Hepatotoxicity Testing

Predictive Strengths and Weaknesses

May 20, 2008

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GSK

Hepatotoxicity

Topic Overview

- **Scope of problem/background**

- **Current approaches**

  - strengths and weaknesses

- **Emerging approaches and potential gap filling opportunities**

- **Summary/discussion**

Scope of Problem

- **Drug induced liver injury**

  - leading cause of acute liver failure

  - major reason for late stage termination or withdrawal

  > patient impact

  > opportunity cost

Scope of Problem

- **Hepatotoxicity detection paradigm**

  - ***preclinical testing***

    > imperfect filter * (Olson *et al*. Reg.Tox.Pharmacol. 2000;32:56-67, Greaves P, *et al*. Nature Rev Drug Disc. 2004;3:226, others)

    > difficulty in detecting impact of modifications, especially if incremental * *

    ***** *however, many true positives discarded***

    ****** *detection endpoints different, databases/assessments very unwieldy***

Background

- **Liver Structure**

  - Lobed with capsule, blood supply arterial and portal, lobular architecture, biliary tree

- **Liver Cells**

  - Kupffer, endothelial, biliary, natural killer (NK), stem (oval), stellate (lipocyte), hepatocyte

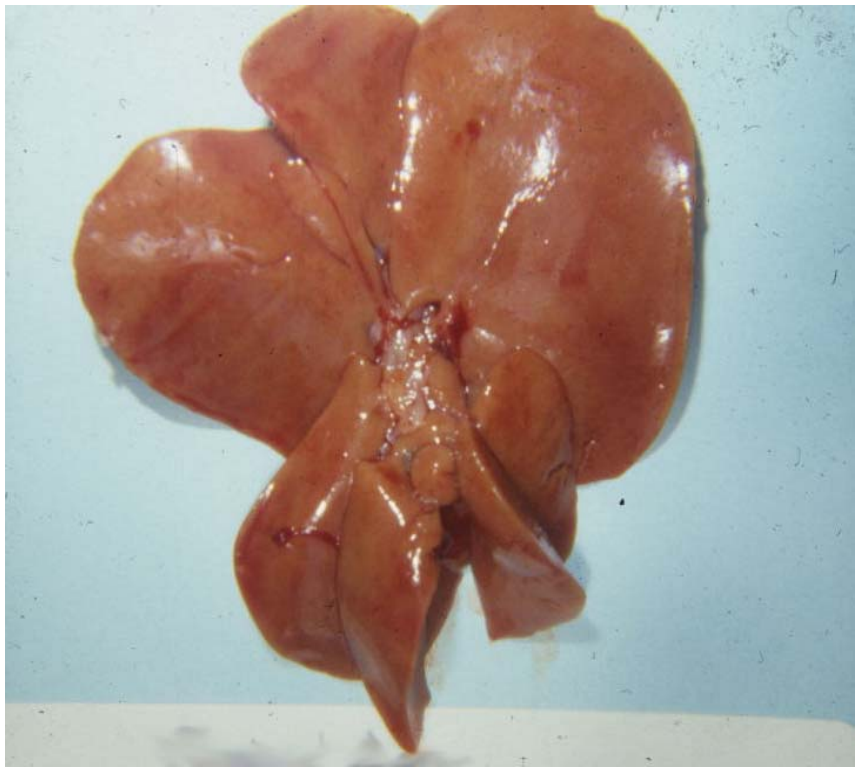
- **Liver Function**

  - Metabolism, detoxification, immune, storage, coagulation, energy, endocrine, bile formation.

