



Biomarker Discovery and Qualification

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Alternative
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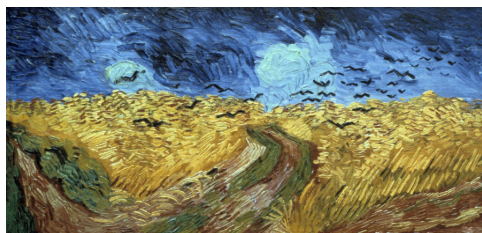
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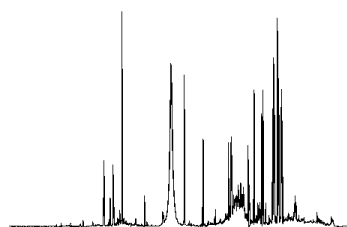
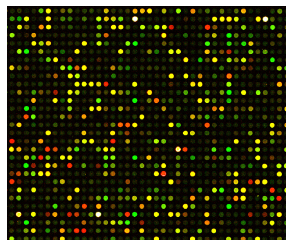
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Outline

- State of the art



- Omics

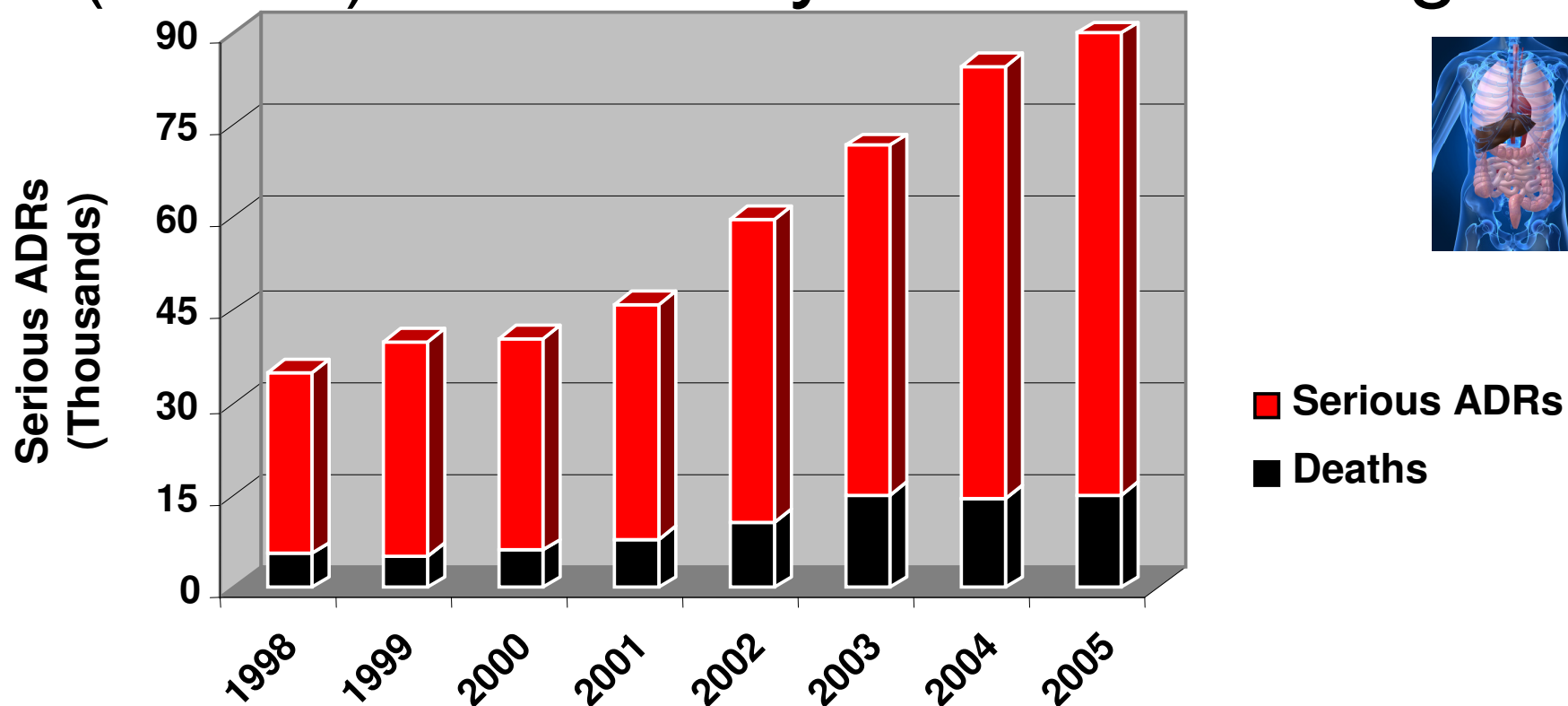


- Biomarker Qualification and Usefulness





Serious Adverse Drug Reactions (ADRs) Caused by Marketed Drugs



Rate of ADRs and death growing faster than # of prescriptions

Moore et al., Arch Intern Med. 167:1752-1759, 2007



Biomarker

A characteristic that is objectively measured and evaluated as an indicator of normal biological processes, pathogenic processes, or pharmacologic responses to a therapeutic intervention

Biomarkers Definitions Working Group, Clin Pharmacol Ther, 69:89-95, 2001



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A characteristic that is objectively measured and evaluated as an indicator of normal biological processes, pathogenic processes, or pharmacologic responses to a therapeutic intervention

Biomarkers Definitions Working Group, Clin Pharmacol Ther, 69:89-95, 2001

