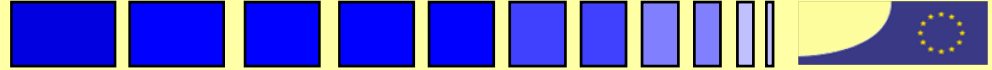


European Framework Programme 6 (2002-2006)  
Innovative Medicines for Europe - InnoMed  
**Integrated Project: Predictive Toxicology – PredTox**

EU FP6: Integrated Project 'PredTox'



# Outcome of PredTox: A Public-Private European Collaboration (FP6)

Laura Suter-Dick, F. Hoffmann-La Roche AG

# The 20 Partners of the PredTox Consortium

PROJECT COORDINATOR, LEADER AND SOUL: Friedlieb Pfannkuch

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<b>Nycomed</b> (former: Altana)
<b>Bayer Schering Pharma</b> (former: Bayer)
<b>Boehringer-Ingelheim</b>
<b>Johnson &amp; Johnson</b>
<b>Lilly (B)</b> (Facility closed) - no EU funds
<b>Merck-Serono</b> (former: Merck KGaA)
<b>Novartis</b>
<b>Novo-Nordisk</b>
<b>Schering-Plough</b> (former: Organon)
<b>Roche</b>
<b>Sanofi-Aventis (D)</b>
<b>Sanofi-Aventis (F)</b>
<b>Bayer Schering Pharma</b> (former: Schering)
<b>Merck-Serono</b> (former: Serono)
<b>Servier</b>
<b>University of Wuerzburg</b>
<b>Univ. College Dublin</b>
<b>University Hacettepe</b>
<b>Genedata</b>
<b>Bio-Rad</b> (former: CIPHERGEN) - no EU funds



# Executive Summary

[www.innomed-predtox.com](http://www.innomed-predtox.com)

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- Project goals met
  - *in vivo* Studies and 'omics investigations performed and reported
  - Bioinformatics evaluation and biological interpretation accomplished
  - Confirmatory experiments performed
- Histopathology examinations by experienced pathologists are still the choice for early safety evaluation / decision making
- '*omics technologies* are instrumental for expanding the mechanistic understanding of drug induced pathologies and the identification of new molecular safety biomarkers – “added value!”
- About 10 potential new candidates biomarker identified
- PredTox is a great experience of collaboration between Pharmaceutical companies, together with Academia and SMEs

# Study Design and Investigations

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16 compounds

Vehicle

Low Dose

High Dose



n=5

Day 1,3,14

Day -3/-4, 1/2, 3/4, 12/13



## Transcriptomics

- Microarrays

## Proteomics

- 2D-PAGE
- 2D-DIGE
- SELDI

## Metabonomics

- LC-MS
- <sup>1</sup>H-NMR

## Conv. Endpoints

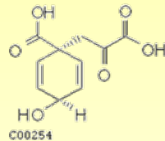
- Histopathology
- Hematology
- Clin. Chem.
- Urinalysis

# Content of the Database

## Sample

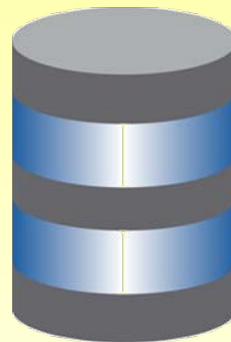
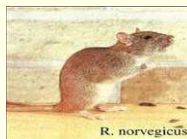
### Treatment and Compounds

- Compound
- Compound class
- Concentration
- Treatment time
- Dosing route
- Dosing frequency
- Vehicle
- Protocols
- ...



### Animals

- Species
- Strain
- Sex
- Age
- Weight
- Husbandry
- Protocols
- ...

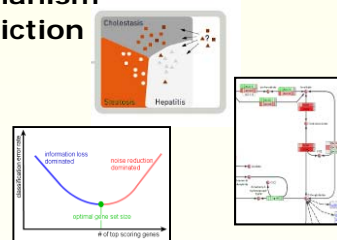


## Measurements and Observations

- Live animal observations
- Sample handling
- Clinical endpoints
- Histopathology
- Serum chemistry
- Urine chemistry
- Hematology
- Protocols
- ...

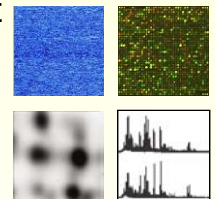
## MOA, Tox Prediction

- MOA classification
- Biomarker candidates
- Tox Mechanism
- Tox Prediction
- ...