Background - structure

- **Glandular organ**

  - One of the largest in the body

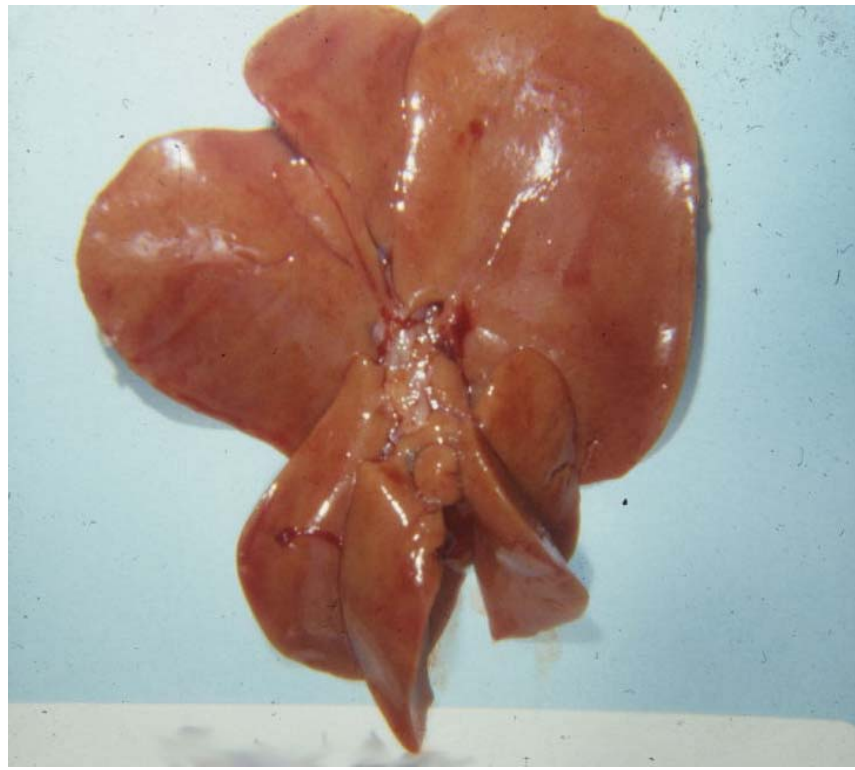


Rat Liver

Background - structure

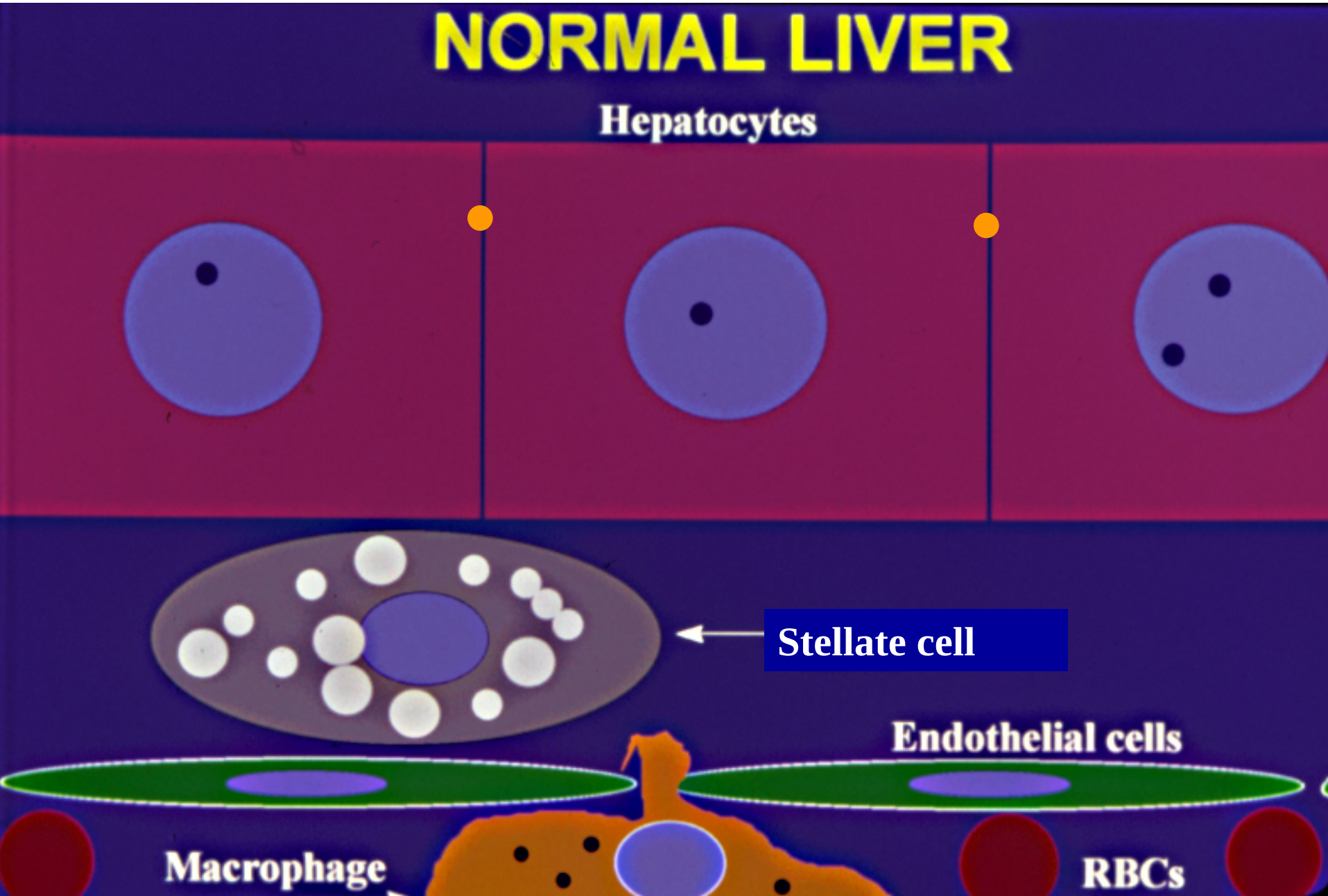
- **Glandular organ**
  - ***One of the largest in the body***

- **Species differences in size**
  - ***dependency on diet***
    - **> carnivores; 3- 4% of BW**
    - **> omnivores; 2% of BW**
    - **> herbivores; 1% of BW**



