



Note - presentation notes may be read by double clicking on the icon to the left

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INSTITUTES FOR HEALTH SCIENCES
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The Application of Metabolomics to Acetaminophen-Induced Liver Injury in Humans

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Hamner-UNC Center for Drug Safety Sciences
Hamner Institutes for Health Sciences, RTP, NC***

Clinical Study Designs of Human of APAP Challenge

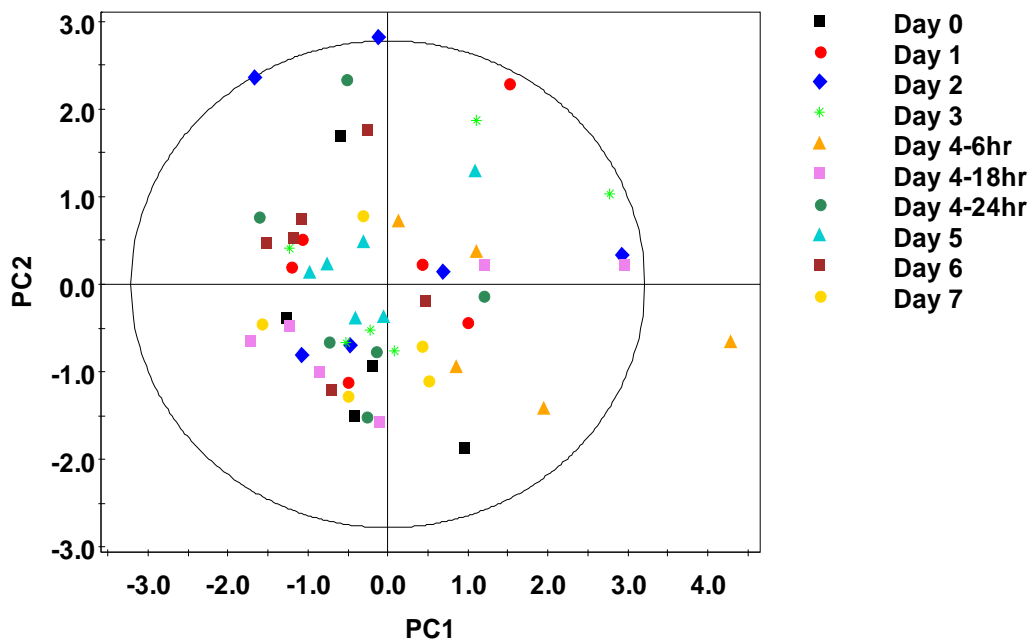
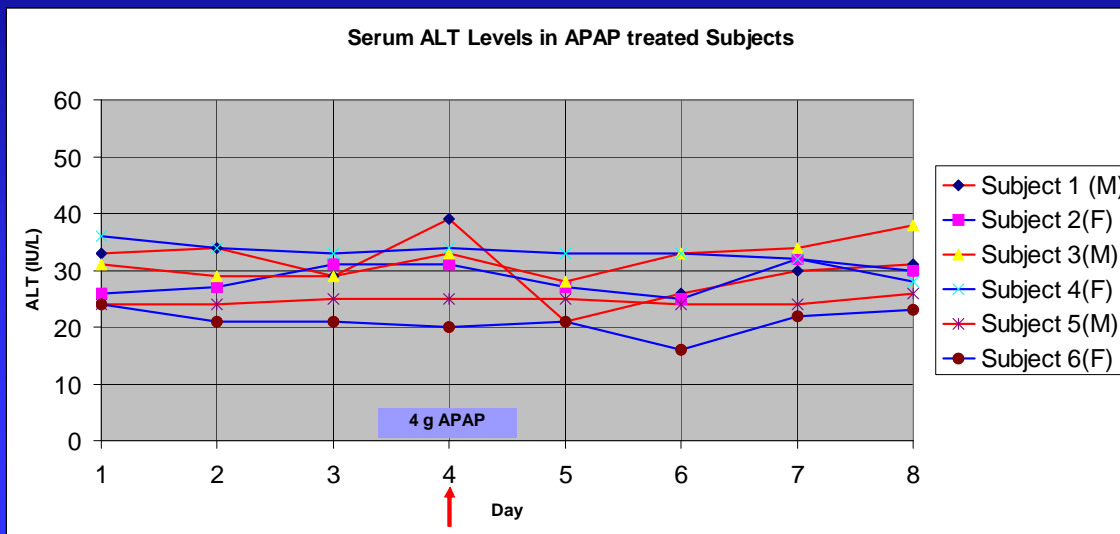
Single dose study

- **9 healthy volunteers, men & women, age 18-55**
- **Inpatient for 7 days & given controlled diet**
- **3 days on a controlled diet**
- **A single 4gm dose given on day 4**
- **Urine, serum collected daily**

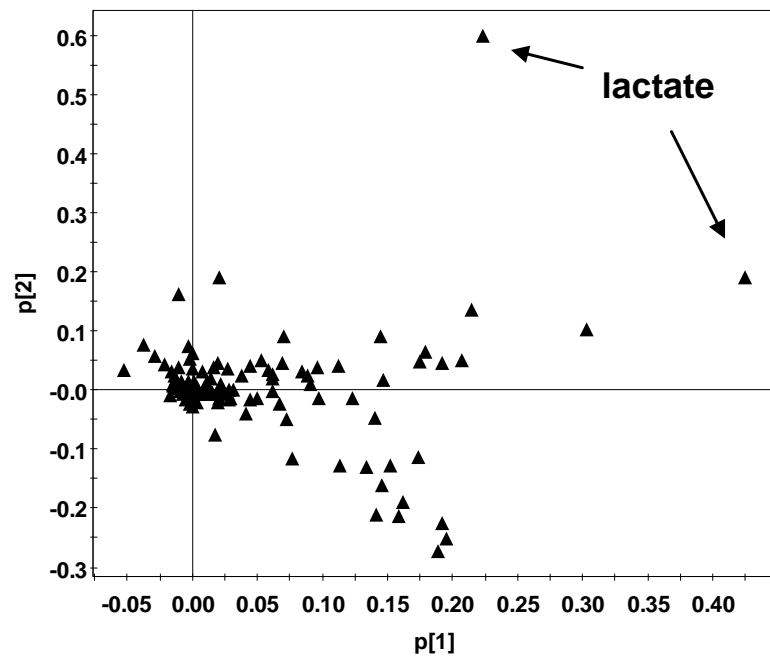
Multiple dose study

- **72 healthy volunteers, men & women, age 18-55**
- **Inpatient for 14 days & given controlled diet**
- **4gm APAP/day (2 500mg tablets 4 times) for 7 days**
- **Urine and serum collected daily**

