An Integrated –Omics Approach Towards a Better Understanding of Drug-Induced Nephrotoxicity and Useful Biomarkers

On Behalf of the InnoMed-PredTox Nephrotoxicity WG

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Agenda

- Goals
- Study Design and Technologies
- Compounds
- Cross-study Analysis
- Cross-Technology, Within Study Analysis
- Conclusions and Next Steps

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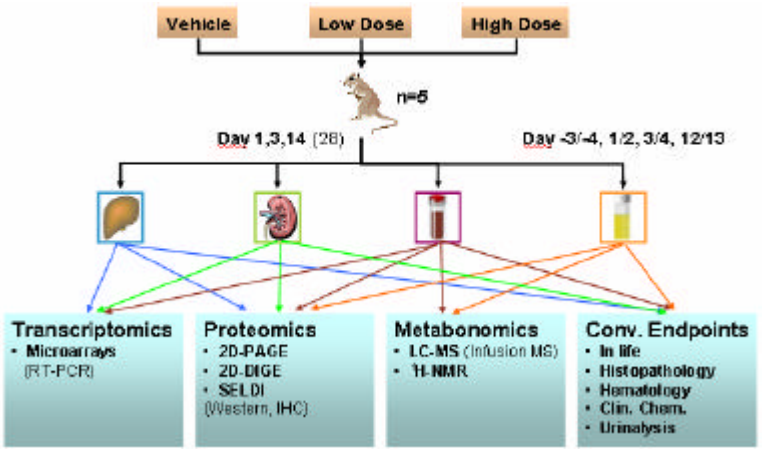
Goals

- InnoMed-PredTox Consortium
  - More informed and earlier decision making in preclinical safety evaluation
    - By combining results from 'omics' technologies in combination with conventional toxicology methods
    - Mechanistic investigation and new, better biomarker identification
- Kidney Working Group
  - Analysis of data from nephrotoxic compounds

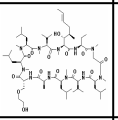
Generic Study Design and Technologies Applied



Compounds

- Proprietary compounds
  - **14 compounds** that failed in later phases of development related to kidney or liver toxicity
- Reference compounds
  - **Gentamicin** (kidney) **and troglitazone** (liver)

Nephrotoxic Compounds

	007SE	Gentamicin	IMM125	006JJ	002BI
Contributor	Boehringer-Ingelheim	Sanofi-Aventis	Novartis	Johnson & Johnson	Boehringer-Ingelheim
MW (Dalton) **Structure**	439.5	477.6 	1263 	/	443.9
Class	/	Aminoglycoside antibiotic	Cyclosporine derivative	/	/

Cross-compound Analysis

Histopathology as Phenotypic Anchor

- Positive group
  - Compound treated animals
  - Histopathology score for terms) of choice = 2 for in both kidneys
- Negative control group
  - Vehicle treated animals
  - Histopathology score of 0 for term of choice in both kidneys
- Selected terms (at least in 3 studies)
  - **Proximal tubule damage (PTD)**
    - Summary term computed of the max. score of three terms
      - Basophilic tubule: proximal
      - Degeneration/regeneration: renal tubule
      - Necrosis: proximal tubule
  - **Infiltrate: mononuclear cells (IMC)**

Representation of Kidney Histopathology Terms

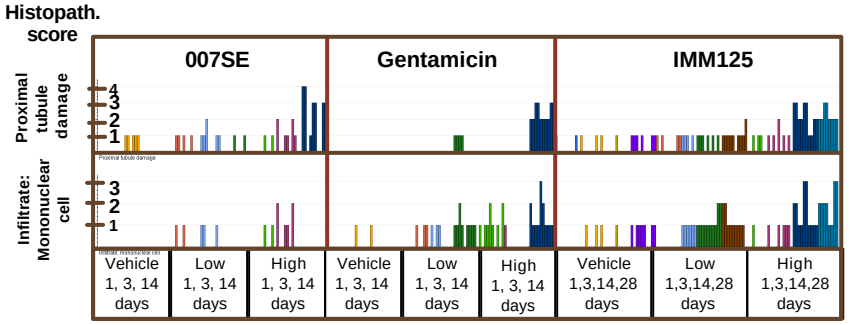
Histopathology terms kidney	007SE	Genta-micin	IMM125	FP006JJ	002BI
Basophilic tubule: proximal	+		+		
Degeneration/regeneration: renal tubule		+			
Necrosis: proximal tubule	+	+			
Infiltrate: mononuclear cell	+	+	+	(+)	
Dilation: renal tubule	+		+		
Mineralization: renal tubule	(+)		+		
Hypertrophy: renal tubule			+		
Cast renal tubule: hyaline	+				
Cast renal tubule:cellular	(+)				
Hyaline droplet: renal tubule					+
Vacuolation: proximal tubule				+	
Vacuolation: distal tubule			+		
Dilation: pelvis		(+)			