Rat Liver

Background - structure

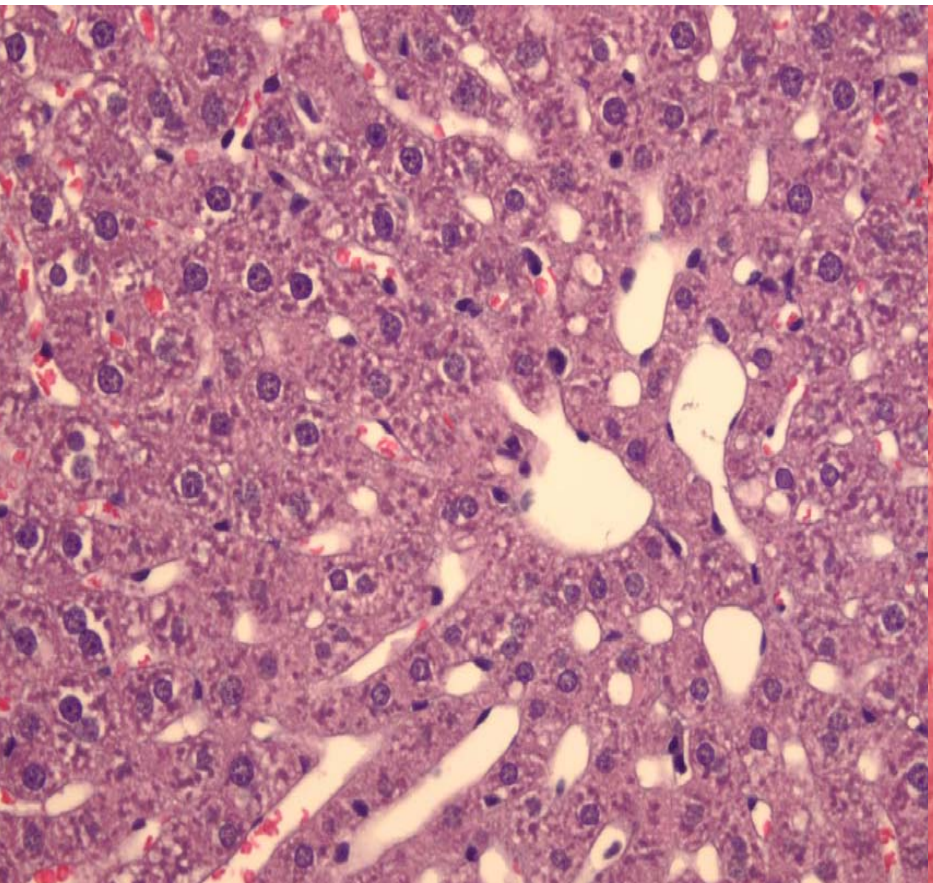


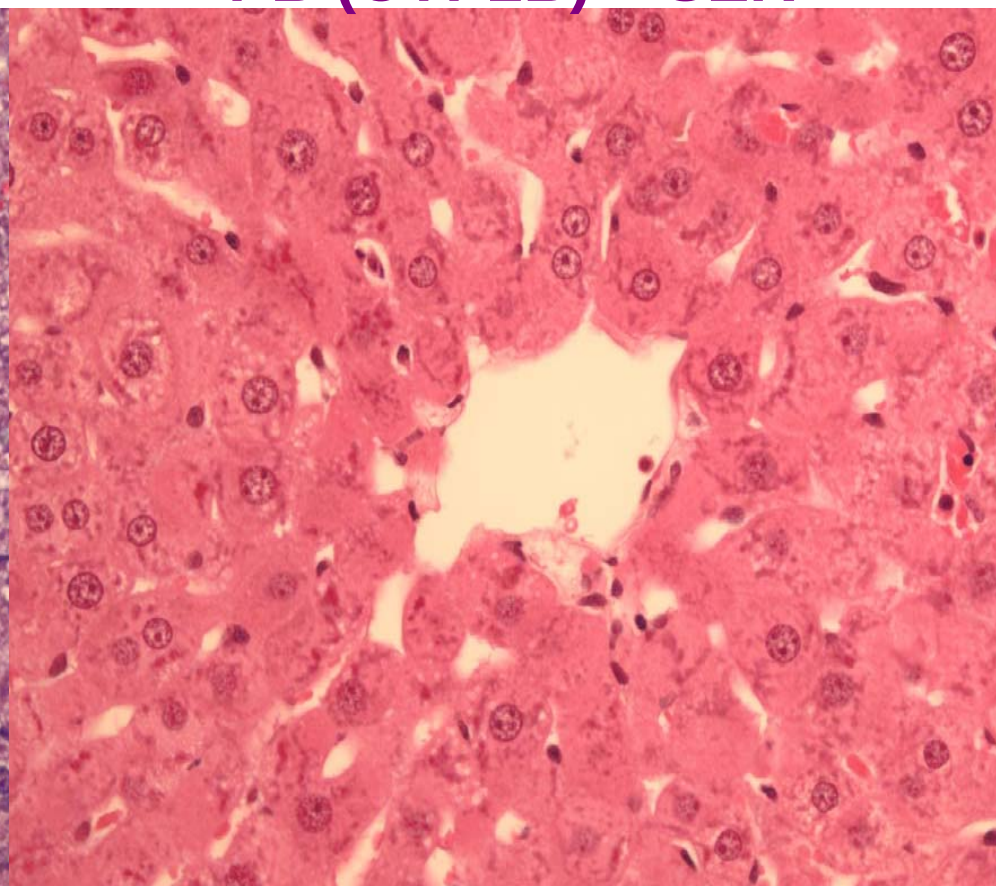
Background – Morphologic (biochemical/molecular) effect

Normal

Centrilobular Hypertrophy

PB (CYP2B) = SER





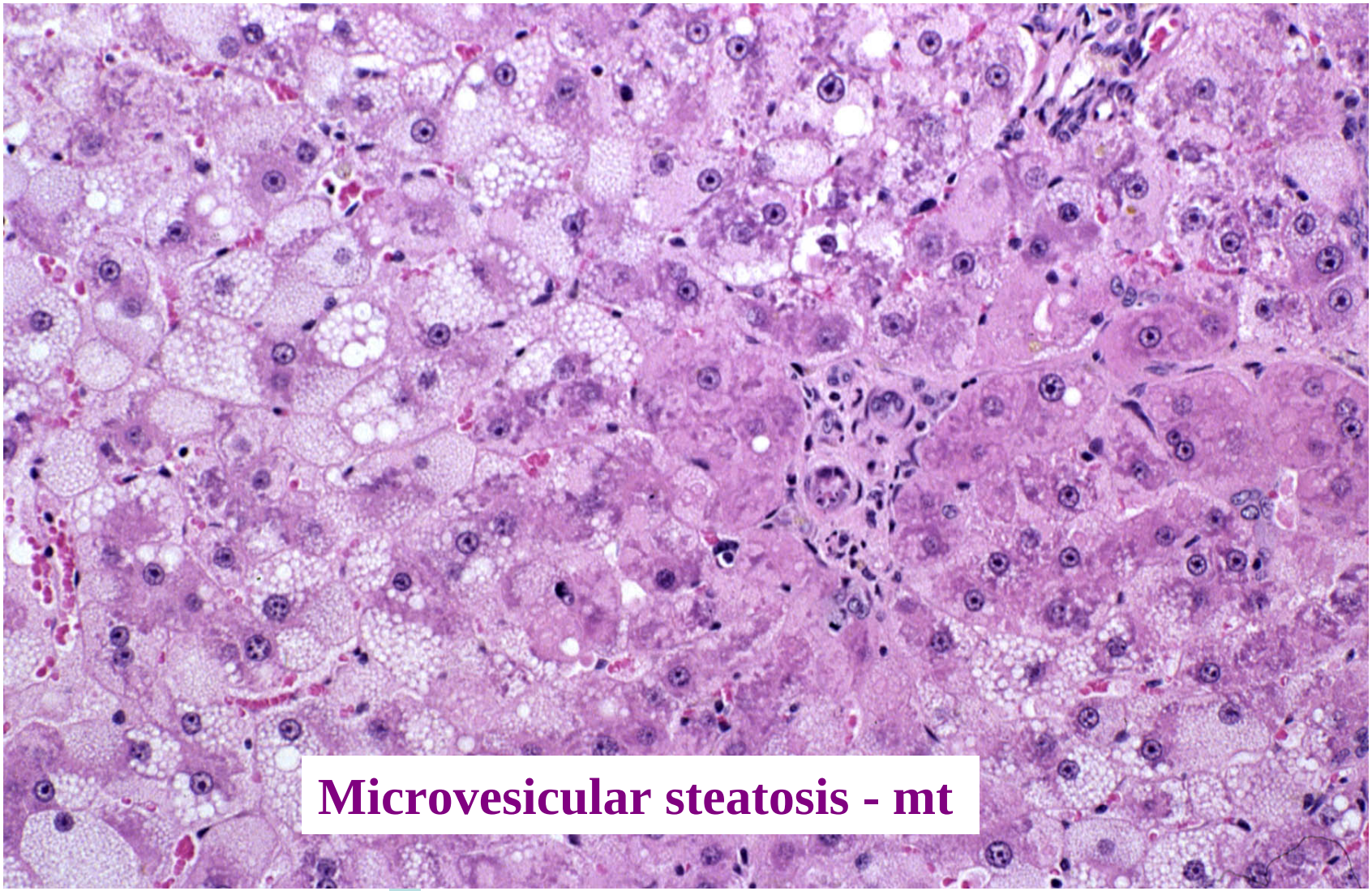
Background – Mechanisms of Toxicity

- **Oxidative stress**
- **Protein synthesis inhibition (multiple levels)**
- **Macromolecular interactions (reactive metabolites)**
- **Fluid/ion imbalances**
- **Calcium shifts**
- **Mitochondrial respiration, permeability**
- **Signal transduction**

Background – pan-species hepatic effects (concordance)

- **APAP**
- **CCl_4**
- **PB**
- **St. John’s Wort**
- **Mushrooms**

Background - manifestations



How are we missing???

- **Subtle (and not so subtle) species and individual variation (patient populations)**
  - ***diet***
  - ***age***
  - ***concurrent medication***
  - ***disease***
  - ***metabolism***
  - ***recreation***
  - ***gender***
  - ***exercise***
  - ***race***
  - ***etc, etc***

How/what are we missing??

- **Subtle (and not so subtle) species and individual variation (patients)**

  - in many cases the differences don't matter (benign effect, adaptation, redundancies, recovery)

  > how can we decipher those cases where it does matter? Or when there is absolutely no signal in routine endpoints?