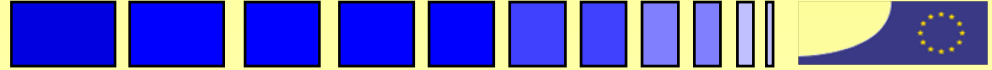
## -omics data

- 1 channel data
- 2D-PAGE, 2D-DIGE
- LC/MS, GC/MS, SELDI
- NMR
- Raw data
- Processed data
- Expression values
- Quality parameters
- Protocols and algorithms
- Annotations
- ...



# Data Analysis & Interpretation

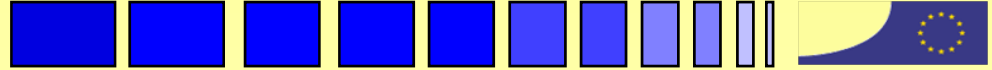
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- **Phenotypical anchoring: definition of 3 EWGs**
  - Liver Hypertrophy: “pure” & “mixed” (SER ± PPAR)
  - Bile Duct Damage/Necrosis, including cholestasis
  - Nephrotoxicity: proximal tubular damage
- **Concordance & complementarity among technologies**
- **“PRE”dictive power (time & dose)**
- **Mechanistic understanding**
- **Identification of biomarkers**

# EWG I: Liver Hypertrophy (LH) - 1

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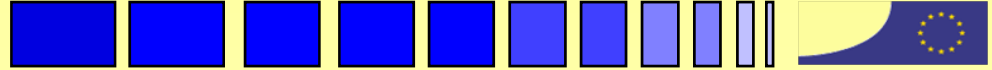


## Toxicogenomics key results

- **“Pure” Liver Hypertrophy phenotype**
  - Association nuclear receptor induction (CAR / PXR / AhR)
  - Up-regulation of genes involved in xenobiotic metabolism (XME)
  - A NRF2-mediated oxidative stress signal (PERK => NRF2)
  - Activation of SER biogenesis machinery due to XBP-1 (UPR sensor protein)
- **“Mixed” Liver Hypertrophy phenotype**
  - Modulation of genes related to FA metabolism and ketogenesis (PPAR $\alpha$  activation)
  - Mild induction of XME's related genes
  - Marked stimulation of peroxisome biogenesis / proliferation => PEX11 $\alpha$  and PEX19

# EWG I: Liver Hypertrophy (LH) - 2

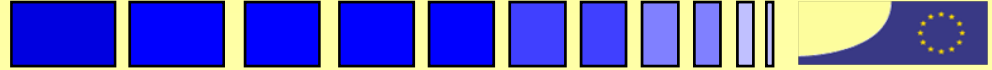
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- **Proteomics key results**
  - 2D-DIGE data: confirmatory & complementary data
  - SELDI data: effects at earlier time points or at lower dose
  - Modulated plasma proteins: CRP fragment, haptoglobin,  $\beta$ 2- $\mu$ Globulin, ApoA1 and ApoM (poor toxicological relevance to LH)
  - 2D-PAGE data: No common urinary proteins
- **Metabonomics-NMR key results (potential markers, urine)**
  - Increase in phenylacetylglycine (PAG) for increased membrane phospholipid synthesis and SER proliferation
  - Decrease in trigonelline as a potential marker for mobilization of oxidative stress signaling in XME studies
  - Decreased glutamine and increased glucose in urine as potential markers for PPAR $\alpha$ / $\gamma$  agonism

# EWG I: Liver Hypertrophy (LH) - 3

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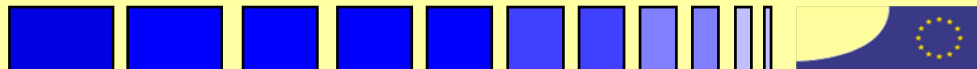


## • Conclusions

- Detailed mechanistic models for liver hypertrophy underlain either by induction of SER biogenesis or peroxisome proliferation could be generated using extensive Toxicogenomics data
- The model was mechanistically improved thanks to Proteomics 2D-DIGE and Metabonomics NMR data
- 2 potential metabolite biomarker candidates (for SER proliferation and oxidative stress, respectively) were identified

