



Life Science Group



Discovery of Markers Associated with Bile Duct Damage in Rat Plasma

*EU PredTox Consortium
Proteomics Working Group*



PredTox Consortium

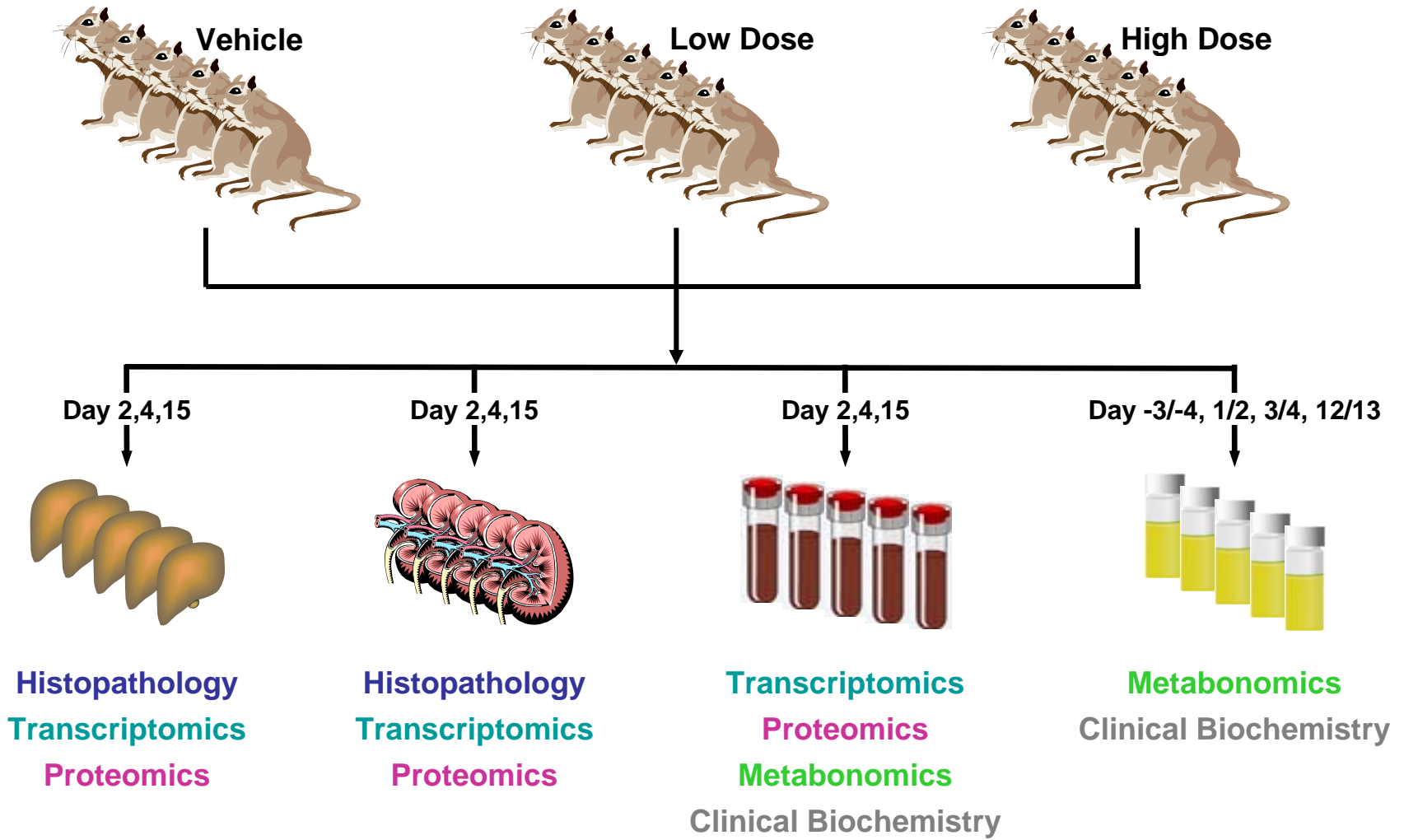
- PredTox (predictive toxicology) consortium
 - Part of Innomed (Innovative Medicines)
 - Funded partly by the EU as part of the 6th Framework program
 - Consists of 18 partners
 - 13 pharmaceutical companies
 - 2 small and medium enterprises
 - 3 universities

- Objectives
 - Improve pre-clinical safety evaluation
 - Combine existing toxicology endpoints with novel “omics” technologies
 - Focus on nephrotoxicity and hepatotoxicity





