Mouse Populations Enable Translational Pharmacogenomic Approaches for Understanding and Predicting Adverse Drug Events

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Disclaimer

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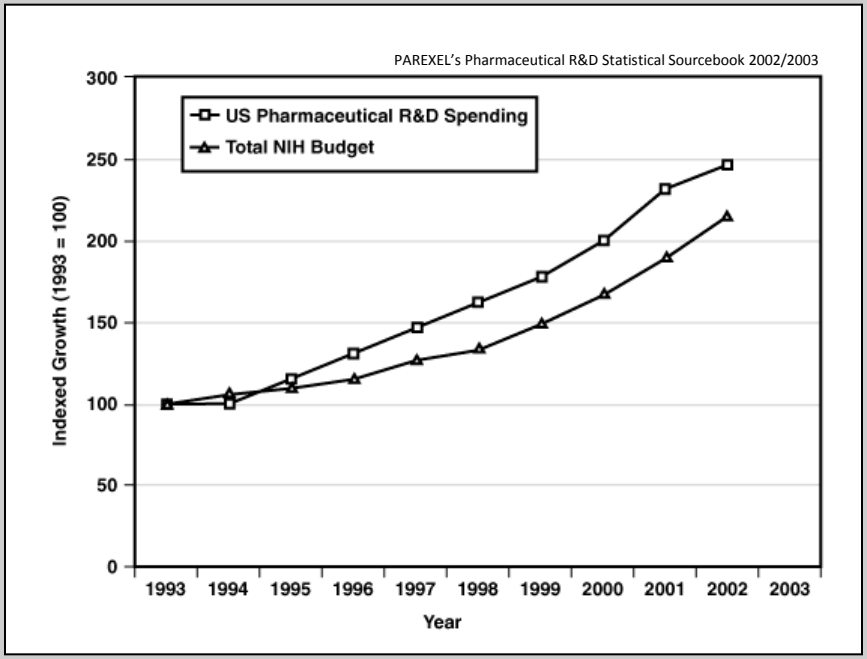
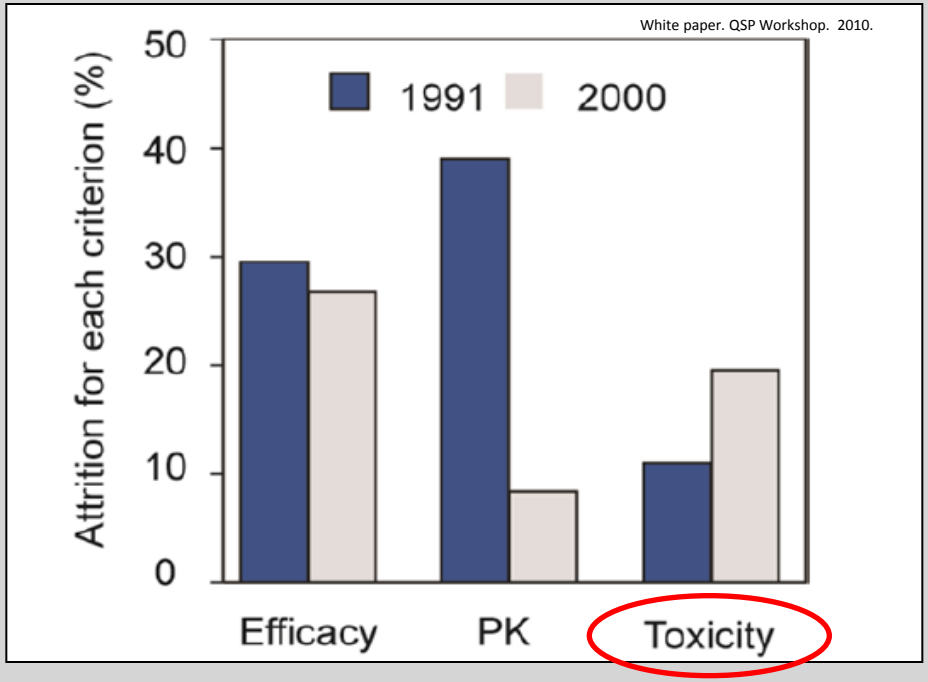
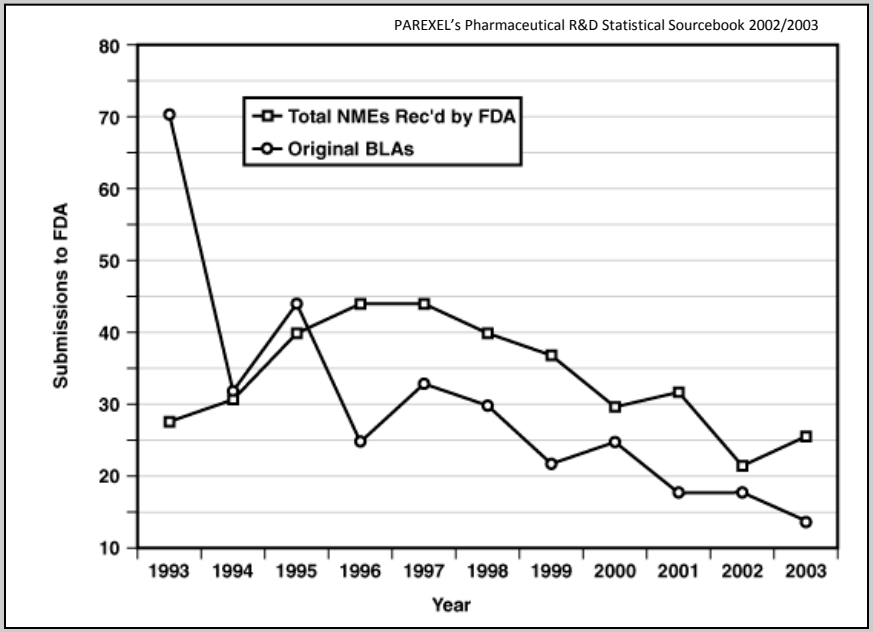






The pharmaceutical industry is facing a *productivity crisis:*

Rate of FDA approvals of new drugs fell by 50% in the past decade

- toxicity a greater attrition factor than in the past

Drug safety becoming a major bottleneck in regulatory approval as the science of drug safety lags behind that of drug efficacy

Even worse – Drugs that appear safe initially may cause severe toxicities in sensitive patients

- Toxicities of (selected) manufactured drugs occurring in the recent modern regulatory era:
  - 1983: Zimelidine (SSRI): Guillain-Barré syndrome
  - 2000: Troglitazone (diabetes): idiosyncratic hepatotoxicity
  - 2004: Vioxx (NSAID): mycardial infarction
  - 2006: Exanta (anticoagulant): idiosyncratic hepatotoxicity
  - 2008: Lumiracoxib (COX-2 inhibitor): idiosyncratic hepatotoxicity
  - 2010: Sibutramine/Meridia (anorexiant): stroke, cardiovascular events

FDA Priorities for Advancing Regulatory Science for Public Health

2011 Strategic Plan

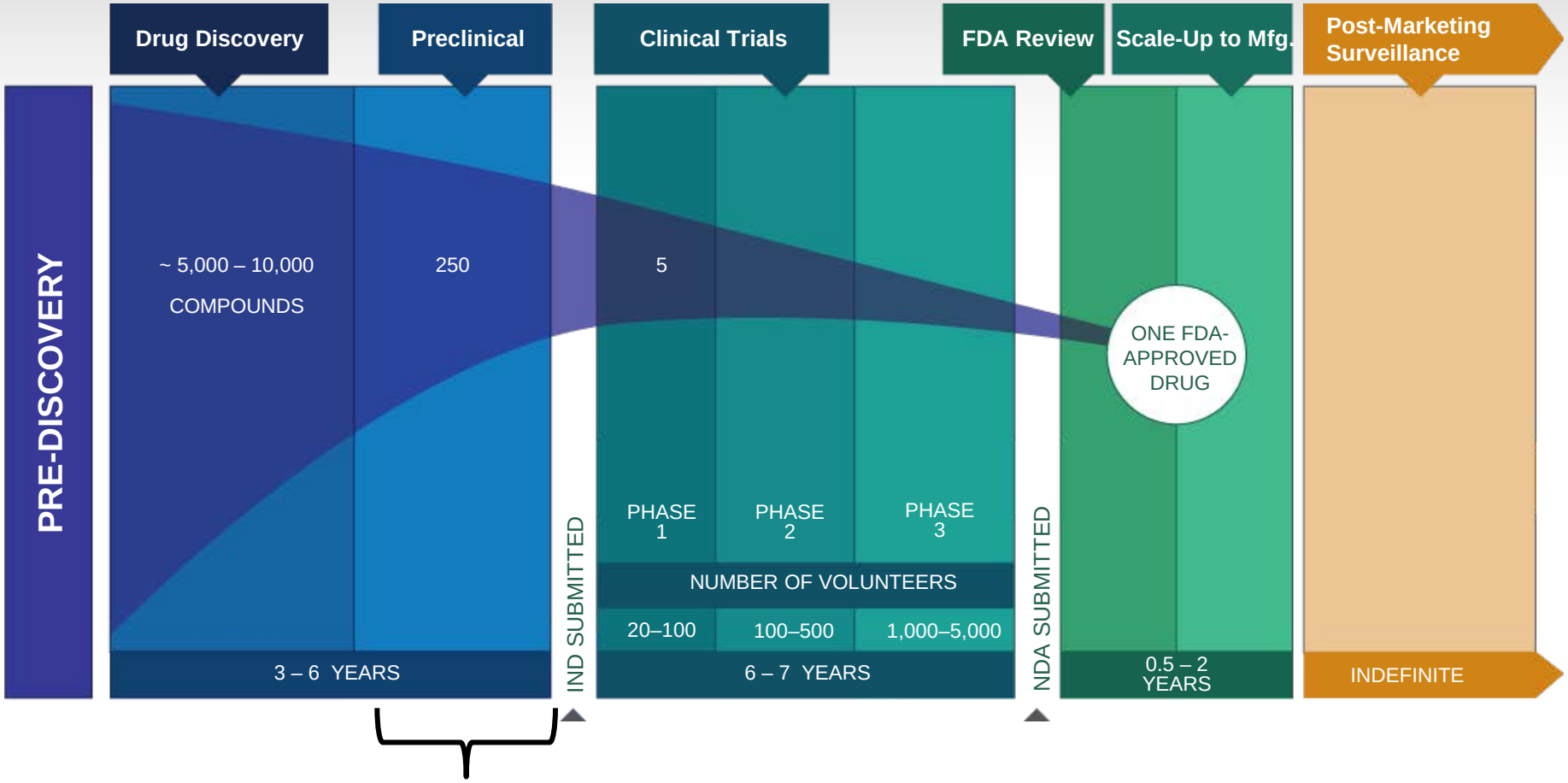
Advancing Innovation with Better Regulatory Science

The FDA is in the middle of an effort to improve regulatory science and how new medicines are approved.

Pharma Voice. May 2013.

