materials for the formation of nanovehicles, since they represent a highly structurally biopolymer. Furthermore proteins have advantages over many synthetic polymers because of their high biodegradability, general biocompatibility, and low toxicity. [4]

We designed a nanoparticle delivery system based on the assembly of surface-modified proteins. This concept is based on the solubility switch

References:
of proteins by high surface-PEGylation. We evaluated the protein-polymer conjugation by using polyethylene glycol that is functionalized with different electrophile linker groups. We found that the introduction of PEG leads to no significant alteration of the secondary protein structure and in many cases the enzymatic activity is still retained. Nanoparticles are prepared by an oil-in-water emulsion method without the need of additional crosslinking steps for stabilization. During the preparation hydrophobic drugs, e.g. doxorubicin (DOX) can be encapsulated in the particle matrix. We prepared empty and DOX-loaded nanoparticles with a size of around 100 nm in diameter. The produced nanoparticles are non-toxic but do not effect the activity of the encapsulated drug. In physiological buffers the particles are stable and only release their payload into the cytosol after cellular uptake. [5,6] This novel method for nanoparticle preparation extends the range of biocompatible materials beyond current known synthetic polymers and polysaccharides and potentially opens up new strategies for therapeutic applications.


POS.110

Bitopic Ligands and their Molecular Fragments for the Study of the M1 Muscarinic Receptors

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GPCR modulation is recognized as a promising option for therapeutic regulation of biological functions. In particular, muscarinic acetylcholine receptors (mACHeRs) control various physiological processes. A high sequence homology between mACHeR subtypes in the orthosteric region has though precluded the development of selective orthosteric ligands. Conversely, allosteric agents are often endowed with pronounced subtype selectivity, due to their conservation of the allosteric site. [1] Recently, aiming to design partial agonists for a G-protein coupled receptor based on dynamic ligand binding, a series of biphasicopharmacophoric ligands[2] composed of the orthosteric building blocks iperoxo linked to allosteric modulators (BQCA-derived compounds) was synthesized. Results demonstrate that iperoxo/BQCA acts as M1 partial agonists.[3]

In order to find out whether the dualistic concept can be regarded as a general one, we are deepening the investigation of iperoxo/BQCA compounds. Novel derivatives characterized by the replacement of the heterocyclic ring of the superagonist iperoxo[4] were synthesized. To investigate the role of the allosteric portion a second series of molecular fragments that consist of the BQCA allosteric moiety linked with a chain of different length to a quaternary ammonium head was synthesized. [5] These modifications should contribute to understand the role of the orthosteric and the allosteric fragment in the pharmacological profile of the dualistic molecules.

The synthesis and the unexpected pharmacological results obtained by means of FRET (fluorescence resonance energy transfer) analyses will be presented in our poster.

References

POS.111

Impurity profiling of N,N'-ethylene-bis-L-cysteine diethyl ester (Bicisate)

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N,N'-Ethylene-bis-L-cysteine diethyl ester (ECD), officially named bicisate, is a chelating ligand for the radiouclide technetium-99m(99mTc) which after labelling with 99mTc is applied in brain perfusion studies using single-photon emission computed tomography (SPECT) [1, 2]. The sulfhydryl groups are essential for complexation of 99mTc whereas the ester functions are essential for the distribution and the retention of the partially or fully hydrolysed complex in the brain [3, 4]. ECD is commercially distributed in so-called labeling kits for preparation of 99mTc labeled ECD under the name Neurulite®. There are a limited number of methods to control the purity of the radiolabeled compound [5-7], but no method for analysis of the unlabeled precursor (ECD) is described in the literature.

A HPLC-UV-CAD method with a HILIC column for impurity profiling of bicisate has been developed and evaluated. Bicisate and its impurities were separated by means of isocratic elution on a zwitterionic stationary phase using a mixture of 7.5 mmol/L trifluoroacetic acid and acetonitrile (47.5:52.5 V/V) as the mobile phase. Five different bicisate batches of a manufacturer were tested using the method and LC-MS experiments were conducted in order to identify the impurities. The predominant impurities found were the oxidation product (disulfide), the monoester of ethylene dicysteine and an unknown compound with an m/z of 293 in ESI positive mode. A new degradation product of bicisate, bicisate lactam, was identified during sample solution stability assessment.

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References

POS.112

Exploiting novel anti-tuberculotics and anti-malarials against DYS by virtual screening

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The enzymes of the non-mevalonate pathway are important drug targets given that pathogens such as Mycobacterium tuberculosis and Plasmodium falciparum use this pathway for the biosynthesis of the essential isoprenoid precursors isopentenyl diphosphate (IPP) and dimethylallyl diphosphate (DMAPP), while humans exclusively utilise an alternative pathway.\(^1\) Our target enzyme 1-deoxy-D-xylulose-5-phosphate synthase (DXS) catalyses the first and rate-limiting step of the non-mevalonate pathway. We aim to develop potent and selective inhibitors of DXS by using both ligand-based virtual screening (LBVS) and structure-based virtual screening (SBVS). LBVS was based on shape similarity to screen the ZINC database based on previously discovered DXS inhibitors as references.\(^2,3\) SBVS was performed on homology model of M. tuberculosis DXS.\(^4\) Biochemical evaluation of the top-scoring compounds against M. tuberculosis DXS led to promising hits with confirmed mode of inhibition. Some of the hits display inhibitory potency in the low micromolar range and promising activities in cell-based assays against P. falciparum and even drug-resistant strains of M. tuberculosis, providing start point for future optimization.

References:

C2-Linked Dimeric Strychnine Analogues: An Application of the Bivalent Concept to the Glycine Receptor

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Strychnine is the major alkaloid from the plant Strychnos nux vomica. Its most pronounced pharmacological action is a strong antagonistic activity at glycine receptors (GlyRs), which are anionic chloride channels composed of five subunits (α or β) and linked to hyperpolarisation and inhibition of neuronal firing.\(^1,2\) Applying the bivalent ligand approach, a series of dimeric strychnine analogues with two glycine units incorporated into the linker and spacer lengths varying from 14 to 24 atoms has been synthesized and pharmacologically evaluated in a functional membrane potential assay at homomeric (α1) and heteromeric (α1)β GlyRs. Several compounds were additionally tested in an electrophysiological patch clamp assay. The findings are useful to assess the optimal spacer length for simultaneous binding of both strychnine pharmacophores to two binding sites of the pentameric GlyR.
POSTERS

4.9 Natural Compounds

Natural products and their de novo mimetics as retinoid X receptor modulators

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Natural products (NPs) modulating the ligand-activated transcription factor retinoid X receptor (RXR) are rare but could serve as innovative lead compounds for drug discovery. To identify RXR targeting NPs, we have virtually screened more than 210,000 NPs from the dictionary of natural products (DNP) and determined the activity of top-ranked virtual hits in a specific RXR hybrid reporter gene assay, confirming RXR agonistic potency of three natural secondary metabolites. RXR (α, β, γ) is a key regulator of human gene expression participating in development and the maintenance of metabolic balance and inflammatory homeostasis. It fulfills a unique role amongst nuclear receptors by serving as hetero-dimeric partner of numerous other members of the nuclear receptor superfamily. RXRs are drug targets for inflammatory and metabolic disorders. However, the available synthetic RXR ligands, such as bexarotene, suffer from high lipophilicity and poor drug-likeness.[1-3] NPs with unique molecular scaffolds and complex geometry might serve as lead compounds for more favourable RXR targeting drug development.[4]

In order to retrieve NPs for the predefined target RXR, we employed a variety of computational methods including self-organizing map consensus and several molecular descriptors considering pharmacophoric features, shape, and partial charges.[5, 6] Five NPs that were top-ranked by diverse descriptors in silico subsequently entered in vitro evaluation in a Gal4-based hybrid reporter gene assay specific for RXRs. Amongst these NPs characterized in vitro, three robustly transactivated RXRα with EC50 values of 26, 27 and 42 µM, respectively, confirming that computer-assisted target-focused discovery of NP bioactivities is feasible. Of note, the three NPs display distinct selectivity profiles amongst the RXR subtypes adding valuable information to the SAR of subtype selective RXR modulators. As entry into early drug discovery, we then employed RXR activating NPs as templates for the de novo design of RXR modulators. By generating novel small molecules with drug-like properties and a desired biological activity, de novo design offers access to potent novel bioactive compounds based on the large chemical space of available building blocks. It can speed up drug discovery by executing several screening rounds and optimization steps in silico before candidates are prepared and tested in vitro.[7, 8] Using all known RXR modulating NPs as templates, we virtually generated roughly 1000 NP mimic de novo designs and used self-organizing maps consensus and various molecular descriptors to rank the designs for RXR modulating activity. The top-ranking de novo designs were synthesized and assayed in vitro for RXR modulation, confirming several actives. One NP mimetic outmatched its template in potency. With its one-pot synthesis, an EC50 value of 18 µM and a high transactivation efficacy, this innovative ligand represents a new lead for the development of potent NP-derived RXR modulators.

References:

POS.115

Influence of food polyphenols on 5-lipoxygenase activity

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Arachidonic acid (AA) can be metabolized to bioactive eicosanoids via three main pathways. Once AA is released from membrane-bound phospholipids it can be converted to prostanoids by cyclooxygenases (COX1-2), to leukotrienes via the 5-lipoxygenase (5-LO) pathway, and to hydroxyeicosatetraenoic acids (HETEs) and epoxy-α (EeETEs) by cytochrome P450 (CYP) enzymes. 5-LO metabolizes AA in a two-step reaction to 5-hydroperoxyeicosatetraenoic acid (5-HPETE) and subsequently to the unstable leukotriene A4 (LT-A4). The latter is further metabolized to the bronchoconstrictive cysteyl LT (cys-LTs) by leukotriene C4 synthase (LTC4-S) or to the chemoattractant LTB4 by LTA4-hydrolase (LTA4-H). Conclusively, targeting the 5-LO pathway displays a reasonable pharmacological strategy for intervention with inflammation and allergy. However, only one drug (i.e. zileuton) directly targeting 5-LO has been approved for therapy, but with restrictive application due to severe side effects. [1]

Several epidemiologic studies showed a correlation between a dietary intake of polyphenols and beneficial health effects. Polyphenols were shown to have anti-inflammatory properties, which are, at least partially, mediated by a modulation of the AA cascade. [2]

Here, we investigated the effects of food-derived polyphenols on 5-LO activity. Inhibition of 5-LO was investigated in Ca2+-ionophore (A23187)-challenged human neutrophils. In order to determine direct effects on 5-LO, polyphenols were additionally tested against purified recombinant 5-LO. The overall effects of the polyphenols on the oxylipin pattern in activated neutrophils were analyzed by LC-MS-based targeted metabolomics.

Amongst a library of food polyphenols, the resveratrol dimer εviniferin was the most potent direct 5-LO inhibitor with an IC50 value of 1.2 µM compared to resveratrol with an IC50 value of 9 µM. In contrast, resveratrol imine analog (RAA) 2[(2-hydroxyphenyl)imethylene]aminophenol (CAS 1761-56-4) showed the highest potency in inhibiting leukotriene formation in isolated human neutrophils with an IC50 value of 0.9 µM. We conclude that εviniferin, in addition to the well-recognized resveratrol, may be of relevance as food polyphenol with anti-inflammatory properties.

References:

POS.116

Effects of a hyperforin-rich extract of Hypericum perforatum root cultures on human keratinocytes and fibroblasts

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Hyperforin (HYP) is recognized as one of the main bio-active constituents of Hypericum perforatum. Among others its spectrum of activities comprises differentiation-inducing [1], anti-proliferative [2] and anti-inflammatory effects [2]. This makes HYP a promising drug candidate for the treatment of diseases characterised by deficient cell differentiation such as psoriasis and hypertrophic scars. Until today, H. perforatum serves as the only source of HYP thus hindering application in the clinical area. To

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meet this need Gaid et al. [3] established an alternative route of HYP production via H. perforatum root cultures. The present contribution examines the activity of the obtained hyperforin- rich root extract (RE). Cell culture experiments on HaCaT and HDF cells were used to determine cytotoxicity and anti-proliferative effects. Therefore, a variety of different test methods such as a CellTox® Green Assay and a Cell Proliferation ELISA were performed. As a result no cytotoxicity against HaCaT and HDF cells was observed during a 72 h incubation up to a concentration of 1.5 µM RE (concentration refers to HYP content), respectively. In contrast to that the proliferation of both examined cell types was reduced by RE in a dose-dependent manner. For 1 µM RE (concentration refers to HYP content) a decrease in proliferation of HaCaT and HDF down to 64 % and 52 % respectively, was detected, whereas treatment with 1 µM pure HYP did not exhibit any effect.

