The Virtual Liver: a Multiscale Systems Biology Platform to Study Liver Function, Dysfunction & Injury

Adriano Henney, Programme Director
Lars Kuepfer, Bayer Technology Services GmbH
Why Focus on the Liver?

- Major organ of homeostasis
  - Innate immunity
  - Acute phase response mediators
- Critical role in detoxification
- Dysfunction -> significant burden on health care budgets
- Dysfunction associated with modern lifestyle
- Toxicity: major hurdle to delivering novel medicines
- Access to data at all level of organisation
Objectives

- Move from the study at the cellular level to consider the whole organ
  - Building on successes of HepatoSys and learning from the Virtual Heart
- Deliver a true multi-scale representation of liver physiology
  - Modular, flexible and modifiable
- Deliver novel tools, processes, technologies and know-how
  - Understanding of dynamics of liver function in normal & diseased states
  - Informed decision making based on network interactions rather than reductionist data
- Deliver tangible evidence of impact on unmet medical needs, through specific, defined objectives aligned to diseases:
  - Non alcoholic fatty liver disease
  - Regeneration
  - Inflammation
The Virtual Liver Network

Complex Organisation:
- 9 Work Packages
- 69 Principal Investigators
- 44 Projects
- >200 contributing Scientists
- 36 Independent Institutions
- Mix of academics & industry

Geographically Dispersed
€50M distributed budget
To create:

- A dynamic mathematical model that represents human liver physiology, morphology and function.
- A model that integrates quantitative data from all levels of organisation, from sub-cellular levels to the whole organ.
- A model that has a specific focus on application to address the needs of the patient and clinician.
- A platform that can be modified, supplemented and improved over time.
## Structure

<table>
<thead>
<tr>
<th>Work Package</th>
<th>Subject</th>
<th>Projects</th>
</tr>
</thead>
<tbody>
<tr>
<td>A1</td>
<td>Cellular Metabolism</td>
<td>3</td>
</tr>
<tr>
<td>A2</td>
<td>Cellular Signalling</td>
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</tr>
<tr>
<td>A3</td>
<td>Cross Linking</td>
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<tr>
<td>B</td>
<td>Cell-Cell Communication</td>
<td>9</td>
</tr>
<tr>
<td>C</td>
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</tr>
<tr>
<td>D</td>
<td>Whole Organ</td>
<td>4</td>
</tr>
<tr>
<td>E</td>
<td>Integrated Model</td>
<td>4</td>
</tr>
<tr>
<td>F</td>
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<td>3</td>
</tr>
<tr>
<td>G</td>
<td>Clinical Translation</td>
<td>4</td>
</tr>
</tbody>
</table>

Levels:
- **CELL LEVEL**: A1, A2, A3
- **TISSUE LEVEL**: B
- **ORGAN LEVEL**: C, D
- **CLINICAL**: E, F, G

*Supported by the Federal Ministry of Education and Research*
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**Vertical Integration across scales**
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**Show Case Studies:**
- Steatosis
- HGF induced regeneration
- LPS induced inflammation
- LIAM: Liver Image Analysis-based Modelling

**Data Management**
- 3 projects

**Clinical Translation**
- 4 projects
Show Cases
HGF-Induced Regeneration (Klingmüller & Drasdo)

Overview
Addresses how proliferation patterns during liver regeneration are controlled by multi-scale spatio-temporal modelling describing events from intracellular to tissue level.

Objectives
To link activation of signalling pathways to hepatocyte proliferation by ODE modelling.
To integrate intracellular and tissue models developed based on proliferation in CCl₄ treated mice.
To Link the multi-scale model to a compartmental pharmacokinetic model of HGF for the whole-body taking into account recirculation of proteins via the blood.
To provide an integrative multi-scale model to gain insights into mechanisms that could promote drug induced liver injury or liver failure after resection.
Steatosis (Holzhütter & Gebhardt)

- Development and validation of a multi-scale organ model to simulate hepatic lipid accumulation, export & degradation
- Dynamic models of metabolism and tissue structure models of liver lobule (Drasdo & Höhme PNAS 2010)
  - a modular backbone of models of the steatotic process bridging spatial and temporal scales.
- Application of the modular backbone:
  - Quantification & Impact of pro- & anti-steatotic factors
  - Propose novel clinical tests to assess individual risk for hepatic steatosis
  - Develop novel dietary & pharmacological strategies for prevention and reversion
  - Impact of steatosis on drug metabolism.
Inflammation (Dooley & Timmer)

Aim

To identify and mathematically model the complex interplay of the different cell types and the most relevant mechanisms that control the inflammatory response of the liver to LPS.