Study Design





SELDI Study Design

- Evaluated 16 Compounds across 3 sites
 - 14 proprietary compounds and 2 reference compounds
 - 3 Toxicity Classes
 - Bile Duct Damage
 - Nephrotoxicity
 - Liver Hypertrophy
 - 5-6 compounds analyzed at each of 3 sites
 - Bio-Rad, Merck KGaA, and University College Dublin
- Sample Preparation
 - Plasma treated to deplete albumin and IgG
 - No pre-treatment for tissue extracts (liver or kidney)
- Profiling
 - Normalized protein load for both plasma and tissue samples
 - Profiled both sample types on CM10 and Q10 ProteinChip arrays
 - 2 mass optimization ranges
 - 4 technical replicates
- *Each compound and tissue type was analyzed under 4 unique conditions, generating 480 spectra*



Statistical Analysis

Peak Screening

- Use statistics to select peaks for further inspection
 - Mann Whitney p -value < 0.02
 - AUC > 0.8
- Calculated statistics for both dose and time-dependent changes
 - Dose dependent comparisons
 - High Dose vs Vehicle at Day 4 and Day 15
 - Low Dose vs Vehicle at Day 4 and Day 15
 - Time dependent comparisons
 - Day 4 vs Day 15 in each treatment group (Vehicle, Low Dose, and High Dose)
- Data also analyzed independently using Genedata Expressionist[®] Software with Refiner MS Module
- Focused on peaks exhibiting differences in the High Dose vs. Vehicle comparison at Day 15
 - Most significant histopathology effects observed at this dose and time point



Final Peak Selection

Secondary Screening Criteria

- Peak quality
 - Can species be measured reproducibly?
- Fold-change (Vehicle vs High Dose at Day 15)
 - > 2 fold increase used as primary criteria
 - > 50% increase used as secondary criteria in conditions with few significant changes
 - Focused primarily on peaks that increased
- Consistency across array types
 - If the peak is observed in CM10 and Q10 data, are the changes consistent in both?
- Consistency across compounds



Final Candidate Marker Selection

- Grouped peaks into three categories
 - Highest upregulated peak(s) in a single compound study
 - Plasma peaks only
 - Generally selected as a marker based on analyses using both Genedata's and Bio-Rad's software
 - Peaks common to plasma and tissue
 - Peaks with similar masses that were modulated in both plasma and tissue could represent the same protein species
 - Restricted to peaks that were up-regulated in plasma but could be either up or down-regulated in tissue
 - Peaks that were modulated in multiple compounds within toxicity groups
 - Plasma or tissue
 - Upregulated in two or more compounds



Plasma Peaks Upregulated in Multiple Compounds Inducing Bile Duct Damage

M/Z	Compound	Vehicle vs High Dose at 4 days		Vehicle vs High Dose at 15 days	
		Mann-Whitney <i>p</i> -value	Median Fold Change	Mann-Whitney <i>p</i> -value	Median Fold Change
11628	004BA	0.2506	1.0	0.1432	2.4
	005ME	0.0090	2.6	0.0090	2.0
	007SE	0.6015	1.2	0.0143	2.7
	014SC	0.1172	2.2	0.0472	1.7
3854	004BA	0.0090	3.9	0.7697	1.1
	005ME	0.0283	2.1	0.0283	2.5
12699	004BA	0.0472	1.5	0.1432	2.2
	005ME	0.0758	1.2	0.0090	1.6