# EWG II: Bile Duct Damage / Necrosis (BDN) - 1

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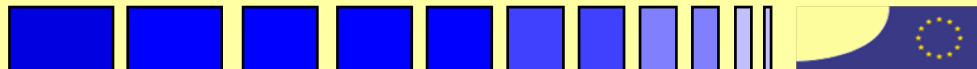


- **Transcriptomics key results**

- Acute damage to bile duct epithelial cells or hepatocytes, characterized by early stress responses
- Regenerative processes
- Inflammation with inflammatory cell immigration
- Fibrosis (not all studies)
- Cholestasis
- No information on localization of damage (bile duct or hepatocytes). Clarified using immunohistochemistry detection

# EWG II: Bile Duct Damage / Necrosis (BDN) - 2

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- **Proteomics key results**

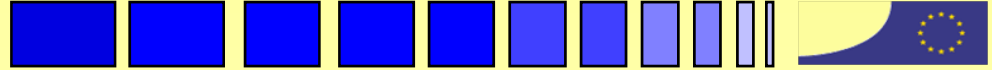
- 2D-DIGE liver: Mainly high abundance proteins. Confirmatory data
- 2D-PAGE urine: Thiostatin increase confirmed by ELISA
- SELDI: Upregulated proteins
  - Liver:  $\beta$ 2- $\mu$ Globulin, GSTA3, Phosphatidylethanolamine binding protein
  - Plasma:  $\beta$ 2- $\mu$ Globulin, APOE, Haptoglobin

- **Metabonomics key results**

- LC/MS: A *targeted bile acid analysis* confirmed hypotheses drawn from classical and transcriptomics data and suggested unconjugated vs. conjugated bile acids as potential mechanism-based markers for hepatocyte vs. bile duct damage
- NMR: Increased Indoxyl sulfate in urine (specificity?)
- *Classification* exercises revealed a potential of NMR in urine to classify samples with respect to bile duct damage.

# EWG II: Bile Duct Damage / Necrosis (BDN) - 3

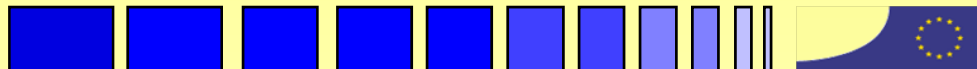
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- **Cross-omics classification analysis**
  - Each technology appears superior to a combined analysis
  - Combination of technologies decreased classification performance
- **Mechanistic investigations**
  - Clusterin, Thiostatin and NGAL protein increased in urine and/or serum => potential non-invasive, general diagnostic markers of tissue injury and/or inflammation
  - Immunohistochemistry studies localized certain overexpressed proteins to damage sites

# EWG II: Bile Duct Damage / Necrosis (BDN) - 4

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## • Conclusions

- Transcriptomics data delivered mechanistic hypotheses to explain classical observations. Predictive abilities need to be tested with more suitable in vivo studies
- Metabonomics (LC/MS) contributed confirmatory information and suggested a potential biomarker candidate for bile duct vs. hepatocyte damage
- Metabonomics (NMR) in urine may be pursued for toxicity-associated sample classification; did not provide mechanistic insight
- Proteomics (SELDI) found a previously described, high abundance general acute stress/inflammation marker in plasma
- Mechanistic investigations delivered details and confirmed 'omics findings, and suggested three diagnostic tissue damage markers

# EWG III: Nephrotoxicity - 1

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## • Transcriptomics key results

- Strong immune and inflammatory response
- Strong activation of the complement system
- Deregulation of “renal damage genes” (like Kim-1, Clusterin, Timp1 etc.)
- Cytoskeleton and extra cellular matrix genes deregulated
- Apoptosis, necrosis, oxidative stress genes deregulated
- A number of genes (7) coming up at *early time points and/or lower doses* could be identified in the defined gene lists (potentially prodromal markers)

# EWG III: Nephrotoxicity - 2

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- **Proteomics key results**

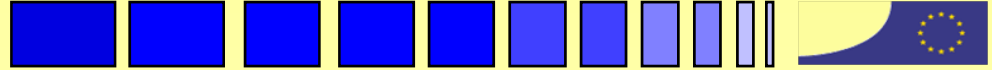
- 2D-DIGE: 6 phenotypically anchored *potential protein biomarkers identified*, mainly involved in oxidative stress, detoxification, energy metabolism
- SELDI: 2 Proteins annotated in Plasma ( $\beta$ -2 Microglobulin and Haptoglobin)
- 2D-PAGE: did not result in any commonly deregulated urinary proteins between the 3 studies