Metabolomics Study of a Single 4 gm Dose of APAP



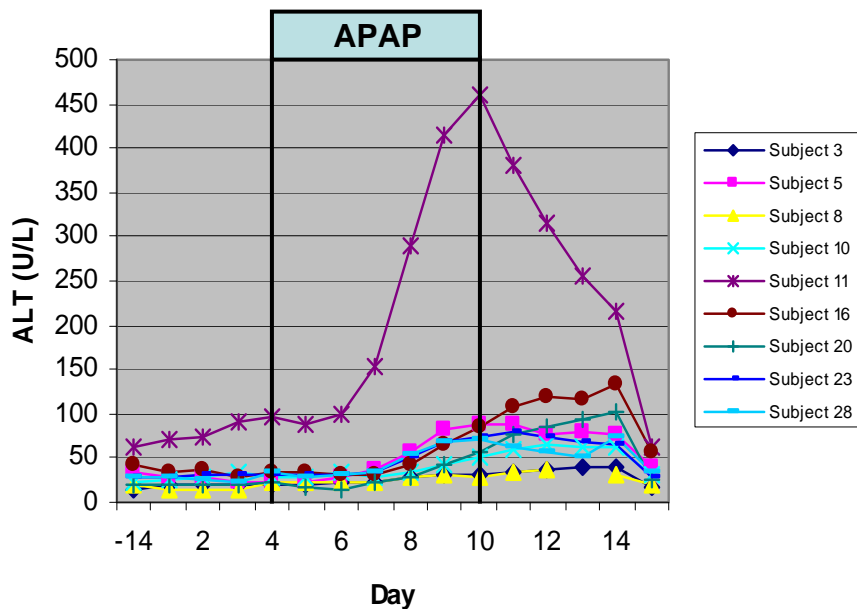
SIMCA-P+ 12 - 2009-02-04 11:42:54 (UTC-5)



SIMCA-P+ 12 - 2009-02-04 11:45:24 (UTC-5)

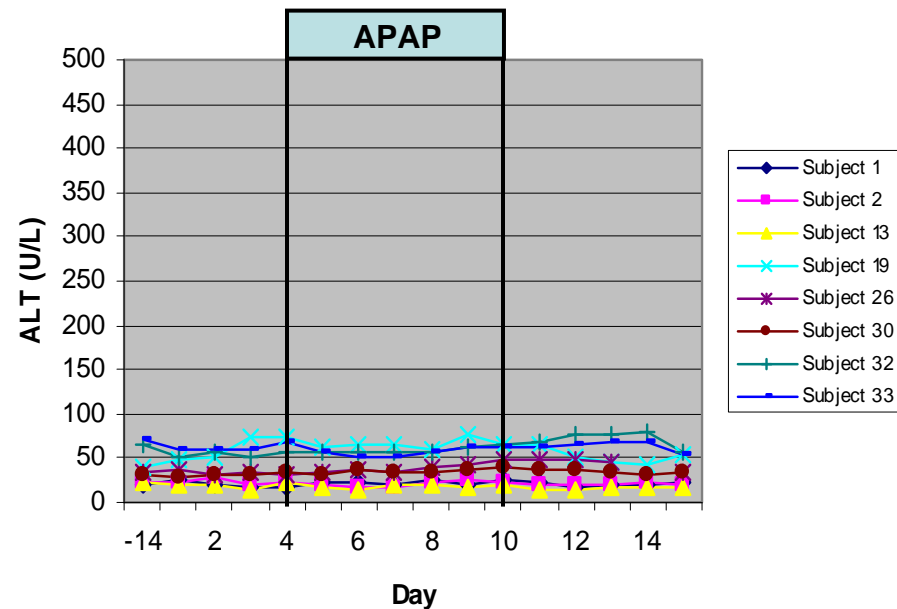
Responders and Non-Responders in the Two Week APAP Study

Responders: Daily ALT (U/L)



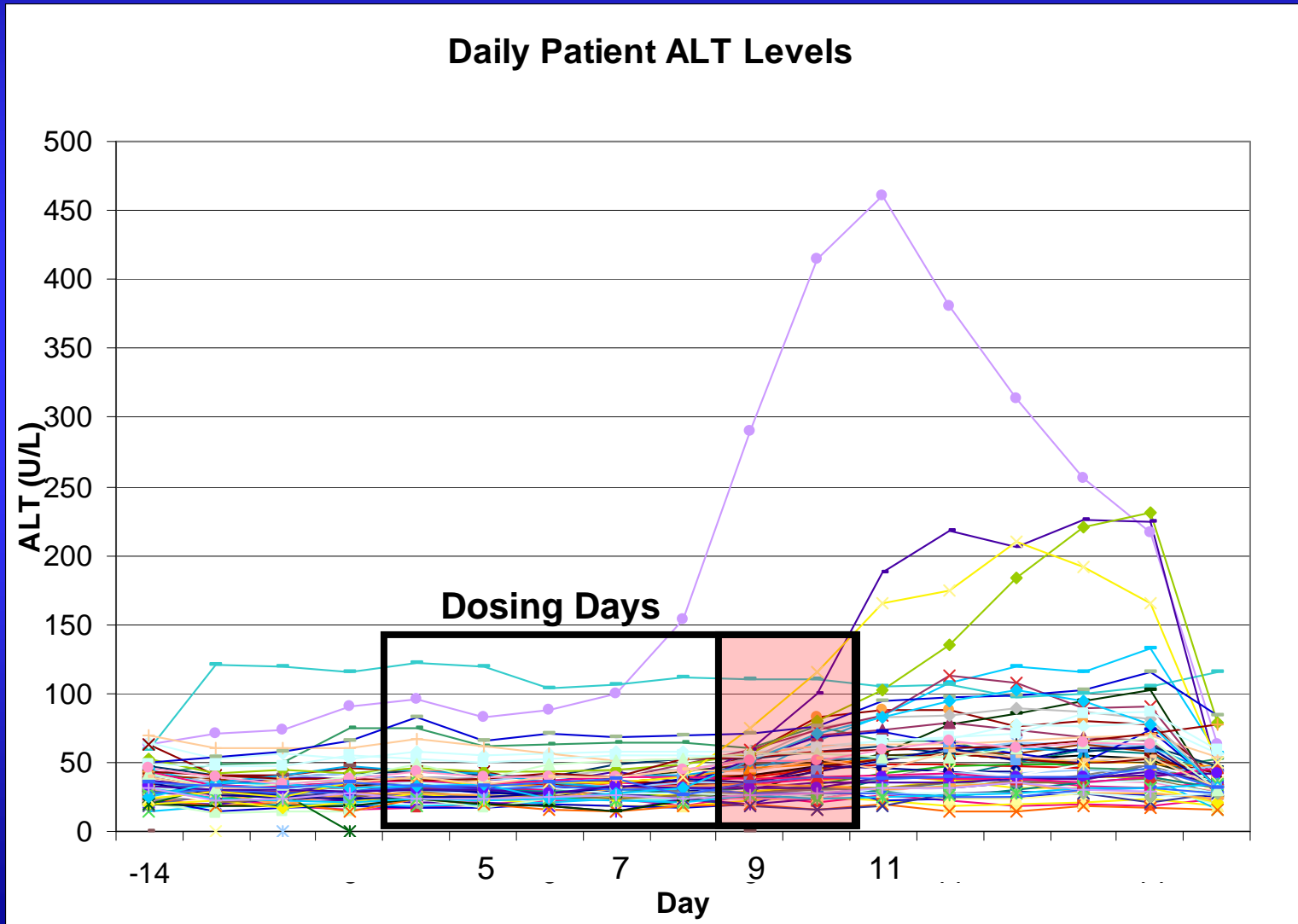
Peak ALT levels $> 2.0 \times$ baseline

Non-Responders: Daily ALT (U/L)