In conclusion a novel H. perforatum root extract has been tested for the first time on cell cultures of HaCaT and HDF cells. In complete absence of cytotoxicity the extract showed the typical antiproliferative effect in a dose dependent manner. In comparison to pure HYP this effect was increased which indicates synergistic effects of HYP and other constituents of the extract, possibly xanthones and lupulones. Finally this novel extract can serve as an alternative source of HYP for future clinical applications. Due to the suggested synergistic effects this unique extract may be more beneficial than formulated HYP alone for the treatment of dermal diseases.

The authors would like to thank Prof. Dr. Fusenig for donating the HaCaT cell line. This study was partly funded by the Niedersächsisches Ministerium für Wissenschaft und Kultur (MWK) in the joint project “SynFoBiA – Novel synthesis and formulation methods for poorly soluble drugs and sensitive biopharmaceuticals”.

References

Historical reports about the traditional uses of medicinal plants can be regarded as documents of ethnopharmacological knowledge. These include printed material like books as well as handwritten material like letters, diaries or travel reports [1, 2, 3]. The documents, including those of Christian missionaries, may be used to unveil forgotten medicinal plant uses and may give, after careful exploration, some hints for further pharmacological investigations [4]. A letter written in 1832 by the English Anglican missionary Joseph John Freeman (1794-1851) is presented as an early and hitherto hardly known document of medicinal plant use on the island of Madagascar in order to explore its role for further ethnopharmacological studies. Information about the traditional uses of 59 medicinal plants, given with their vernacular names only, is documented. Botanical names were preliminarily assigned to the species according to standard literature. The indications mentioned most often are fever, pain, and skin diseases. For 12 out of 59 medicinal plants described by Freeman (20.3%), not any PubMed entity could be identified. For a variety of species, including Emilia grairneana DC., Kotschya strigosa (Benth.) Dewit & P.A. Duvign. (Sarcobotrya strigosa), Ipomoea wightii (Wall.) Chiosyo, Cissus microdonta (Baker) Planch., and Gladiolus garnieri A. Klaat, a considerable knowledge gap between traditional reports and current study results has been detected and thus, they are recommended for further investigation of their traditional uses and their potential as medicinal plants. In conclusion, the letter is an important, early and hardly known document about ethnopharmacology in Madagascar. Information from its analysis may be further confirmed and eventually lead to purposeful pharmacological screening, as is known from other historical sources.
Various computational tools are available to detect similarities between proteins which can be used to cluster related proteins into families [1].

But the crucial step to elucidate functional differences, such as substrate specificity, protein-protein interaction and co-factor interaction are often encoded in the small differences between family members. It can be cumbersome work for a researcher to find those differences.

Therefore, we implemented a discrimination rule pipeline which allows simple detection of sequence differences between protein groups with known distinct functions. Currently the pipeline can create detection rules for three different methods: Specific hidden markov profiles based on HMMER3 [2], optimized position specific scoring matrices (PSSMs) and sequence alignment analysis using classification and regression trees (CART [3]). Additionally, the pipeline enables the user to implement own detection rules in a straightforward manner.

The algorithms are automatically cross-validated for a given dataset and the most effective can be used to classify novel sequence. An update function facilitates the integration of new protein sequences into the dataset.

The power of the pipeline was tested to predict iteration steps for iterative polyketide synthases (type 1) and showed promising results.

It is planned to integrate the pipeline into the SeMPI web-server [4] for fast and accurate structure prediction of natural compounds.

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References:
Market entry, power, pharmacokinetics - what makes a successful drug innovation

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Innovations promise economic success and improvement of patient care. Depending on the time point of market entry radical innovations can be distinguished from incremental innovations. While a radical innovation typically is the first available derivative of a drug class, incremental innovations are drugs which are being launched thereafter and that show a certain benefit compared to the radical innovation. [1-5] This work uses historical market data to investigate which derivatives within certain drug classes were most successful on the market. [6] Therefore respective pharmacokinetic, pharmacodynamic and other drug related properties were evaluated. It is suggested to call the most successful drug "overtaking innovation", as this innovation exceeds all other derivatives' market shares. Seven drug classes showed exemplarily that the overtaking innovation is never a radical innovation, but rather an early incremental innovation, which shows advantages in manageability and/or tolerance.


RESULTS

Results: During the ALERTS study, 26,958 patients in the first and 35,219 patients in the second surveillance period were admitted to the cohort at Jena University Hospital. 1,643 and 2,474 HAIs were identified prospectively in the first and second periods, respectively. Results of proportional hazard for death and discharge alive showed that patients with HAIs exhibited significantly increased hazard of hospital death compared with non-infected patients. Extra LOS (± standard error) for HAI patients exclusively treated in general wards was 8.45 ± 0.80 days in the first period and 9.63 ± 0.67 days in the second period. Additional LOS decreased from 8.09 ± 0.91 days in the first period to 7.31 ± 0.60 days in the second period for infected patients treated in both Intensive care units (ICUs) and general units. Excess costs (range) due to HAIs in general units were €6,118 (€5,539 – €6,697) and €6,972 (€4,487 – €7,457) in the first and second periods, respectively. Total reduced excess costs due to HAIs among infected patients treated in both ICUs and general units were from €11,653 (€10,346 – €12,969) in the first period to €10,534 (€9,669 – €11,398) in the second period.

Conclusions: Health care-associated infections prolong LOS and can increase the hazard of hospital mortality and costs of care.

Acknowledgments: The CSCC and this research were supported by the German Ministry of Education and Research (BMBF) under grants 01EO1002 and 01EO11002.

REFERENCES

POS.119

POS.120

Estimating the health and economic impacts of healthcare-associated infections in Jena University Hospital

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Objective: Healthcare-associated infections (HAIs) have a substantial effect on morbidity and mortality and increase the length of hospitalization and associated costs [1,2]. We investigated the effect of a hospital-wide prevention program on mortality, additional length of stay (LOS) and economic costs due to HAIs.

Methods: We analysed data on HAIs from the ALERTS study, a prospective, pragmatic quasi-experimental interventional cohort study, at the Jena University Hospital, a tertiary care medical center in Germany [3]. HAIs were identified among patients hospitalized for 48 hours with at least 1 risk factor for HAI and new antimicrobial therapy; the diagnosis was confirmed by U.S. Centers for Disease Control and Prevention criteria [4]. To compare infected and uninfected patients’ daily risk of reaching the endpoint, we used cox proportional hazards models [4]. A multistate model was used to estimate additional LOS [2.5], and excess costs were calculated using average per diem costs for patients with HAIs in both the first and second surveillance periods.

Results: During the ALERTS study, 26,958 patients in the first and 35,219 patients in the second surveillance period were admitted to the cohort at Jena University Hospital. 1,643 and 2,474 HAIs were identified prospectively in the first and second periods, respectively. Results of proportional hazard for death and discharge alive showed that patients with HAIs exhibited significantly increased hazard of hospital death compared with non-infected patients. Extra LOS (± standard error) for HAI patients exclusively treated in general wards was 8.45 ± 0.80 days in the first period and 9.63 ± 0.67 days in the second period. Additional LOS decreased from 8.09 ± 0.91 days in the first period to 7.31 ± 0.60 days in the second period for infected patients treated in both Intensive care units (ICUs) and general units. Excess costs (range) due to HAIs in general units were €6,118 (€5,539 – €6,697) and €6,972 (€4,487 – €7,457) in the first and second periods, respectively. Total reduced excess costs due to HAIs among infected patients treated in both ICUs and general units were from €11,653 (€10,346 – €12,969) in the first period to €10,534 (€9,669 – €11,398) in the second period.

Conclusions: Health care-associated infections prolong LOS and can increase the hazard of hospital mortality and costs of care.

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REFERENCES

POS.121

Triple Resonance 15N-detected NMR Experiments for Backbone Assignments of Dynamic and Intrinsically Disordered Proteins (IDPs)

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The functional significance of intrinsically disorder proteins (IDPs) has been shown by a number of studies over the past decade. [1,2] Structural studies on these proteins have been mostly dominated by NMR spectroscopy due to their conformational heterogeneity and inherent dynamics. IDPs present several unique challenges to traditional 1H-detected biomolecular NMR experiments. We overcame the limitations of conventional assignment strategies by developing a suite of 3D 15N-detected pulse sequences that take advantage of 15N spins’ slower transverse relaxation, narrower linewidth and greater chemical shift dispersion compared to 1H and 13C resonances (see Figure). [3,4]
The four experiments enabled complete backbone assignment of the transcription factor "Nuclear Factor of Activated T cel lS" (N-FAT), which contains 17% proline residues, located in numerous serine-proline repeats. The experimental suite should be broadly applicable for backbone assignment of proteins that are disordered, dynamic, or possess unfavorable amide proton exchange rates.

References:

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The PubPharm search engine can be found here: www.pubpharm.de

More information about SIS Pharmacy and the PubPharm system can be found in the PubPharm blog: https://blogs.tu-braunschweig.de/pubpharm/

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POSTERS

Figure: A: Transverse relaxation rates of different nuclei as a function of a protein’s rotational correlation time. B: Resulting narrower line widths of 15N-detected peaks compared to 1H-detected peaks.

The four experiments enabled complete backbone assignment of the transcription factor "Nuclear Factor of Activated T cells" (N-FAT), which contains 17% proline residues, located in numerous serine-proline repeats. The experimental suite should be broadly applicable for backbone assignment of proteins that are disordered, dynamic, or possess unfavorable amide proton exchange rates.

POSTERS

New tools in PubPharm, the search engine for pharmacy-specific literature

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PubPharm is a pharmacy-specific search engine that is being developed by the Specialised Information Service (SIS) Pharmacy (Fachinformationsdienst Pharmazie). The project aims at sustainably improving the supply of literature for academic pharmaceutical research in Germany [1]. It is being funded by the German Research Foundation (Deutsche Forschungsgemeinschaft) since January 1st 2015. SIS Pharmacy is a collaboration project between Braunschweig University Library and the Institute for Information Systems at Braunschweig University University.

PubPharm provides access to pharmacological journals free of charge. It can be used to search within 45 Mio. publications. The underlying database index goes well beyond Medline (PubMed) data (27 Mio.) to provide a comprehensive set of publications for pharmaceutical research. Hence, using one single query, many different literature resources can be found, for example, interesting Medline articles, articles in chemical journals (that are not contained in Medline), pharmaceutical e-books and PhD theses. The PubPharm search engine contains an availability check which, for users from universities in Germany, is personalized based on location. Through this, the full text of many publications can be directly accessed. The PubPharm search engine can be found here: www.pubpharm.de

A new feature in PubPharm is structure search which allows to search for a compound not only by its (textual) name but also by its molecular structure. Both similarity search and substructure search can be performed. Structure search features a web interface for accessing PubChem data. PubChem contains 60 million unique chemical structures. For similarity search the minimum Tanimoto score is 90 using a 880-bit fingerprint. The hit list contains brief information about each compound from DrugBank and PubChem. Prospective, hits will also be combined with data from Protein Data Bank. For each hit, links to relevant literature sources and patent documents are provided.

PubPharm undergoes a continuous development process. Since its launch in October 2016 several new features have been added. At the moment, integration of authority data is under development. It allows query expansion and thereby retrieval of additionally relevant documents. For example, if the search query is the brand name of a pharmaceutical drug, also publications containing only the INN or IUPAC name will be retrieved. Furthermore, content expansion is planned: Data about pharmacy-related patents and ongoing clinical trials will be integrated.

More information about SIS Pharmacy and the PubPharm system can be found in the PubPharm blog: https://blogs.tu-braunschweig.de/pubpharm/

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Life paths of sudeten german pharmacist after the second world war

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The fate of the expulsion as a result of the Second World War affected 14 million Germans who had to leave their area of origin in the former East German territories and the settlements in Central, Southeast and Eastern Europe. The expelled pharmacists lost their employment rights with the expulsion and had to start a new career in the host areas. In the Federal Republic of Germany, the concession system at that time did not allow a large number of new pharmacies to be admitted, due to the growing population as a result of the influx of millions of home-displaced persons.

In the DDR, with the new arrangement of the pharmacy system on 22 June 1949, new citizens no longer had the opportunity to lead pharmacists in private ownership. If they did not want to work as employees, they could only run a federal pharmacy as a tenant.

The poster shows life paths of Sudeten German pharmacists after the expulsion.

Nationalwide MCQ state exams as performance indicators in the basic study period of German pharmacists’ education: How to make institutions comparable?