...understanding the signal

Hepatic Biomarkers

- **Hepatocellular integrity**

  - ALT, AST, SDH, GDH, LDH, αGST

- **Hepatobiliary (biliary)**

  - ALP, γGT, 5'Nucleotidase

- **Hepatic functional indicators**

  - bilirubin, bile acids, clearance tests, clotting cascade (APTT, PT), metabolism/secretion (urea, protein, cholesterol, triglycerides)

Hepatic Biomarkers

- **As with any biomarker consider…**

  - sensitivity
  - specificity (tissue, species)
  - utility in clinical trials/practice
  - physiologic variations (diet, strain, age, diurnal variation, gender, timing of food consumption)
  - value of pre-test assessment (especially if low n)
  - handling (animal, sample)
  - source of specimen

Hepatic Biomarkers

- **As with any biomarker consider…**

  - timing of sampling relative to known or suspected onset of liver injury

  > half-life

  > severity of effect

Hepatic Biomarkers

- **Further considerations;**

  - ALT induced by steroids
  - Muscle can be significant source of ALT (particularly primates)
  - AST is in muscle and rbcs (ratio to ALT can be helpful)
  - LDH not very specific, may be falling out of favor
  - ALP is inducible (dog), intestinal form detectable (rat)

Hepatic Biomarkers

- **What does it mean?**
  - ***adverse versus physiologic effect/induction***

> integrated interpretation

} concordant clinical pathology endpoints

} anatomic pathology data

} exposure

} previous experience

} chemical/target knowledge

} injury or other effect in other tissues with relevant enzymes

Hepatic Biomarkers

- **Species differences**

  - in rat; intestinal form of ALP is major constituent

  - in dog; bilirubin readily excreted in urine

  - in human; ALT half life much longer than rat

  - in dog; corticosteroid inducible form of ALP (stress rather than hepatic injury)

Hepatotoxicity

- **Chemical, species, and individual specific mechanisms**

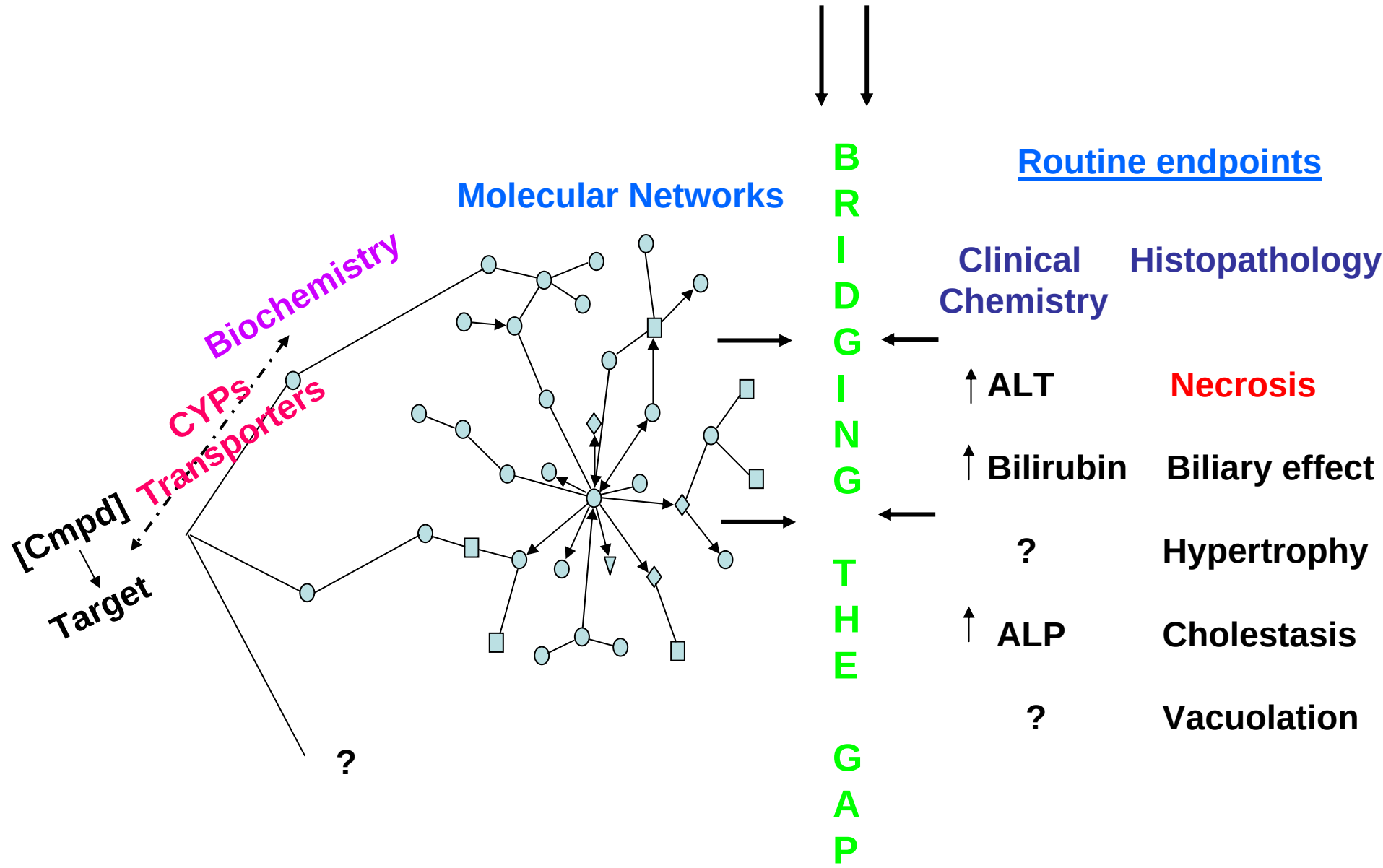
  - suggests multiple approaches needed to enhance predictivity