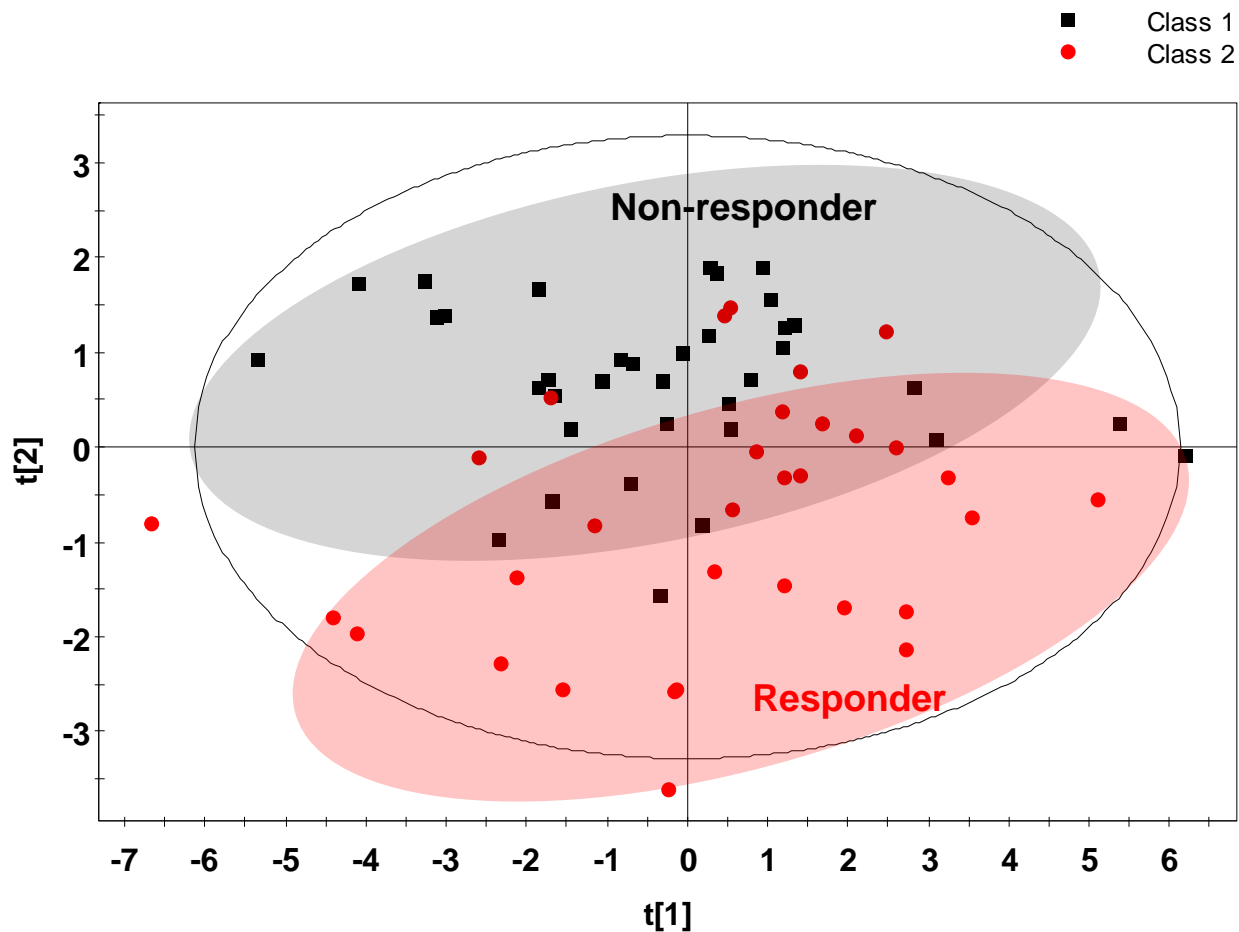


Peak ALT levels $< 1.5 \times$ baseline

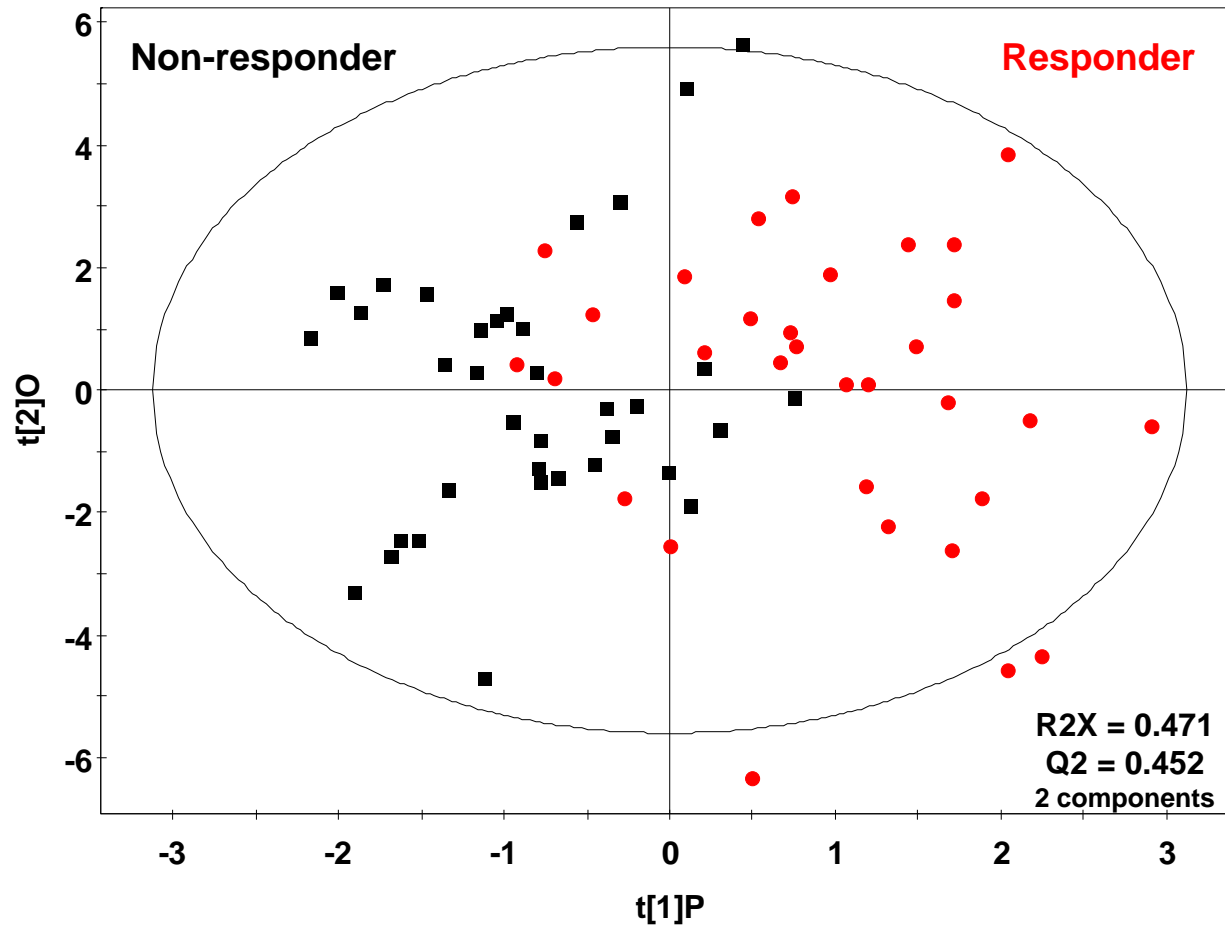
Can Metabolomics Distinguish Responders from Non-Responders?