Peaks Modulated in both 004BA & 007SE

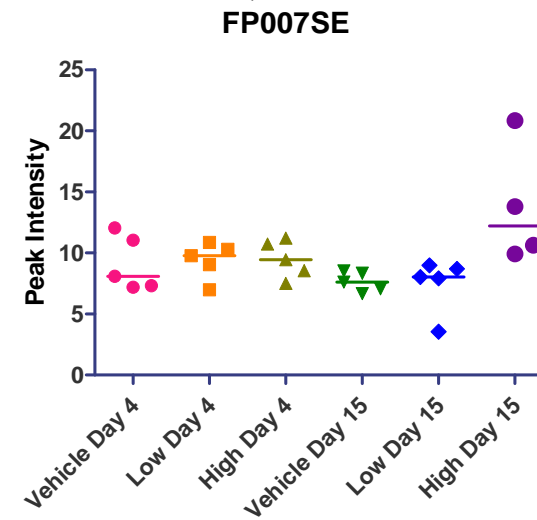
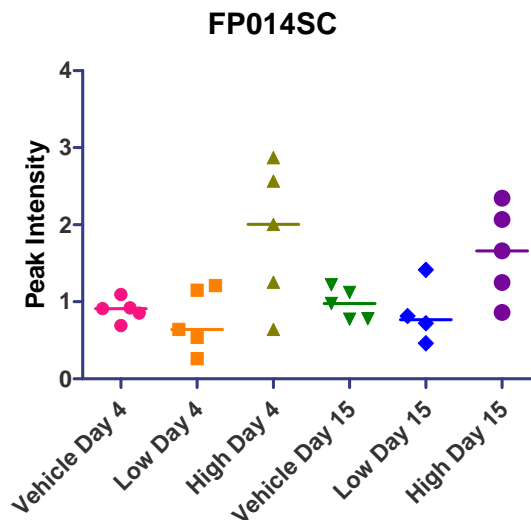
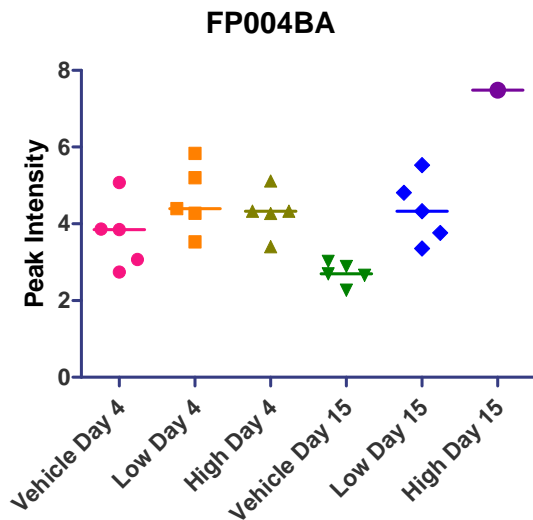
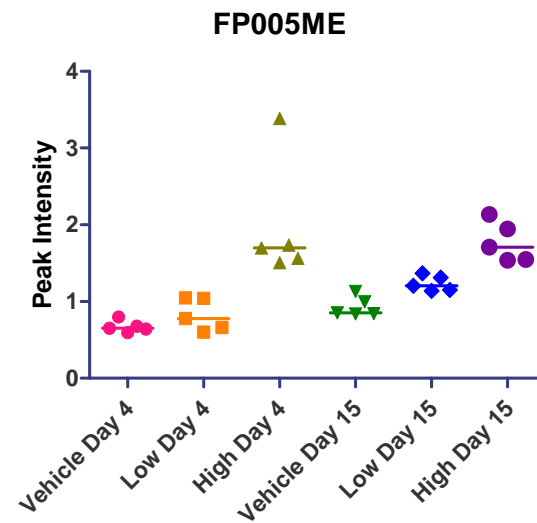
Bile Duct Damage

M/Z	Compound	Vehicle vs High Dose at 4 days		Vehicle vs High Dose at 15 days	
		Mann-Whitney <i>p</i> -value	Median Fold Change	Mann-Whitney <i>p</i> -value	Median Fold Change
5816	004BA	0.9168	0.9	0.1432	2.1
	007SE	0.7540	1.2	0.0143	2.1
6174	004BA	0.0090	1.3	0.1432	2.1
	007SE	0.0090	1.9	0.0143	5.2
9944	004BA	0.0090	1.5	0.1432	0.7
	007SE	0.3472	1.1	0.0275	5.8
11840	004BA	0.9168	1.0	0.1432	2.2
	007SE	0.6015	1.2	0.0143	2.2
12827	004BA	0.0090	5.7	0.1432	12.2
	007SE	0.0758	2.0	0.0275	2.7
13062	004BA	0.0090	3.7	0.1432	7.1
	007SE	0.3472	1.1	0.0143	1.6



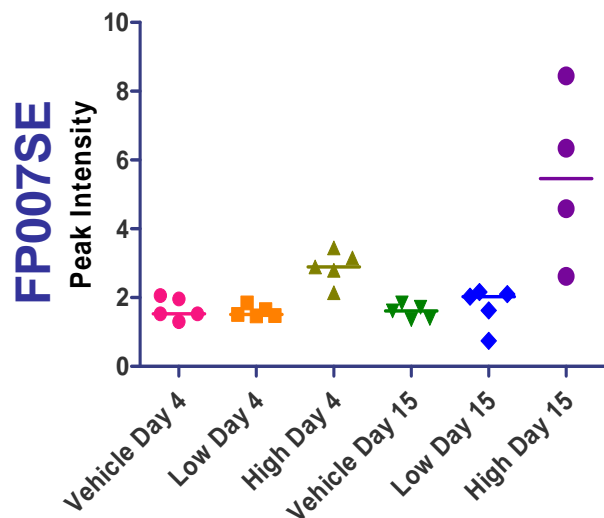
11630 Da Peak Is Upregulated in Four Compounds