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Statistical analyses from the medical faculties in Germany as well as the approximately 20 German institutions that are educating pharmacists indicate that the major factor that affects the exam outcomes is the
composition of the candidate population at the respective institution with respect to socio-demography and educational biography, rather than faculty-related factors (such as teaching program / organizational and didactic aspects).

Socio-demographic and education-biographic characteristics of the candidates as significant predictors of exam performance are not evenly distributed within the institutions in Germany. Hence, only adjusted averaged exam results would represent a measure that provides comparability of institutions, while non-adjusted results include a comparability bias.

Aim of the current retrospective study was to define and evaluate differences and prognostic factors in the test populations (candidate groups), i.e., the potential relevance of influencing factors, such as school grades as well as non-native German language skills, and to apply the procedure of covariate adjustment, in order to compensate for such differences and provide adjusted and comparable results for the different institutions.

For the analyses, a linear multivariate model was employed. The analyses are based on the results obtained from the 1st pharmaceutical state exam within the period of fall 2007 to spring 2017. Within the German pharmaceutical state exam, the semiannual and for all candidates simultaneous 1st state exam represents a series of 4 subsequent written (MCQ) exams in 4 subject clusters (I-IV). Data from these 4 exams were evaluated separately.

The analyses of the respective exam candidate populations at the different institutions yielded intrinsic differences, e.g. in pre-university biographies, other nationalities, school grades. As a major difference between institutions, the final school grades were identified. The respective 8-year-average numbers for the institutions range from 1.68 to 2.41 (school grades as university entrance parameter). Results of the adjustment procedure yields normalized outcomes for P1/I-IV, which are characterized by a reduced range of the exam grades for the majority of the exam days, i.e., the covariate-adjusted results may show smaller inter-institutional variability.

It is concluded that covariate adjusted data are a more appropriate basis for inter-institutional comparisons and that such adjustments may affect the institutional ranking.
4.11 Pharmaceutical technology and biomaterials

**POSTERS**

Challenging the hydrophilicity of bacterial nanocellulose

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The renewable bipolymer bacterial nanocellulose (BNC), is characterized by a unique three-dimensional network of nanostructured fibers, high purity and over 90% water content. Moreover, the excellent biocompatibility and outstanding mechanical and biological properties as well as the enormous internal surface area, provide an excellent basis as a biomaterial for artificial blood vessels, scaffolds for tissue engineering and wound dressings. Although many promising attempts were made using BNC as a drug delivery system [1], loading with lipophilic substances is still considered as one of the main challenges to develop further applications. In this study, bearing lipophilic drugs (coenzyme Q10 (Q10) and alpha-Tocopherol) with the hydrophilic BNC network utilizing several innovative, dermal friendly and flexible colloidal carrier systems was investigated.

BNC fleeces were produced by strains of Komagataeibacter xylinus (DSM 14666) in Hestin-Schrarrm medium under static conditions in 24-well plates, which were harvested, alkaline purified [2] followed by an optional freeze-drying. Lipophilic drugs were encapsulated by Hydro-Tops (w/v/nanomulsion), Lipo-Tops (o/w emulsion) and liposomes, which were produced by high pressure homogenization. The stability of the carrier systems regarding hydrodynamic diameter and zeta potential were measured at three different temperatures (4 °C, room temperature °C, and 37 °C) over up to 90 days. The carrier systems with Q10 and alpha-Tocopherol were loaded into BNC by a standard sorption method [3] and other post synthetic loading techniques such as injection and reswelling. Release was studied in purified water at 32 °C using the Franz cell diffusion system. The determination of the antioxidant activity of alpha-Tocopherol was investigated using the semi stable 1,1-diphenyl-2-picryl-hydrazil (DPPH) whose reaction with alpha-Tocopherol is detected by electron spin resonance spectroscopy (ESR) [4]. Different carrier systems were successfully prepared and revealed hydrodynamic diameters of about 65-130 nm, negative zeta potentials and an excellent stability over 90 days. The incorporation of the carrier systems into BNC fleeces was performed using the standard sorption method with an efficacy of about 9%. Controllable Q10 release behaviour was detected, depending on the type of carrier systems, the BNC conditions (native or freeze dried) and the type of loading technique. Furthermore, alpha-Tocopherol maintained its anti-oxidative activity after loading into the BNC structure. Lipophilic substances could be successfully loaded into the hydrolymph bacterial nanocellulose, which accomplished a wide range of applications using BNC. Moreover, variation of the parameters facilitates the adjustment of drug load and release for custom-designed application.

Acknowledgement: We would like to thank JenaCell GmbH especially Elena Pfaff for providing the Komagataeibacter xylinus culture.

References

**POS.125**

**POS.126**

**POSTERS**

Photosensitizer-loaded nanoparticles: Promising drug delivery systems for photodynamic therapy of intestinal cancer?

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Photodynamic therapy (PDT) is a promising therapy approach in oncology to treat several types of cancer [1]. It is based on the interaction of three elements – a light-responsive drug called photosensitizer (PS), a light-source that emits a specific wavelength of light, and the presence of oxygen in the target region. After its enrichment in cancer cells, the PS is activated by irradiation of the tumor. Active PS molecules react with oxygen and subsequently, reactive oxygen species (ROS) are generated. Those interact with cell components like DNA, lipids, or proteins and thus cause apoptosis or necrosis of cancer cells [2,3]. Despite many advantages of PDT like only slight invasiveness and the convenience for patients and therapists, there are also some drawbacks: Most PS are insoluble in water and need to be administered intravenously. After injection, they tend to aggregate and the remaining PS increases skin-photosensitivity up to six weeks [4].

To exploit the advantages of PDT and circumvent side effects of systemic application, new application routes for PS have to be investigated. Therefore we developed polymeric nanoparticles as promising oral PS delivery systems for gastrointestinal cancer therapy. Crucial for this therapy is a high concentration of PS in the intestinal epithelium. Achieving this aim, a main hurdle – the mucus barrier in GI-tract - has to be overcome. Nanoparticle drug delivery systems enable mucus-penetration [5], overcome water-insolubility of PS and thus lead to efficient drug transport.

We established different preparation methods for nanospheres composed of the biodegradable and biocompatible polymer poly(lactic-co-glycolic acid) (PLGA) and the model-API THPP 5,10,15,20-tetakis(3-hydroxyphenyl)-porphyrin (mTHPP). mTHPP is structural related to mTHP (temoporfin®), which is EMA-approved for the treatment of head and neck cancer. To examine influence of the nanoparticle surface on mucus penetration, we modified the surface of our established mTHPP-PLGA-nanoparticles, e.g. with polyethylene glycol (PEG).

To ensure high product quality, all prepared nanoparticle formulations were fully characterized for their physicochemical properties. Photon correlation spectroscopy (PCS) was used to measure particle diameter, polydispersity index, and zeta potential. In order to determine drug load and in vitro release of PS, HPLC analysis was performed, using the fluorescence properties of mTHPP. Mucus penetration behavior of the nanoparticle formulations was investigated by several in vitro experiments, like penetration studies using a ‘biosimilar mucus’ [6]. Furthermore, we performed cell culture experiments to identify interactions of nanoparticle drug formulations with intestinal cancer cells.

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References:

**POS.127**

**Development and optimization of novel NIR-emitting gold nanoclusters-loaded gelatin nanoparticles**

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Visualization of nanoparticles (NPs) in tissues is considered as an important tool to gain knowledge about NPs’ tissue distribution, therapeutic effect and toxicity. However, deep tissue imaging is challenging and requires the development of fluorescent probes emitting light in the optical window of tissues (NIR) [1]. The available fluorescent probes, mainly dyes and quantum dots, have limiting shortcomings represented in photobleaching, toxicity and fluorescence independence of the carrier. This is urging the development of new fluorescent probes.

Gold nanoclusters (AuNCs) may offer great promises in this regard. The objective of the present work was to develop NIR-emitting AuNCs incorporated into gelatin NPs as imaging nanocarriers for skin applications. The clusters were formed directly by aggregation of reduced gold atoms in coordination with the polymer chains [2]. This offers potentials for deep tissue imaging and precise tracking of NPs without fluorescent probe leaching.

Gold nanoclusters were synthesized using gelatin B and the effects of the gold to gelatin ratio, pH and the reactants concentration on the fluorescence intensity and the emission wavelength have been investigated. For tuning the emission wavelength, different concentrations of glutathione (GSH) were added to gelatin in one pot preparation. As a step toward the formulation of labelled cross-linked gelatin NPs, the change in gelatin structure after AuNCs formation was monitored by FT-IR. Further, the free amino groups in gelatin were quantified over different time intervals of the reaction with gold. Finally, AuNCs-loaded gelatin NPs from AuNCs-gelatin B and AuNCs-GSH-gelatin B were produced by cross-linking using EDC/NHS as a non-toxic cross-linker. The NPs were characterized for their morphology by SEM and for AuNCs loading by TEM. Furthermore, confocal microscopy was applied to optically visualize the particles.

Gold nanoclusters emitting light at 640 nm were successfully fabricated by gelatin B. The intensity and wavelength of fluorescence were controlled by variation of the reaction conditions. Gold nanoclusters with higher emission wavelengths up to 720 nm were prepared by the aid of GSH. Cross-linked AuNCs-loaded gelatin NPs were successfully prepared by controlling the cross-linking time and pH. The labelled NPs showed a well detectable fluorescence for imaging by confocal microscopy.

In conclusion, AuNCs-loaded gelatin NPs emitting light in the optical window of tissues were developed for skin application. Moreover, theranostic NPs could be developed by loading the AuNCs-gelatin NPs with therapeutic agents.

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References:

Template-Assisted Fabrication of Aspherical and Nanostructured PLGA Microparticles for Pulmonary Drug Delivery

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The replication of nature-inspired forms can lead to unprecedented advantages for the interaction with biological systems [1]. For the lung, targeted drug delivery and controlled release are still current challenges. Non-spherical particles such as fibres or cylinders seem to elude these physiological hurdles. It was shown that phagocytosis is strongly influenced by the shape of the object and aspherical particles have the potential to influence clearance processes [2]. Furthermore, fibres and cylinders are showing a higher probability of depositing in the deep lung in comparison to spheres of identical volume [3].

So far, the production of aspherical particles is not straightforward and most fabrication methods lead to aggregated or non-biodegradable particles. This greatly limits the use of aspherical particles for pharmaceutical research, in which individual particles with good colloidal dispersability as well as biocompatible and biodegradable properties are necessitated. Therefore, we have developed a new technique for the production of cylindrically and nanostructured microparticles, called the template-assisted particle infiltration [4, 5]. We are infiltrating materials of interest in form of nanoparticles rather than single molecules into porous membranes and then stabilizing the arranged microstructure by electrostatic attraction or physical entrapment (Figure A).

Hitherto, for harvesting the microparticles, the template membrane was dissolved in organic solvents. Thus, the material repertory is limited and pharmaceutical essential polymers (e.g. polyactic-co-glycolic acid) aren’t utilizable. The use of an adhesive polymer layer allows a non-destructive technique for the release. By using polyvinyl alcohol (PVA) as adhesive layer we have several advantages: The polymer film is highly water soluble, it already exists in our nanoparticulate system and it is biocompatible. Furthermore, the adhesion of the PVA layer is strong enough to capture the microparticles without damaging the membrane (Figure B) allowing for easy release in aqueous media.

In summary, we have developed a novel method for the fabrication and harvest of isolated and aspherical microparticles. Due to this non-destructive and organic solvent-free approach, we are able to use hydrophilic polymers for the first time. In addition, we can easily modify the particle geometry by changing the pore diameter or thickness of the membrane.

Acknowledgments: Ministry of Higher Education, Cultural Affairs and Missions Sector (MOHE-CASM), Egypt for financial support.

References:

Biorelevant dissolution testing of different IR formulations of n-acetylcysteine using the dynamic open flow through cell

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PURPOSE

One aspect of modern in vitro dissolution testing is the prediction of the in vivo performance of delivery systems. The imitation of physiological conditions in the gastrointestinal tract (GIT) is one possibility for the realization of more predictable in vitro dissolution testing methods. In this study we investigated the application of the dynamic open flow through test apparatus (DFT) for the understanding why in a phase I clinical trial the intake of n-acetylcysteine (NAC) either as tablet or granule with and without water resulted in comparable plasma concentration profiles.

METHODS

In order to simulate physical stress on the dosage forms and different water kinetic schemes the dynamic open flow through test apparatus (DFT, figure 1) was used. The probe chamber was filled with 50 mL of SGFsp and perfused with 150 mL table water. Compendial dissolution testing was carried out with the paddle apparatus and the media volume of 900 mL SGFsp.
The aim of the test scheme was to simulate a first-order like water emptying kinetic for 150 mL in the first 20 minutes with a gentle agitation after 10 minutes. Afterwards a gastric emptying with an increased flow rate and three pressure waves was applied. In comparison a scheme equal to the first one but with a constant flow rate of 2.5 mL/min for the first 20 minutes was performed to simulate a drug administration without water. In this scenario the 2.5 mL/min was assumed as the sum of basal gastric and salivary secretion.