- **Metabonomics key results**

- NMR urine (but not serum): promising mechanistic metabolite candidates were identified by a multivariate data analysis
- Some of the identified metabolites (1 study) associated with nephrotoxicity or immune response

# EWG III: Nephrotoxicity - 3

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- **Cross-omics analysis**

- Intersection of transcriptomics and proteomics 2D-DIGE data: revealed additional *biomarker candidates*, some of them, like Coro1A and Capg showed high specificity in for the 3 Nephrotoxicity studies
- Ranking analysis: Among the top 100 ranked genes some new identified biomarker gene candidates like Coro1a and genes previously described as associated with kidney toxicity could be identified (e.g.: Kim-1 was ranked at place 3, Osteoactivin at place1)

- **Mechanistic investigations**

- KIM1 and Clusterin confirmed by ELISA and IHC analysis.
- Lipocalin 2 not confirmed
- Some proteins identified by 2D-DIGE analysis could be confirmed by Western blot analysis

# EWG III: Nephrotoxicity - 4

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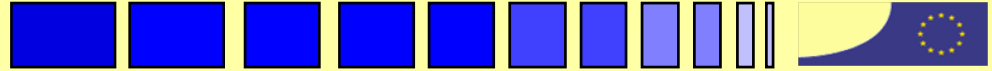


## • Conclusions

- Toxicogenomics delivered mechanistic insight to explain classical toxicological observations
- Candidate biomarkers were identified using transcriptomics (tissue), proteomics (tissue and plasma) and metabonomics (urine) data
- Cross 'omics and Cross study analysis (mainly protein and mRNA tissue data) in combination with histopathology added value for identification of at least 11 new mRNA and protein biomarker candidates for PTD

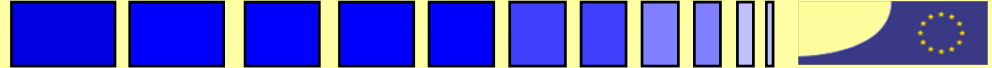
# Data Analysis

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- **Focused on mechanistic interpretation rather than assessment of 'predictivity'**
  - **Lead question: Can we explain / substantiate histopathological observations with 'omics data?'**
- **Data organization based on histopathology:**
  - **Expectation of histopathological findings at high concentration / 'late' time point**
    - Focus on interpretation of histopathological findings and less on analysis of earlier time points / lower concentration
    - Focus on the most efficient combination of classic endpoints with selected 'omics

# Conclusions



- ***Histopathology* examinations by experienced pathologists remains currently the most reliable approach for early safety evaluation and decision making**
- ***'omics technologies* are instrumental for expanding the mechanistic understanding of drug induced pathologies and the identification of new molecular safety biomarkers – “added value!”**
- **At present**
  - **A combination of *transcriptomics* and classical endpoints delivers most relevant results. In some cases, proteomics and metabonomics provided confirmatory and / or complementary information**
- **In the future**
  - **Provided further maturation of *proteomics* and *metabonomics*, these technologies will provide considerable added value**

# Project Goals met ...

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✓ **More informed decision making earlier in preclinical safety evaluation by combining results from**

- 'omics Technologies together with conventional toxicology methods

✓ **Development of scientists within Systems Toxicology**

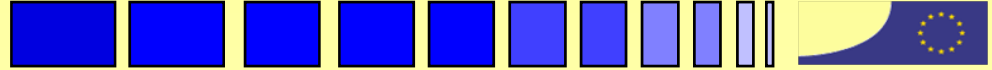
- Due to collaborative approach and intensive networking

✗ **Regulatory Authorities to review the approach**

- (Attempts to get EMEA-CHMP Safety Working Party representation were not successful)

# Acknowledgements

EU FP6: Integrated Project 'PredTox'



- **Prof. Friedlieb Pfannkuch**
- Consortium WP-leaders
- EWG-Leaders: Eric Boitier, Heidrun Ellinger-Zielgelbauer, Katja Arnold
- All consortium scientists
- Technology providers: Affymetrix, Genelogic, Entelos (Iconix), Waters, Biorad (CIPHERGEN), etc
- EFPIA, European Commission