PCA Analysis of Responders & Non-responders at Days 9-10

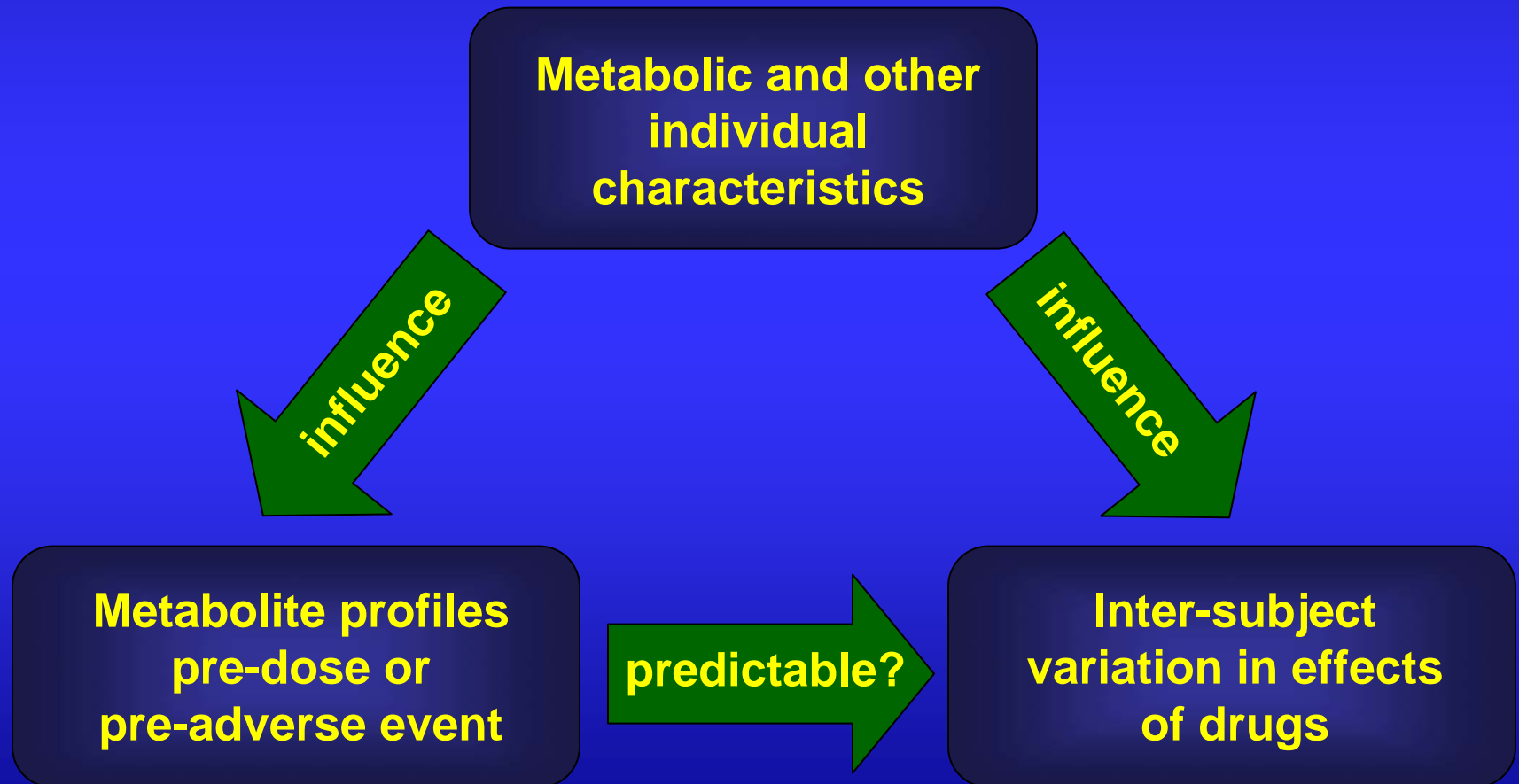


OPLS Analysis of Responders vs. Non-responders at Days 9-10

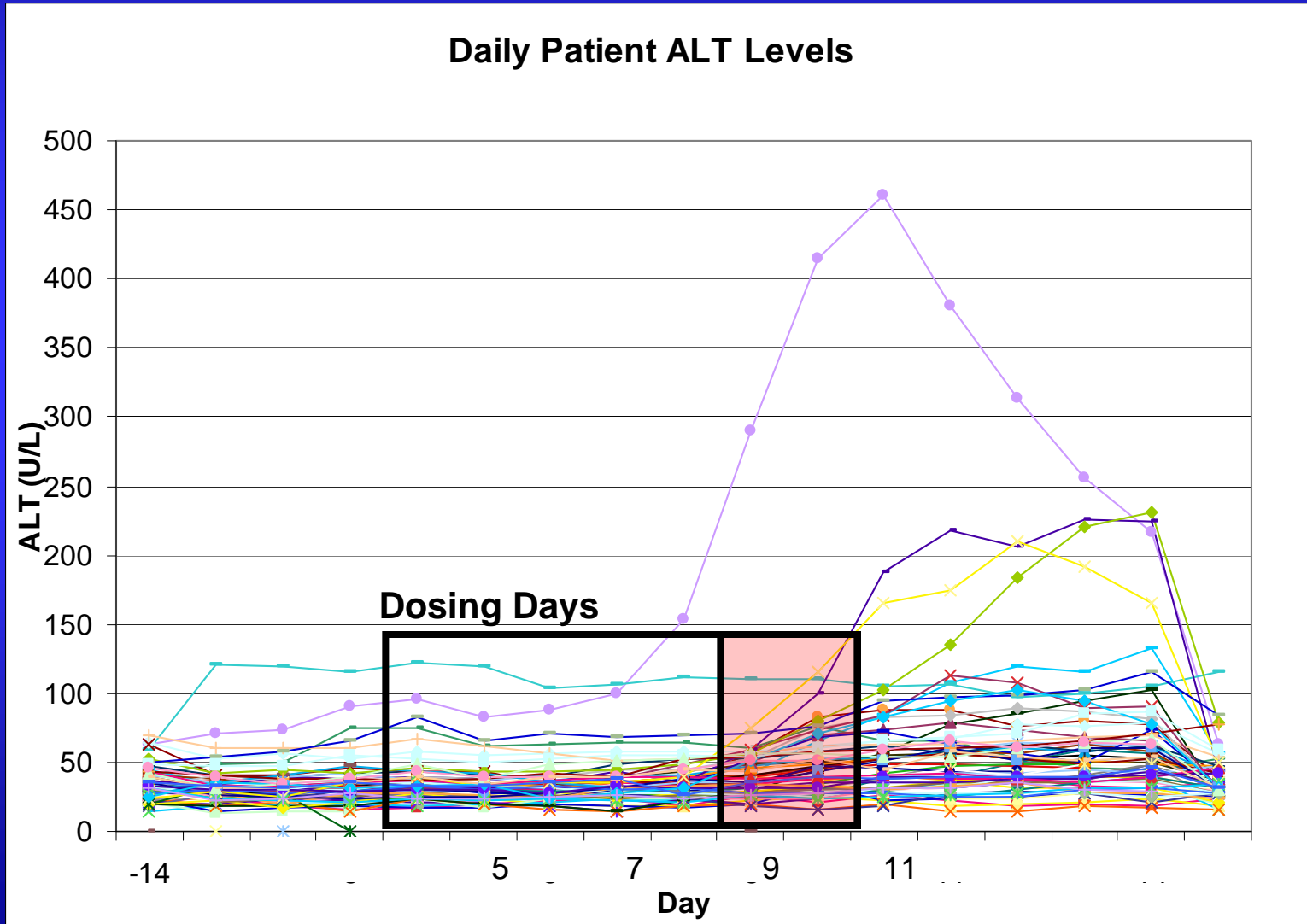


Pharmaco-Metabonomic Hypothesis

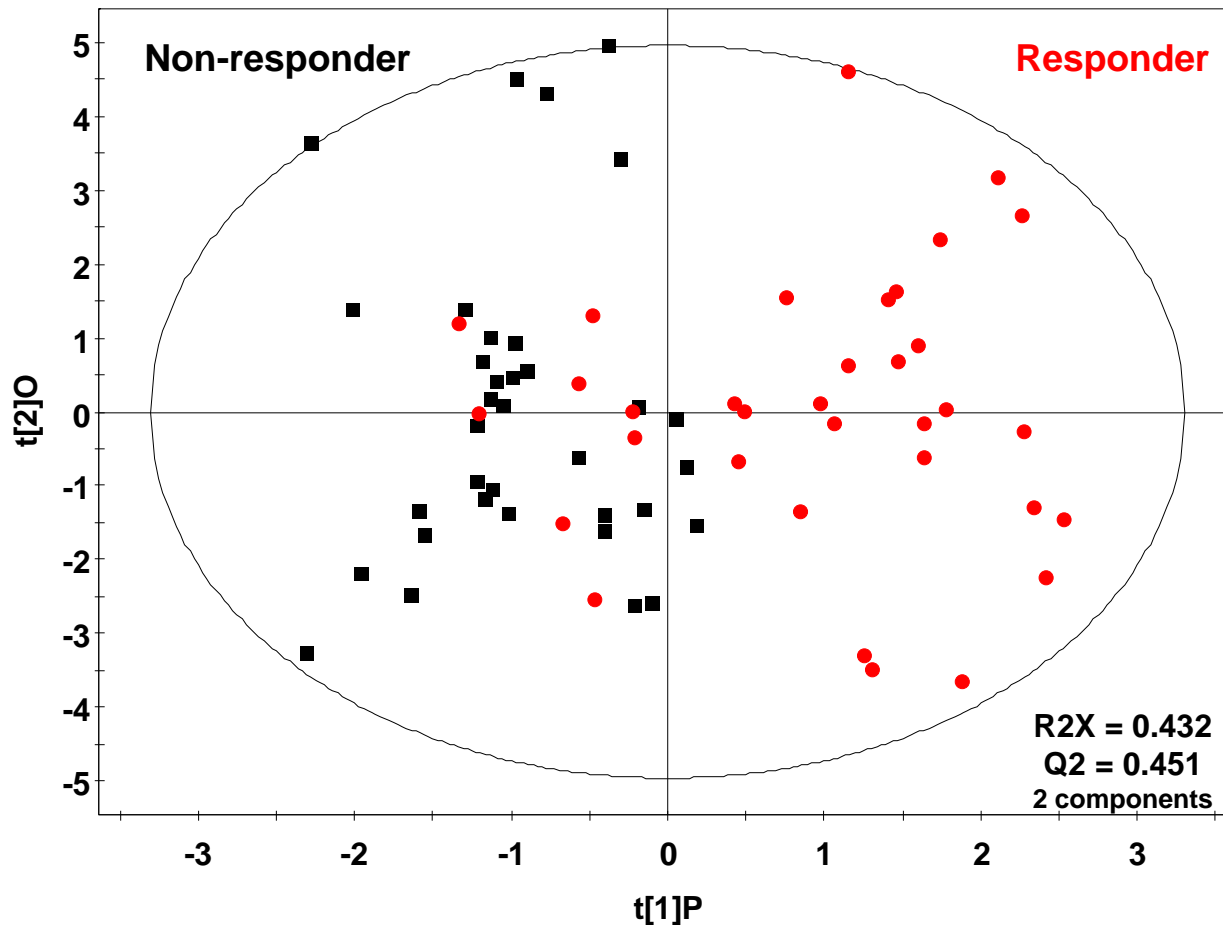