Figure 1  Charting of the experimental setup.

RESULTS
For dissolution testing in the DFT a drug release of around 30% until simulated gastric emptying (20 minutes) was observed. The terminal part of the program is for verifying a complete drug release. We could not observe great differences in release profiles of the three different test scenarios. The scenario “granule without water” showed a slightly delayed drug release, due to its initially slower pump rates. But at simulated gastric emptying, drug release was higher than for the other two. The tablets showed a slightly faster drugs release. We relate this to supersdesintegrate exipient formulation and a poor wetting of the granule. This assumption is supported by compendial dissolution tests.

CONCLUSION
The in vitro test setting revealed a slow dissolution until the simulated gastric emptying for all cases. This finding suggests that the administered water is not able to wash out the drug from the stomach in high amounts. To our understanding gastric emptying is the main mechanism which influences NAC bioavailability.

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POS.131
Synthesis and Characterization of Gelatin Nanoparticles for the Delivery of Hydrophilic Macromolecules

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Hydrophilicity is an important feature of gelatin which makes it an attractive biopolymeric vehicle for hydrophilic active pharmaceutical ingredients (APIs). In addition to this feature, it is biodegradable and biocompatible. But from a biopharmaceutical point of view, there are also some demerits of the hydrophilic nature of gelatin. On contact with biological fluids, gelatin-based delivery systems are beginning to swell and thus cannot maintain its structural integrity. This consequently, leads to the release of incorporated hydrophilic cargos. Therefore, to maintain the structural integrity and sustain release, gelatin-based nanoparticles have been stabilized by crosslinking with glutaraldehyde, glyoxal, carbodiimide, genipin, transglutaminase and reduced sugars [1-6]. The main disadvantage of these hydrophilic crosslinkers is that they penetrate the gelatin colloidal interface and cause crosslinking of the loaded macromolecules with the gelatin or intramolecularly. Thus, causing a potential interference in the release of incorporated hydrophilic cargo from the gelatin matrix.

The aim of this project is to physicochemically stabilize the gelatin nanoparticles, using zero-length crosslinker, divinyl sulfone (DVS). Gelatin-NPs with a mean diameter of 200 nm and a critical micellization concentration and a positive charge, and PVA was used for steric stabilizer followed by a covalent coupling reaction with targeting ligands [2]. In combination with suitable ligands such as i.e. apolipoproteins it will be possible to achieve NPs which are capable to cross the BBB by receptor-mediated transcytosis. Another promising approach is the use of a positively charged systems, which may enable transport across the BBB by adsorptive transcytosis.

PLGA-NPs were prepared either by emulsiﬁcation-diﬀusion (ED) or solvent displacement (SD) method. In the case of the ED method an outer aeous phase containing the preformed VS-PVA as steric stabilizer was used for particle preparation, whereas for the SD method the PLGA-NPs were prepared in the presence of unmodiﬁed PVA as stabilizer followed by a covalent modiﬁcation of the PVA-coated NPs with divinyl sulfone (DVS). PLGA-NPs with a mean diameter of 200 nm and 120 nm were achieved for the ED and SD method, respectively. For the preparation of cationic PLGA-NPs an ED method in combination with didodecylammonium bromide (DAB), a surfactant with a low critical micellization concentration and a positive charge, and PVA was used for steric stabilisation [3]. This process led to NPs with a diameter below 100 nm. After successful preparation it is the future goal of the study to discover, if the obtained NP’s characteristics are sufficient to overcome the BBB.

In summary, we have developed a validated and optimized novel nano-sized formulation consisting of gelatin nanoparticles (200-300 nm, PDI < 0.2, see Fig. 1). The GNPs are selectively and specifically crosslinked on their colloidal interface. These selectively croslinked gelatin nanoparticles provide a promising opportunity to encapsulate hydrophilic macromolecular entities.

In light of this observation a nanosuspension stabilized with a nanoscale benzoyl peroxide. This observation is in good agreement with the supposed induction of amorphous fractions since an increase in solubility is a direct consequence of amorphisation. As the solubility of benzoyl peroxide in hydrous solvents expressed in absolute terms is beneath 1 per mill [2] even the increased solubility of the nano suspension does not seem to evoke any stability problems. Although the fluid nanosuspension chemically as well as physically seems to be sufficiently stable freeze drying experiments utilizing varying additives were conducted. They lead to two formulations which showed excellent redispersibility so that a long term stable storage formulation is available too.

In conclusion the phospholipid-stabilized nanosuspension proved to be a stable and easy to handle intermediate for the manufacture of solid lipid microparticles. And what is more it might very well be a promising formulation strategy for APIs per se.

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POS.132

Amphiphilic polysaccharide block copolymers for pH-responsive micellar nanoparticles

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Dextrins are linear polysaccharides, composed of 1-6 glycosidic linked glucose units (AGU). They are produced from renewable resources and can be used as an alternative biopolymer building block to artificial polymers in macromolecular drug delivery systems.[1-3] A full polysaccharide amphiphilic block copolymer was prepared from end group-functionalized dextrins using copper mediated azide-alkyne click chemistry. Sufficient modification of the reducing end in both blocks was achieved by microwave-enhanced reductive amination in a borate-buffer/methanol solvent system. The combination of a hydrophobic dextrin block with a hydrophobic acetalated dextrin block results in an amphiphilic structure that turns water-soluble upon acid treatment. The material has a low CMC and self-assembles in water to spherical micellar nanoparticles. The formed nanoparticles have a narrow size distribution below 70 nm in diameter and disassemble in slightly acidic conditions. The amphiphilic polysaccharide system shows low toxicity and can stabilize the hydrophobic model drug curcumin in aqueous solutions over extended time periods.

Sporopollenin is a biopolymer consisting of carotenoids or polyunsaturated fatty acids that can be found in the outer cell walls of spores and pollen of plants. This porous natural material can be isolated from Lycopodium clavatum spores by a chemical process including defatting, alkaline lysis and acidolysis of the raw material followed by different washing steps and drying [1]. Sporopollenin has caused increasing interest as drug delivery system due to remarkable biocompatibility and high stability against temperature, pressure and most chemicals. The large cavities of sporopollenin enable the microencapsulation of several materials with a high loading capacity [2]. Two sporopollenin samples obtained by different isolation and purification procedures were used. They were investigated by elemental analysis and scanning electron microscopy (SEM) for composition and morphology. The sporopollenin samples were analysed concerning cytotoxicity using the CellTiter-Glo™ luminescent cell viability assay using L-929 cells. Additionally, hemocompatibility was tested by red blood cell aggregation and hemolysis. Proof-of-concept encapsulation studies were performed with fluorescein isothiocyanate (FITC) labeled dextran (MW: 21,200 Da) using a vacuum loading technique and evaluated with fluorescence microscopy. The two evaluated sporopollenin samples showed a porous structure with large cavities as demonstrated in SEM picture (Figure 1). Both sporopollenin samples showed a notable biocompatibility even in high concentrations of 6.25 mg/mL and incubation times up to 72 h independent of the preparation technique. Hemocompatibility and pH-value of the sporopollenin samples was shown to be dependent on the isolation technique. Successful loadings could be demonstrated in proof-of-concept encapsulation studies with FITC-dextran. In conclusion, sporopollenin possesses promising properties as non-toxic and biologically inert drug delivery system with high loading capacity.

![SEM picture of sporopollenin obtained with a streamlined preparation method as described in literature][1]

**Acknowledgments:**
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**References:**

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**Sporopollenin isolated from Lycopodium clavatum spores as drug delivery system**

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The usage of mucoadhesive polymers is a promising approach to develop a peroral solid dosage form for a local tumor therapy in the small intestine. In this study different polymers were screened regarding their mucoadhesive properties in different media.
For the mucoadhesion test tablets were manufactured containing 5% mucoadhesive polymer, 1% silicon dioxide, 1% magnesium stearate and 93% lactose. A negative control was made by exchange of the mucoadhesive polymer by lactose. Following polymers were tested: Carbopol 971P NF, Chitosan HCl, Chitosan 90/30, Chitosan 90/400, Hypermellose, CEKOL 700, CEKOL 2000, CEKOL 30000.

The mucoadhesion was measured by the Texture Analyzer (TA.XT plus) with a 30% mucin solution [1]. The pulling force was measured while the tablet was lifted from the mucin solution after a defined incubation time. This force represents the mucoadhesion force.

Disintegration tests were performed to investigate the influence of the polymers on the disintegration time of the tablets. A longer disintegration time due to a gelling effect of the tablets indicates a higher mucoadhesion polymers on the disintegration time of the tablets. A shorter disintegration time represents a lower mucoadhesion effect. The disintegration time in phosphate buffer pH 6.8, representing the stomach, was compared.

The results indicate that a suitable method was found to evaluate the mucoadhesive properties of polymers. Carbopol 971P NF is the best candidate for further investigations in a solid dosage form. It showed the highest mucoadhesion and a great retard effect in the phosphate buffer pH 6.8 which indicates a mucoadhesion in the intestinal fluid. Chitosan AA-1 showed also promising mucoadhesive properties. The chitosans are not suitable for mucoadhesive effects in the small intestine but in acid areas like the stomach. Hydroxypropyl methylcellulose and carboxymethyl cellulose showed retardation in phosphate buffer pH 6.8. The chitosans exhibit a retard effect only in 0.1 N hydrochloric acid, representing the stomach, was compared.

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References
Results and Discussion: Rutin nanocrystals possessed a size of about 400nm. After addition of the nanocrystals to the CAM a strong agglomeration of the particles was observed. Hence the particles were transformed into larger agglomerates, making an evaluation of the irritation potential of the nanocrystals itself impossible. The reason for the agglomeration is the saline solution, which is used to moisture the CAM and for washing off the samples. It reduces the zeta potential of the nanocrystals and thus reduces the repulsive forces of the suspension. To avoid agglomeration, the standard test protocol was slightly modified by exchanging 0.9% NaCl solution to an isotonic 2.2% (w/w) glycerol solution. Results revealed that agglomeration could be circumvented by this. Further tests revealed after the test, that nanosized samples could not be washed off completely from the CAM, as they stuck closely to it. The reasons for this are the increased adhesive forces of nanosized materials, when compared to larger sized materials. The washing procedure could not be improved, because more aggressive washing would destroy the sensitive CAM. Therefore, to evaluate the irritation potential of nanosized material, it is important to consider that the test cannot be stopped as it is possible with standard test materials. Therefore, in order to obtain comparable results, the times for evaluating the CAM after the washing must be standardized. By using the modified test protocol emulsions with identical composition but different sizes (150nm to >10µm) were tested. Results revealed an increase in irritation potential with decreasing size (Fig. 1A+B). The increase in irritancy can be explained by the increase in attaching points with decreasing size (Fig. 1C), hence the smaller the size, the more surfactant is in contact with the CAM, which increases the irritation potential.

Figure 3: Comparison of irritation potential via HET-CAM test of a large sized emulsion (A) and a nanoemulsion (B) with identical composition and scheme of mechanism (C).

Conclusion: Nanocosmeceuticals possess special properties when compared to larger sized material. The increased sensitivity against electrolytes, the increased surface area and increased adhesiveness must be considered when using HET-CAM tests and the protocol needs to be modified accordingly to obtain reliable results. A decrease in particle size was found to increase the irritation potential of the surrounding stabilizer. Therefore, when developing nanocosmeceuticals, especially skin-friendly stabilizers should be used.

References:

Investigating the preparation of Eudragit® RL and RS nanoparticles via a solvent displacement method

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The glass transition temperature (T_g) is a well-known parameter for pure polymers, which are used as starting material for nanoparticles. However, the determination of T_g is rarely applied. T_g describes the temperature range of amorphous substances from a highly viscous glassy state to a low viscous liquid state. There are various influencing factors on the T_g of polymeric nanoparticles, for example the incorporated drug. Relevant properties of this factors on the T_g are the Flurbiprofen (FBP) and 5,10,15,20-tetra(1-C), hence the smaller the size, the more surfactant is in contact with the

Clinical doses of therapeutic proteins (e.g. monoclonal antibodies) are in a range of 5-700 mg per patient. For subcutaneous injection with typical volumes of 1 to 1.5 ml, highly concentrated antibody formulations are needed. Compared to more diluted protein formulations, an increased concentration accompanies with some different challenges in the development. At high antibody concentrations above 150-200 mg/ml the solution becomes crowded and protein-protein interactions become more relevant [1]. These conditions are associated with altered protein-stability and high viscosity inducing problems for filtration processes and drug delivery [2].

