Intelligent Drug and Product Profiling: *in vitro* und *in vivo* Teststrategien zur effizienten präklinischen Entwicklung

**Across Barriers GmbH**

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**Company profile Across Barriers**

- Foundation by Prof. C.-M. Lehr and Dr. E. Haltner in 1998
- 1,000 m² working area
- S1 labs for biological and radioactive operations
- 37 Employees (pharmacists, chemists, biologists)
- Permissions and quality standards
  - 11/00 Permission for radioactive work
  - 07/01 GLP certified, 3 categories (Good Laboratory Praxis)
  - 07/02 test of drugs and drug products; GMP
  - 02/03 Permission to work with Narcotic Agents
  - 10/07 biological safety level S1 (GMOS)
  - independent quality assurance, double check of all data, frequent training for our employees
### Product Development Steps

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<th>Phase</th>
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<td>Klinische Entwicklung</td>
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<td>Patienten od. erkrankte Probanden</td>
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<td>Monitoring nach Markteinführung</td>
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#### Entwicklungsphasen
- Synthese
- biologische Tests
- pharmakologisches Screening
- toxikologische und pharmakokinetische Studien
- Tierstudien
- chemische Entwicklung
- pharmazeutische Entwicklung

#### Anzahl zu untersuchender Substanzen
- >10,000 (klassisch)
- 8-15
- 4-8
- 2-3
- 1

### Introduction

Some facts about oral dosage forms...:

- About 50% of the marketed drugs are still oral dosage forms e.g.
  - high compliance
  - line extension
  - long stability
  - low production costs

- Estimated cost > 500 million USD or more
- Estimated time up to 12 years (also we have tremendous innovation)
- High costs/timelines reflect the high rate of failure in early phases of drug development

- Attribution of failure:
  - 10% lack of efficacy
  - 5% commercial reasons
  - 39% poor pharmacokinetics or inadequate bioequivalence for generics

- Major efforts are directed to identify and eliminate compounds that are not likely to have “drug-like” properties or to reach bioequivalence

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Outline – Intelligent Drug and Product Profiling

Oral Dosage Forms
- Relevance of efflux transporters
- Drug – Drug Interaction Studies

Topical Dosage Forms
- Skin Permeation with patches
- NSAIDs in vitro and in vivo Penetration

Pulmonary Delivery
- pBCS – pulmonary Biopharmaceutics Classification System
- Formulation selection

Compatibility testing
- Summary
- Outlook and potential Collaborations

Different in vitro Methods to Predict Intestinal Absorption

Discovery
- In silico models
- logP or logD
- pka
- Solubility
- BBMV
- Pampa

Development
- Caco-2 MDCK-WT MDCK-mdr
- Ussing
- Gut loop or perfusions
- In vivo animal

Golden Standard
- Fa % in humans

Prediction, costs, time, expenditure

Automation, Reproducibility
Factors Influencing Oral Absorption

- **Disintegration**
  - Tablet
  - Aggregates
  - Fine particels

- **Dissolution**
  - Drug in solution
  - Drug in blood

- **Permeability**
  - Biochemical factors
    - Metabolism, efflux, active transporters
  - Physicochemical factors
    - Size, solubility, molecular weight, charge (pKa), H-bonding potential, molecular surface area, stability

- **Formulation factors**
  - Dosage form, absorption enhancers, coatings, stability, solubility enhancement

- **Biological factors**
  - Stomach emptying time, intestinal motility, lumenal conditions (pH, content, metab. activity), diseases

Know more about your API
Benefits of biopharmaceutical classification system

I. High Solubility – High Permeability
   - The behaviour of drug and excipient are independent
   - GI permeability is not the limited factor
   - Absorption dependent on gastric emptying
   - Interaction and stability are the two main points
   - IVIVC correlation
   - **Conventional formulations**

II. Low Solubility – High Permeability
   - **Solubility enhancers** should be included in formulations for efficient bioequivalency
   - IVIVC might be possible

III. High Solubility – Low Permeability
   - **Permeability enhancers** should be included in formulations for efficient bioequivalency
   - In some cases IVIVC