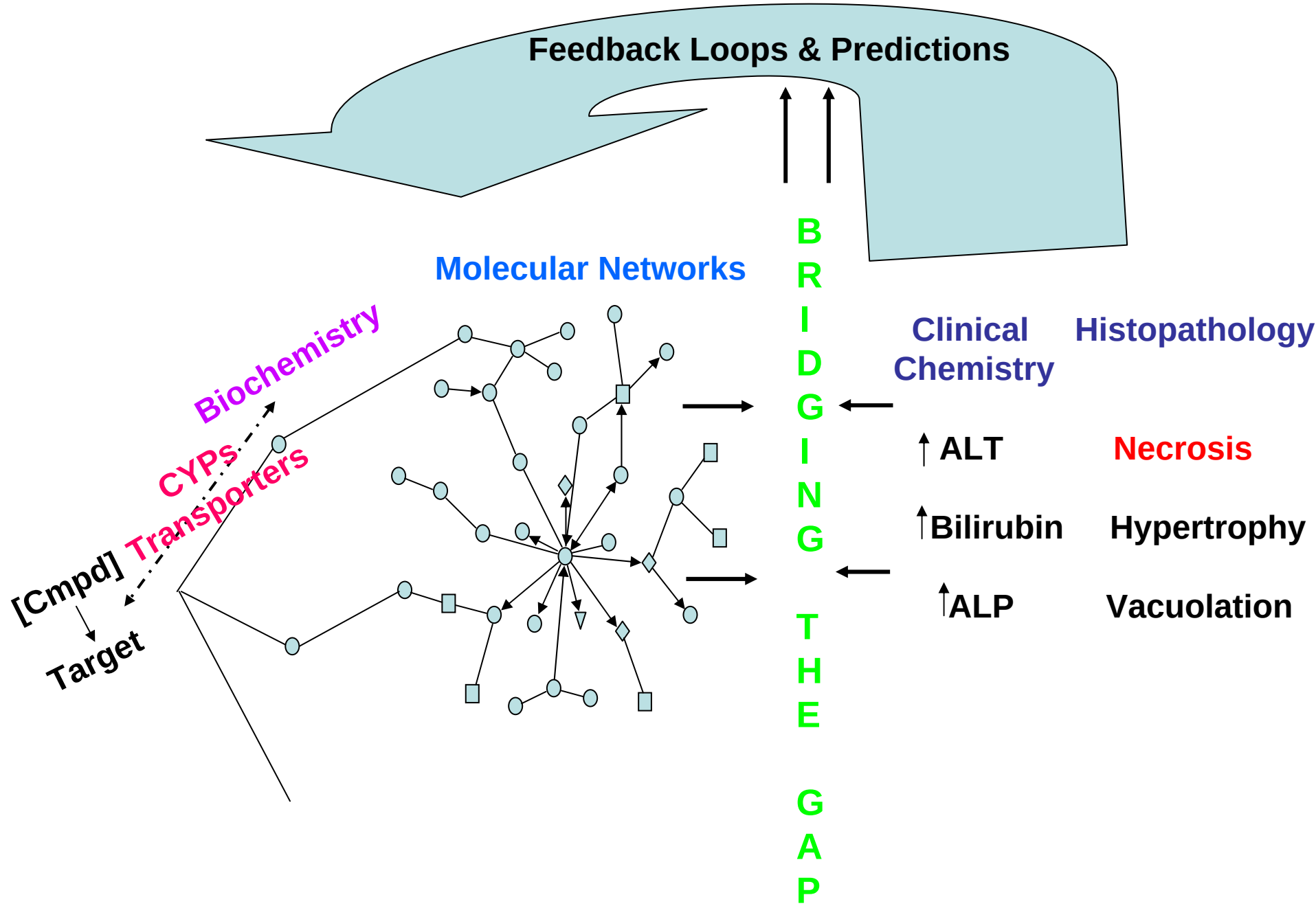
What can be done?

- **Extract more from routine approaches**
  - ***e.g., transcriptomics, other biomarkers, imaging***

- **Supplemental approaches**
  - ***in vitro***
    - **> cell systems, enzymes, GSH trapping, CYP inhibition, mitochondrial assays, transporters**
  - ***in vivo***
    - **> LPS, Transgenics/KO (CYP), GSH depletion**

- **Further understand the patients**
  - ***steatosis***
  - ***pharmacogenomics***


Molecular Networks
Biochemistry
CYPs
Transporters
[Cmpd]
Target
?
BRIDGING THE GAP
Routine endpoints
Clinical Chemistry
Histopathology
↑ ALT
Necrosis
↑ Bilirubin
Biliary effect
?
Hypertrophy
↑ ALP
Cholestasis
?
Vacuolation


Feedback Loops & Predictions
Molecular Networks
Biochemistry
CYPs
Transporters
[Cmpd]
Target
BRIDGING THE GAP
Clinical Chemistry
Histopathology
ALT
Necrosis
Bilirubin
Hypertrophy
ALP
Vacuolation

Enhancing Liver Effect Predictivity

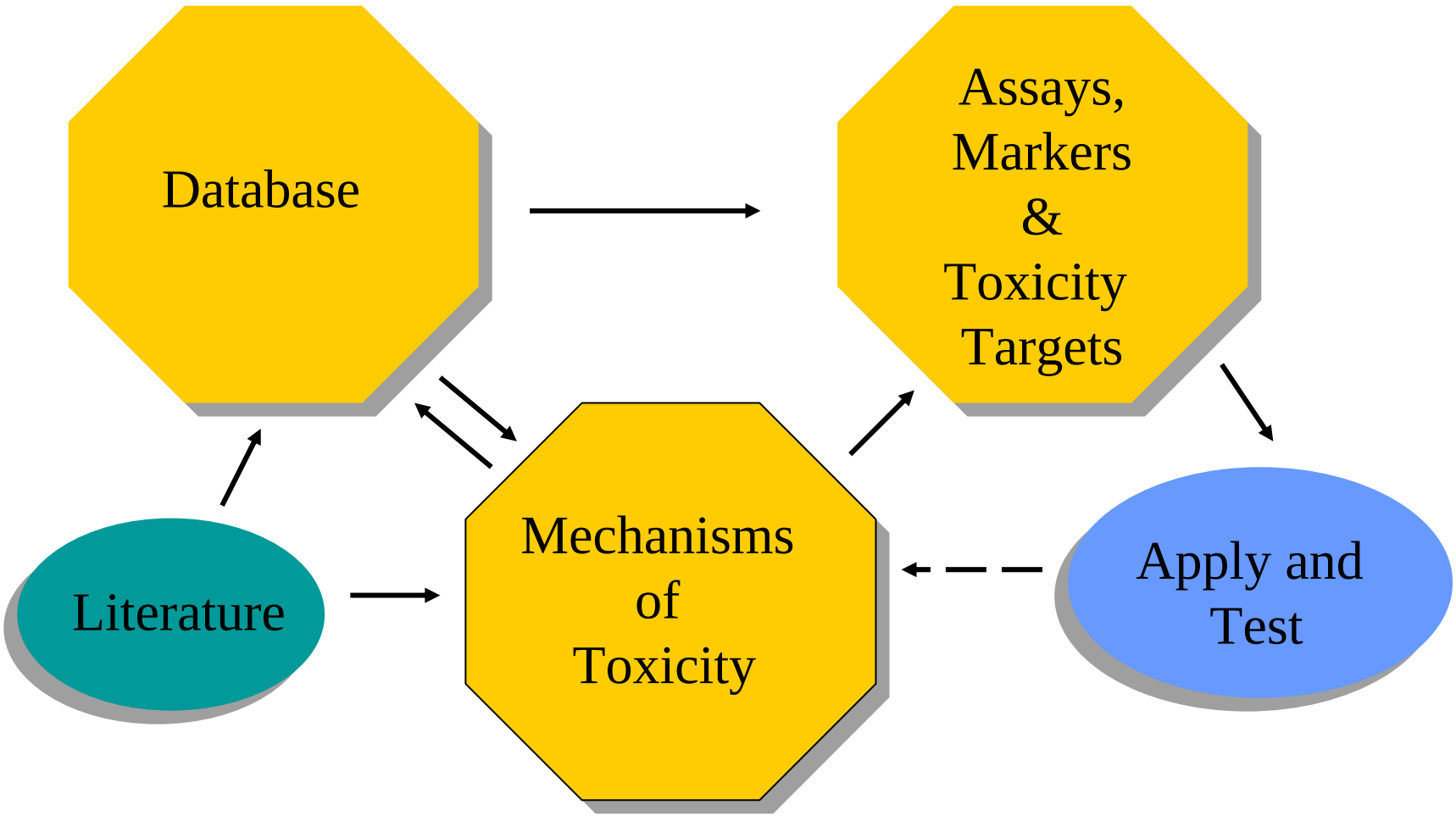
- **Objective**

  - apply novel and conventional technologies to create assays/tools that enhance decision making capability in non-clinical safety assessment