Pairwise and higher-order protein-protein interactions, clustering, and protein-excipient interactions are crucial parameters to predict protein-stability and modulate the rheological behaviour [3]. Our working hypothesis is that a complex microscopic description of solution viscosity in combination with measured complex rheological and protein-protein interaction parameters can explain differences in viscosity of different antibody candidates. Non-ideality parameters, such as the second virial coefficient B2 and the interaction parameter kD measure intermolecular protein interactions in the MHz range, complex colloid theories can be extended to explain multiple protein-protein interactions. Fits of the frequency dependence of the elastic modulus G(ω) and the loss modulus G''(ω) being used the Maxwell-model result in a solution relaxation time, τ and a complex shear modulus at the inverse relaxation time G* at the "cross-over frequency" ωc = 1/τ [5]. The solution relaxation time τ is able to describe the protein heterogeneity and was found to correlate with non-ideality parameters and protein cluster-size. The complex shear modulus G*(ω) consists with entropy-driven interactions and describes the stiffness (κ) of the intermolecular protein-protein interactions. By determination of both

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**Characterizing Protein-Protein-Interactions in Highly Concentrated Monoclonal Antibody Formulations with the Quartz Crystal Microbalance**

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Clinical doses of therapeutic proteins (e.g. monoclonal antibodies) are in a range of 5-700 mg per patient. For subcutaneous injection with typical volumes of 1 to 1.5 ml, highly concentrated antibody formulations are needed. Compared to more diluted protein formulations, an increased concentration accompanies with some different challenges in the development. At high antibody concentrations above 150-200 mg/ml the solution becomes crowded and protein-protein interactions become more relevant [1]. These conditions are associated with altered protein-stability and high viscosity inducing problems for filtration processes and drug delivery [2].

Pairwise and higher-order protein-protein interactions, clustering, and protein-excipient interactions are crucial parameters to predict protein-stability and modulate the rheological behaviour [3]. Our working hypothesis is that a complex microscopic description of solution viscosity in combination with measured complex rheological and protein-protein interaction parameters can explain differences in viscosity of different antibody candidates. Non-ideality parameters, such as the second virial coefficient B2 and the interaction parameter kD measure intermolecular protein interactions in dilute solutions. However at high protein concentrations, higher order terms of multi-body interactions contribute to aggregation processes and solution behaviour (shear viscosity) [4]. By studying the behaviour of concentrated protein formulations under high-frequency shear excitation in the MHz range, complex colloid theories can be extended to explain multiple protein-protein interactions. Fits of the frequency dependence of the elastic modulus G(ω) and the loss modulus G''(ω) being used the Maxwell-model result in a solution relaxation time, τ and a complex shear modulus at the inverse relaxation time G* at the "cross-over frequency" ωc = 1/τ [5].

The solution relaxation time τ is able to describe the protein heterogeneity and was found to correlate with non-ideality parameters and protein cluster-size. The complex shear modulus G*(ω) consists of entropy-driven interactions and describes the stiffness (κ) of the intermolecular protein-protein interactions. By determination of both
parameters, the strength and order of multiple protein-protein interactions can be characterized. Also low frequency viscosity can be predicted by $G^*$ and $\gamma$.

The study will describe the novel principle of the determination of a multi-body interaction parameter ($G^*$) and the solution heterogeneity ($\gamma$). Both parameters were correlated to the non-ideality parameters ($R_b$ and $k_D$) and shear viscosity with respect to clustering and the influence of ionic strength, $\phi$, different exipients and buffers in highly concentrated antibody-solutions.

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Erythromycin nanocrystals for topical application

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Introduction: The skin is colonized by a diverse milieu of microorganisms that is known as the microbiome of the skin. Even though the knowledge about the skin microbiome is still in its infancy, there is strong evidence that some skin disorders have an underlying microbial aetiology, such as caries [1,2], periodontitis and gingivitis [3,4]. This dosis form is well accepted by patients [5] and in contact with saliva it falls apart in a few seconds, but the probiotics should remain in the oral cavity and especially on the mucosa and gingiva for some time to exert their positive effects. Moreover, the constant flow of saliva and eating or drinking are limiting the residence time in the oral cavity. The addition of mucoadhesive polymers is an effective strategy to prolong the adhesion of probiotics to the oral mucosa. Unfortunately, these polymers prolong the disintegration time of the tablets and they do not meet the acceptance level from the FDA (30 s). Simultaneous granulation of mucoadhesive polymer and probiotic bacteria was shown to aid fast disintegration on the one hand and to provide an intimate contact between both materials on the other hand.

For this study three different batches of L. plantarum 299v (Lp299v) were used and tablets with pure probiotics, with granulated probiotics, and with probiotics granulated after the addition of Carbopol, as a mucoadhesive polymer, were prepared. Interestingly, the disintegration time differed markedly depending on the used batch of Lp299v (6535295, 6538131, or HF0988). A clear trend showed up for the tablets with ungranulated probiotics and those tablets with granulated probiotics plus Carbopol, whereas the effect was not measurable in the very fast disintegrating tablets with granulated probiotics.

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POS.141

Influence of particle size of probiotic bacteria on disintegration time of orodispersible tablets

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Orodispersible tablets (ODTs) are a promising formulation strategy for the delivery of probiotic bacteria to the oral cavity to fight disease with a microbial aetiology, such as caries [1,2], periodontitis and gingivitis [3,4]. This dosage form is well accepted by patients [5] and in contact with saliva it falls apart in a few seconds, but the probiotics should remain in the oral cavity and especially on the mucosa and gingiva for some time to exert their positive effects. Moreover, the constant flow of saliva and eating or drinking are limiting the residence time in the oral cavity. The addition of mucoadhesive polymers is an effective strategy to prolong the adhesion of probiotics to the oral mucosa. Unfortunately, these polymers prolong the disintegration time of the tablets and they do not meet the acceptance level from the FDA (30 s). Simultaneous granulation of mucoadhesive polymer and probiotic bacteria was shown to aid fast disintegration on the one hand and to provide an intimate contact between both materials on the other hand.

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This behaviour seemed to be related to the particle size of the tested Lp299v batches as batches with smaller particles revealed tablets with prolonged disintegration time. This hypothesis could be confirmed by preparing tablets from distinct particle size fractions of a singular Lp299v batch. Again, the smaller the particle size of the probiotics was, the slower was the disintegration. This effect might be attributed to the hydrophobic nature of the probiotic bacteria, which restricted the
absorption and further transport of water into the tablet. Consequently, the disintegration process was negatively affected and was more pronounced in material with a small particle size. Hence, the particle size of the probiotic material, which is defined by the milling process after freeze-drying, has to be carefully controlled for reproducible tablet characteristics.

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References:

of the samples. In most cases a small size and a narrow size distribution is desired, because typically larger sized particles possess different properties (e.g. less bioactivity) and can impair the physical stability of the nanosystem, e.g. due to Ostwald ripening. Therefore if particle size analysis is performed, it is of uppermost importance to yield results that enable the reliable discrimination of narrowly and broadly distributed samples. The aim of this study was to evaluate the discrimination efficacy of size analysis of polydisperse samples by using two different sizing methods.

Materials and Methods: Standard particles of defined size were mixed in a ratio 1:1 (v/v) to artificially create polydisperse samples. The used standards possessed a size of 170 nm, 250 nm, 550 nm and 1000 nm (Silica, Sedistest, Dr. Lerche KG, Germany) with a particle concentration of 0.11% (m/m), except the 1000 nm standard that possessed a concentration of 0.06% (m/m). The particles were mixed in six bimodal, four tri-modal and one tetra-modal sample. Every mixture was analysed by dynamic light scattering (DLS) by using the standard measuring setup [1]. In addition an analytical centrifuge, which measures space and time related transmission profiles, was used to characterize the samples.

Results and Discussion: In all samples DLS measurements could not detect the larger particles besides the small sized main population. The polydispersity index (PDI), which is known to be a sensitive measure for the broadness of the size distribution, was always found to be below 0.1. Hence, no discrimination between the different samples was possible.

The reasons for these findings are probably the low total number of larger particles, e.g. a 1:1 (v/v) mixture of 170nm and 1000nm results in a numeric distribution of > 99% small sized particles and < 1% larger particles. Hence, the scattering intensity of the low number of larger particles seems to be not sufficient to be detected during the measurement. The results obtained from the analytical centrifuge yielded space and time resolved extinction profiles. Depending on the number of particle fractions within the sample, corresponding high numbers of sedimentation profiles were detected for all samples analysed. For example, the tetra-modal sample containing a mixture of particles with size of 170nm, 250nm, 550nm and 1000nm, yielded 4 different sedimentation layers, respectively [Fig.1].

Conclusion: Dynamic light scattering could not discriminate between different polydisperse samples and also the polydispersity index gave no hint to the existence of the larger sized populations, which is probably due to the small number of particles and the resulting low scattering intensity of these particle fractions. The analytical centrifuge could discriminate the polydisperse samples due to its different measuring principle (Stokes law) and is therefore more recommended if high resolution size distribution profiles are required. If a fast determination of the main size of a sample is sufficient the fast and convenient DLS measurement should be preferred.

References:

Figure 4: Measurement of the tetra-modal sample with an analytical centrifuge. No. 1-4 represent the sedimentation curves of the four different particle fractions in the sample.

A novel approach for nanoparticle-loaded, inhalable microparticles using smart excipients as a matrix

Cystic fibrosis is a genetic disease which causes the human body to excrete highly viscous and adhesive mucus in various organs throughout the body. One of the highest risk factors of this disease is the lung in which the viscous mucus serves as a habitat for several bacteria like Pseudomonas aeruginosa. This often leads to heavy infections that are difficult to treat due to drug inactivation and problems for the drug to even reach the infection site. Nanoparticle technology promises to enhance the efficacy of drugs by protecting the drug from inactivation, increasing mucus penetration due to the small particle size and the possibility to change the physicochemical properties by using different polymers. This could heavily increase drug delivery, reduce strong side effects of antibiotics to the human body while increasing the direct interaction with the pathogenic microorganisms.

However, nanoparticles are too small to apply them directly to the lung. Because of this, the nanoparticles in this work where incorporated in microparticles (MP) which consist of a matrix made out of smart excipients. The used excipients were chosen because of their properties to increase mucus penetration, mucolytic potential and osmotic quality.

The nanoparticles where produced by nanoprecipitation using PLGA (Poly(lactic-co-glycolic acid) for its biodegradable and biocompatible properties. The obtained nanoparticle suspensions were mixed with the smart excipients (Poly-N-(2-Hydroxyprolyl) acrylamide), N-AcetylcySTEINE, Mannitol) and then spray dried. For analysis of the microparticles flight characteristics and their aerodynamic properties they were marked using the fluorescent dye Rhodamine B. Scanning electron microscopy (SEM) was used to analyze their morphology. Dynamic light scattering (DLS) was used to investigate the size of the NPs.

The next step is to incorporate nanoparticles produced by microfluidic techniques which might enhance the in-build quality due to the possibility of exactly tuning the nanoparticle properties.

The approach of using a matrix made of smart excipients as a carrier for drug-loaded nanoparticles could heavily enhance the future treatment for cystic fibrosis by increasing the drug delivery within the lung and the local effect of applied drugs while lowering the used dose. This would also decrease the overall exposition of the drug to the whole body and could therefore decrease unwanted side effects.

The success of chemotherapy is limited by poor selectivity of active drugs combined with occurrence of tumor resistance. New star-like structured N-(2-hydroxypropyl) methacrylamide (HPMA) copolymer-based drug delivery systems containing doxorubicin attached via a pH-sensitive hydrazide bond were designed and investigated for their ability to overcome chemotherapy resistance. These conjugates combine two strategies to achieve a high drug concentration selectively at the tumor site: (i) high accumulation by passive tumor targeting based on enhanced permeability and retention effect and (ii) pH-sensitive site-specific drug release due to an acidic tumor microenvironment. Mice bearing doxorubicin-resistant xenograft tumors were treated with doxorubicin, PBS, poly HPMA (pHPMA) precursor or pHHPMA-doxorubicin conjugate at different equivalent doses of 5 mg/kg bodyweight doxorubicin up to a 7-fold total dose using different treatment schedules. Intratumoral drug accumulation was analyzed by fluorescence imaging utilizing intrinsic fluorescence of doxorubicin. Free doxorubicin induced significant toxicity.
but hardly any tumor-inhibiting effects. Administering at least a 3-fold dose of pHMA-doxorubicin conjugate was necessary to induce a transient response, whereas doses of about 5- to 6-fold induced strong regressions (Fig. 1). Tumors completely disappeared in some cases. The onset of response was differential delayed depending on the tumor model, which could be ascribed to distinct characteristics of the microenvironment. Further fluorescence imaging—based analyses regarding underlying mechanisms of the delayed response revealed a related switch to a more supporting intratumoral microenvironment for effective drug release. In conclusion, the current study demonstrates that the concept of tumor site-restricted high-dose chemotherapy is able to overcome therapy resistance.