Objectives

A multi-scale understanding of the role of liver and hepatocyte derived factors in the LPS response of the organism to:

- bridge intra- to intercellular to whole-body scale
- integrate experimental data into a mathematical model, which relies on models within several submodules
- test the hypothesis that response of the liver predominantly includes communication between hepatocytes, Kupffer cells, sinusoidal endothelial cells & hepatic stellate cells.
LIAM (Zerial & Drasdo)

- Regulation and control of bile flow in normal and diseased liver based on detailed study of liver micro-architecture
- Flow, zonation & osmotic gradients control cellular uptake
- Interplay of factors controlling flow integrated in a multi-scale model reflecting physical constraints involved in function.

**IMAGING**

- 3D-structure of liver tissue and its dynamics in vivo.
- high- & super-resolution light & electron microscopy, intra-vital imaging
- multi-scale quantitative understanding of tissue organisation

**MODELLING:**

- A tissue structure model based on 3D organisation
- A fluid mechanics model: blood flow, bile flux and processes important in flow control.

**VALIDATION:**

- Intra-vital imaging, Genetic perturbations, Pharmacological perturbations
In Vivo Translation (Küpfer, Hengstler, Kerb)

preclinical translation clinical

<table>
<thead>
<tr>
<th>Level</th>
<th>Surgery</th>
<th>Volunteer</th>
<th>Therapy</th>
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<tr>
<td>organism</td>
<td>Whole-body Model</td>
<td>PK</td>
<td>Serum Markers</td>
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<tr>
<td>organ</td>
<td>Liver Organ Model</td>
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<tr>
<td>cell</td>
<td>(Sub-)Cellular Model</td>
<td>(Biopsy) „Omics“</td>
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Data Mining

Clinical Trial Design & Personalised medicine

Minimal Invasive Surrogate Markers Clinically Applicable

Experimental Markers Proof of concept
Physiology-based pharmacokinetic (PBPK) modelling
Physiology-based pharmacokinetic (PBPK) modelling

Physiology-based pharmacokinetic (PBPK) models

- organs are structurally included
- distribution models for generic description of mass transfer (passive and active processes)
- substance specific model-parameters are calculated from few physicochemical data
- extensive data collections of prior biological and physiological data included
- comprehensive representation of experimental data from different scales of biological organization

A unified model representation of prior physiological and (pre-)clinical knowledge.
Using gene expression data as a surrogate for tissue-specific protein abundance

PK-Sim® Express-Expression Database: ArrayExpress UniGene Literature
Vertical model integration
Vertical model integration

cell
tissue
organ
whole-body
population
Vertical model integration

Integrating hepatic metabolism into a whole-body model

first-pass perfusion of the liver

cell

tissue

organ

body

population
Integrating hepatic metabolism into a whole-body model
Integrating hepatic metabolism into a whole-body model


Gille et al., MSB, 2009
Integrating hepatic metabolism into a whole-body model

Integrating hepatic metabolism into a whole-body model

Allopurinol treatment of hyperuricemia

**Single dose**

![Graph showing concentration vs. time for single dose](image1)

**Multiple dose**

![Graph showing concentration vs. time for multiple dose](image2)

**Cellular response**

![Graph showing relative enzyme activity vs. time](image3)
Allopurinol treatment of hyperuricemia

**Single dose**

- Concentration [µM] vs. time [h]

**Multiple dose**

- Concentration [µM] vs. time [d]
- * diseased
- ** healthy

- Uric acid venous plasma
- Uric acid liver cell
Integrating hepatic metabolism into a whole-body model

Paracetamol intoxication

1 g paracetamol

15 g paracetamol

A toxic dose of paracetamol significantly affects the correct execution of metabolic functions of the hepatocyte.
Spatio-Temporal Simulation of First Pass Drug Perfusion in the Liver
A spatio-temporal model of the liver

- geometry of the organ and the vascular tree
- organ & vascular geometry
- physiologically based pharmacokinetics
- mass transfer within hepatic tissue

A spatially-resolved model of the liver
Spatio-temporal distribution within the liver

Visualization of a 2 second pulse of carboxyfluorescein diacetate succinimidyl ester (CFDA SE) within the liver
Visualization of pathophysiological states in the spatially resolved liver model

- **Steatosis**
  - Heterogeneous steatosis
  - Homogeneous steatosis

- **Necrosis**
  - CCl4-induced necrosis
Simulation of an isolated perfused liver

Spatio-temporal concentration profiles in an isolated perfused liver

Schwen et al., PLoS Comp Biol, 2014
Translational Studies
PBPK-based Cross-Species Extrapolation
Cross-species extrapolation in clinical development

First in man studies are a critical step in drug development:
- limited physiological comparability of different species
- different genetic/enzymatic setup of different species
- caution needed in terms of patient safety
Cross-species extrapolation

Which information in PBPK models is the most important?