- **Expectation**

  - better markers of safety in use
  - better problem solving tools in place

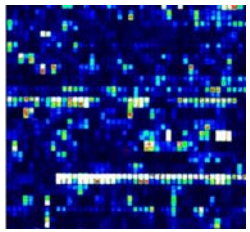
Strategy


Database
Assays,
Markers
&
Toxicity
Targets
Literature
Mechanisms
of
Toxicity
Apply and
Test

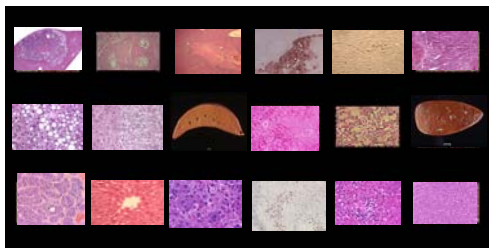
Modification to Holistic 'omic' Approach

Reference compounds

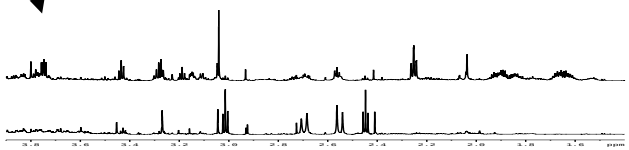
Liver Microarray



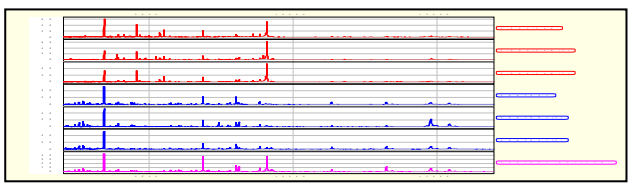
Clinical & Histopathology



Urine NMR



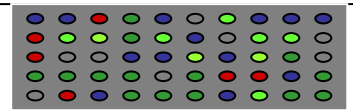
Serum SELDI



Analyses: Teams & Technology

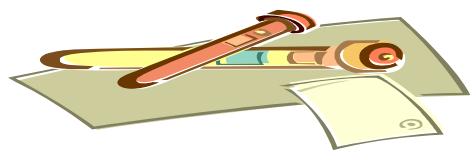
Output: Practical Applications

Liver Tox gene panel
Select Genes
Interpretive Rules
Reference Information



Liver Tox Target panel
Selected transporters, receptors, enzymes for compound screening

Liver Tox biomarkers
Serum, urine



HepatoTaq™: Rat Liver Gene Panel

16 specifically focused subpanels
Each subpanel has its own set of genes (4-18 genes per subpanel) and interpretative guidance

Manifestations of Hepatotoxicity

- **Hepatic Fibrosis**
- **Hepatic Phospholipidosis**
- **Hepatocellular Apoptosis**
- **Zonal Hepatocellular Necrosis**
  - **Hepatocellular Cell Cycle**
- **Cholestasis**
- **Biliary Hyperplasia**
- **Hepatic Peroxisome Proliferation**
- **Acute Phase Response**

Potential Modes of Hepatotoxicity

- **Glutathione Depletion**
- **Lipid Peroxidation/ Mitochondrial Dysfunction**
- **Reactive Metabolites**
- **AhR-type inducer**
- **PXR-type inducer**
- **CAR-type inducer**
- **Increased Hepatic Thyroid Hormone Clearance**

Understanding the signal

- **Biliary effects**

  - degeneration (probably not good)

  - hyperplasia (good or not good?)

Understanding the signal

- **Biliary effects**

- degeneration (probably not good)

- hyperplasia (good or not good?)

> response to previous biliary cell injury
> response to hepatocellular injury
> mitogenic response
> aging change (background lesion)
> cholestasis

Acute Response of Biliary Epithelium to ANIT-induced Injury

- **Model toxicant**
- **SD rats**
- **50 mg/kg ANIT orally, once**
- **2, 6 and 48 hr time points**

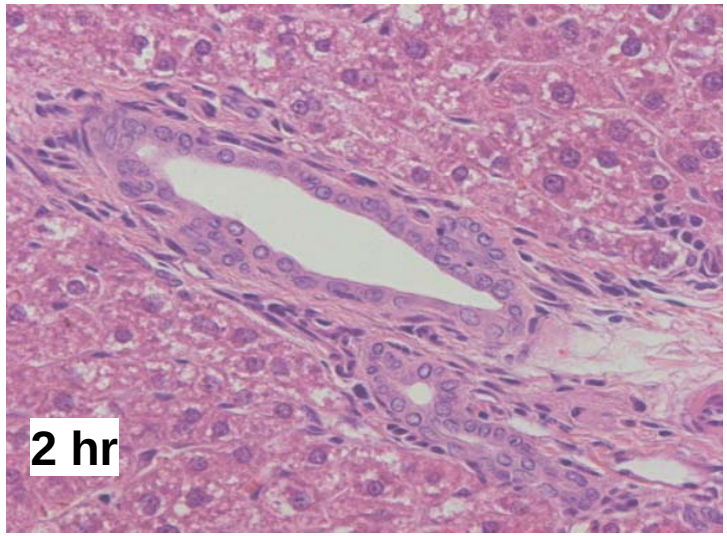
Acute Response of Biliary Epithelium

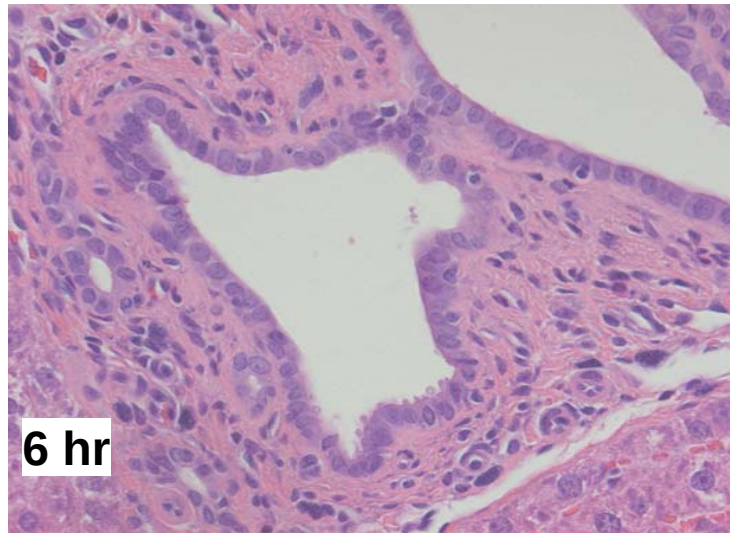
- **Study designed to address cell specific response to bile duct toxicant**