Fig.1: Impact of treatment on tumor volume of a doxorubicin resistant tumor model. By means of the repeated application of the pH-stimulus sensitive pHMA-Dox conjugate, tumor regression was achieved. In contrast, free doxorubicin did only slightly retard the tumor growth and caused high toxicity.

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Visualization of mucus under native conditions

Mucin is a viscous secretion, consisting of ~95% water and other components, such as mucins, lipids, proteins and DNA, which functions as a barrier to protect the underlying cellular surfaces. The mucin glycoproteins are responsible for the gel-like structure of mucus. [1, 2] Understanding of the formation and the relevant parameters for the structure of mucus is of special interest regarding particle penetration. The network properties which were found by cryoSEM [3, 4] are directly relevant for its size filtering barrier properties. [5] Our aim was to visualize the structure of mucus under native conditions using Confocal Laser Scanning Microscope (CLSM) and Stimulated Emission Depletion Microscope (STED), which provides an improved, nano-scale resolution. Gel forming glycoproteins were labeled with fluorescent wheat germ agglutinin (which binds to N-acetylgalactosamine) and specifically labeled with fluorescent antibodies directed to specific mucins. We observed that the latter labeling method provided a better visualization of the mucus structure. In addition to mucins, also other components of the mucus structure were complementarily labeled, in order to improve the understanding of mucus structural composition. Drying and dilution during the handling process were considered as important factors that could affect the native state of mucus. Our results also show a porous, foam-like structure [6] in the native state morphologically similar to the images obtained by cryoSEM. This successfully visualization of the structure of native mucus reveals pore-like structures ranging from some nm to several micrometers. In the next steps, this investigation will be extended by correlating these data to other relevant microscopic methods. This study improves the knowledge about the structure of native mucus and can be used as a method to study mucus interaction with inhalation pharmaceuticals.

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POS.150
Towards biomaterial development for local release of siRNA in bone tissue engineering applications

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RNA interference (RNAi) is a promising technique to down-regulate disease related proteins on a pre-protein level. We and others postulate that the release of nanoparticle-complexed small interfering RNA (siRNA) from implanted biomaterials could provide structural support for tissue repair, combined with local siRNA transfer and the reduced therapeutic efficiency of BMP-2. Hence, these antagonists are interesting targets for gene silencing in order to improve local bone regeneration. We could show that silencing of BMP-antagonists (chordin and noggin) as well as WiT-antagonist sclerostin have positive effects on osteogenic differentiation of human adipose derived stromal cells (hASC) or osteoblast-like osteosarcoma cells.

Therapeutic application of bone inducing BMP-2 is known to stimulate antagonistic proteins, such as noggin and chordin as well as sclerostin and reduce the therapeutic efficiency of BMP-2. Hence, these antagonists are interesting targets for gene silencing in order to improve local bone regeneration. We could show that silencing of BMP-antagonists (chordin and noggin) as well as WiT-antagonist sclerostin have positive effects on osteogenic differentiation of human adipose derived stromal cells (hASC) or osteoblast-like osteosarcoma cells. In order to realize these concepts for application in bone regeneration, we set out to develop implant systems for local delivery of siRNA. To this end, we employed gelatin-based hydrogels cross-linked with anhydride-containing oligomers (cGEL) in combination with different polymeric nanoparticles. With the aim to generate siRNA complexes with different zeta potentials, we used a low molecular weight branched polyethyleneimine (PEI F25-LMW) resulting in strongly positive zeta potentials, a tyrosine-derivative of PEI (P10Y) causing an intermediate zeta potential and PEI complexed siRNA in combination with neutral liposomes forming almost neutral lipopolyplexes. Release from cGEL hydrogels was a multifactorial process, as diffusion, hydrogel degradation and nanoparticle decomposition overlapped over time. Depending on cGEL formulation, we found sustained release of siRNA for up to 21 days.

Other concepts pursued in the lab involve 3D printing of ceramic materials in combination with crossed siRNA for controlled release.

3loth, T., Hietz, R., Kaschelke, C., Anderegg, U., Schulz-Mie miser, M., Hacker, M. (2014) Gelatin-based biomaterial engineering with anhydride-containing oligomers (cGEL) in combination with different polymeric nanoparticles. The aim of the present investigation was the systemic comparison of genipin and glutaraldehyde as crosslinking agents in the preparation of HSA-NPs. Therefore, we used varying crosslinking degrees for both agents subsequently to the desolvolution process with ethanol. For crosslinking reaction, the NPs were either incubated with glutaraldehyde (GA-NPs) for a minimum of 12 h at room temperature [4] or with genipin (Ge-NPs) for at least 3 d at 37°C [5]. After crosslinking, the NPs were purified via centrifugation and following redisopem. The purified NPs were then further investigated regarding their stability by measuring particle diameter, size distribution and zeta potential. In order to compare the cytotoxic properties of GA-NPs and Ge-NPs, a WST-1 assay was performed after NP incubation of HEK 293T cells. We were able to successfully develop genipin crosslinked HSA-NPs. The results show that even small crosslinking degrees of genipin are sufficient for a satisfying stabilization of the NPs with particle diameters ranging from 100 – 130 nm, a Pdi < 0.1 and a zetapotential below -30 mV displaying an electrochemical stable system. The WST-1 assay revealed that even high concentrations of the Ge-NPs did not lead to any detectable cytotoxicity. It can be stated that genipin was not inferior to glutaraldehyde in any way. Hence, genipin proposes a favorable organic alternative for crosslinking HSA-NPs in comparison to classical chemical crosslinking agents such as glutaraldehyde.


POS.152
Effect of different serum types on the formation of the nanoparticle-protein corona

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Nanoparticles (NPs) exhibit a great potential in different biomedical applications including drug targeting, disease diagnosis and transfusions. However, when NPs are applied in a biological fluid, due to their high surface energy, proteins bind rapidly on the NPs surface resulting in the formation of a protein corona. The structure and composition of the protein corona is determined by the physico-chemical properties of the NPs (e.g. size, shape, surface charge, surface functional groups, hydrophobicity) and the biological environment (e.g. blood serum or plasma, different serum types) [1,2]. Consequently this results in a new biological identity and affects the NPs body distribution [3]. In our study we examined the influence of different biological media on the composition of the protein corona. Therefore, we prepared NPs using the biodegradable polymer poly(DL-lactide-co-glycolide) stabilized with polyvinyl alcohol (PLGA-NPs). The PLGA-NPs were incubated with either fetal bovine serum (FBS) or human serum (HS) to induce the formation of a protein corona. After incubation PLGA-NPs were purified by several centrifugation steps. The supernatants were discarded and the pellet was resuspended in purified water to remove excess protein. Hence, the isolated nanoparticle protein complex was ready for further investigations. The molecular composition of the adsorbed protein layer was determined by SDS-PAGE and the total protein amount of the corona was quantified using Bradford protein assay after alkaline sample hydrolysis. The qualitative identification of the proteins was performed after protein desorption and tryptic in-solution digestion by mass spectrometrical detection of peptides (LC-MS/MS).

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POS.151
Genipin as a bioalternative crosslinker for the stabilization of HSA nanoparticles

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The crosslinking of human serum albumin nanoparticles (HSA-NPs) is an essential parameter regarding the stability of colloidal systems. However, commonly used chemical crosslinking agents such as glutaraldehyde bear a potential risk of cytotoxicity due to unreacted residues remaining in the particle samples. Thus, the selection of a more appropriate crosslinking agent is crucial for the applicability in vivo. Genipin displays an organic alternative to chemical crosslinking agents, since it is a naturally occurring crosslinker extracted from the plant Gardenia jasminoides [1]. It has recently been proposed that genipin is superior to established aldehyde crosslinkers due to its low cytotoxicity and efficient capability of crosslinking biopolymers containing primary amine groups [2,3].

The aim of the present investigation was the systemic comparison of genipin and glutaraldehyde as crosslinking agents in the preparation of HSA-NPs. Therefore, we used varying crosslinking degrees for both agents subsequently to the desolvolution process with ethanol. For crosslinking reaction, the NPs were either incubated with glutaraldehyde (GA-NPs) for a minimum of 12 h at room temperature [4] or with genipin (Ge-NPs) for at least 3 d at 37°C [5]. After crosslinking, the NPs were purified via centrifugation and following redisopem. The purified NPs were then further investigated regarding their stability by measuring particle diameter, size distribution and zeta potential. In order to compare the cytotoxic properties of GA-NPs and Ge-NPs, a WST-1 assay was performed after NP incubation of HEK 293T cells. We were able to successfully develop genipin crosslinked HSA-NPs. The results show that even small crosslinking degrees of genipin are sufficient for a satisfying stabilization of the NPs with particle diameters ranging from 100 – 130 nm, a Pdi < 0.1 and a zetapotential below -30 mV displaying an electrochemical stable system. The WST-1 assay revealed that even high concentrations of the Ge-NPs did not lead to any detectable cytotoxicity. It can be stated that genipin was not inferior to glutaraldehyde in any way. Hence, genipin proposes a favourable organic alternative for crosslinking HSA-NPs in comparison to classical chemical crosslinking agents such as glutaraldehyde.

completed using PEAKS® software and UniProtKB database. For all experiments the ratio of total particle surface area to serum was kept constant to ensure comparability between the obtained results. Our results show that the composition of the protein corona not only depends on the physico-chemical properties of the nanoparticle system but also on the biological media. SDS-PAGE analysis suggested that composition and amount were different for the two serum types. Quantification confirmed that the amount of protein bound onto the surface of PLGA-NPs incubated with HS is significantly higher than the protein amount bound onto the same particles after PBS incubation. LC-MS/MS analysis indicated that the protein coronas consisted of numerous different proteins. The main components take part in immune response. In conclusion, it can be stated that the protein corona around PLGA-NPs is strongly affected by the type of serum used for nanoparticle incubation.

References:

POS.153

Influence of size and urea on dermal penetration efficacy of hesperetin from nanocrystals

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Introduction: Nanocrystals are obtained by milling large sized crystalline bulk material to sizes below 1µm. Due to the decrease in size the surface area increases and with this the dissolution velocity and the kinetic solubility of the active increases. Therefore nanocrystals are known to be an effective formulation strategy to overcome poor solubility and associated poor bioavailability of poorly soluble actives [1]. Nanocrystals can be used for oral administration, but were also shown to be beneficial to improve the dermal penetration of poorly soluble actives. However until now no systematic study was done to prove the influence of size on the penetration efficacy. Therefore the aim of this study was to produce differently sized nanocrystals and to determine their efficacy to improve the penetration of a poorly soluble active upon topical application. In addition the influence of urea, which is known to be a moisturizer and a penetration enhancer on the penetration efficacy, was investigated.

Methods: Hesperetin drug nanocrystals of different sizes were produced by high pressure homogenization, bead milling and combinations of these methods. The particles were characterized regarding size by using dynamic light scattering, laser diffraction and light microscopy. The differently sized nanosuspensions were applied on the skin of fresh pig ears and the penetration of the active was analysed by tape stripping and subsequent HPLC analysis. The influence of urea was investigated by applying different concentrations of urea on the skin prior to the application of the nanosuspensions. In addition to these tests, biophysical skin parameters, e.g. transdermal water loss (TEWL), skin hydration and pH were analysed on untreated and treated skin, respectively.

Results and Discussion: Nanocrystals with sizes of about 200nm, 400nm, 600nm and 800nm were obtained. The polydispersity index increased with increasing size, which was due to the decreased milling times or homogenization cycles when compared to classical production parameters for the larger sized suspensions. This resulted in a decreased physical stability of the suspensions and thus required an immediate testing of these formulations after the production. Penetration studies proved an increase in the penetration efficacy with decreasing size of the nanocrystals. Interestingly, urea did not increase the penetration of hesperetin, independent on the size of nanocrystals used. This was unexpected, because in general urea is used as moisturizer and known to improve the penetration of many actives due to the improved hydration of the stratum corneum [2]. However, studies by Neubert and co-workers suggest that urea can only enhance skin penetration for polar actives via improving the polar route of penetration, but will not improve the penetration of apolar actives [3]. As hesperetin is a non-polar active, this might explain our results. This theory is further supported by the finding that the application of 5% and 15% urea solution hampered the penetration of hesperetin more than it was found for the application of 10% urea. TEWL measurements and measurements of skin hydration confirmed less increase in TEWL and less skin hydration of the skin which was treated with the 10% urea solution in comparison to skin treated with 5% and 15% urea solution, proving that less hydrated skin led to improved penetration of the apolar active hesperetin from nanocrystals.