The prediction of the outcome of a drug or xenobiotic intervention based on a mathematical model of pre-intervention metabolite signatures.



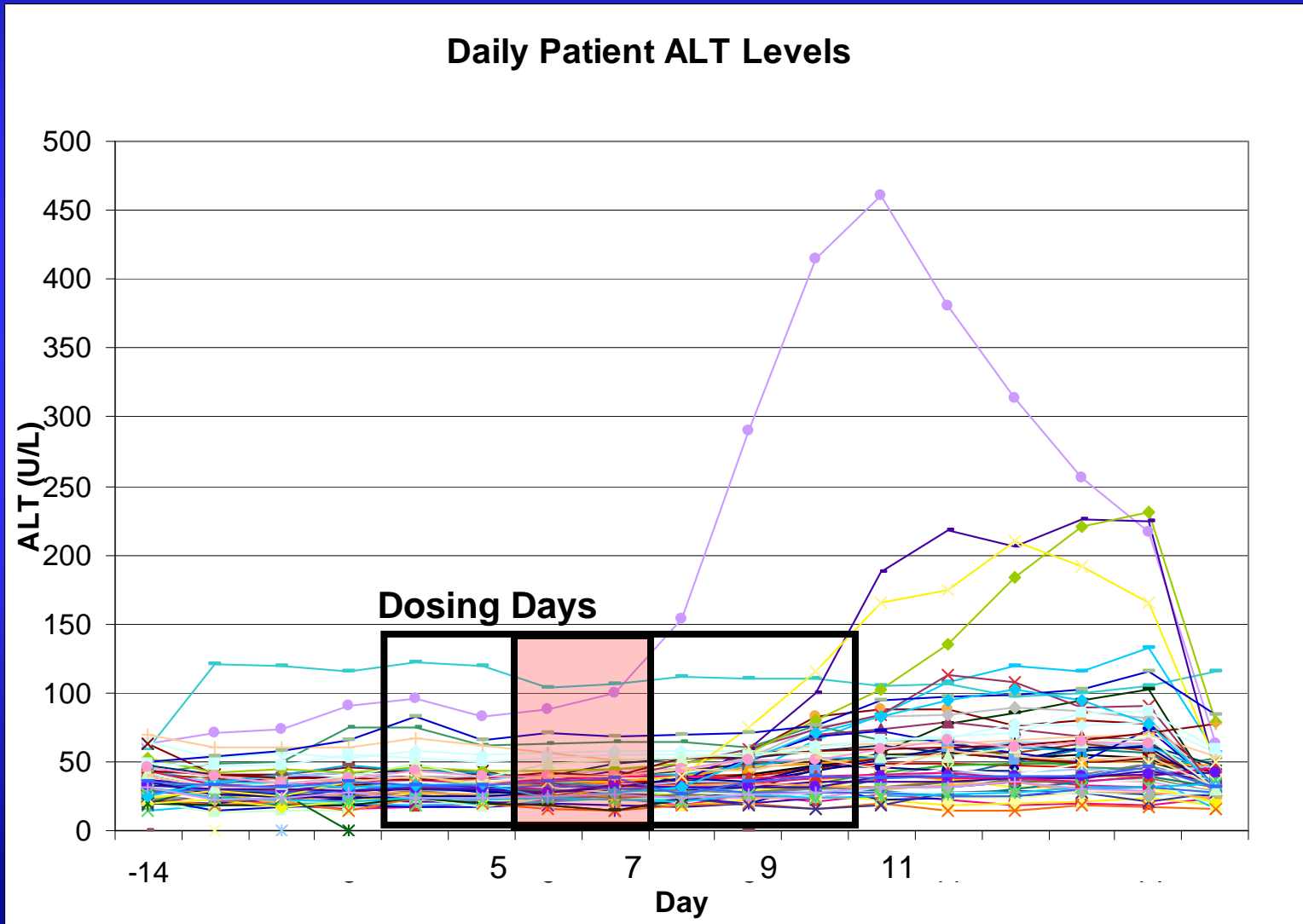
Metabonomic Prediction of Hepatotoxicity Prior to ALT Rise