1. Modernize toxicology to enhance product safety
2. Stimulate innovation in clinical evaluations and personalized medicine
3. Support new approaches to improve product manufacturing and quality
4. Ensure FDA readiness to evaluate emerging technologies
5. Harness diverse data through information sciences to improve health outcomes
6. Implement a prevention-focused food safety system to protect public health
7. Facilitate development of medical countermeasures
8. Strengthen social and behavioral science to help make informed decisions about regulated product

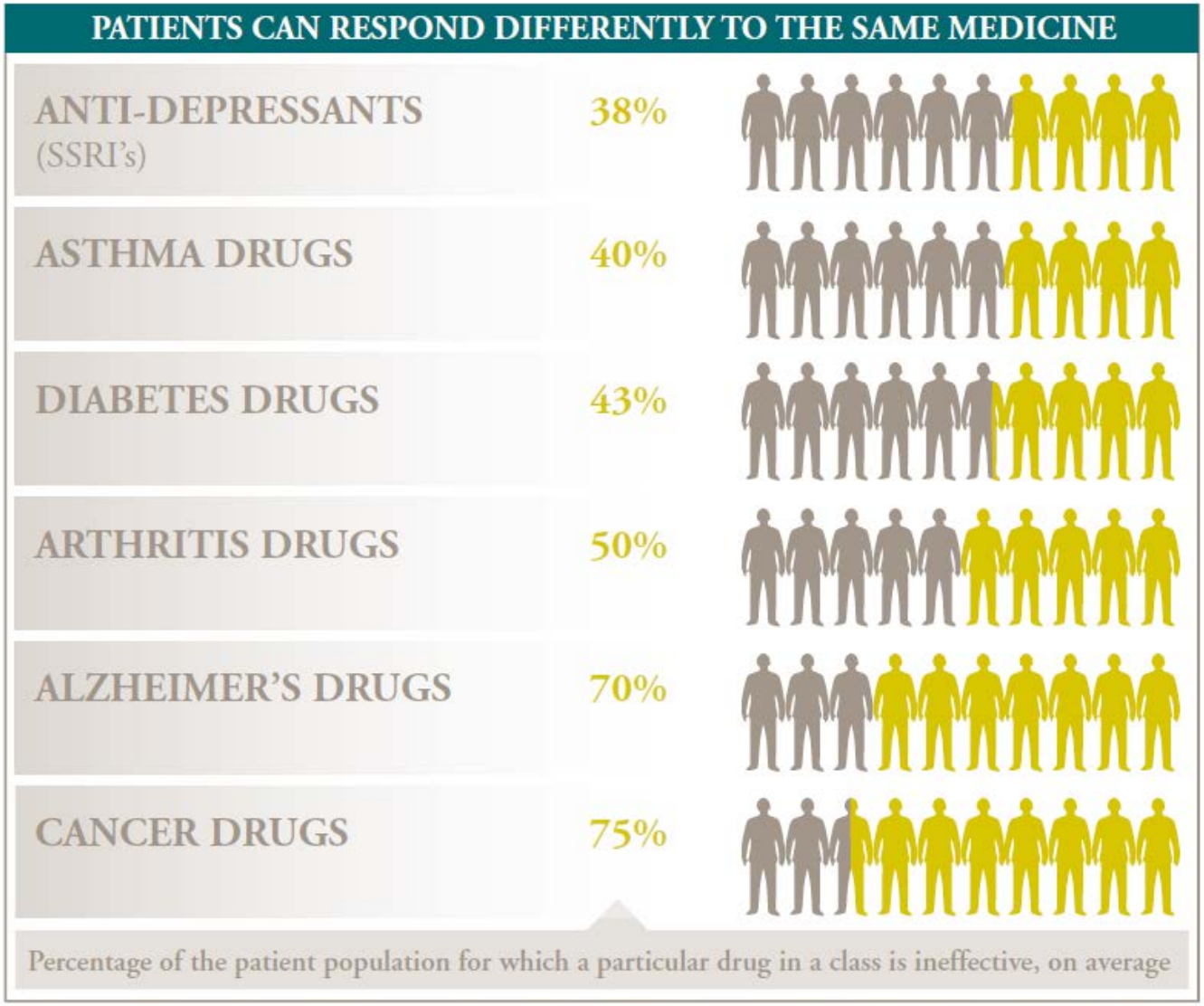
Attrition During Drug Development is Costly



Standard animals: How much like humans are they?

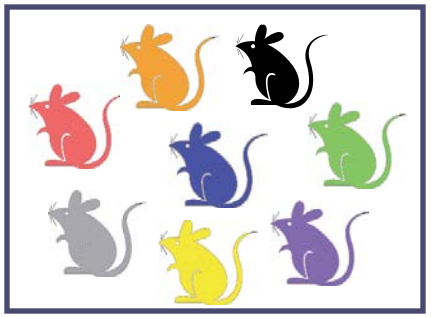
Sources: Drug Discovery and Development: Understanding the R&D Process, www.innovation.org; CBO, *Research and Development in the Pharmaceutical Industry*, 2006.; [1]. DiMasi and H. Grabowski, "The Cost of Biopharmaceutical R&D: Is Biotech Different?," *Managerial and Decision Economics*, 2007

Even Humans Aren't a Good Model for Humans!



Source of data: Brian B. Spear, Margo Heath-Chiozzi, Jeffrey Huff, "Clinical Trends in Molecular Medicine, Volume 7, Issue 5, 1 May 2001, Pages 201-204.

Using Genetically Diverse Mouse Populations to Identify Safety Concerns and Genes Associated with Toxicity



Genetically Diverse Mouse Population

Translate results →

Genetically Diverse Human Population

Genetically defined rodent populations may provide ability to:

1. Better predict safety
2. Identify genetic factors that predict an individual patient's risk (or benefit), thereby:
   - Allowing otherwise efficacious drugs to remain on the market
   - Providing insight into mechanisms that guide design of next-in-class compounds

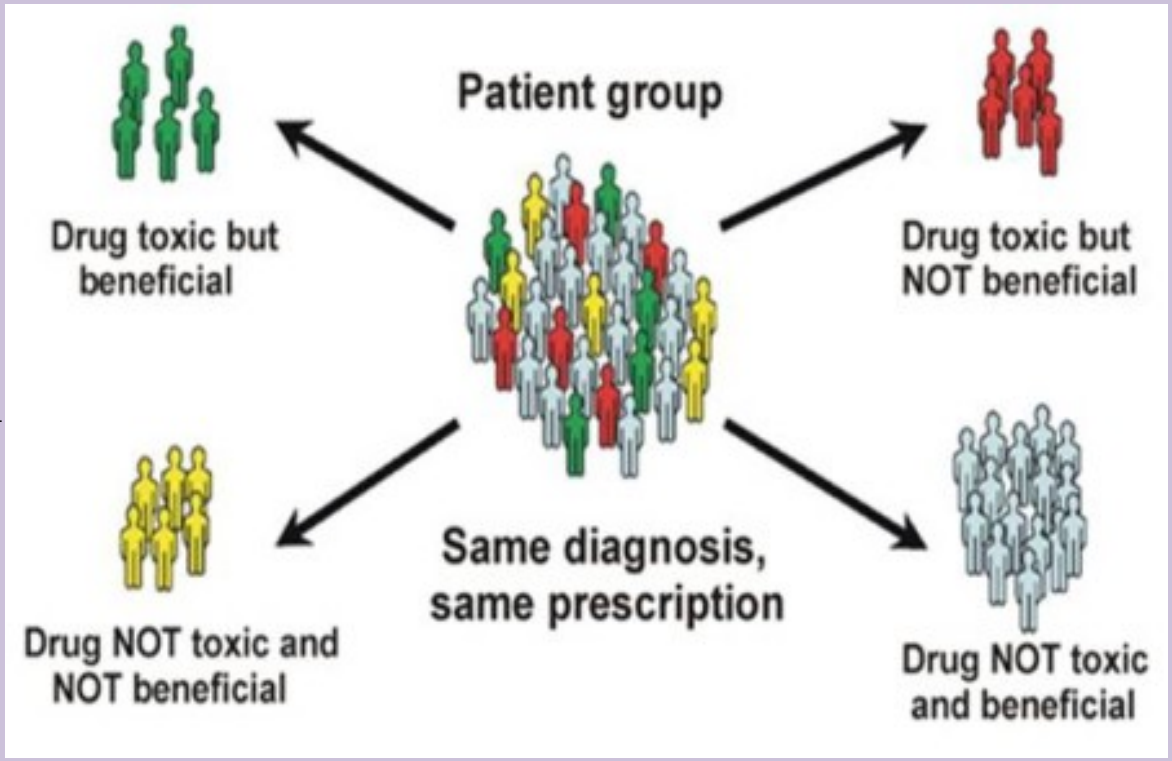
Additional benefits:

- Ethical concerns might preclude treating humans with a compound known to be toxic, but for which the mechanisms remain to be elucidated
- Some adverse reactions are so rare that it is a major challenge to identify and catalog samples from enough patients to sufficiently power GWAS

Genetically Diverse Mouse Populations – Implementation #1:

Identification of Genetic Risk Factors for Drug Toxicity

Personalize Medicine for Each Patient

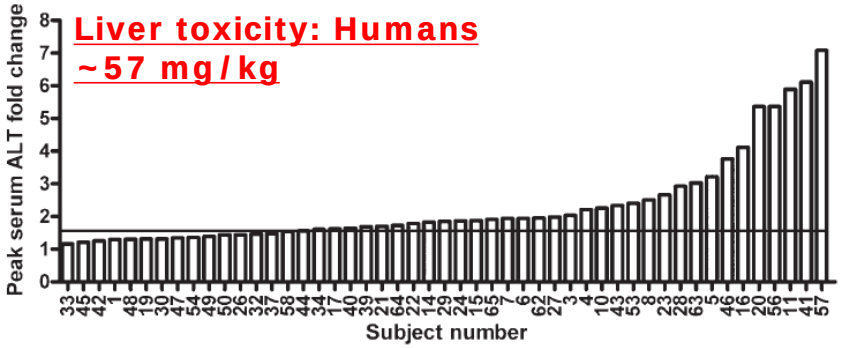


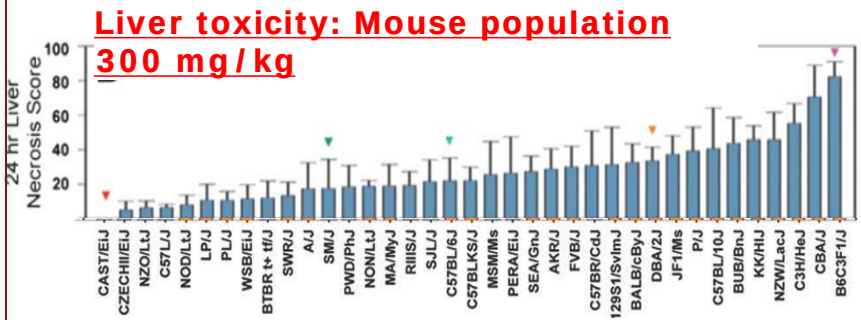
www.pharmainfo.net

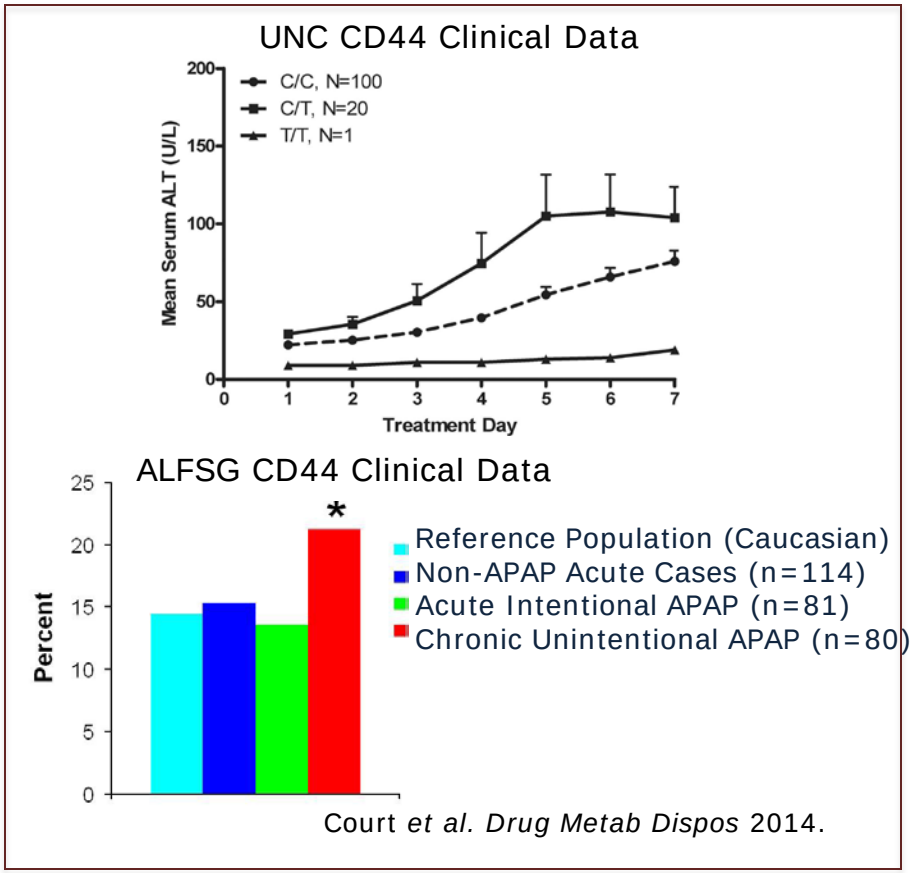
Molecular Taxonomy of Disease: Classifying the patient's disease by molecular or genetic signature instead of symptoms

Acetaminophen Toxicity in Mouse and Human Populations

Acetaminophen-induced liver injury



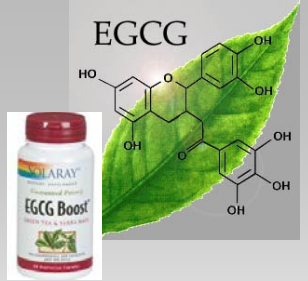




Court *et al. Drug Metab Dispos* 2014.