Consideration of four PBPK parameter domains:
- Species specific physiology (SP)
- Gene expression, i.e. tissue-specific protein abundance (EX)
- Kinetic parameters (KP)
- Fraction unbound (FU)

Consideration of 10 exemplary drugs

Exemplary consideration of ten different drugs (i.v.)

Route of degradation is governed by a single reaction

<table>
<thead>
<tr>
<th>Drug</th>
<th>Log P</th>
<th>MW (g/mol)</th>
<th>pKa</th>
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</thead>
<tbody>
<tr>
<td>Torsemide</td>
<td>0.57</td>
<td>348.40</td>
<td>7.10</td>
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<tr>
<td>Talinolol</td>
<td>2.30</td>
<td>363.56</td>
<td>9.43</td>
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<tr>
<td>Midazolam</td>
<td>2.70</td>
<td>325.77</td>
<td>6.04</td>
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<td>Caffeine</td>
<td>-0.07</td>
<td>194.20</td>
<td>10.40</td>
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<td>Morphine</td>
<td>0.89</td>
<td>265.30</td>
<td>8.20</td>
</tr>
<tr>
<td>Docetaxel</td>
<td>2.92</td>
<td>807.88</td>
<td>10.96</td>
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<tr>
<td>Dextromethorphan</td>
<td>3.60</td>
<td>271.39</td>
<td>9.85</td>
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<tr>
<td>Cyclosporine</td>
<td>3.64</td>
<td>1202.60</td>
<td>11.83</td>
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<tr>
<td>Erythromycin</td>
<td>3.06</td>
<td>733.92</td>
<td>8.88</td>
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<tr>
<td>Pravastatin</td>
<td>1.66</td>
<td>424.53</td>
<td>4.56</td>
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</table>

<table>
<thead>
<tr>
<th>Drug</th>
<th>Primary Route of Degradation</th>
<th>Species</th>
<th>Log P</th>
<th>F_U</th>
<th>Enzyme/Transporter</th>
<th>K_M (μmol/L)</th>
<th>v_max (μmol/Lmin)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Torsemide</td>
<td>Metabolic</td>
<td>Human</td>
<td>0.57</td>
<td>0.025</td>
<td>CYP2C9</td>
<td>11.20</td>
<td>12.22</td>
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<td>Thalidomide</td>
<td>Renal</td>
<td>Mouse</td>
<td>0.57</td>
<td>0.065</td>
<td>CYP255</td>
<td>5.20</td>
<td>2.71</td>
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<tr>
<td>Midazolam</td>
<td>Metabolic</td>
<td>Human</td>
<td>2.30</td>
<td>0.300</td>
<td>ABCB1</td>
<td>0.699</td>
<td>2.13</td>
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<td>Caffeine</td>
<td>Metabolic</td>
<td>Human</td>
<td>0.07</td>
<td>0.700</td>
<td>CYP1A2</td>
<td>400.00</td>
<td>30.40</td>
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<td>Morphine</td>
<td>Metabolic</td>
<td>Human</td>
<td>0.07</td>
<td>0.850</td>
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<td>Mouse</td>
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<td>UGT2B7</td>
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<td>0.100</td>
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<td>Cyclosporine</td>
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<td>Mouse</td>
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<td>CYP2D6</td>
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<td>Erythromycin</td>
<td>Metabolic</td>
<td>Human</td>
<td>3.06</td>
<td>0.145</td>
<td>CYP3A4</td>
<td>44.00</td>
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<tr>
<td>Pravastatin</td>
<td>Biliary, renal</td>
<td>Mouse</td>
<td>1.65</td>
<td>0.730</td>
<td>OATP1B1</td>
<td>11.50</td>
<td>5.50</td>
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<tr>
<td></td>
<td></td>
<td>Human</td>
<td>1.65</td>
<td>0.730</td>
<td>Aocc2</td>
<td>223.00</td>
<td>405.90</td>
</tr>
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PBPK models of 10 different drugs for both mouse and humans