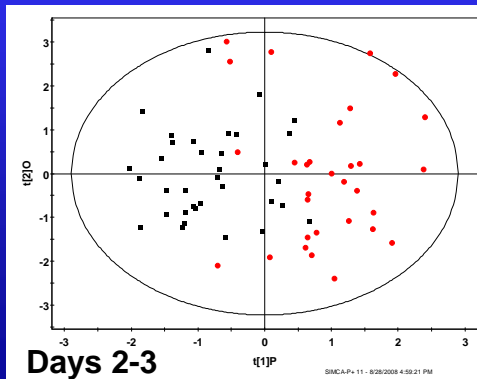
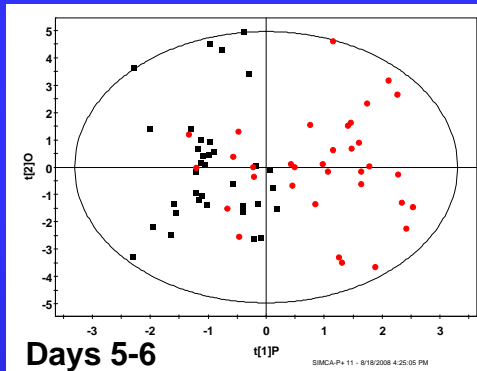
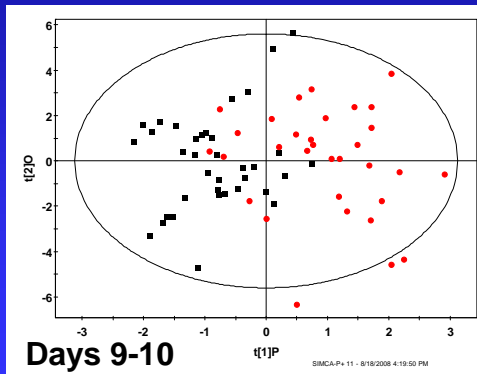
OPLS Analysis of Responders vs. Non-responders at Days 5-6



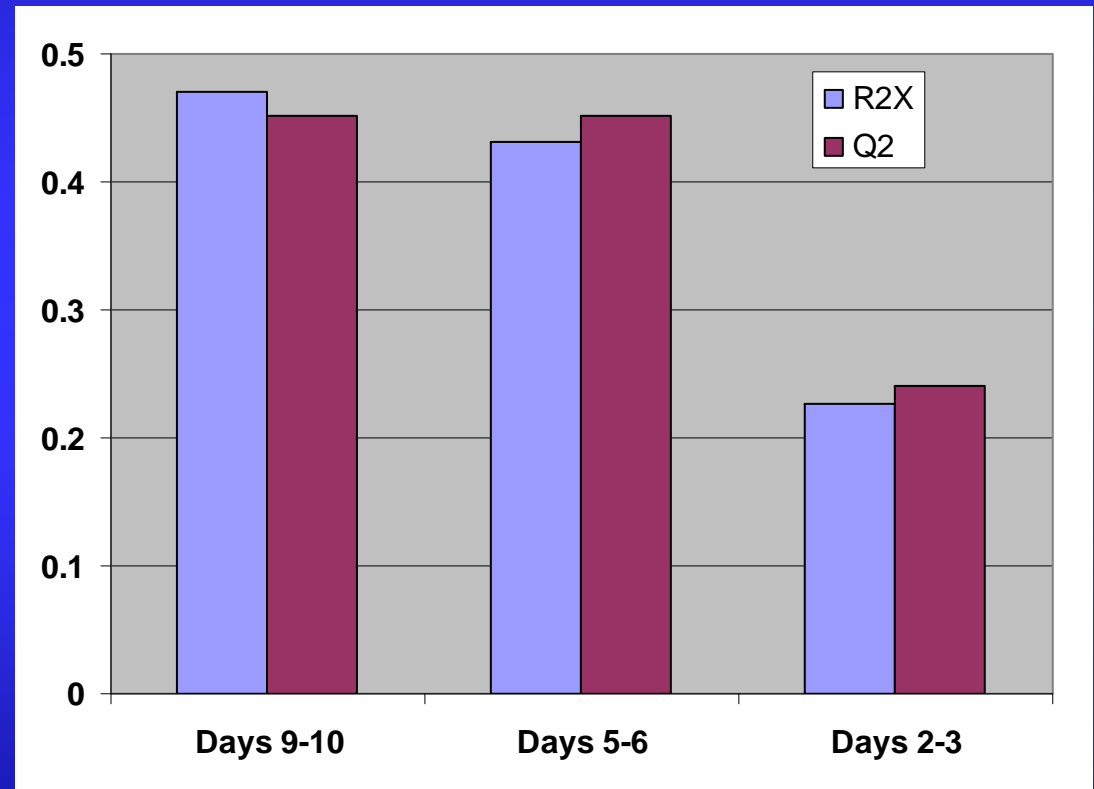
Pharmaco-Metabonomic Approach for the Hepatotoxicity in Humans