RESULT: Enrichment of CD44 variant in ALF cases due to chronic acetaminophen use.

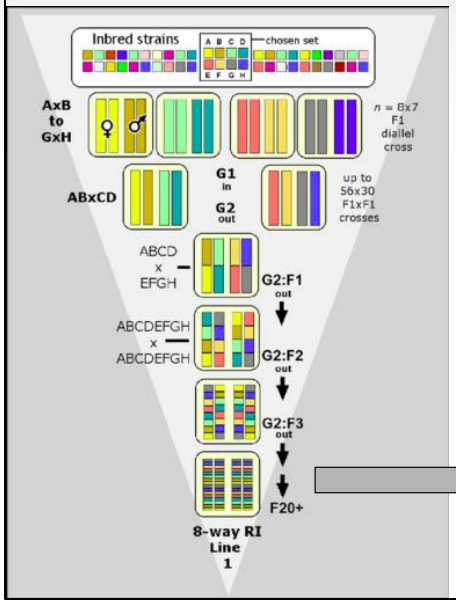
Harrill, AH *et al. Genome Res*. 2009.

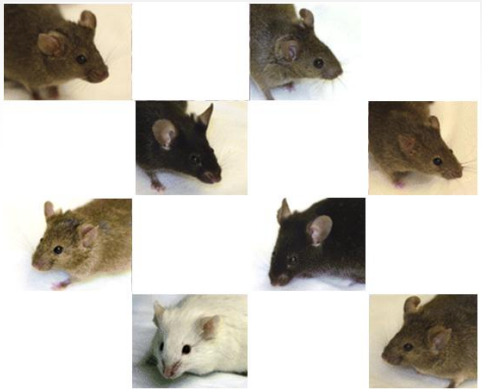


Pharmacogenetics of EGCG DILI

- Epigallocatechin gallate (EGCG) is a minor component of green tea with antioxidant properties
- Concentrated green tea extract herbal supplement use has led to rare cases of severe DILI with no obvious link to dose
- **Key collaborators**: Gary Churchill and Dan Gatti (Jackson Laboratory); David Threadgill (NC State), Yi Xang, Qiang Shi (NCTR), Paul Watkins, Tom Urban (DILIN), Rachel Church (former postdoc)

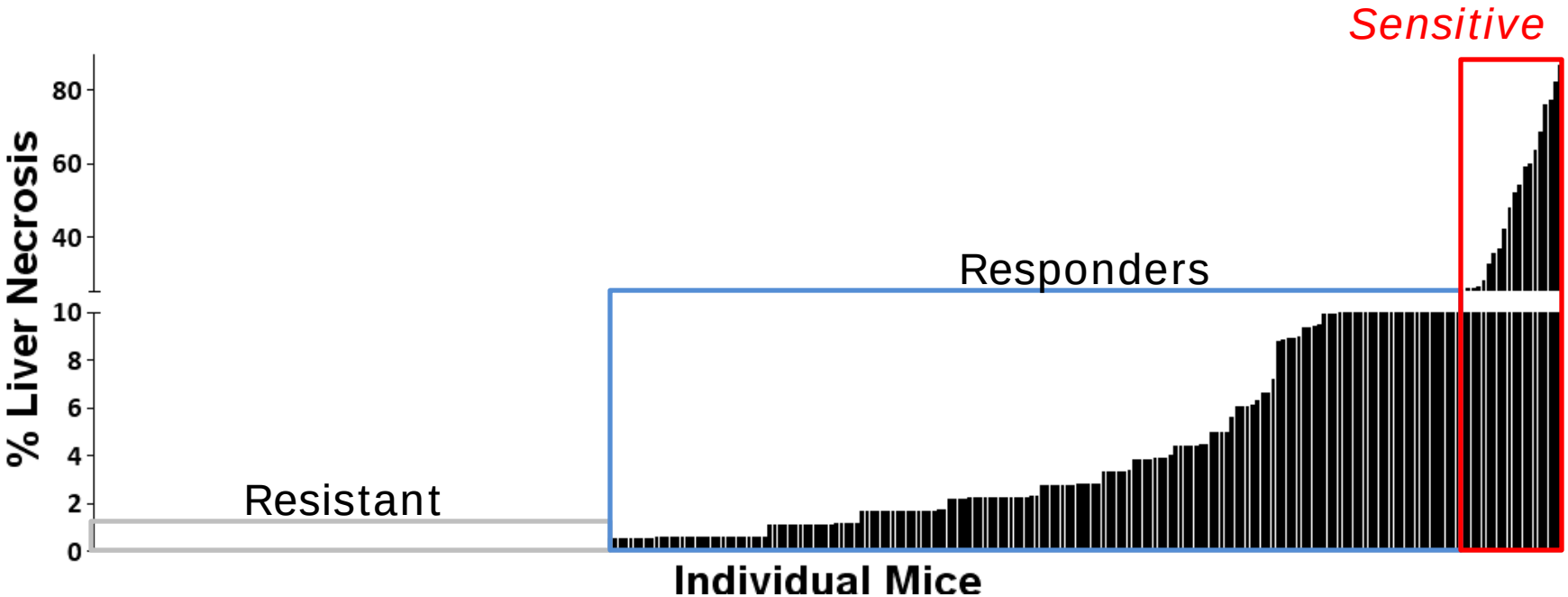
Collaborative Cross → Diversity Outbred



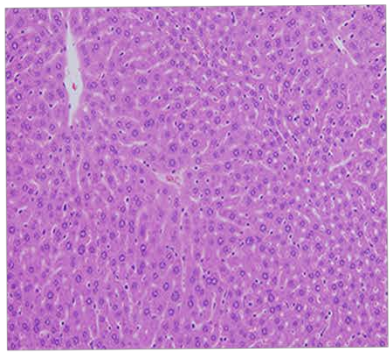


DO Initiation
144 CC lines between G2:F4 & G2:F12

A Sensitive Subpopulation Identified Using Diversity Outbred Mice

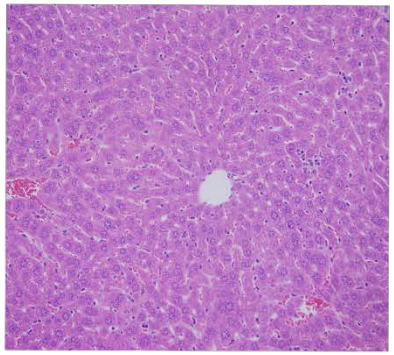


Vehicle



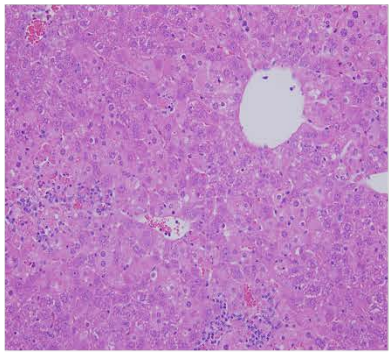
0 mg/kg

Resistant



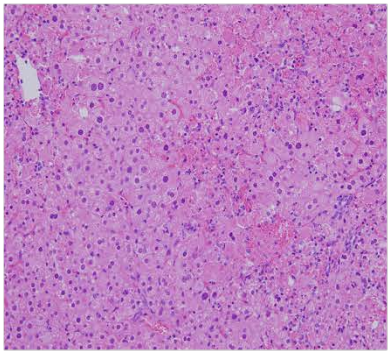
50 mg/kg

Responder



50 mg/kg

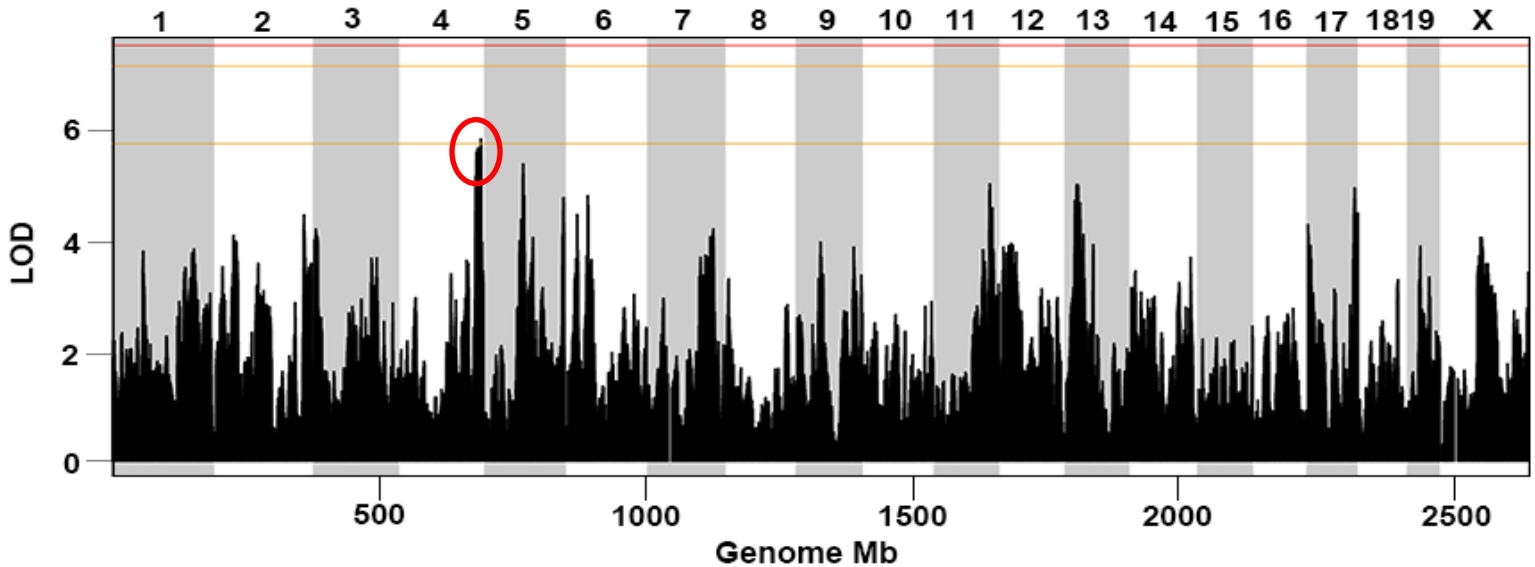
Sensitive

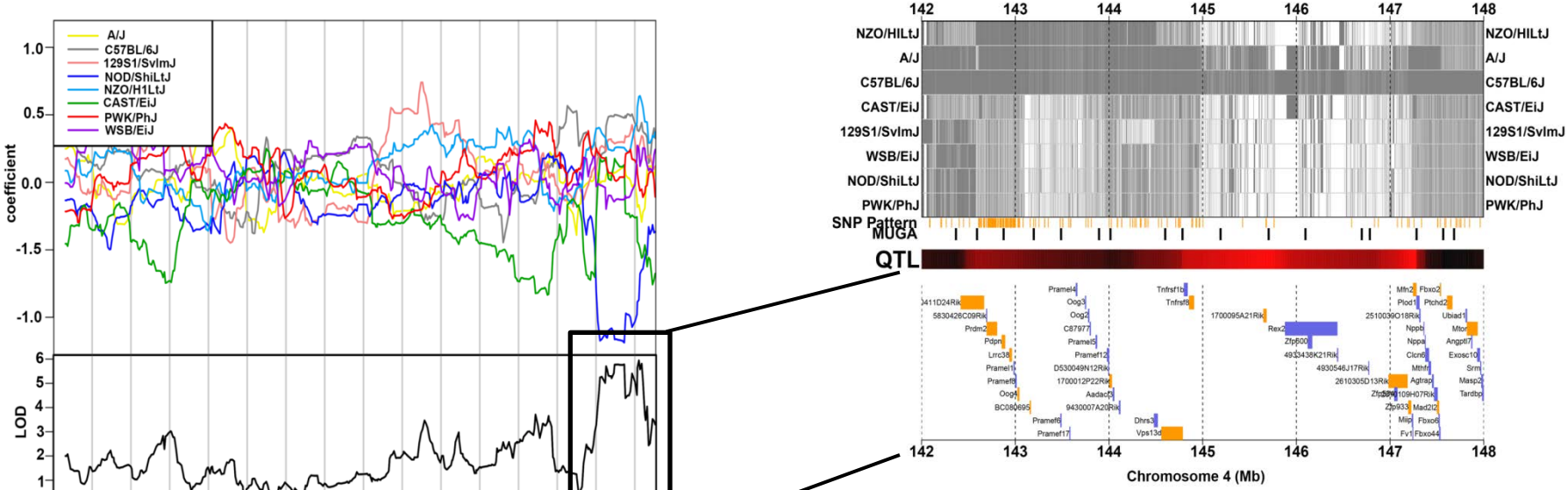


50 mg/kg

Church et al. Tox Sci. 2014

Identification of Genes that Modulate EGCG DILI Outcomes





Church et al. Tox Sci. 2014

Translation to Human Patients

Gene Symbol	SNP (Array)	Gene Name	Chromosome	Position	P value	Risk/Protective allele	Effect
RNF207	exm8990	ring finger protein 207	1	6278414	0.00478	G/A	
RNF207	exm8997	ring finger protein 207	1	6279370	0.006987	G/C	
PER3	exm10762	period circadian clock 3	1	7887234	0.004937	T/C	
MFN2	exm15928	mitofusin 2	1	12069692	0.0067	A/G	Missense (I/V)
GPR153	exm9066	G protein-coupled receptor 153	1	6310562	0.016703	C/G	
VPS13D	exm16480	vacuolar protein sorting 13 homolog D (S. cerevisiae)	1	12343493	0.043064	A/T	