Mouse  Human  Mouse  Human

Erythromycin
Midazolam
Caffeine
Tainolol
Dextrometorphan
Morphine
Pravastatin
Docetaxel
Torsemide
Cyclosporine

Cross-species extrapolation

Consideration of four PBPK parameter domains:
- Species specific physiology (SP)
- Gene expression, i.e. tissue-specific protein abundance (EX)
- Kinetic parameters (KP)
- Fraction unbound (FU)

15 combinations × 10 drugs × 2 directions (m2h, h2m) = **300 cases considered**

Benchmarking the benefit of using different combinations of parameter domains

PBPK models of reference & target species

1. Naive Extrapolation

Naive extrapolation (benchmark): dose/(BW weight)

2. Knowledge-Driven Extrapolations

15 combinations of parameter domains

Statistical analysis

Evaluating the benefit of using additional degree of prior information (i.e. PBPK parameter domains)
Cross-species extrapolation in clinical development

The relative change (RC) of using different combinations of parameter domains relative to using the naive approach (benchmark) shows significant variability for the ten drugs, yet obvious trends.
Statistical analysis of the results identifies the following key results:

1. Species-specific physiology is of major relevance.
2. If using all available information, 83.5% of model agreement can be reached.
3. Expression data must only be used together with corr. kinetic parameters.
Translational Studies
Statin pharmacogenomics

Physiology

Hepatic uptake by OATP1B1

Statin PK

Genotype-phenotype correlation (c.521T>C SNP)

GWAS of myopathy

SEARCH Collaborative Group

A mechanistic explanation, however, is lacking as of now
Model-based risk assessment in pharmaceutical R&D

1. establishment of reference PBPK models
2. model evaluation at relevant scales
3. simulation of virtual populations and model evaluation
4. calculation of toxicodynamic (TD) markers
5. evaluation of the safety risk
6. prediction of drug safety
   a. dose to dose
   b. drug to drug
   c. patient to patient extrapolation

Lippert et al., *CPT:PSP, 2012*
Validating the predictive power for PK genotypes

Model is predictive for pharmacokinetic phenotypes
Model-based risk assessment in pharmaceutical R&D

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6. prediction of drug safety
   a. dose to dose
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Lippert et al., *CPT:PSP, 2012*
Population simulations

Genotype-specific virtual patient populations (n=1000 individuals).

Pasanen et al., *Pharmacogenet Genomics*, 2006
Niemi et al., *Clin Pharmacol Ther*, 2006
Model-based risk assessment in pharmaceutical R&D

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Lippert et al., *CPT:PSP*, 2012
Calculation of a toxicodynamic (TD) marker for statin toxicity

Model-based estimation of drug exposure in the target tissue

\[ TD = \frac{C_{\text{max}}}{IC50} \]

An in vivo marker for statin toxicity

IC50_{Simvastatin} = 3.99 \mu M

IC50_{Pravastatin} = 4890 \mu M

In vitro toxicity (IC50 values) in embryonal rhabdomyosarcoma cells

Kobayashi et al., Life Sciences, 2008
The toxicodynamic marker is considerably higher for simvastatin, which is in agreement with observations in clinical practice.
Model-based risk assessment in pharmaceutical R&D

1. establishment of reference PBPK models
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   a. dose to dose
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Lippert et al., *CPT:PSP, 2012*
Prediction of clinical incidence rates for the rare CC-subpopulations.

A model-based approach to safety assessment in clinical development.

Lippert et al., CPT:PSP, 2012
SEARCH Collaborative Group, NEJM, 2010
Bayesian PBPK
PBPK model for pravastatin

Pravastatin:
- low lipophilicity
- degradation by sulfotransferases in various tissues
- uptake and secretion by active transport processes in various tissues: OATP1B1, OAT3, MRP2

→ protein abundance was estimated by using tissue-specific gene expression levels as a surrogate
Variability of pravastatin pharmacokinetics

200 parameter vectors from the posterior distribution obtained with Bayesian PBPK were used to perform population PK simulations

⇒ Quantification of inter-individual variability
The distribution of OATP1B1 transporter efficacy ($k_{cat}$) shows a bi-modal behavior indicating the existence of specific patient subgroups!
Plotting OATP1B1 transporter efficacies for patients with known OATP1B1 genotype can be assigned to the two different subgroups

⇒ Huge potential for (early) clinical development
Summary

- models of the liver as the key detoxification organ in the human body

- representation of the liver within the context of the organism

- clinical translation
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