(+): minimal effec; both kidneys

Selected Studies

- Studies
  - 07SE
  - Gentamicin
  - IMM125
- Data type to start with
  - Transcriptomics
  - Pathway enrichment analysis

Individual Animal Histopathology Scores of Two Histopathlogy Terms Represented in 3 Studies



PTD: Top Up-regulated Transcripts

Fold Change	Symbol	Name
42.5	HAVCR1	**hepatitis A virus cellular receptor 1 (KIM1)**
41.7	GPNMB	glycoprotein (transmembrane) nmb
20.2	CXCL13	chemokine (C-X-C motif) ligand 13 (B-cell chemoattractant)
8.8	PILRA	paired immunoglobin-like type 2 receptor alpha
7.7	FCGR2A	Fc fragment of IgG, low affinity IIa, receptor (CD32)
7.5	FCGR3A	Fc fragment of IgG, low affinity IIIa, receptor (CD16a)
5.8	LCN2	**lipocalin 2 (oncogene 24p3)**
5.8	EMR1	egf-like module containing, mucin-like, hormone receptor-like 1
5.8	PLA2G2D	phospholipase A2, group IID
5.6	MS4A7	membrane-spanning 4-domains, subfamily A, member 7
5.3	ATF3	activating transcription factor 3

PTD: Top Down-regulated Transcripts

Fold Change	Symbol	Name
15.6	CYP2C18	cytochrome P450, family 2, subfamily C, polypeptide 18
4.2	CALB1	**calbindin 1, 28kDa**
3.9	GC	group-specific component (vitamin D binding protein)
3.3	SNCA	synuclein, alpha (non A4 component of amyloid precursor)
3.1	MLCK	MLCK protein
2.9	NEFM	neurofilament, medium polypeptide 150kDa
2.9	RGN	regucalcin (senescence marker protein-30)
2.8	ALDH1B1	aldehyde dehydrogenase 1 family, member B1
2.7	MLANA	melan-A
2.5	ACSM3	acyl-CoA synthetase medium-chain family member 3
2.4	MLC1	megalencephalicleukoencephalopathy with subcortical cysts 1

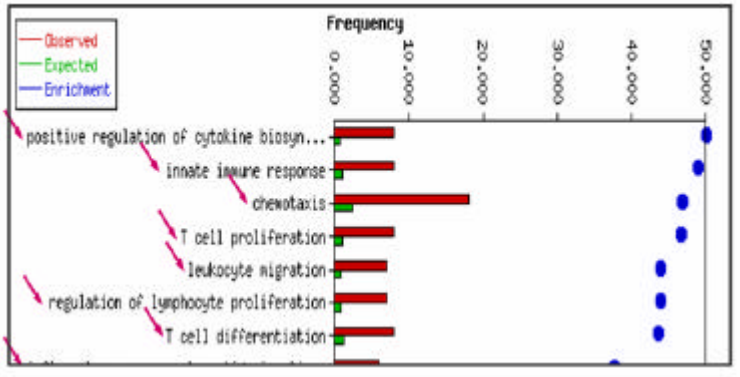
IMC: Top Up-regulated Transcripts

Fold Change	Symbol	Name
20.2	CXCL13	chemokine (C-X-C motif) ligand 13 a B-cell chemoattractant
14.2	GPNMB	glycoprotein (transmembrane) nmb
12.6	HAVCR1	**hepatitis A virus cellular receptor 1 (=KIM1)**
8.8	PILRA	paired immunoglobin-like type 2 receptor alpha
7.5	FCGR3A	Fc fragment of IgG, low affinity IIa, receptor (CD32)
5.7	PLA2G2D	phospholipase A2, group IID
5.6	MS4A7	membrane-spanning 4-domains, subfamily A, member 7
4.8	CD53	CD53 antigen
4.7	CD68	CD68 antigen
4.5	ATF3	activating transcription factor 3

IMC: Top Down-regulated Transcripts

Fold Change	Symbol	Name
5.3	CYP2C18	cytochrome P450, family 2, subfamily C, polypeptide 18
2.8	GC	group-specific component (vitamin D binding protein)
2.3	APOC2	apolipoprotein C-II
2	ACSM3	acyl-CoA synthetase medium-chain family member 3
2	PPFIA4	protein tyrosine phosphatase, receptor type f polypeptide (PTPRF), interacting protein (liprin), alpha 4
2	SNCA	synuclein, alpha (non A4 component of amyloid precursor)
2	MLCK	Myosin-light-chain kinase
1.9	SLC25A25	solute carrier family 25 (mitochondrial carrier; phosphate carrier), member 25
1.8	CALB1	**Calbindin 1 (28kDa)**
1.8	ETV5	ets variant gene 5 (ets-related molecule)