- In this case, whole genome SNP genotyping had been performed on patient samples for another study

- Mitofusin 2: Involved in mitochondrial fusion and autophagy

- Previous study confirmed EGCG inhibits respiratory complexes in stressed mitochondria Weng et al. Biochem Biophys Res Commun 2014

Genetically Diverse Mouse Populations – Implementation #2:

Improve Prediction of Human Toxicity Risk & Mechanistic Analysis

PF-04287881, Novel antibiotic that failed Phase I Clinical Trials due to Hepatotoxicity

Key Collaborators: Karissa Adkins & Hong Wu (Pfizer); Merrie Mosedale (former postdoc fellow)

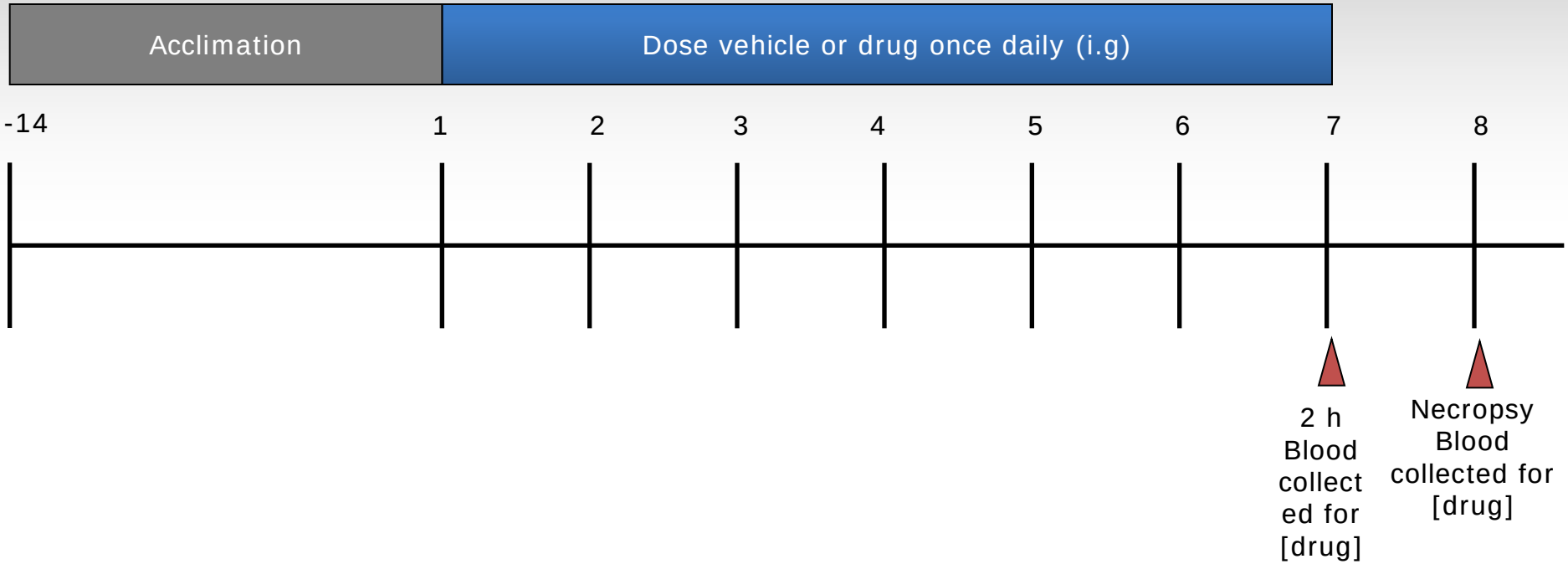
- PF-04287881 -
  - Ketolide antibiotic under development at Pfizer
  - Like other cationic amphiphilic antibiotics, there were indications of the presence of phospholipidosis and liver injury in rodents and dog
    - Other examples: azithromycin and erythromycin
    - Well tolerated at 30X the anticipated clinical exposure
  - Development discontinued following mildly increased LFTs in Phase 1 trial
    - Inter-individual variation observed

Mosedale *et al. TAAP* 2014.

PF-04287881, Novel antibiotic that failed Phase I Clinical Trials due to Hepatotoxicity

- **Goal 1**: Identify sensitive and resistant strains of mice to 881 hepatotoxicity
- **Goal 2**: Characterize the injury
- **Goal 3**: Elucidate molecular pathways that distinguish responders from nonresponders

PF-04287881, Novel antibiotic that failed Phase I Clinical Trials due to Hepatotoxicity



- Dose range finding study in CD-1 mice
- 600 mg/kg (i.g) or vehicle for 7 days
- 34 inbred strains (classical MDP)

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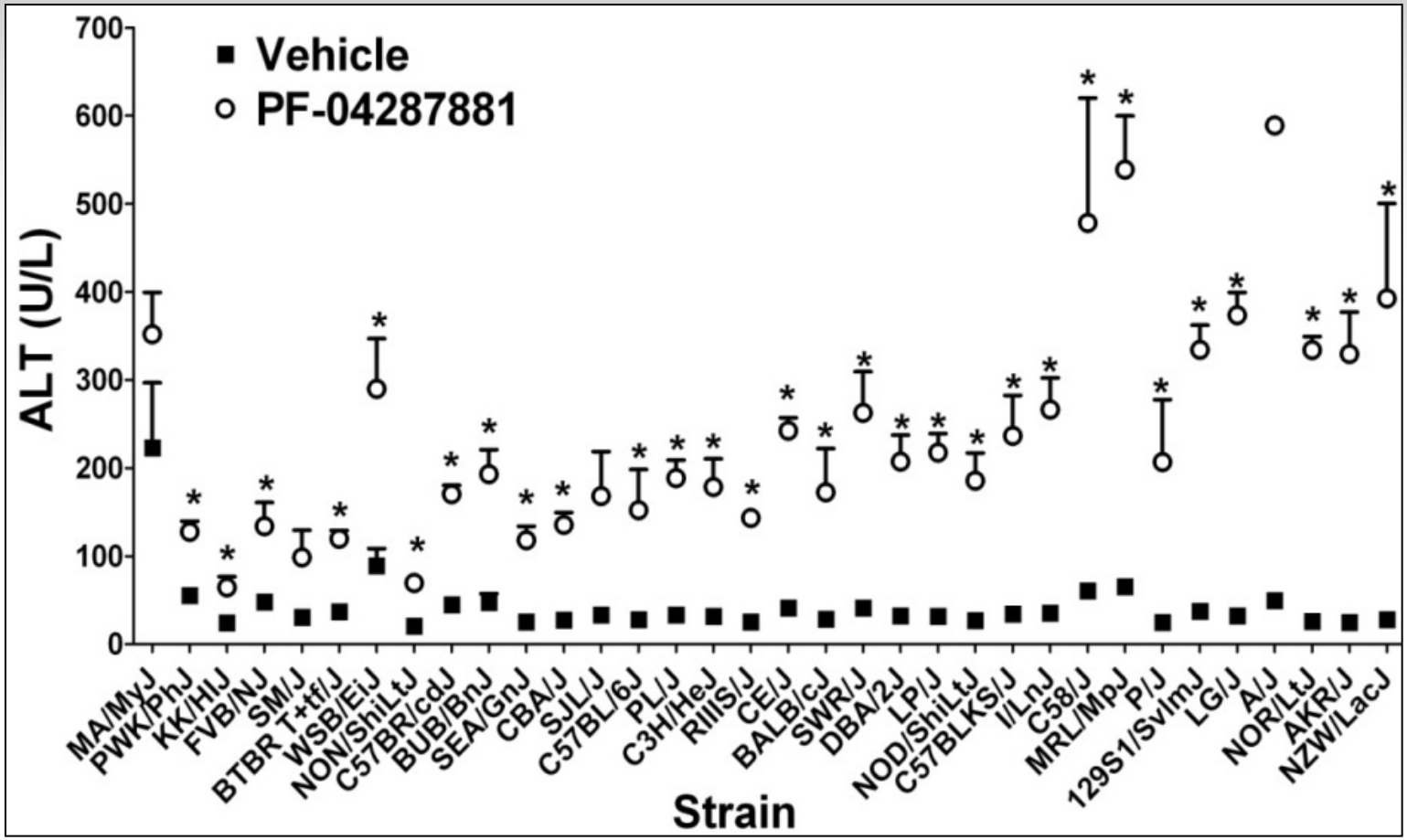
PF-04287881, Novel antibiotic that failed Phase I Clinical Trials due to Hepatotoxicity



RESULT: 881 elicited liver toxicity in a majority of strains tested. Identified responder and nonresponder strains.

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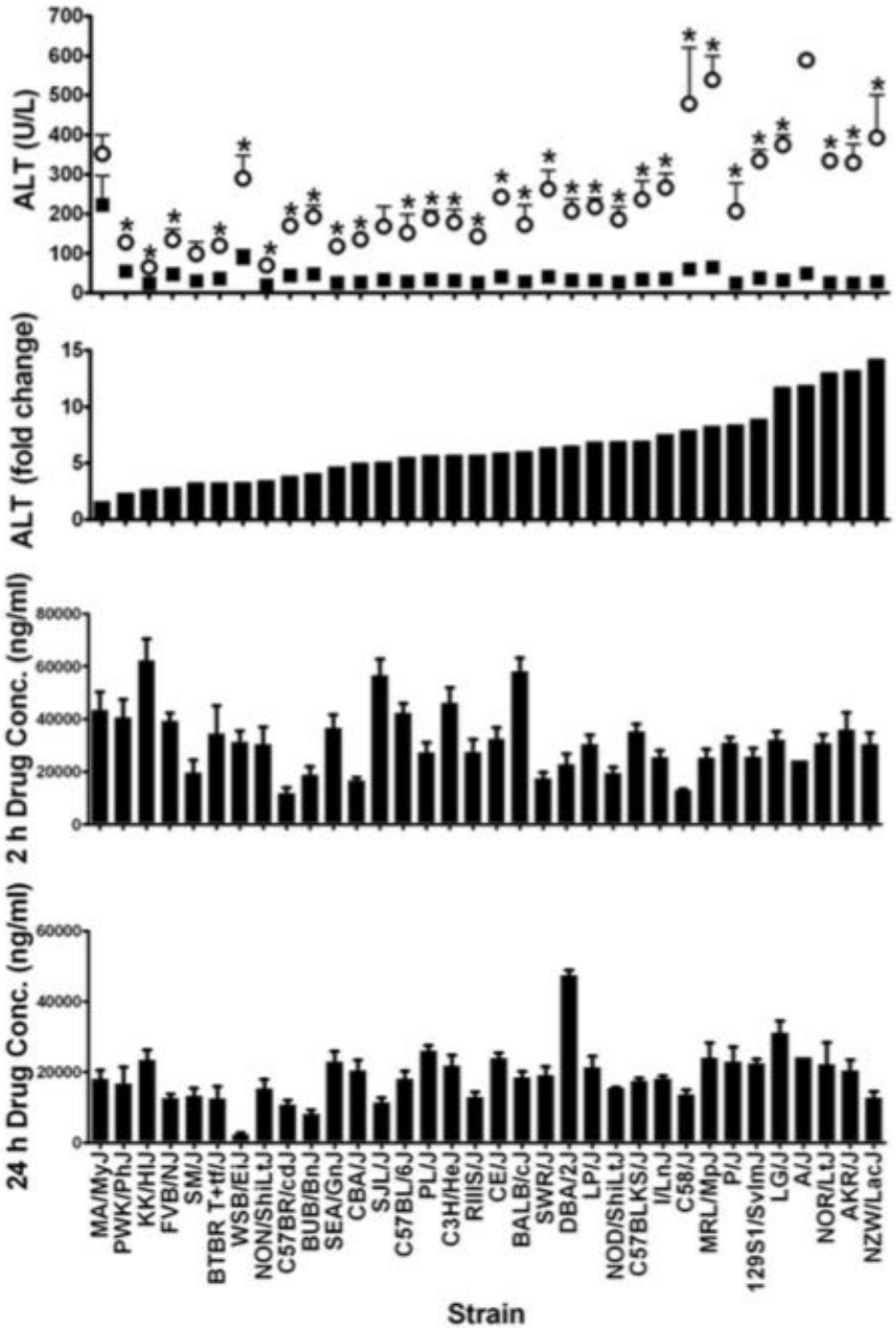


RESULT: ALT did not correlate with plasma drug concentration or AUC

Mosedale *et al. TAAP* 2014.