Pre-dose Metabolite Models Were Much Weaker

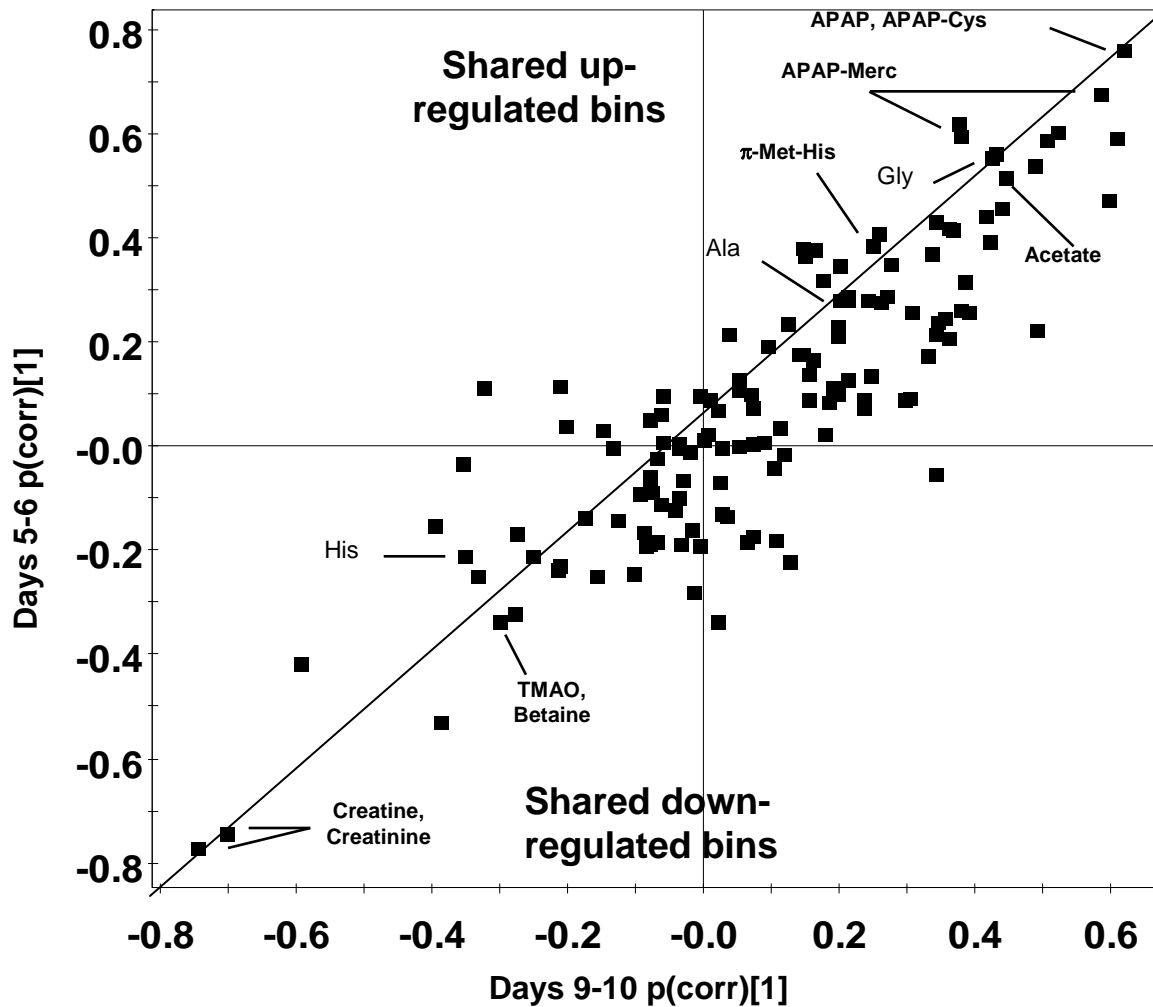


2-Component OPLS Model Statistics



Shared & Unique Biomarkers for Days 5-6 vs Days 9-10

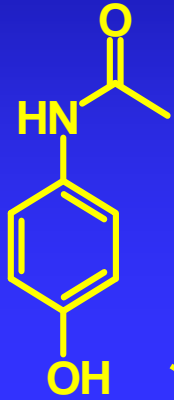
Coefficients from Days 5-6



Coefficients from Days 9-10

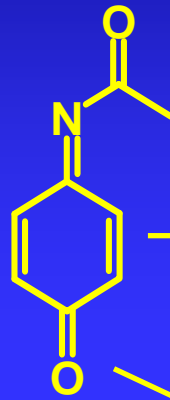
APAP Metabolite Levels

APAP



NADPH, O₂
Cytochrome P450

NAPQI



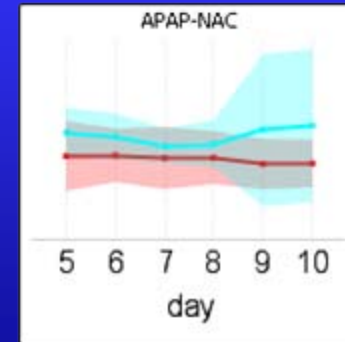
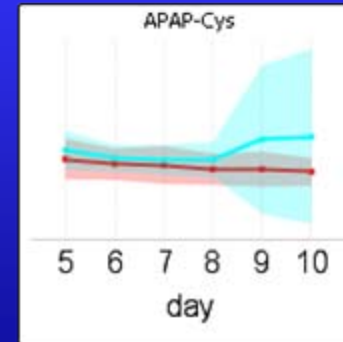
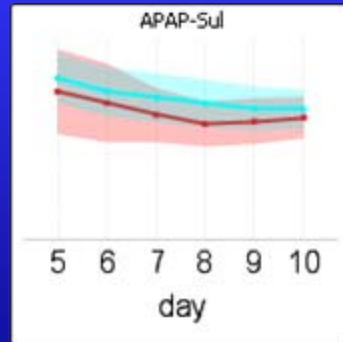
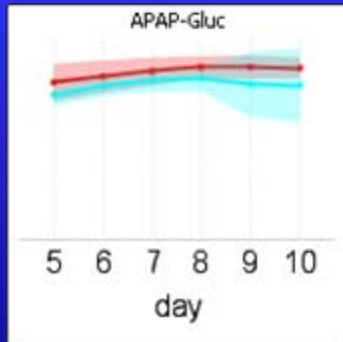
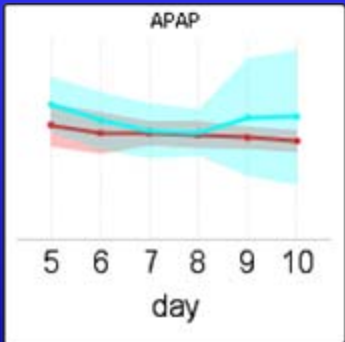
Protein
binding