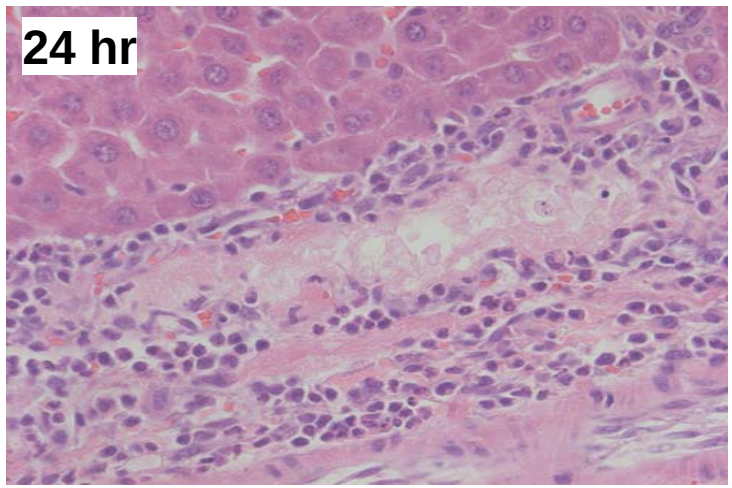
- **Combine LCM with microarray analysis to analyze specific cell populations**

  - novel approach to evaluate biliary toxicity
  - acquire baseline gene expression data for biliary epithelium
  - time course to characterize acute response versus repair/progression

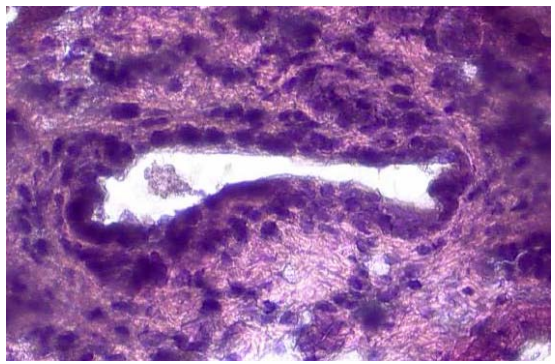
Histopathology

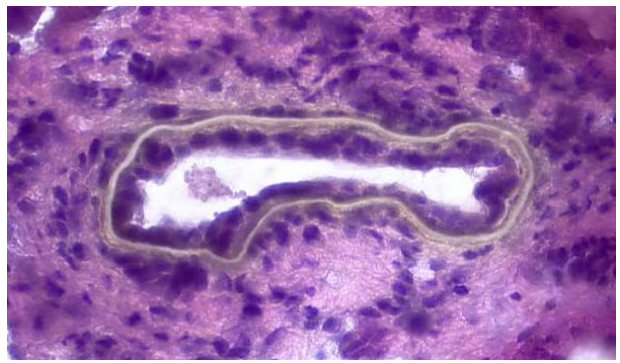


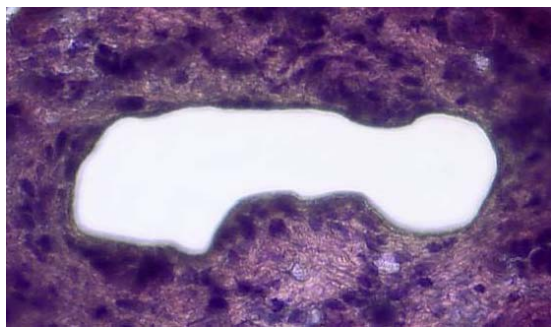


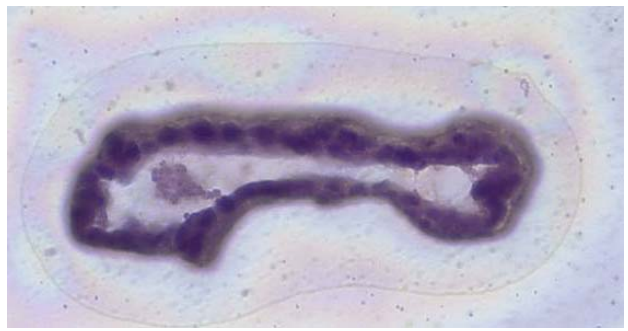


Laser Capture Microdissection









Selected large rat bile ducts only >15 μm (Marzioni et al. 2002, *Seminars in Liver Disease*)