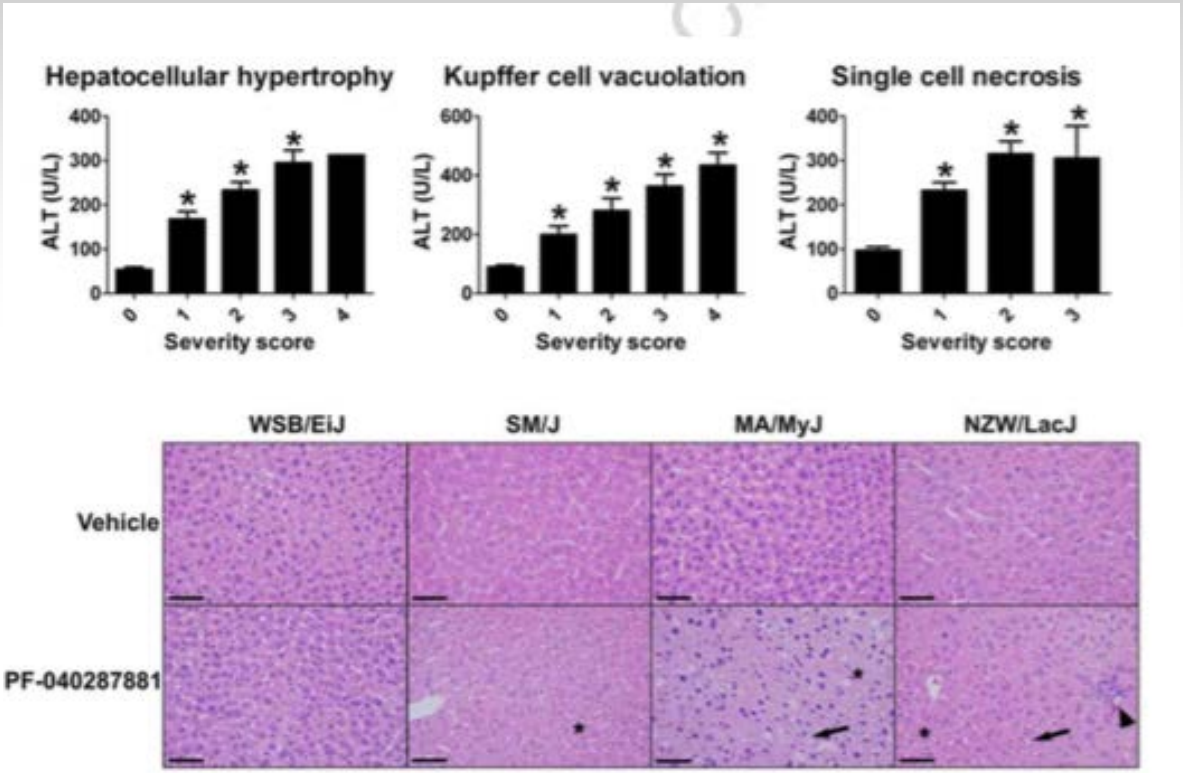
Selection of Strains for Gene Expression Studies

COMMON MISCONCEPTION: You have to do every mouse population-based experiment in 300-400 animals.

FACT: Not necessarily the case. Once you have determined sensitive genetic backgrounds, you can focus your follow up studies in selected strains.

This is also true for the DO mice once the genetic basis for the phenotype has been established.

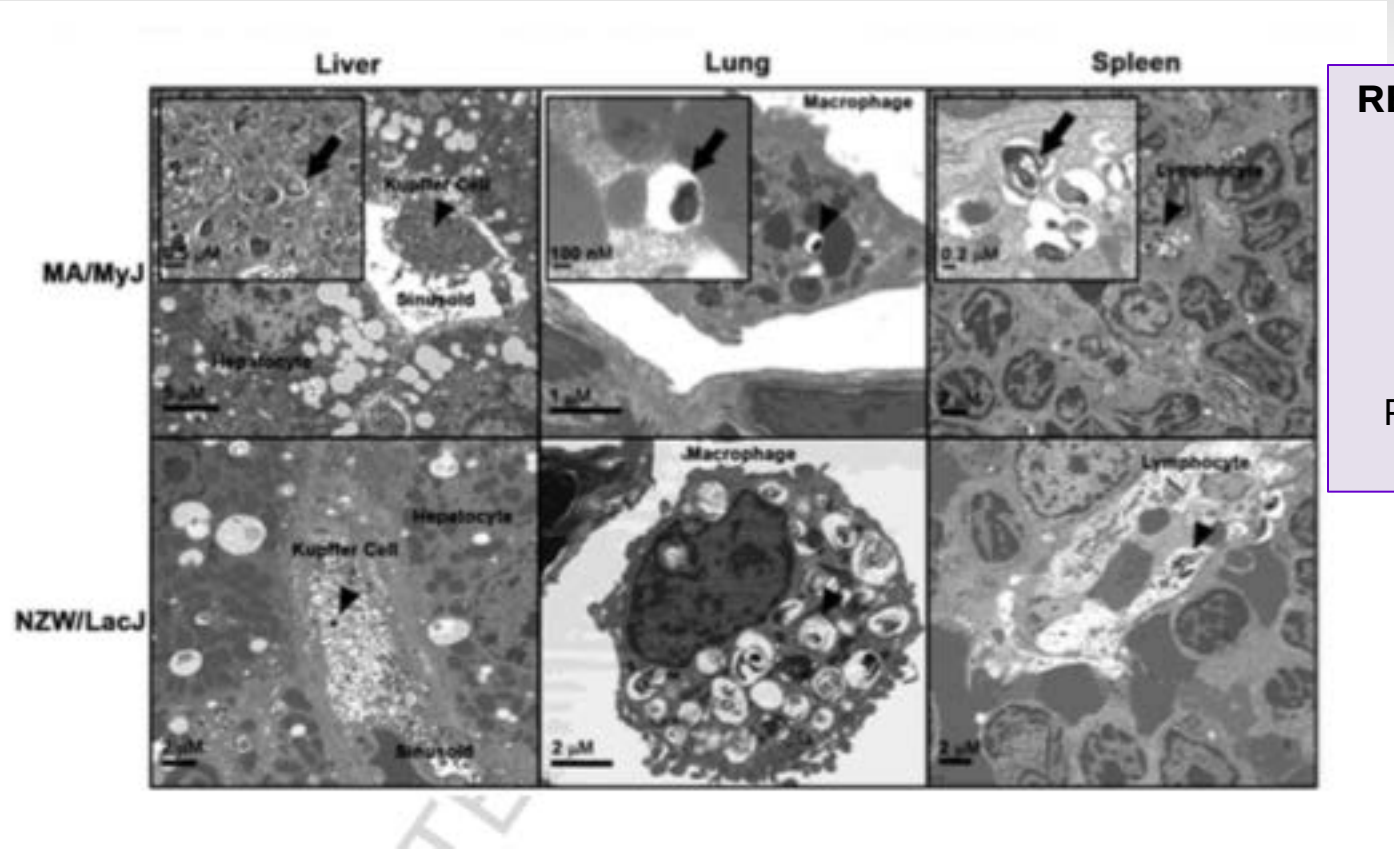
RESULT: Degree of KC vacuolation is correlated with ALT.



Finding	WSB/EiJ	SM/J	MA/MyJ	NZW/LacJ
Hepatocellular Hypertrophy	-	+	+	+
Kupffer Cell Vacuolation	-	-	+	+
Single Cell Necrosis	-	-	-	+

Mosedale *et al. TAAP* 2014.

Characterization of histologic vacuoles as phospholipidosis



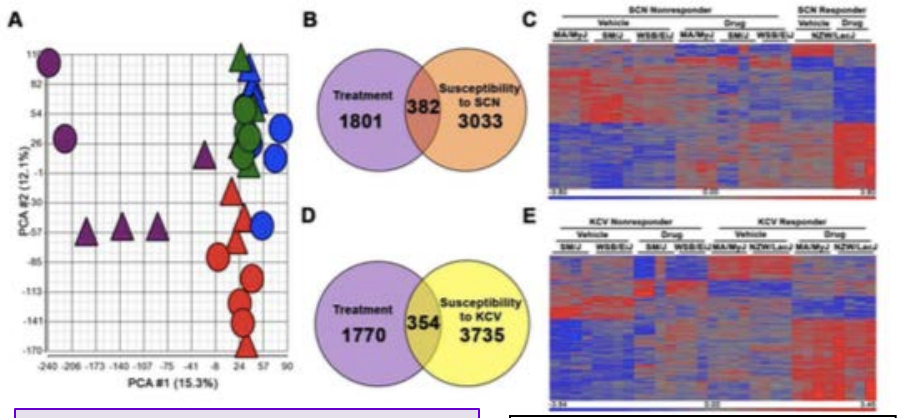
RESULT: Of 4 strains tested

The two strain with KC vacuolation (H&E) had characteristic phospholipidosis (EM)

Present in liver, lung, and spleen

Mosedale *et al. TAAP* 2014.

PF-04287881 Caused Single Cell Necrosis in Sensitive Strains that was Correlated with ALT



RESULT: miR-122 release into plasma confirmed liver origin of ALT.

Transcripts for which expression changes were significant by responder status and by treatment group were highly associated with protein ubiquitination and lysosomal function.

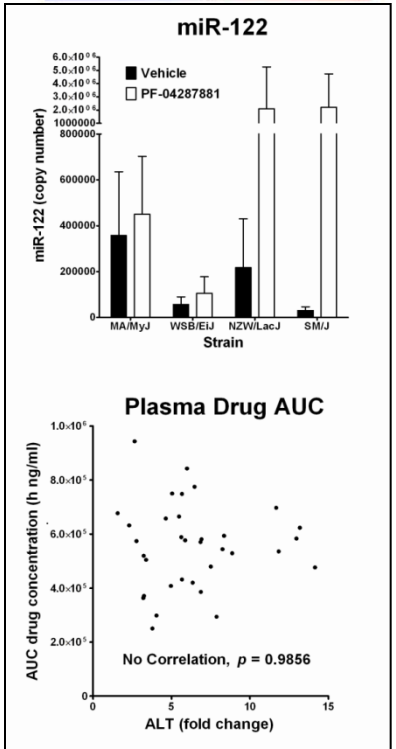


Table 1
Genes in the protein ubiquitination pathway that are altered with PF-04287881-induced hepatocellular single cell necrosis.

Gene symbol	Gene title
Decreased	
Psmb5	proteasome subunit, beta type, 5
Psmc5	proteasome 26S subunit, ATPase, 5
Psmc3	proteasome 26S subunit, ATPase, 3
Psmc4	proteasome 26S subunit, ATPase, 4
Psma6	proteasome subunit, alpha type, 6
Ube2g2	ubiquitin-conjugating enzyme E2G 2
Psmc6	proteasome 26S subunit, ATPase, 6
Hspa9	heat shock 70kDa protein 9
Usp48	ubiquitin specific peptidase 48
Psmd6	proteasome 26S subunit, non-ATPase, 6
Psma5	proteasome subunit, alpha type, 5
Psmd4	proteasome 26S subunit, non-ATPase, 4
Psmc2	proteasome 26S subunit, ATPase, 2
Hspa1a/Hspa1b	heat shock 70kDa protein 1A
Hspb6	heat shock protein, alpha-crystallin-related, B6
Psmd3	proteasome 26S subunit, non-ATPase, 3
Psmd12	proteasome 26S subunit, non-ATPase, 12
Psmd7	proteasome 26S subunit, non-ATPase, 7
Increased	
Psmb8	proteasome subunit, beta type, 8
Hla-B	major histocompatibility complex, class I, B
Dnajc16	DnaJ (Hsp40) homolog, subfamily C, member 16
Ube4b	ubiquitination factor E4B
B2m	beta-2-microglobulin

ACCEPTED MANUSCRIPT

Mosedale *et al. TAAP* 2014.