Compound	Statistic	Vehicle vs Low Dose		Vehicle vs High Dose	
		Day 4	Day 15	Day 4	Day 15
FP004BA	<i>p</i> -value	0.0758	0.0163	0.2506	0.1432
	fold change	1.27	1.37	0.98	2.41
FP005ME	<i>p</i> -value	0.2506	0.0090	0.0090	0.0090
	fold change	1.19	1.42	2.61	2.01
FP007SE	<i>p</i> -value	0.9168	0.9168	0.6015	0.0143
	fold change	1.21	1.06	1.17	2.74
FP014SC	<i>p</i> -value	0.6015	0.4624	0.1172	0.0472
	fold change	0.70	0.78	2.20	2.20

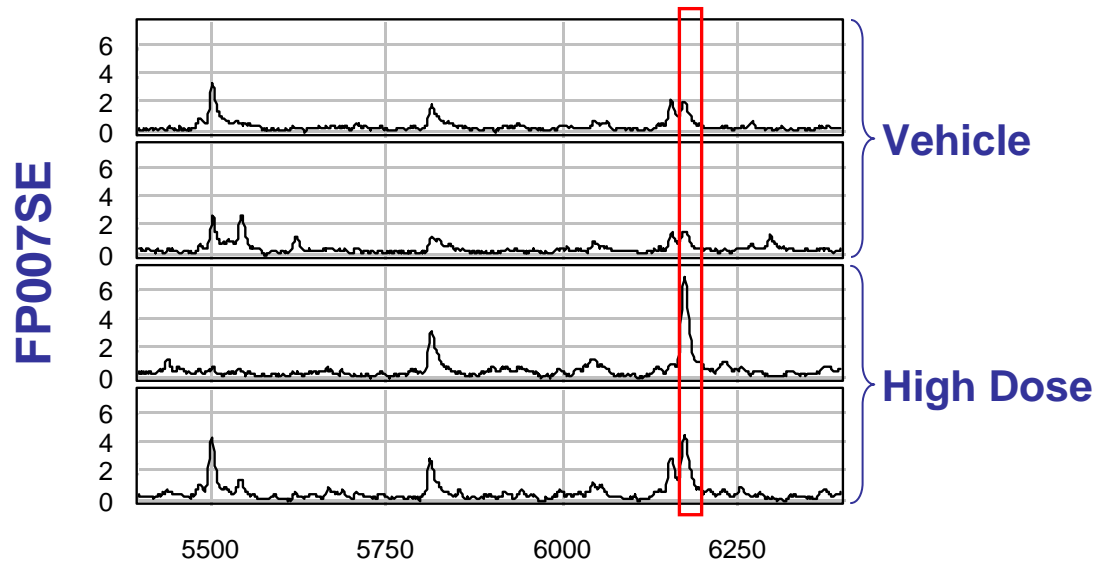
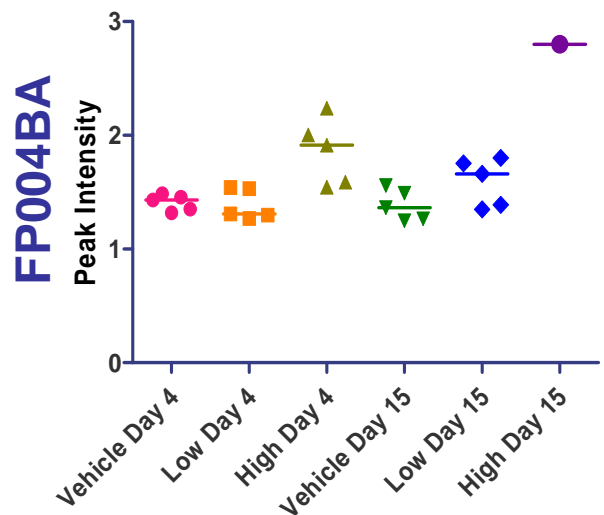




6175 Da Peak Is Upregulated in Two Compounds at High Dose



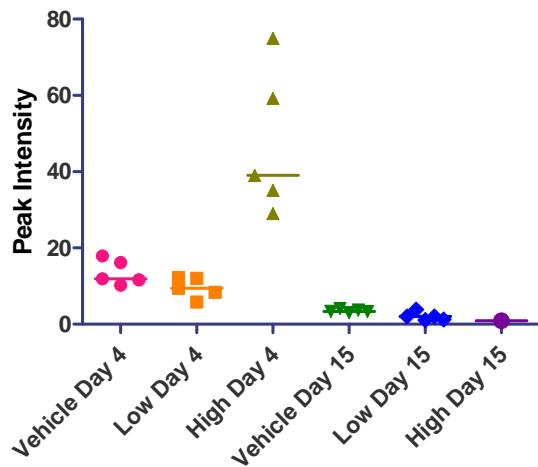
Compound	Statistic	Vehicle vs Low Dose		Vehicle vs High Dose	
		Day 4	Day 15	Day 4	Day 15
FP004BA	<i>p</i> -value	0.6015	0.1172	0.0090	0.1432
	fold change	0.91	1.22	1.34	2.05
FP007SE	<i>p</i> -value	0.4647	0.4647	0.0090	0.0143
	fold change	0.99	1.26	1.89	5.24