Toxicity biomarker = disease biomarker = efficacy biomarker



Why Do We Need New Biomarkers?

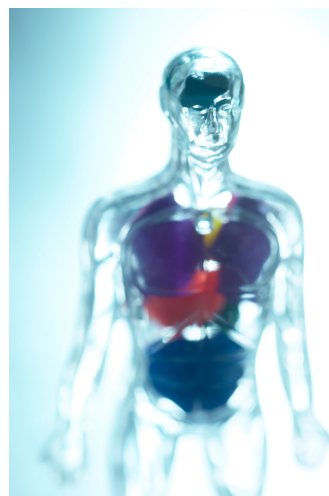
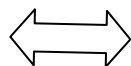
- Improve accuracy of animal and clinical assessments during drug development
- Empower personalized medicine to better manage patients

Concerns

- <100% accuracy in predicting toxicity of even single compounds
 - Nonclinical test species identify many dangerous drugs/chemicals so are not tested in humans but...
 - Even when using multiple species of nonclinical animals, still miss ~30% of drug-induced adverse events seen in humans
- Individual patient susceptibilities (personalized medicine)
 - Relatively small numbers of humans (Phase I-III)



Translational Biomarkers



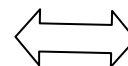
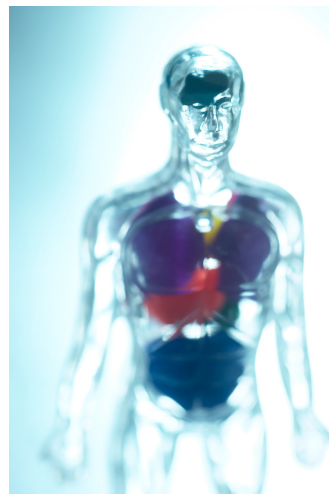
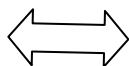
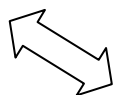
Omics (miRNA, proteomics, metabolomics, etc.) in body fluids from patients treated with APAP, following CPB (children and neonates?), etc.



Translational Biomarkers



Omics in animal models of liver, kidney and heart damage

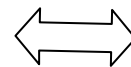
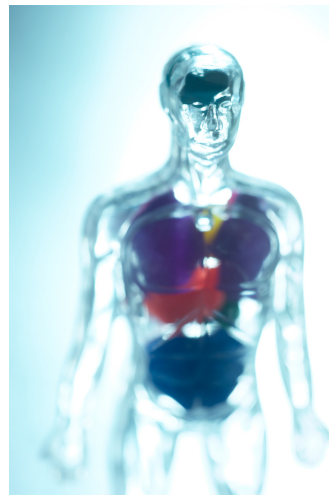
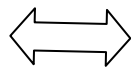
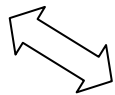