CPDD
Consortium FOR
Parasitic Drug Development

THE UNIVERSITY
of NORTH CAROLINA
at CHAPEL HILL

DB289 - Test case for improving prediction of human-relevant adverse events

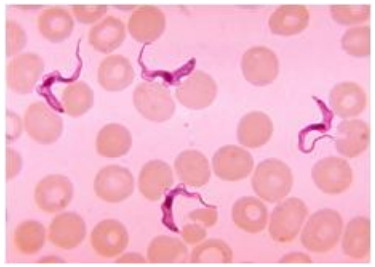
- DB289 – promising new drug to treat infection of trypanosomal parasites (sleeping sickness), a fatal disease endemic to sub-Saharan Africa

Consortium FOR Parasitic Drug Development

THE UNIVERSITY of NORTH CAROLINA at CHAPEL HILL

DB289 Clinical Trials Efficacy

- Phase II
  - Democratic Republic of Congo (DRC), Angola
  - 93% cure rate at 3 mo. post-treatment
  - No safety concerns
- Phase III
  - DRC, Angola, Sudan
  - Hepatotoxicity of DB289 <<< pentamidine
    - (7 vs. 77%)
  - 84% cure rate 24 mo. post-treatment



DB289 Clinical Trials Expanded Phase I Safety

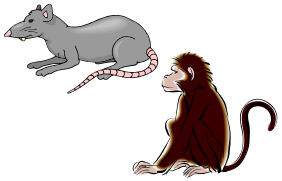
- Conducted in South Africa

- Frequent hepatobiliary adverse events put study on hold
  - 28 volunteers (35%) with ALT ≥ 3X ULN
  - 5 volunteers (6%) with bili ≥ 1.5X ULN

- Renal toxicity in 6 volunteers terminated development
  - Acute renal insufficiency
  - 5 hospitalized/ 1 outpatient
  - 1 volunteer required prolonged dialysis

Could the MDP Approach Detect the Kidney Toxicity Liability?

Preclinical testing

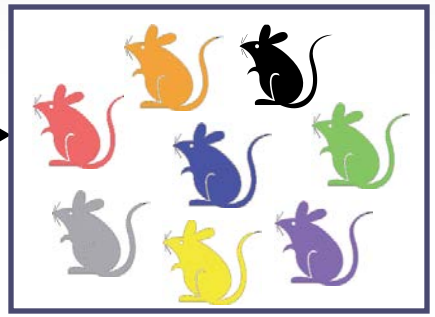


Predicted: efficacy, hepatotoxicity
Nor predicted: renal toxicity

Clinical Testing

Hepatotoxicity,
Renal insufficiency in expanded safety Phase I

Mouse Diversity Panel

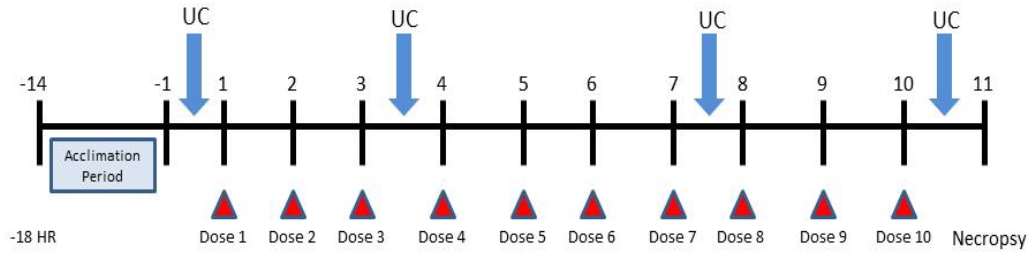


Hypothesis:
Susceptible strains will detect renal toxicity liability,
Mechanistic insight, biomarkers

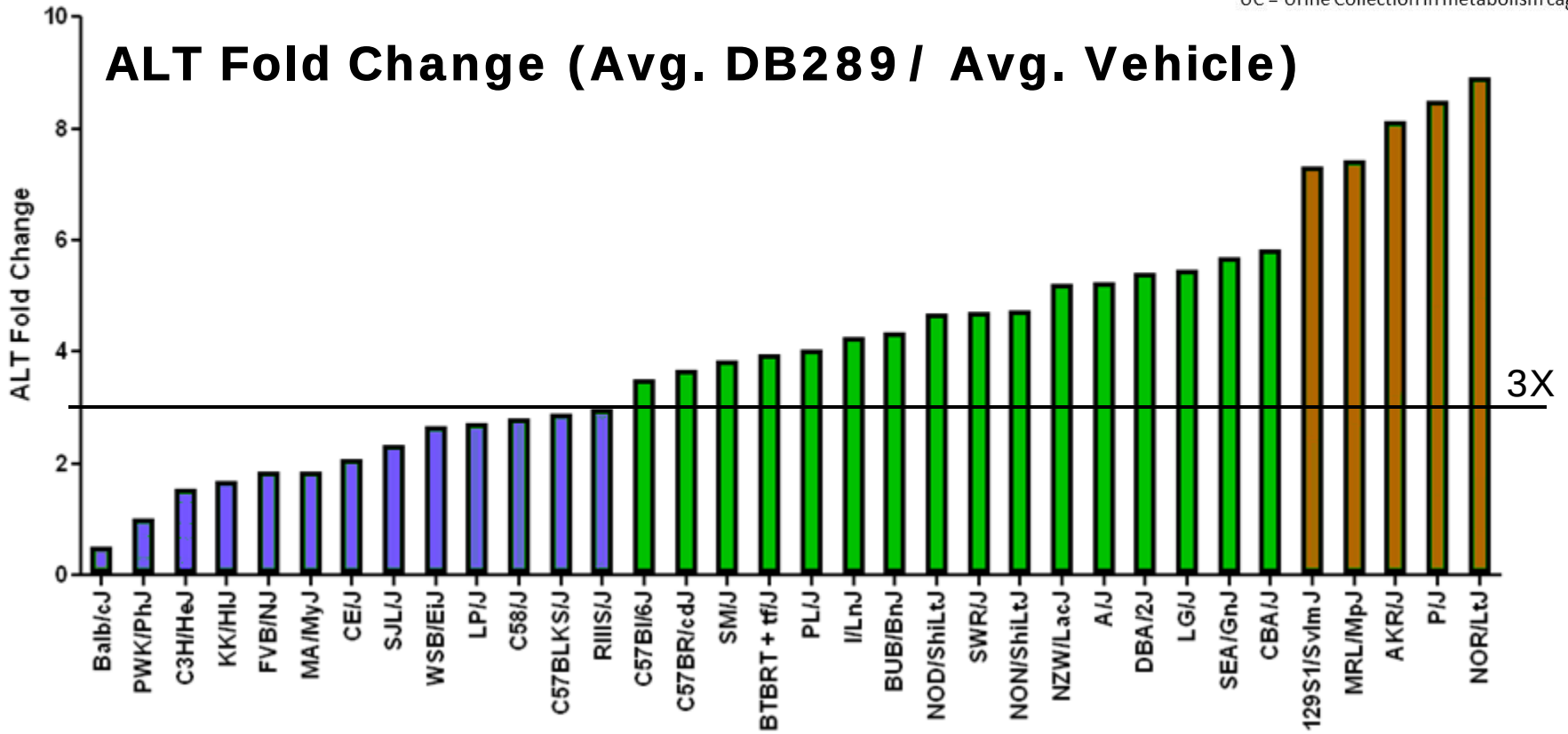
Inform rational design of next-in-class

Provide biomarkers or sensitive strains to test

Liver Injury: ALT

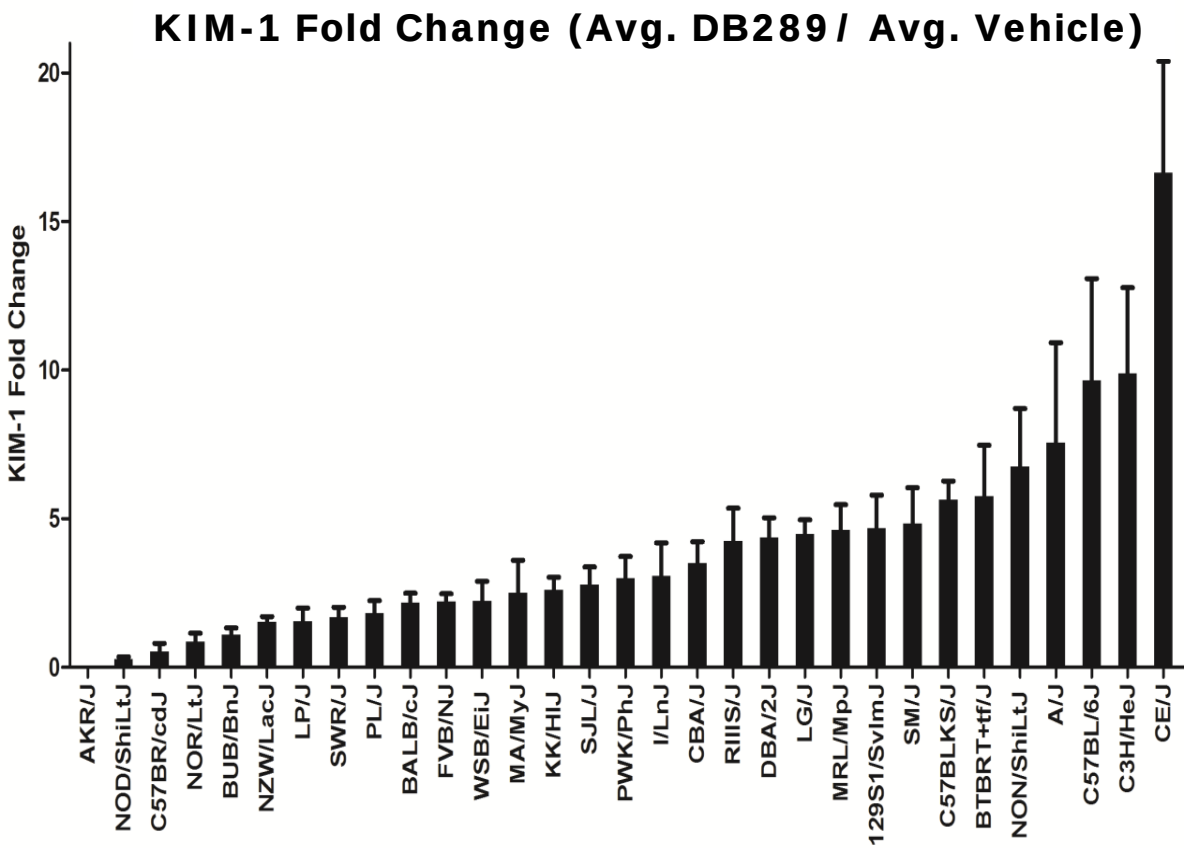


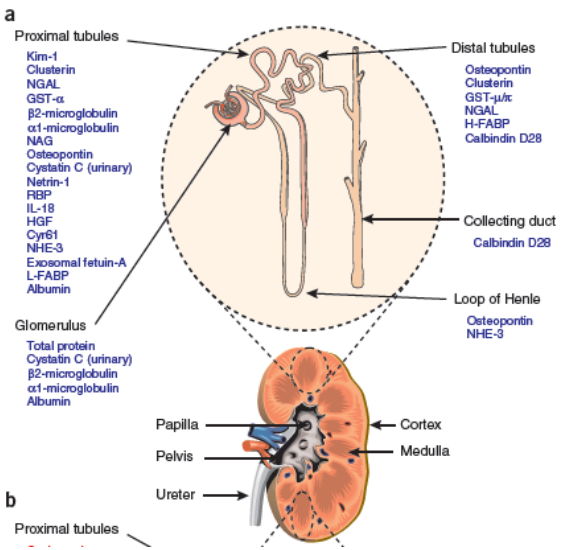
UC = Urine Collection in metabolism cages



Harrill et al. Tox Sci. 2012

Kidney Injury: KIM-1





MDP approach enabled detection of clinical liver and kidney toxicity risk. Kidney toxicity risk would not have been detected in circulation without the use of sensitive biomarker KIM-1.