IV. Unknown

- **Increase permeability**
- **Increase solubility or solubility rate**
Active Carriers: P-Glycoprotein (P-gp) as example for active transport

- Transmembrane protein
- Active efflux of chemically diverse compounds
- Physiological function: detoxification of cells
- Key role in barrier properties

**Effects of efflux mediated transport of drug molecules**

- Species dependent bioavailability
- Large interspecies variability of absorption due to differing expression of efflux systems
- Induction of efflux systems resulting in decreasing absorption or faster elimination
- Interactions with phytopharmaca (e.g., St. John’s Wort)
- Interactions with a wide range of drugs
- Interactions with food (e.g., herbal tea; fruit juice...)

Pgp-marker Compounds and Inhibition Studies

- Rhodamine 123 ab and ba
- Digoxin ab and ba (Fa: 81%)

Inhibitors

- Verapamil (VER)
- Cyclosporin A (CsA)
Guideline on Drug – Drug Interactions

Guidance for Industry

Drug Interaction Studies — Study Design, Data Analysis, and Implications for Dosing and Labeling

DRAFT GUIDANCE

U.S. Department of Health and Human Services
Food and Drug Administration
Center for Drug Evaluation and Research (CDER)
Center for Biologics Evaluation and Research (CBER)

September 2006
Clinical Pharmacology

- Quinidine across Caco-2
- LY335979 (Zosuquidar trihydrochloride)
  - Highly specific P-gp inhibitor
  - Ki of 59 nM
  - Full inhibition 150 nM

An in vivo drug interaction study with a P-gp substrate such as digoxin is recommended.

An in vivo drug interaction study with a P-gp substrate is not needed.

[I]/IC50 (or Ki) > 0.1

[I]/IC50 (or Ki) < 0.1
### Dermal ex vivo Systems (human und porcine)

<table>
<thead>
<tr>
<th>Permeation</th>
<th>Penetration</th>
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<td>Stratum corneum</td>
<td>viable epidermis</td>
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<tr>
<td>dermis</td>
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#### Skin permeation in vitro

- Human skin (full thickness, dermatomized skin, heat-separated skin or trypsin isolated SC)
- 36mm punch
- Transferred into Franz diffusion cells (20 mL acceptor volume, 3 cm² skin surface)
- Assembling and performance at 32°C
Permeation of estradiole from matrix TTS across human skin in vitro

- Dermatomized human skin (500 µm)
- 96 hours, n=3, 32 °C
- 6 individuals
- Age from 40 to 60 years
- Female
- Knowledge of SC thickness
- Knowledge of caffeine permeability
- Documented skin color
- Performed according to SOP M 005

In vivo Penetration in accordance to FDA Guideline

- Test formulation
- Left and right arms
- finite dose
- 2-3 mg/cm²
- Control(s)
- Kinetics
- up to 8 positions per arm

In vitro [µg/cm²]
- 3 h incubation
- Stratum Corneum (SC) 37.7 ± 12.1
- Deep Skin 27.3 ± 8.5

In vivo [µg/cm²]
- 3 h incubation, only SC
- Person 1: 97.0 ± 14.8
- Person 2: 81.1 ± 17.8
- Person 3: 62.2 ± 11.2

The Company
Cell & Tissue based Systems
Analytical Support
Portfolio


Abbildungen
Abb. 5: Penetration von Ketoprofen in humaner Haut (Stratum corneum) in vitro (3 Stunden) und in vivo (3 Personen) nach 3-stündiger Inkubation mit Test-O. Die angegebenen Werte sind Mittelwerte ± Standardabweichung von 3 Biomerfolgen. Jede Testung wurde mittels Absorption im Dunkeln durchgeführt.