Oxidative
stress
macromolecule

TOXICITY

conjugation

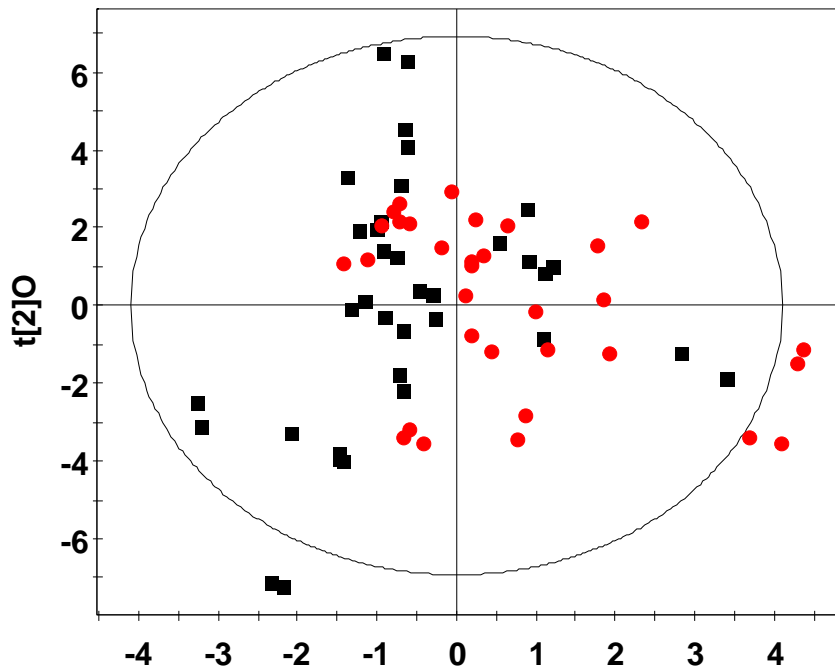
GSH
binding



— non-resp

— resp

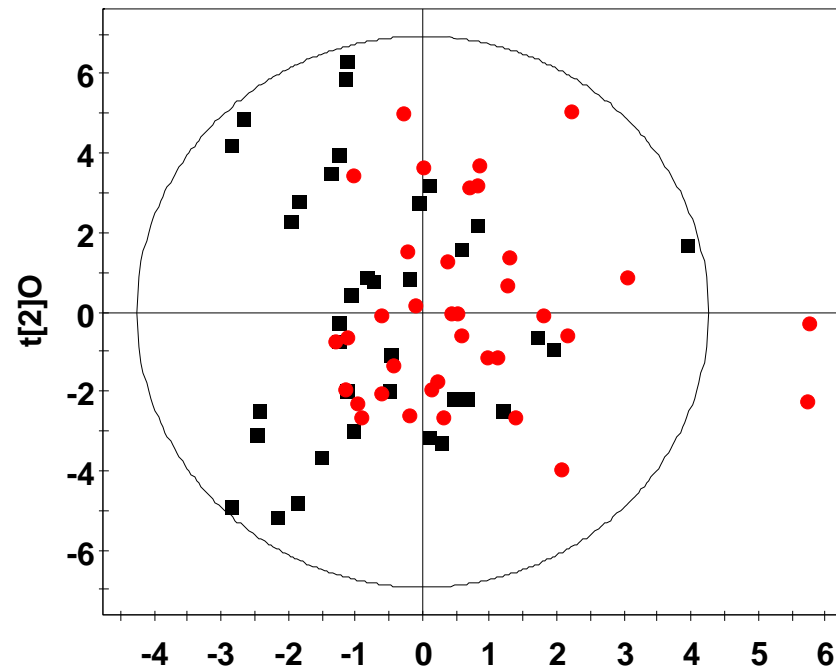
OPLS Days Using Only APAP Metabolites



Day 9-10

t[1]P
SIMCA-P+ 11 - 8/21/2008 11:52:33 AM

$R^2X = 0.91$
 $R^2Y = 0.16$
 $Q^2 = 0.09$



Day 5-6

t[1]P
SIMCA-P+ 11 - 8/21/2008 11:53:37 AM

$R^2X = 0.88$
 $R^2Y = 0.16$
 $Q^2 = 0.11$

Prediction Accuracy of OPLS Models

Confusion matrices

Days 9-10	Predicted	
Actual	Resp	Non-resp
Resp	23	9
Non-resp	9	27

Days 5-6	Predicted	
Actual	Resp	Non-resp
Resp	24	10
Non-resp	10	25

Days 2-3	Predicted	
Actual	Resp	Non-resp
Resp	19	12
Non-resp	11	24

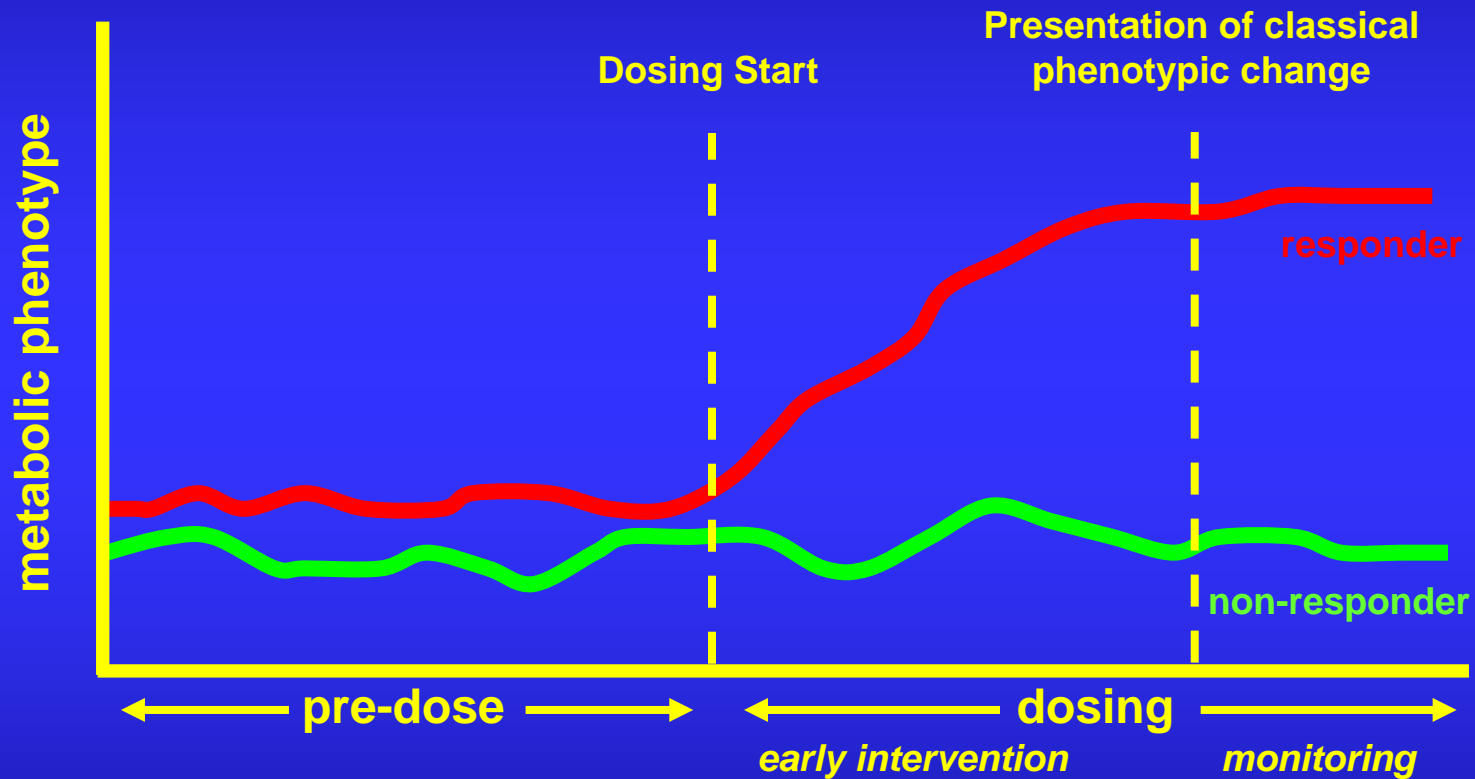
Predictive Accuracy

Accuracy	73.5%	(0.0004)
avg data	72.7%	(0.015)

Accuracy	71.0%	(0.038)
avg data	74.3%	(0.007)

Accuracy	65.2%	(0.153)
avg data	61.8%	(0.300)

Early Intervention Pharmaco-metabonomics



- Pre-dose profiles may not be predictive in many cases
- Early doses may evoke the predictive metabolic phenotype

Conclusions

- Metabolomics can distinguish responders from non-responders in urine
- Early biomarkers of toxicity (pre-ALT rise) are largely the same as the later biomarkers (during-ALT rise)
- Significant predictive capacity arises from combined endogenous and exogenous metabolite changes
- Early Intervention pharmaco-metabolomics is practical for predicting potential hepatotoxicity in *humans*

Acknowledgements

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