Harrill et al. Tox Sci. 2012

Isoniazid Hepatotoxicity

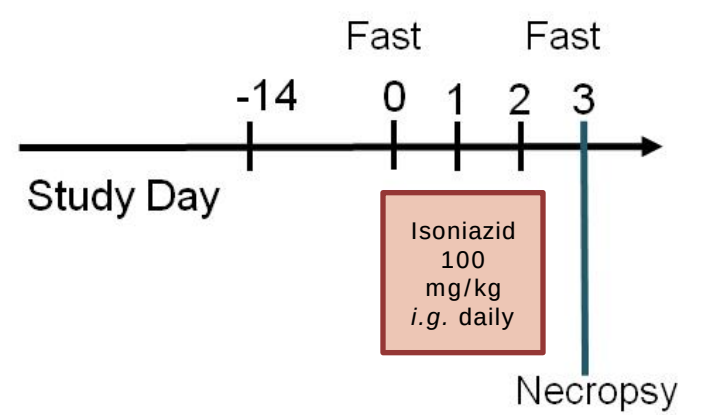
- **MILD: 10-20% patients;** ATs < 100 U/L, focal hepatocellular damage on biopsy (Nolan et al. *JAMA* 1999); mild AT elevations resolve despite continued therapy
  - In some cases, physicians opt to "treat through" AT elevations

- **SEVERE: 1-2% patients;** hepatitis and liver failure, combination therapy for active TB infection increases incidence (i.e. rifampin)

  - Most common cause of acute liver failure due to DILI in some countries (ex. India)

Isoniazid *in vivo* Experimental Design

Drug Exposure (3 Days):



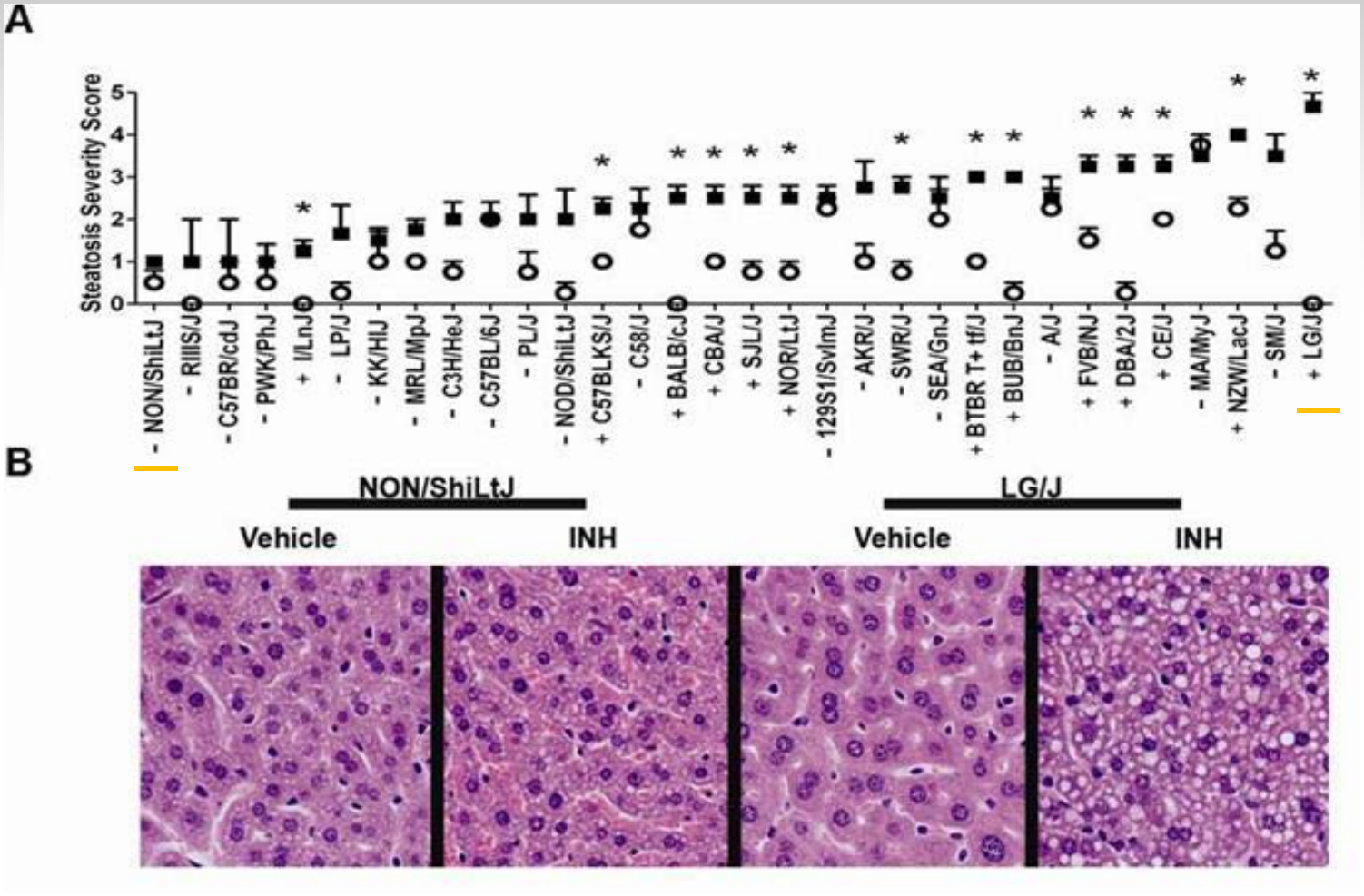
Collaborations with HESI Genomics Technical Committee

Collaborations

Endpoints of Interest:

1. **Histopathology, Adipophilin** – Hamner/EPL, Pfizer, NIH(NIDDK)
2. **Cytokine profiles** – J&J (Ameesha Batheja, Monica Singer)
3. **miRNA (miR-122) Expression -** J&J (Ameesha Batheja, Monica Singer)
4. **Transcriptomics–** Hamner (Affymetrix arrays donated by Lilly, AstraZeneca, and Pfizer)
5. **Metabolomics** – RTI (Susan Sumner)
6. **Genomics** (GWAS): Hamner, Pfizer
7. **Cholesterol/Triglycerides:** Hamner, Pfizer

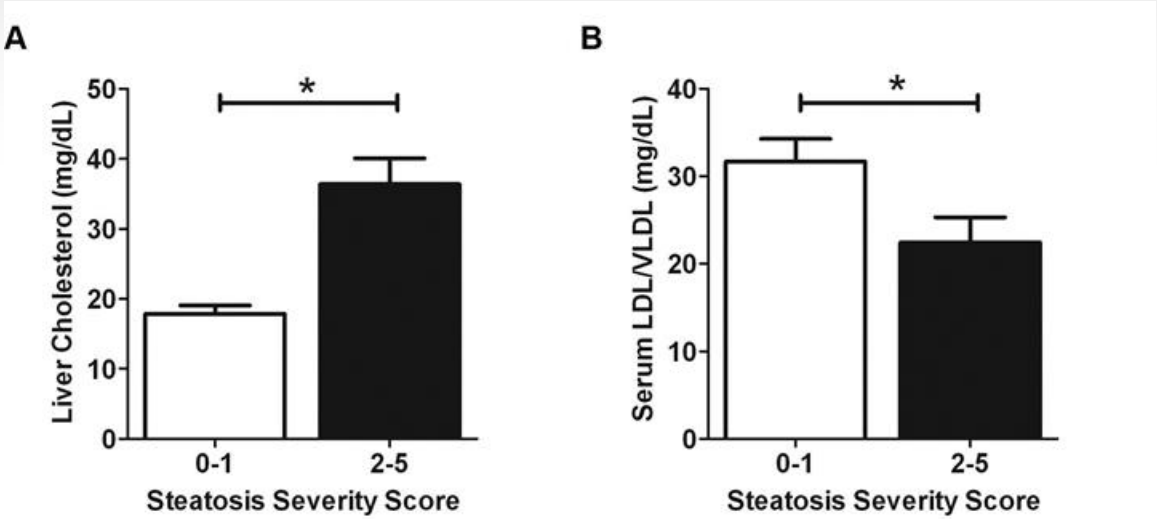
Isoniazid-Induced Steatosis

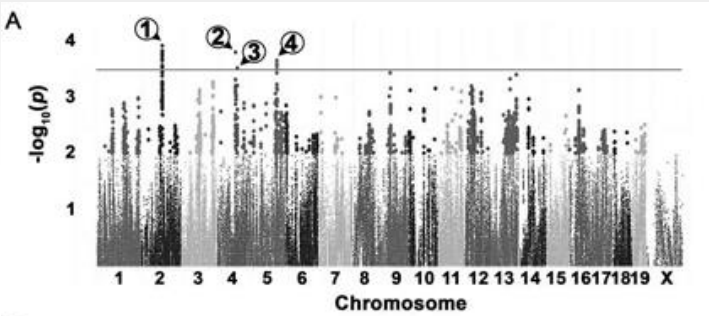


RESULT: About half of strains tested experienced a positive steatotic response.

Church et al. Tox Sci. 2014

QTL Mapping of INH Steatosis





B

Peak	-log(*p*)	Genome Position	Genes in Region
1	3.90	Chr 2: 96765876-97931800	*Lrrc4c*
2	3.79	Chr 4: 86299052-86507962	***Plin2***, *Dennd4c, Rps6*
3	3.51	Chr 4: 94973736-95178980	*No genes*
4	3.65	Chr 5: 116304968-116629195	*Cit, Prkab1, Tmem233, Ccdc60*

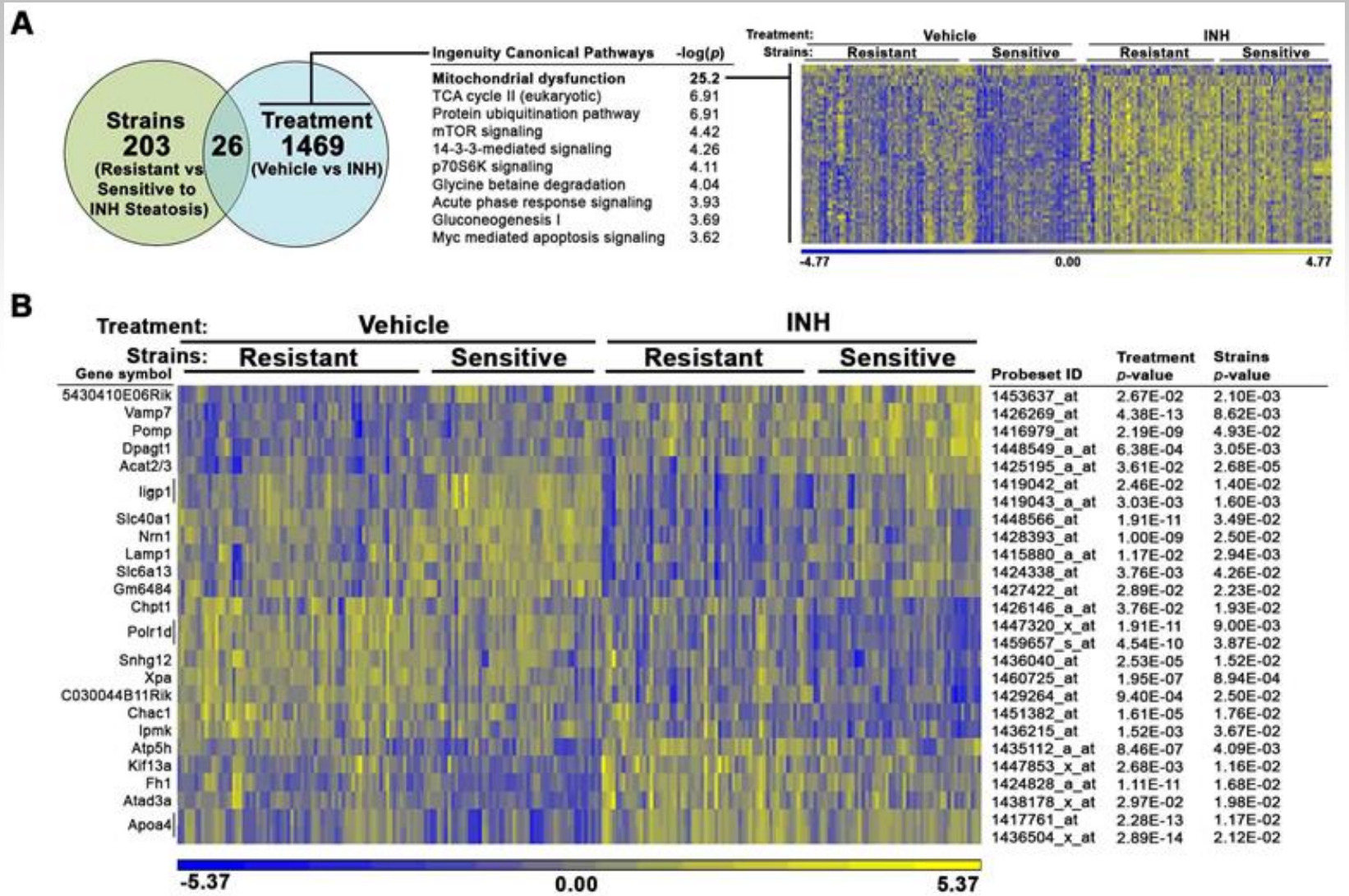
RESULT: Liver cholesterol and Serum LDL levels correlated with the extend of liver steatosis.