Conclusion: Nanocrystals improve passive dermal penetration of poorly soluble actives in a size dependent manner, i.e. smaller sizes lead to improved penetration. Urea influences the penetration efficacy in a negative way, as it increases skin hydration and thus hampers the diffusion of the apolar hesperetin. Therefore, for improved dermal delivery of apolar actives from nanocrystals, excipients and vehicles that improve the apolar penetration pathway, e.g. oils, should be used as vehicles.

References:

POS.154

BergaCare SmartLipids Retinol – industrial suspension concentrates for dermal retinol products

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Retinol is of high interest for dermal application, but is a highly sensitive molecule requiring chemical stabilization. In addition, it can cause skin irritation, thus requiring ideally prolonged release to avoid too high irritating concentrations on the skin. In addition, efficient skin penetration is required. One approach is to incorporate retinol into a solid particulate carrier, providing all 3 features: protection, option of modulating the release and increasing dermal bioavailability. Also, the particulate carrier should be industrially feasible (regularly accepted excipients, large scale feasible etc.) and commercially as bulk available, SmartLipids – the 3rd generation after SLN and NLC – were chosen as carrier, being particles in the submicron range (>0.1 and <1 µm).

Nanocrystalline Lipid Carriers (NLC) are typically produced from 1 solid lipid and 1 liquid lipid (oil). SmartLipids are the successor generation. They are lipid submicron particles being composed of a complex lipid mixture, using either a priori commercial complex lipid mixtures or blending typically up to 10 different lipids [1, 2]. This has the advantage of creating a highly unordered particle matrix with many imperfections, thus increasing drug loading. For retinol, the loading could be increased from 1% (SLN) and 5% (NLC) to 15% in SmartLipids (calculated on particle weight). Additionally, the complex lipid mixture of SmartLipids has difficulties to re-order during storage. This avoids/minimizes formation of highly ordered lipid modifications in the particle matrix [3], which can cause expulsion of loaded actives. Retinol is firmly enclosed during the shelf life of a product, and thus better chemically protected.

An optimal retinol formulation was systematically developed. The chemical stability of incorporated actives was found to depend on the lipid mixture used for particle production, but also on the type of stabilizer used. Based on an initial lipid screening, in a first step 3 different promising lipid mixtures were used for production of retinol loaded particles, the suspensions stabilized with about 10 different stabilizers. These 30 formulations were stored over 6 months (5°C, room temperature, 40°C), to identify the formulation composition being most stable (= pure effect of the solid particle matrix). In the next step, physical protection by the particle matrix was combined with chemical protection by an anti-oxidant. The three best formulations were produced with varying anti-oxidants, and again stored over 6 months at the 3 temperatures (n=3). In the next step, the 2 best lipid particle suspensions entered the final study to identify the most suitable preservatives or preserving agents, again in a 6 months study. As reference, retinol nanoeumulsions were produced.
The resulting best 2 formulations were composed of retinol C50 BASF (4.5%), 17 particles from lipid mixture, Miranol ultra as stabilizer, Hydrolite 5 and chemically stabilized either by BHT or Tinogard TT. By using Hydrolite 5, the particle suspensions are preservative-free (not on preservative list). The stability was clearly improved compared to the nanoemulsions. After just 4 months of storage at room temperature, only 78% of retinol was left in the nanoemulsion. In contrast, 94% retinol remained in the SmartLipids suspension after even 6 months of storage, 100% remained when storing at 5°C. In a human case study it could be shown, that the nanoemulsion remained more on the surface of the skin whereas retinol form SmartLipids had lower surface concentration and penetrated deeper into the skin.

The aqueous retinol suspensions are commercially available as product BergaCare SmartLipids Retinol®. The suspension concentrates can be simply admixed at the end of the production process of dermal gels or creams, admixing ratio depends on the final retinol concentration desired in the product. In addition to the benefits for the retinol, the BergaCare SmartLipids possess the general beneficial skin effects of all the previous SLN and NLC solid lipid particles described in the literature: e.g. repair of damaged protective lipid films on the skin, thus having an anti-inflammatory effect, increasing skin hydration related to anti-wrinkle, and normalization of moisture skin conditions being beneficial for skin cells.

References:

An in vitro model of the Gram-negative bacterial cell envelope to predict its permeability for anti-infectives

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Fighting infectious diseases caused by Gram-negative bacteria is a challenging task also taking into account the increasing resistance of pathogens as P. aeruginosa and K. pneumoniae [1]. Unfortunately, the discovery of new classes of antibiotics for these bacteria has almost stopped since the discovery of lipopeptides in 1987 [2]. In order to keep Gram-negative infections still under control, it is necessary to develop new tools, which can help to discover new compounds with anti-infective activity. As a starting point, we selected the Gram-negative bacterial cell envelope, which is known for its complexity, and developed a membrane model to assess the permeability of anti-infective drugs across this peculiar biological barrier that might limit their bioavailability at the intra-bacterial sites of action. This model is based on commercially available Transwell®-8μm inserts, following an approach that has already been successfully described to model permeability across intestinal epithelial cells [3]. Guideline for the architecture of our model is the structure of the actual Gram-negative bacterial cell envelope, which can be divided into an inner membrane, the periplasmic space and the asymmetric outer membrane, which consists of an inner leaflet, composed of phospholipids and an outer leaflet made of lipopolysaccharides. The composition of phospholipids used for the inner membrane and inner leaflet of our model resembles the composition of those in E. coli and P. aeruginosa [4]. An aplanar layer consisting of the inner leaflet from the outer membrane serves as substitute for the periplasmic space. Stereomicroscopy and confocal laser scanning microscopy did not only reveal the presence of separate layers of the model membrane, but also the presence of an asymmetric bilayered structure of the outer membrane. As a further step, transport studies were performed with quorum sensing inhibitors. Their results were compared to their ability to inhibit HHQ - an important signaling molecule for quorum sensing of P. aeruginosa [5]. It could be shown, that there is an association between the reduction of HHQ in bacteria and the permeation of those substances across our in vitro model. Ongoing work is about to further validate the predictivity of this model by generating permeability data for both established and novel antibiotics, as well as to miniaturize and automate the system towards high throughput screening applications. Another focus will be on refining the model by including further permeation delimiting factors.

References:

Development and characterization of indomethacin nanosuspensions prepared by high-pressure homogenization

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An increasing number of newly developed drugs exhibit low water solubility which leads to poor bioavailability [1]. A promising approach is the development of pharmaceutical nanosuspensions to increase the solubility, dissolution rate, and oral absorption of poorly soluble drugs [2]. Nanosuspensions are submicron colloidal systems. They can be prepared by either a bottom-up method or a top-down method. Since bottom-up methods are limited in terms of scaling up, top-down methods are frequently used to prepare nanosized products. Popular top-down methods are wet ball milling (e.g. Rapamune®) and high-pressure homogenization (e.g. TRIGLIDE®) [3]. These processes use mechanical energy to break down drug particles. During high-pressure homogenization, drug particle diameter is decreased by the collision of particles and by cavitation [4]. Preparation of nanosuspensions correlate with an increase of Gibbs free energy and result in thermodynamically unstable systems. Stabilizers are used to prevent agglomeration and crystal growth due to Ostwald ripening [5].

The model drug indomethacin is a nonsteroidal anti-inflammatory drug (NSAID) and represents a BCS class II substance, exhibiting low water solubility but high permeability. Indomethacin nanosuspensions were prepared by high pressure homogenizer (EmulsiFlex-C3, AVESIN GmbH) using different steric stabilizers. Polyvinylpyrrolidone, (hydroxypropyl)methyl cellulose, and D-α-tocopherol polyethylene glycol 1000 succinat were used in different concentrations and resulting particles were compared in terms of particle size, PDI, zeta potential, and storage stability. Additionally, the influence of various surfactants (sodium dodecyl sulfate, poloxamer, poloxarbate) was investigated. The particles were used to develop an asymmetric flow field flow fractionation (AF4, Postnova Analytics GmbH) method. AF4 is used to fractionate, separate, and characterize nanoparticles relating to particle diameter and size distribution.

Overall, stabilizers in appropriate concentrations were found to prepare indomethacin nanosuspensions. The nanosuspension were stable for at least 7 days. No significant particle growth could be observed. Promising results regarding nanonized indomethacin suspensions were obtained. Technology advantages of the nanosuspensions in relation to unprocessed drugs are expected.

References:
Drying of flavonoid nanosuspensions – freeze drying versus spray drying

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Introduction: Antioxidants are known to be beneficial for the prevention and treatment of oxidative stress related diseases. Especially natural antioxidants, e.g. flavonoids, are promising candidates to fight against oxidative stress in the body. Unfortunately, these compounds possess poor solubility and consequently poor bioavailability. Nanocrystals are the pure, nanosized active pharmaceutical ingredient, which can enhance bioavailability through the increase in dissolution velocity and kinetic solubility [1]. However, production techniques, e.g. bead milling or high pressure homogenization, lead to aqueous nanosuspensions, which is not very convenient for patients to apply. Therefore the aim of this study was to transform flavonoid nanocrystals into dry powders, that can be filled into capsules, transferred into tablets or can be used as powder for pulmonary application. To form solid products, state of the art is freeze and spray drying of nanosuspensions. Therefore, in this study both methods were compared in regard to suitability and efficacy.

Materials and Methods: Rutin nanocrystals were produced by high pressure homogenization and stabilized by Tween 80 or Poloxamer 188 and with or without albumin as co-stabilizer in a ratio 1:1 [2]. Prior to the freeze drying process mannitol was added to the suspensions as cryoprotectant and carrier matrix. Spray drying was performed at different temperatures and at different air flows by using a Büchi Mini Spray Dryer B-190 (Switzerland). Freeze drying was performed for 2h by using a Christ alpha 1-4 LSC freeze dryer (Germany). Nanocrystals and re-dispersed microparticles were characterized by light microscopy as well as by dynamic and static light scattering.

Results and Discussion: The best nanosuspension was obtained by using as stabilizers Tween 80 and albumin in a ratio 1:1. This formulation however could not be spray dried, due to the low melting temperature of Tween. Therefore, in the next step the formulation, stabilized by Poloxamer 188 and albumin, was spray dried. Also these formulations could not be dried, because albumin denatured at higher temperatures but lower temperatures, preventing the denaturation of albumin, were not sufficient to dry the suspension completely. To overcome this, a formulation containing only Poloxamer 188 as stabilizer was spray dried. This resulted in a dry and freely flowable powder, which was easy to disperse in water, yielding to particle sizes being similar to the original non-dried nanosuspension, with a size of about 600 nm. In contrast to this, no denaturation of albumin was observed during the freeze drying process of the suspensions. Best results were obtained for the suspensions being stabilized with Poloxamer 188 and albumin. The formulations were re-dispersible and led to sizes below 300 nm after re-dispersion. However, the disintegration time of the freeze dried products was slower, when compared to the spray dried formulation and could not be improved by varying the amount of the cryoprotectant.

Conclusion: Rutin nanocrystals for antioxidative treatment were successfully transferred into a solid form by spray and freeze drying. Spray drying was more efficient but was not suitable for heat sensitive excipients. In case of using heat sensitive excipients, freeze drying should be used to transfer the nanocrystals into dry powders.

References:
**POS.160**

**Development and characterization of targeted liposomes for non-invasive diagnostic molecular imaging of liver fibrosis**

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Fibrosis is a pathological reaction that could arise in various tissues and organs as a response to acute or chronic injury. It is defined as an accumulation and deposition of extracellular matrix released by activated myofibroblasts. If remaining untreated, this process can lead to organ malfunction and death [3]. Particularly, liver fibrosis derived from non-alcoholic steatohepatitis, drug abuse-induced- or viral hepatitis followed by the activation and transdifferentiation of hepatic stellate cells (myofibroblasts) is responsible for a high morbidity because of the development of cirrhosis and hepatocellular carcinoma. Due to the high prevalence and mortality of hepatic fibrosis it is of paramount importance to develop a targeted diagnostic molecular imaging technique for early diagnosis and comprehensive prognostic to improve treatment and management strategies of this chronic fibro-proliferative disease. Indeed, nowadays a definite diagnosis can only be achieved by means of liver biopsy, an invasive methodology that often implicates high risk for complications and misjudgement [2].