Biliary cell analyses/results

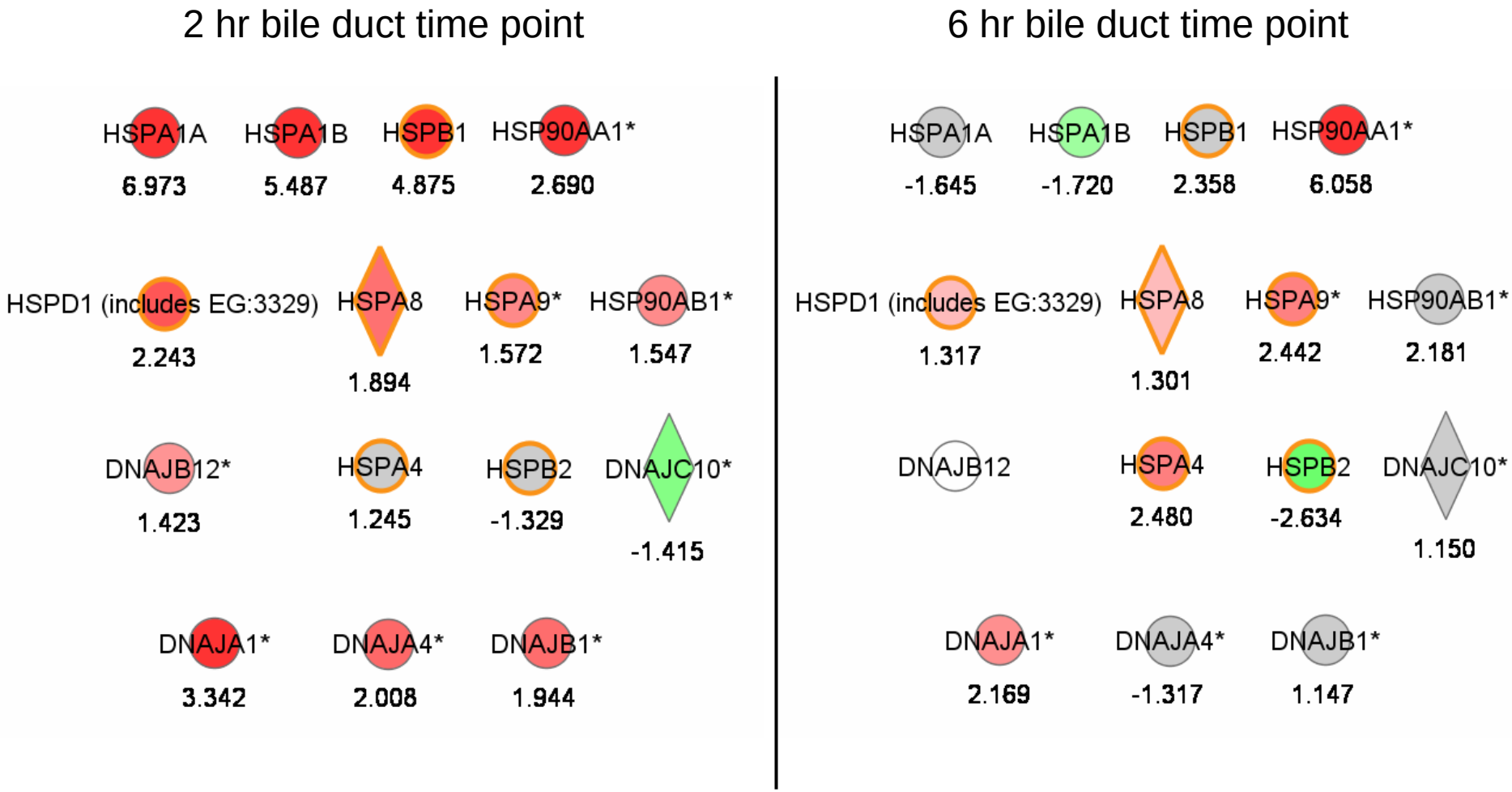
- **LCM**

- **Gene array**

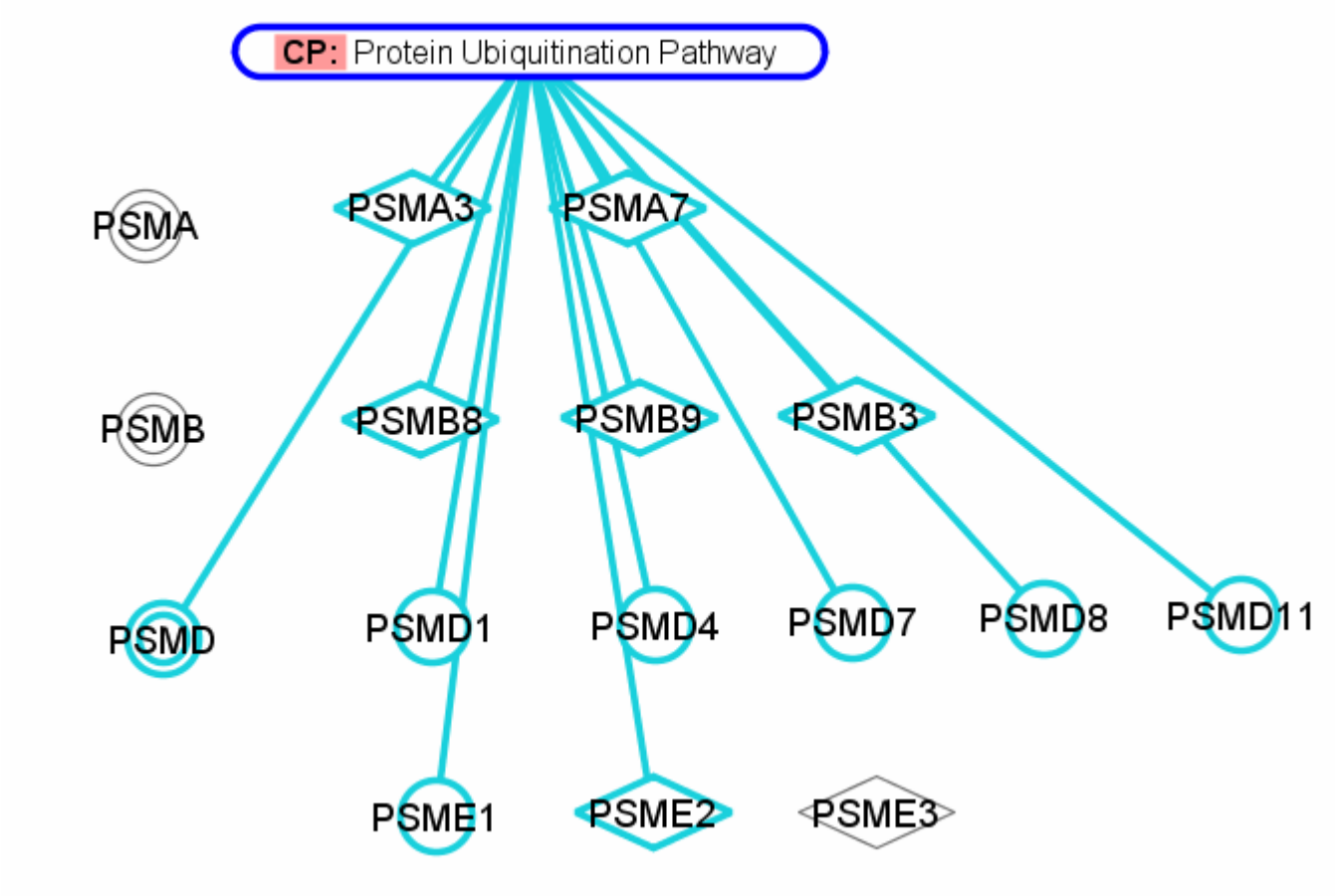
  - Biliary cell gene expression alterations occurred prior to onset of clinical and anatomic pathology changes

  > heat shock protein
  > oxidative stress
  > protein folding/Ubiquitination
  > ER stress response genes

Heat shock chaperones



Proteasomal Genes in Protein Ubiquitination Pathway



Potential Application

- **IHC or ISH in practice**

- **Gene panel**

> add to weight of evidence

Understanding the signal

- **Immunologic effects**

- many hepatotoxicities in people have immunologic component

- very few hepatotoxicities in preclinical species have an immunologic component (we think!)

> could this be “better” adaptation in preclinical models (and can we detect it?)?

} IL-6, IL-17, Th17 (penicillamine model)

Summary

- **Difficulty and complexity of hepatotoxicity requires a multi-faceted approach**

  - Biologic rationale for additional endpoints or assays may be sufficient to employ

- **Further data/experience sharing**

- **Further collaborative research**

- **Bridge preclinical clinical knowledge**

ACKNOWLEDGMENTS

Brenda Faiola, Holly Jordan, Greg Falls, Beth Romach, Roger Brown, Neal Cariello, Julie Holder, John Cullen, Daniela Ennulat, Lawrence Yoon, Rick Hailey, Chris Hunt