IMC: Pathway Enrichment Analysis



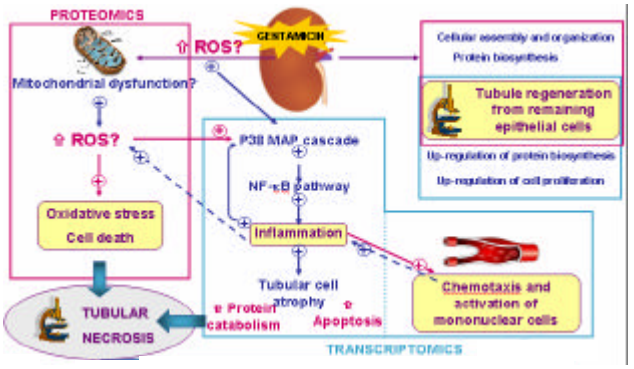
Results Pathway Mapping

- PTD
  - Renal damage
  - Apoptosis, necrosis, inflammation, oxidative stress
  - Cytoskeleton and extra cellular matrix
- IMC
  - Immune and inflammatory response
    - Activation, proliferation, chemotaxis, infiltration of blood cells
  - Renal damage

Cross-technology, Within-study Analysis

Gentamicin: Integrated Transcriptomic and Proteomic Evaluation of Nephrotoxicity in Rats

- E.Com; E. Boitier; J. Marchandeau; M. Courcol; J. Leonard; M. Duchesne; B. Genet; S. Schroeder; M. Wendt; J. Gautier (676)

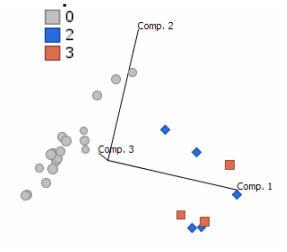


IMM125

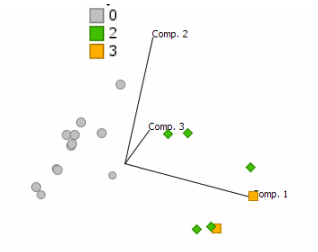
- Grouping of animals
  - Histopathology based approach as for the cross-compound analysis
- Data types included
  - Transcriptomics – Kidney
  - Proteomics - 2D-DIGE – Kidney
  - LC/MS – Urine
  - Infusion MS - Serum