In preclinical and clinical diagnostics, the use of highly sensitive nano-tools is gathering growing interest, owing to the high surface area available for chemical modifications and for specific interactions with biological targets in vivo. Among the various targeted nanocarrier-based contrast agents, liposomes present distinctive characteristics that make them ideal to encapsulate contrast agents with different chemical properties and to transport them directly into the area of interest. In this study, a recently described peptide specific for binding on the insulin growth factor 2 receptor (IGF2R) [3], overexpressed on activated hepatic stellate cells [4], was evaluated as a potential new targeting ligand to decorate liposomal surface with previously conjugated lipid-peptide-conjugates. At first, the in vitro binding efficiency and cell uptake of the IGF2R-specific peptide and its scrambled version were validated by flow cytometry and fluorescence microscopy and optimized on adherent immortalized human hepatic stellate cells (LX2) in different conditions. A superior binding efficiency of the targeting peptide compared to the scrambled peptide could be observed. A good stability (~90% after 30 min incubation) of both peptides in 50% (v/v) fetal bovine serum (FBS) in phosphate buffered saline at 37 °C was assessed by high performance liquid chromatography. Hence, the IGF2R-specific peptide and a PE-Glucose functionalized with an N-hydroxysuccinimide-anchor were conjugated in dimethyl sulfoxide (about 50% for both peptides) and after purification and lyophilisation added to the lipid films. Control liposomal dispersions were generated similarly replacing the IGF2R-specific peptide with the scrambled one. By means of dynamic light scattering the stability at different temperatures of the targeted dispersions obtained by extrusion was determined, pointing out that the formulations retained a hydrodynamic diameter of 130 nm and a polydispersity index of < 0.15 for one month. The binding to LX2 cells of targeted liposomes and non-targeted liposomes, both labelled with 1.33'- dioctadecylxocarbocyanine perchlorate, was tested in the optimized conditions. Further investigations regarding the improvement of the in vitro binding efficiency by modification of the liposomal composition and of the peptide grafting density are currently ongoing.

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References

**POS.161**

**Characterization of extracellular vesicles from LX-2 human hepatic stellate cell line**

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Liver fibrosis is the wound-healing response to chronic hepatic insults, and it is characterized by the excessive deposition of scar tissue, a process driven by activated hepatic stellate cells (HSC) [1]. The broader aim of this project is to assess the impact of lipid-based therapeutics on extracellular vesicles isolated from the LX-2 human hepatic stellate cell line, used as an in vitro model for the progression of liver fibrosis. Extracellular vesicles (EVs) were successfully isolated from conditioned cell culture medium (CCM) harvested from untreated LX-2 cells (2.6-2.8 x10^6 viable cells, 94-96 % cell viability) after differential centrifugation...
followed by an ultracentrifugation step (300, 10'000, and 100'000 g) [2].

The re-suspended EV-pellet was either directly purified by size exclusion chromatography (SEC), or upon storage at -80 °C for 24 h. The concentration and size distribution of EVs in the 1 mL fractions collected after SEC were determined by nanoparticle tracking analysis (NTA). The fractions collected after SEC from the directly analyzed and stored samples had 5.40 ± 0.85 x10⁸ vesicles/mL (size: 215.5 ± 85.8 nm) and 5.67 ± 1.43 x10⁸ vesicles/mL (size: 198.7 ± 68.1 nm) respectively. These preliminary results indicate that LX-2 cells produce EVs, while also suggesting that those EVs can be stored at -80 °C for at least 24 h without affecting their yield or size distribution. Our results create an important basis for further EV characterizations in the context of hepatic liver models.

References:
4.12 Pharmacology

POS.162

The effect of genetic polymorphism in MDR1 gene (G2677T/A, C3435T, and C1236T) on Tacrolimus dose among kidney transplant recipients.

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Tacrolimus is the main component in the immunosuppression therapy of kidney transplant in Jordan. This agent has high efficiency in prevention of acute rejection, but high degree of inter-individual variability of blood levels [1]. Genetic makeup of transporter protein (P-glycoprotein) has been expected to play important role in determining blood level and dose requirements of tacrolimus [2]. The study aimed to evaluate the effects of genetic polymorphism in MDR1 (G2677T/A, C3435T, and C1236T) on tacrolimus dose requirement among kidney transplant recipients.

Blood levels of Tacrolimus were measured using microparticle enzyme immunoassay (MEIA). Genotyping analysis for detection of MDR1 polymorphism (G2677T/A, C3435T, and C1236T) not statistically affected tacrolimus dose requirements among kidney transplant recipients.

Analysing data based on the median dose-adjusted tacrolimus level (ng/ml per mg/kg), wild genotypes of G2677A/T, C3435T and C1236T did not significantly differ from mutant genotypes regarding tacrolimus dose requirement. The effect of haplotype based on median dose of tacrolimus adjusted level was not statically different.

Current study showed that MDR1 polymorphism at different positions (G2677T/A, C3435T and C1226T) not statistically affected tacrolimus dose requirements among kidney transplant recipients.

Acknowledgement

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References:

POS.163

Ketamine metabolites with antidepressant effects: fast, economical, and eco-friendly enantioselective separation based on SFC and single quadrupole MS detection

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Increasing evidence accumulates that metabolites of the dissociative anesthetic ketamine contribute considerably to the biological effects of this drug and could be developed as next generation antidepressants, especially for acute treatment of patients with therapy-refractory major depression [1]. Analytical methods for the simultaneous determination of the plethora of hydroxylated, dehydrogenated and/or demethylated compounds formed after administration of ketamine hydrochloride are a prerequisite for future clinical investigations and a deeper understanding of the individual role of the isomers of these metabolites. In this study, we present development and validation of a method based on supercritical-fluid chromatography (SFC) coupled to single quadrupole MS detection that allows the separation of ketamine as well as all of its relevant metabolites detected in urine of healthy volunteers. Inherently to SFC methods, the run times of the novel protocol are four times shorter than in a comparable HPLC method [2], the use of organic solvents is reduced and we were able to demonstrate and validate the successful enantioselective separation and quantification of (R)- and (S)-ketamine, (R)- and (S)-norketamine, (2R,6R)- and (2S,6S)-hydroxy norketamine in one run. The developed method may be useful in investigating the antidepressant efficacy of ketamine in clinical trials.

References:

POS.164

Cytosplasmic LPS-mediated inflammatory responses are suppressed by anti-endotoxin peptides

Pfizergraff, A.1, Heinbockel, L.1, Su, Q.1, Brandenburg, K.1, Weindl, G.1
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Toll-like receptor (TLR) 4-independent recognition of cytosplasmic lipopolysaccharide (LPS) by inflammatory caspases triggers pyroptosis and non-canonical inflammasome activation [1]. LPS-mediated hyperactivation of caspases is critically involved in endotoxic shock and therefore the discovery of this novel mechanism has potential implications for the development of effective drugs to treat sepsis [2]. Previously, we have demonstrated that the synthetic anti-endotoxin peptide Pep19-2.5 efficiently neutralises bacterial pathogenicity factors and protects against sepsis in vivo [3]. Here, we show that Pep19-2.5 blocks the effects of intracellular LPS in human myeloid cells and keratinocytes. In THP-1 monocytes and macrophages, the peptide strongly decreased intracellular LPS-induced IL-1β and LDH secretion. In contrast, the TLR4 signalling inhibitor TAK-242 reduced LPS-induced TNF and IL-1β secretion, but not pyroptosis. In THP-1 monocytes, Pep19-2.5 further suppressed LPS-induced HMGB-1 production and caspase-1 activation. Consistent with this observation, we found impaired IL-1β and IL-1α release in LPS-stimulated primary monocytes and reduced LDH release and IL-1β and IL-1α expression in LPS-transfected HaCaT keratinocytes in the presence of Pep19-2.5. In addition, Pep19-2.5 completely abolished IL-1β release induced by LPS/ATP in THP-1 macrophages via classical inflammasome activation. Notably, anti-endotoxin peptides reduced IL-1β and LDH secretion induced by outer membrane vesicles which mediate LPS access to the cytosol under physiological conditions [5]. In conclusion, we provide evidence that anti-endotoxin peptides inhibit the inflammasome-1 axis induced by cytoplasmic LPS sensing in myeloid cells and keratinocytes and activation of the classical inflammasome by LPS/ATP which may contribute to the protection against bacterial sepsis and skin infections with intracellular Gram-negative bacteria.
St. John’s wort and phytocemical constituents thereof attenuate gene expression in neuronal cells after dexamethasone induced stress

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Background: It is well known that dysregulation of the HPA axis plays an important part in the development and maintenance of depressive symptoms. Glucocorticoids affect cellular and molecular events in brains by modulating the expression of many genes during stress. In the present study we evaluated the effects of a St. John’s wort extract (STW 3-VI), hyperforin, miquelianin and the SSRI citalopram on the expression of genes relevant to HPA axis function in human neuronal cells.

Methods: SH-SY5Y cells were treated with STW3-VI (20 µg/mL), hyperforin (10 µM), miquelianin (10 µM) or citalopram (10 µM) in the presence or absence of the glucocorticoid receptor agonist dexamethasone (DEX 10 µM) for 6 h and 48 h, respectively. Quantitative real time PCR was used to determine the expression of FKBP5, CREB, GRIK4, VEGF, NET, and ARRB, which have been shown to be meaningful biomarkers in the treatment response for depression. Relative expression values were determined by using the −ΔΔCt method.

Results and Discussion: Using DEX to mimick stress conditions, we were able to show the responsiveness of the selected genes. It was shown that the gene expression pattern of FKBP5, CREB, GRIK4, VEGF, NET, and ARRB2 in SH-SY5Y neuronal cells is time and treatment dependent. Most pronounced effects were observed for FKBP5, which was upregulated after 6h (1.3 fold) but an even stronger increase in mRNA expression was observed after 48h (1.8 fold). While after 6h of co-incubation only STW3-VI could reverse the dexamethasone induced increase in FKBP5 expression, after 48h cytokoram, miquelianin and hyperforin also reversed the glucocorticoid induced increase in FKBP5 mRNA expression.

The effects observed on FKBP5, CREB, GRIK4, VEGF, NET and ARRB2 are in good correlation with published data, suggesting that this in vitro model can be used to screen the responsiveness of antidepressants under stress conditions.

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Modeling tolerance development for the effect on heart rate of the selective S1P1 receptor modulator ponesimod

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2 Clinical Pharmacy, Saarland University, Campus C2 2, 66123 Saarbrücken, Germany

Objective:
Development of a population pharmacokinetic/pharmacodynamic (PK/PD) model to characterize the effect of the selective S1P1 receptor modulator ponesimod on heart rate (HR), including the development of tolerance upon repeated dosing.

Methods:
ECG HR data from 280 subjects in 9 phase 1 studies (42500 measurements in total) were pooled (single doses of up to 75 mg and multiple once-daily doses of up to 100 mg). The PK/PD model was built sequentially. Based on the PK model (L), PD model selection started with the analysis of placebo data. Presence of a circadian rhythm and placebo effect(s) were investigated. Subsequently, all data were used to include the drug effect and the development of tolerance. With HR as safety parameter, particular focus was placed on adequate capturing of the variability to predict the occurrence of bradycardia (HR < 40 bpm).

Results:
A direct effect model with tolerance compartment and circadian rhythm was found to best describe the effect of ponesimod on HR. Baseline HR was estimated as 66.2 bpm and found to vary with an amplitude of 6.3% during the day. The circadian maximum was estimated to be reached at 6 PM. Although suggested by individual subjects’ data, a placebo effect could not be identified. The maximum possible reduction in HR was estimated as 45% (from baseline), decreasing with development of tolerance with multiple doses. The appearance of tolerance was fast (0.0056/hr) compared to its decrease (0.011/hr) indicating rapid onset and sustained maintenance of tolerance, allowing for multiple days of treatment interruption without complete loss of tolerance.

The effect of the first dose of ponesimod on HR including inter-individual variability was simulated using the final model. The median HR (10th to 90th percentile) at the time of maximum decrease was estimated as 56 (49-66), 52 (44-63), 49 (40-60), and 46 (38-57) bpm for doses of 2, 5, 10, and 20 mg, respectively. These results show that the risk of eliciting HR values < 40 bpm is minimal following an initial dose of 2 mg, the dose selected as starting dose for phase 3 clinical development (2-4 mg), followed by gradual up-titration.

Conclusion:
This analysis quantifies the effect of ponesimod on HR including the development of tolerance. A large number of measurements from 9 phase 1 studies provide robust data, allowing to simulate up-titration regimens to minimize the risk of bradycardia. In turn, these regimens can be clinically investigated in patients.

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