Translational Biomarkers



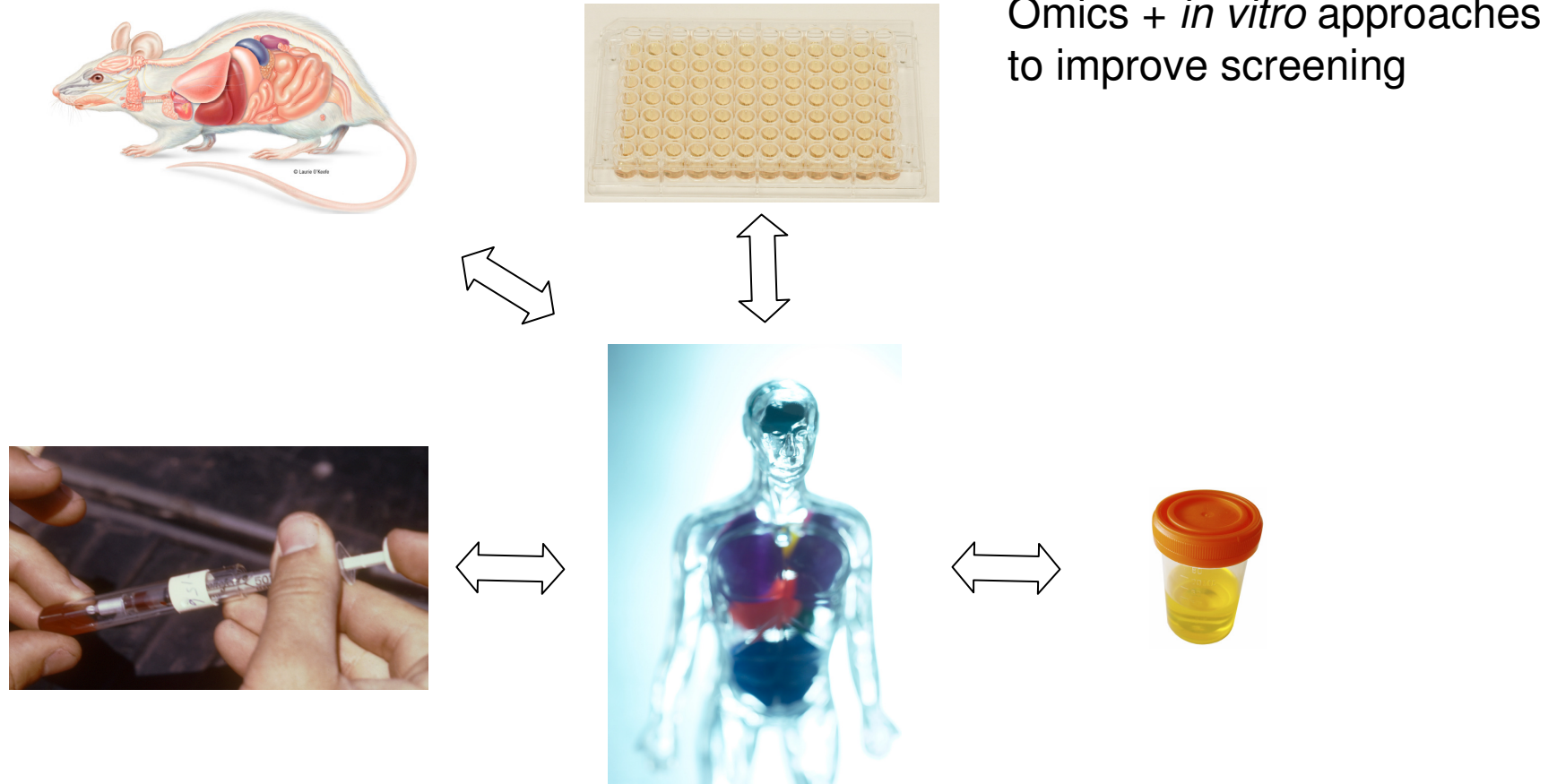
Omics in animal models of liver, kidney and heart damage

e.g., biomarkers (metabolomics and miRNA) from patients and rodents following APAP exposure