RESULT: GWAS identified a few significant peaks, one contained *plin2*, the gene encoding adipophilin.

Adipophilin itself is often used as an IHC marker of steatosis.

Church et al. Tox Sci. 2014

mRNA Transcript Expression



RESULT: Transcripts changed by treatment and differentially expressed in liver in sensitive strains are largely associated with lipid packaging (i.e. *lamp1*).

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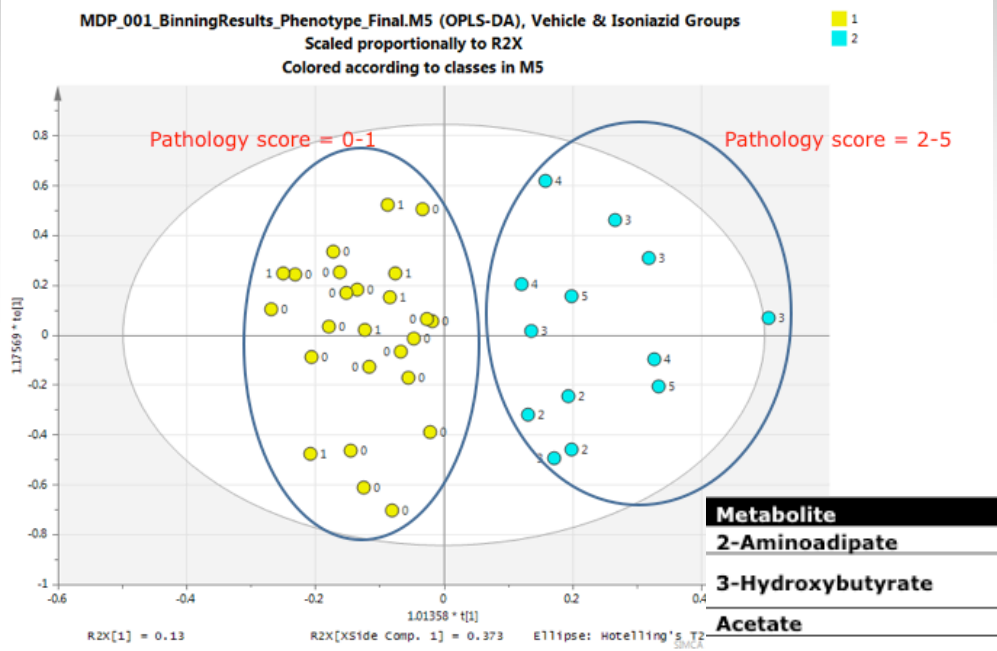
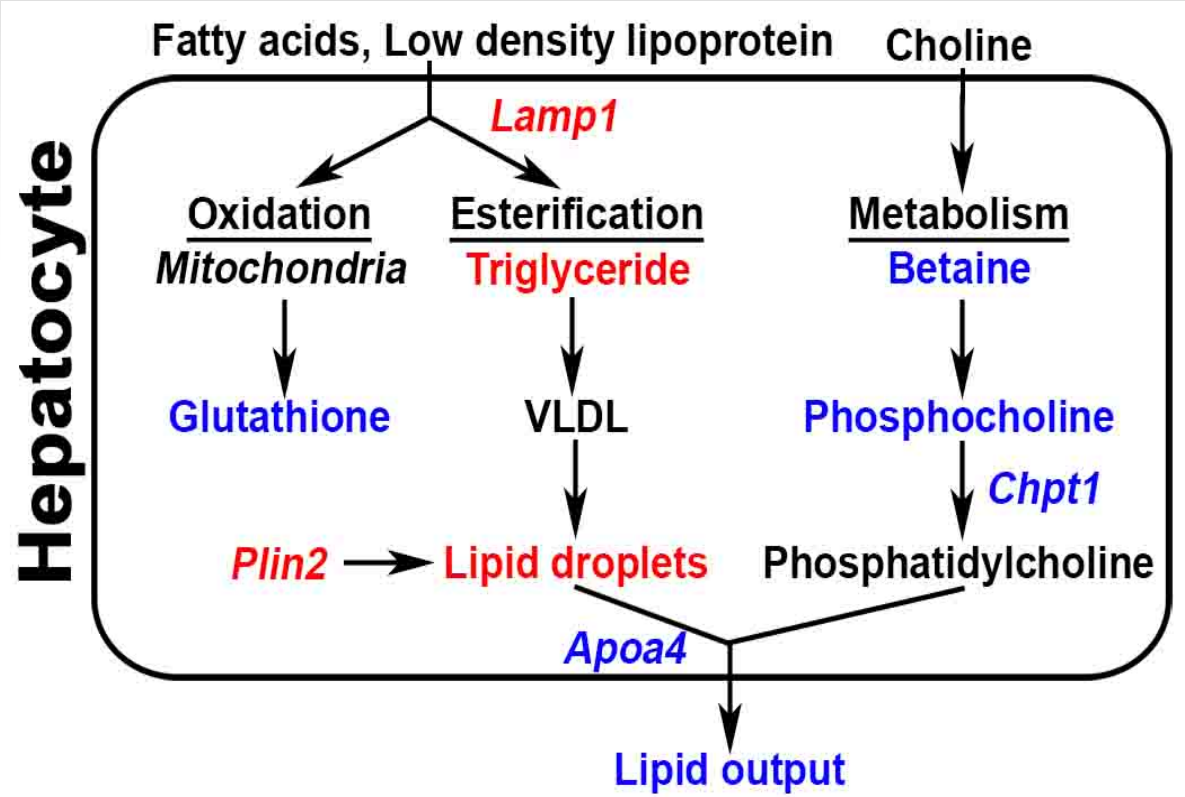
All Samples: Metabolites important for the separation of pathology groups (0-1 vs 2-5)



RESULT: Liver metabolites that distinguished responders enriched for metabolites associated with betaine homocysteine methyltransferase pathway

Metabolite	pValue	Incr/Decr	Metabolite	pValue	Incr/Decr
2-Aminoadipate	<0.050	Incr	Lactate	<0.100	Decr
3-Hydroxybutyrate	<0.100	Decr	Leucine	<0.100	Decr
Acetate	<0.100	Decr	Lipoproteins	<0.100	Incr
Adenine	<0.100	Decr	O-Phosphocholine/ O-Acetylcholine/sn-Glycerophosphocholine / Carnitine	<0.005	Decr
Alanine	<0.050	Decr	Ornithine	<0.100	Decr
Ascorbate	<0.010	Decr	Phenylalanine	<0.005	Decr
Betaine/ TMAO	<0.050	Decr	Serine	<0.100	Decr
Cadaverine	<0.005	Decr	Taurine	<0.100	Decr
Fumarate	<0.001	Decr	Tyrosine	<0.100	Decr
Glucose	<0.010	Decr	Unknown (0.79, 0.83 ppm)	NS	Incr
Glutamine	<0.100	Incr	Unknown (3.16ppm)	<0.005	Incr
Glutathione	<0.100	Decr	Unknown (3.28ppm)	<0.100	Incr
Glycylproline	<0.005	Decr	Unsaturated lipids	<0.100	Incr
Homoserine	<0.100	Incr	Valine	<0.100	Decr
Isoleucine	<0.050	Decr			

Effect of Isoniazid Treatment on Lipid Export in Genetically Sensitive Mice



Key:
Increased
Decreased

RESULT: All omics data combined together into one unifying hypothesis regarding the mechanism of INH-induced steatosis

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Next Steps for the Harrill Lab

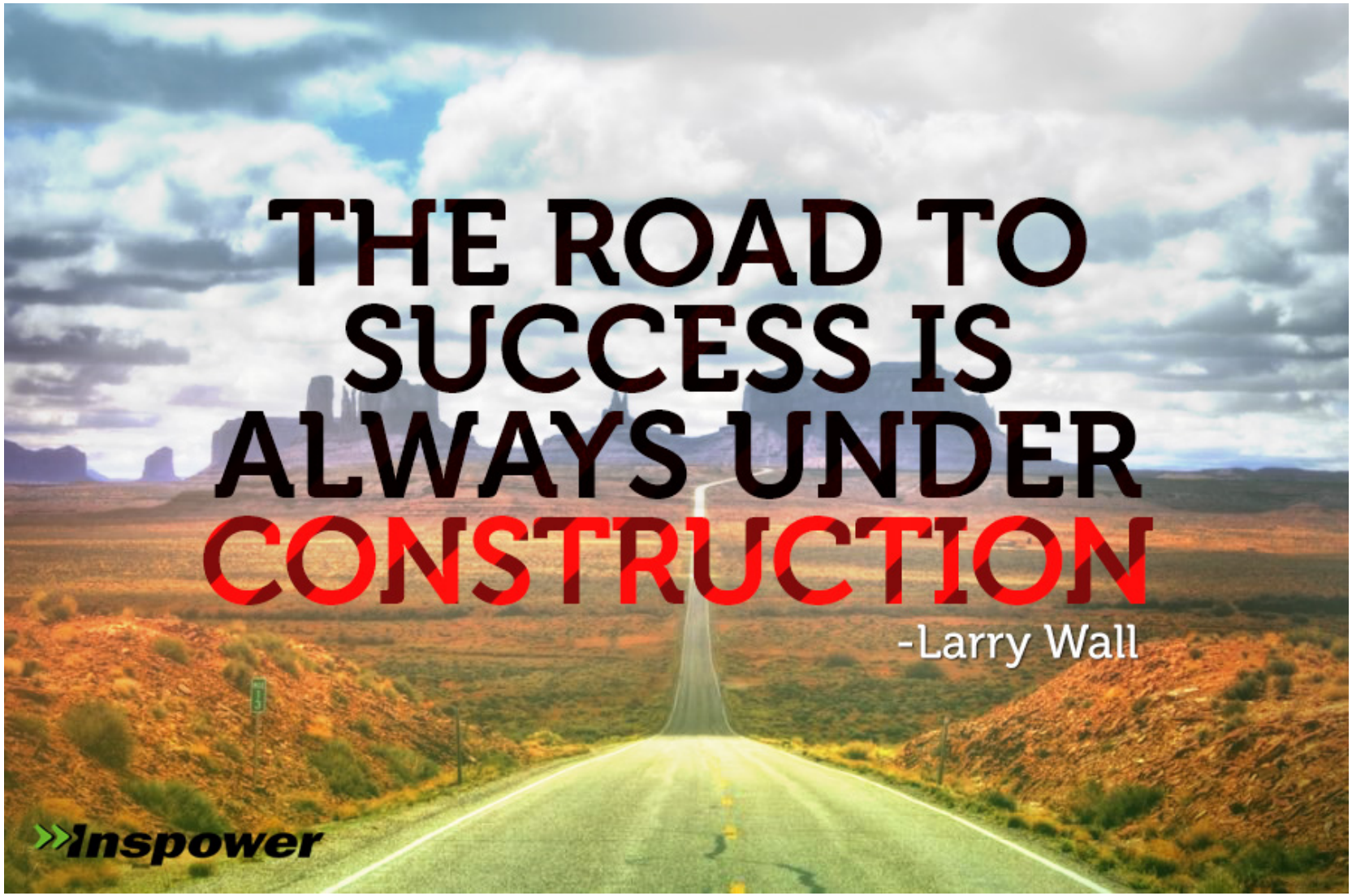
- Investigation of the DO mice as a tool to detect and predict DILI (Collaboration with Jax and FDA)
  - Screen drugs known to cause idiosyncratic DILI
  - Screen drugs that failed in clinical development
  - GWAS on selected candidates
- Mechanistic analysis of the DILI reaction for various drugs (now that we have a model – we can study it!)

Visit Lascelles Lyn-cook's poster to see our preliminary data on using the DO for drug safety assessment.

Conclusions

- Genetically diverse mouse population-based approach was able to detect a genetic variant associated with DILI sensitivity for:
  - Dose dependent hepatotoxic acetaminophen
  - Idiosyncratic hepatotoxin EGCG

- Genetically diverse mouse populations may offer improved ability over classical models to detect human toxicity risk:
  - 881 – liver injury
  - DB289 – renal injury

- Genetically diverse mice offer insights into mechanisms of toxicity that occur within sensitive individuals
  - Isoniazid – hepatic steatosis
  - 881 - phospholipidosis

In the world of drug safety science,