Abb. 6: Penetration von Ketoprofen in humaner Haut (Stratum corneum) in vivo (3 Personen) nach 3-stündiger Inkubation mit Test-O. Die in vivo Untersuchungen wurden mittels Inkubation im Dunkeln durchgeführt.
Aerolized CsA for prevention of organ rejection in lung transplant - 
Trammer et al., EJPB, 2008;70(3):758-64

Chamber 1: human lung 
Homogenate

Chamber 2: human blood

Dialysis membrane

RPMI 2650

9HTE16o-

Calu-3

NHBE 16HBE14o-

A549 (build no tight monolayers)

Primary cells (porcine)

High yield, anatomically, physiologically and biochemically similar to human

Results at Calu-3

Papp of CsA formulations across Calu-3 monolayers (in vitro model)

- CsA is low permeable
- CsA transport has no directionality
- L-CsA shows lower permeability than CsA-PG

![Graph showing permeability](graph.png)
Results at the ex vivo model

- CsA diffusion towards the blood compartment (ex vivo model)
  - CsA diffusion across the dialysis membrane
  - Area under the curve (AUC) and diffusion rate (DR) determined
  - CsA-PG is absorbed by the blood compartment faster than the liposomal formulation
  - The model did not allow calculation of $c_{\text{max}}$, $t_{\text{max}}$, and $t_{1/2}$ (drug transfer was not completed): the model mirrors the absorption phase

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Stress stability and pre-formulation screening for liquid, solid and semisolid formulations

- Benefit – one mg one day approach
  - Low sample amounts (approx. 30 mg of API for a set of 20 excipients)
  - Very fast result (usually within 10 working days full report available)
  - Chromatographic Impurity Profile available for each API-excipient mixture

- Information we get
  - Purity of drug
  - Influence of conditions (acid, base, oxidation)
  - Influence of API/ excipient ratio
  - Influence of excipient quality resp. purity
  - Influence of the granulation/tableting process
  - No information about kinetic

- Two step procedure
  - Assessment of API stability
  - Assessment of API-excipient interaction
Example of API forced degradation: Acetylsalicylic acid

Compatibility screening of Raloxifene with selected excipients

L-HPCs as preferable excipients identified (instead of commonly used Povidones) to reduce oxidative degradation
Across Barriers’ in vitro ADMET models

In vitro permeability (standardized and customized assays)
- Investigation of transport mechanisms
  - identification of active transport mechanisms
  - identification of efflux mediated transport
  - interaction of drug substances
  - influence of excipients on permeability and/or transport mechanism
  - proof of drug delivery concepts
- Dermal Barrier (porcine, human, reconstructed skin)
- Gastrointestinal Barrier (Caco-2, porcine gut)
- Blood Brain Barrier (primary endothelial cells)
- Pulmonal Barrier (bronchial cells, lung cells)
- Buccal and nasal Barrier (excised tissue)
- Human vaginal and cervical barrier (excised tissue)
- Classification of drug substances according Biopharmaceutics classification system (FDA and EMEA)

How to work with us

Fee for Services
Strategic Alliances
Consulting
  - support with authorities
  - regulatory support
  - customized study design
  - project planning
  - project management
R&D Cooperations
Frame Work Agreements
MAARS - Magnetic Active Agent Release System

Our Techniques

- MAARS Capsule
- Release process
- Patent: DE 10302614.2-44

Future Projects

- Phase 1: Oral intake
- Phase 2: Tracking
- Phase 3: Release
- Phase 4: Plasma concentration
- Phase 5: Evaluation
- Phase 6: Decision

Path of the capsule

Magnetic tracking

© Across Barriers GmbH 2009 – page 27, 28
MAARS - Magnetic Active Agent Release System

MAARS – Selection of services
- Testing of drug-drug interactions
- Inhibition studies with efflux transporters (e.g. Pgp, MRP-2, BCRP)
- Side specific absorption windows (e.g. Duodenum, Ileum, Jejunum, Colon)
- Impact of polymorphism, pH value or salt forms on gastrointestinal absorption
- Testing of principle of drug delivery approaches
  - Mucoadhesion
  - Micro- and nanoparticles
  - Enzyme inhibition gastrointestinal absorption
- Selection of solubilizer (BCS II compounds) or Penetration enhancer (BCS III compounds)

Thank you for your attention!

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