Example I: Transcriptomics in Kidney

- PCA, all doses and time points, 31099 transcripts

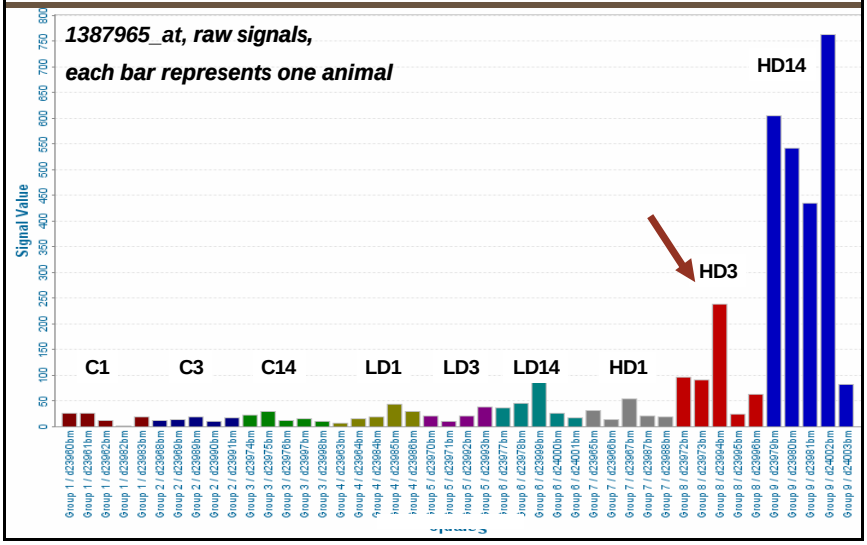


PTD

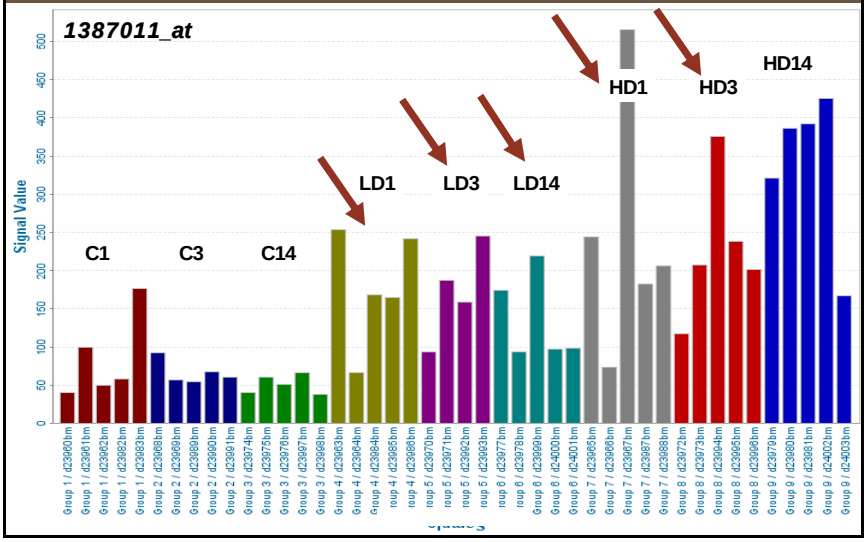


IMC

HAVCR1 (Kim-1) – Kidney

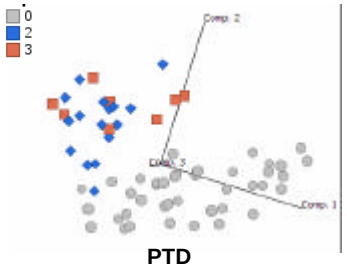


Lipocalin-2 (Lcn2) - Kidney

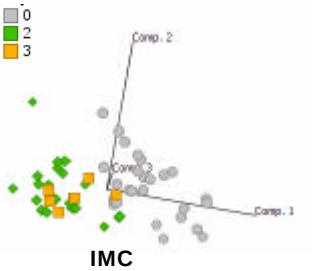


Example II: Infusion MS - Serum

- PCA, all doses and time points, 98474 points, 433 (PTD) or 360 (IMC)



PTD



IMC

Cross-Validation: Predict Control or Positive Grp.

- Classifiers from each data type and all ‚omics'-data by supervised learning approach

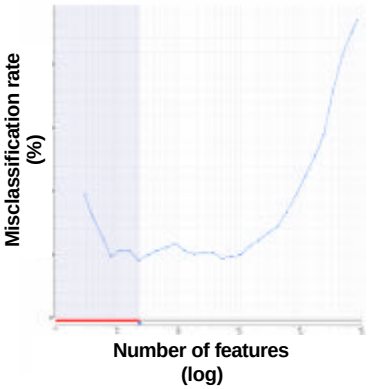
Cross Validation Error Rate

		PTD	IMC
Transcriptomics	Kidney	2.1	1.4
LC-MS	Urine	15.8	22.2
Proteomics	Kidney	4.4	41.8
Infusion MS	Serum	0.3	0.5
Cross-omics	All	1.6	43.4
Cross-omics	*Optimized	0	0

*Ranking Method: Recursive Feature Elimination

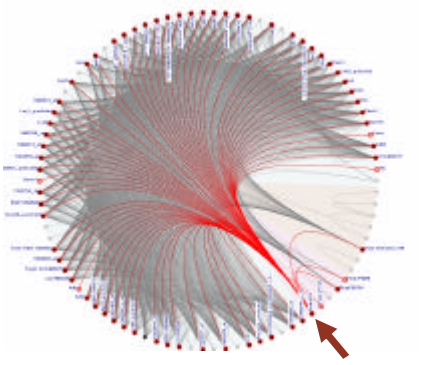
Example: Cross-Omics Classifier for IMC

- Ranking method: recursive feature elimination
- Cross-validation error ~ 0 % with 23 items
  - 6 Transcripts - Kidney
  - 3 Peaks from LC - MS Urine
  - 14 Peaks from I - MS Serum



Example: Cross-omics Correlation Analysis - IMC

- Top ranked 100 IMC features
  - 9 peaks from I-MS - Serum
  - 7 peaks from LC-MS - Urine
  - 84 Transcripts
- Example: peak 878 from urine
  - Correlates with
    - Other urine peaks
    - 1 peak from serum
    - Many transcripts



Conclusions

- Cross-study analysis revealed
  - Mechanistic insight
  - Known and new biomarker candidates
  - Some of these BM candidates are earlier and more sensitive than traditional endpoints (histopathology)
- Cross-technology analysis revealed
  - Mechanistic insight
  - Good concordance for some of the technologies
  - Great potential for new, optimized cross-technology biomarkers with options to bridge more easily into clinical applications

Envisaged Next Steps

- Assess specificity and predictivity of results
  - Different grouping, e.g. by kidney function markers (BUN, creatinine)?
  - Non-target organ effects as controls?
  - Apply to lower dose and earlier time point animals
  - Technical confirmations/ localizations of BM candidates (started)
  - Compare performance of molecular biomarkers vs. conventional biomarkers (ROC)
  - Extend cross-omics correlation analysis
  - Judge on contribution of each individual technology
- Propose candidates for further BM qualification (PSTC?)
- Use lessons learned for more informed decision making in integrated preclinical safety assessment

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