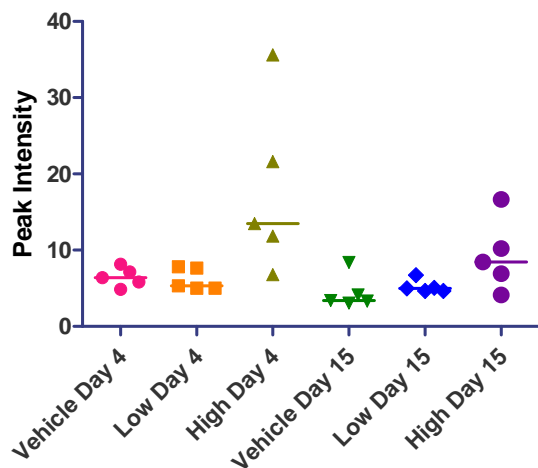


3860 Da Peak Is Upregulated at Day 4 in Two Compounds

FP004BA

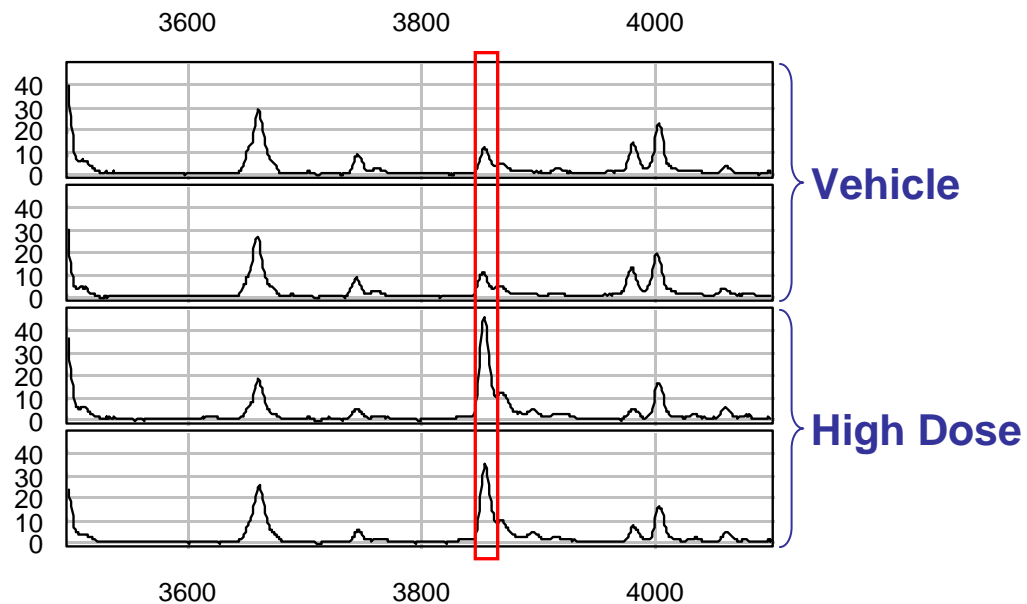


FP005ME



Compound	Statistic	Vehicle vs Low Dose		Vehicle vs High Dose	
		Day 4	Day 15	Day 4	Day 15
FP005ME	<i>p</i> -value	0.7540	0.1172	0.0283	0.0283
	fold change	0.83	1.47	2.11	2.49
FP004BA	<i>p</i> -value	0.1745	0.0758	0.0090	0.1432
	fold change	0.79	0.60	3.28	0.27

FP004BA
(4 Days)





Conclusions

- 9 candidate biomarkers in rat plasma were upregulated upon treatment with two of more compounds that caused bile duct damage
- Although the number of samples per group was lower than typically recommended, rigorous study design strengthened confidence in markers
 - Changes were observed in multiple compounds analyzed at different sites
 - Individual compound study included multiple doses and time points
- Next steps:
 - Purification and identification of selected candidate biomarkers
 - Currently underway
 - Integration of SELDI data with other “omics” data
 - Genomics
 - Metabonomics
 - 2D-DIGE



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