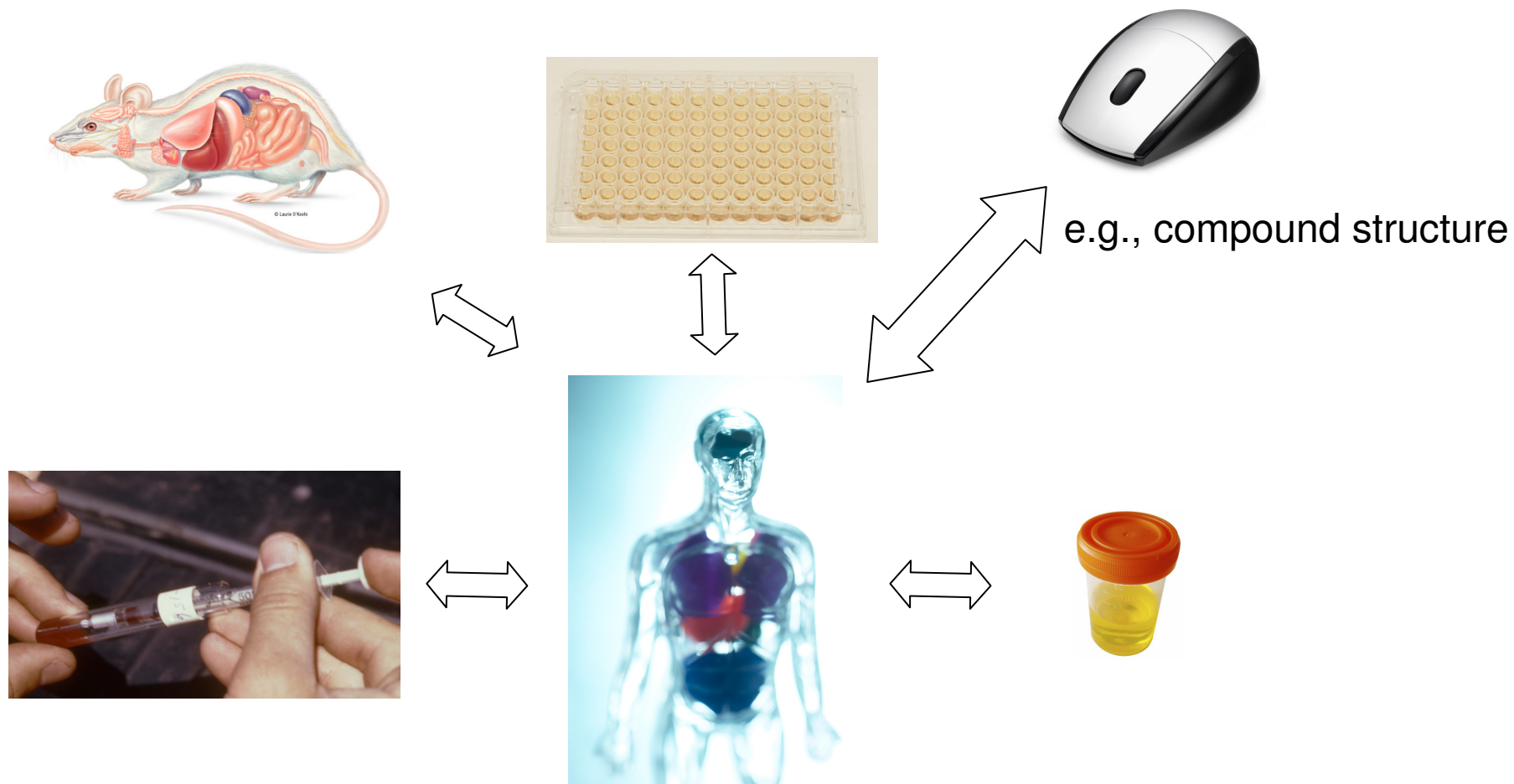


Translational Biomarkers





In Silico Biomarkers



Hepatotoxicity



- Testing in animals identifies many toxic drugs but not a complete safety net
 - ~50% of drugs that cause human hepatotoxicity were not detected in preclinical animal testing
- ~1% of hospitalized patients develop drug-induced liver injury (DILI)
- Liver injury in humans linked to ~1000 drugs
- Some dietary supplements known to cause hepatotoxicity

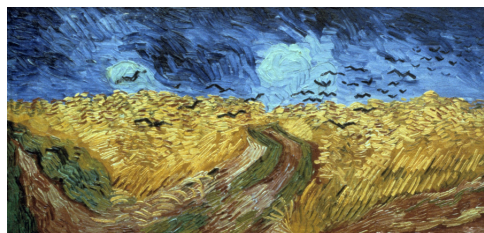
Biomarkers

- Biomarkers of liver injury
 - Need to improve translational medicine
 - Serum ALT is a very sensitive biomarker but not specific enough
 - Bilirubin is a measure of liver function. Serum elevation may occur too late
 - Need biomarkers that predict if a patient's liver injury will progress and provide some information as to severity
- Biomarkers of liver recovery
 - Some patients in acute liver failure recover and some die without a transplantation. How to manage patients?

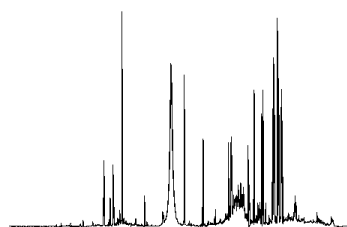
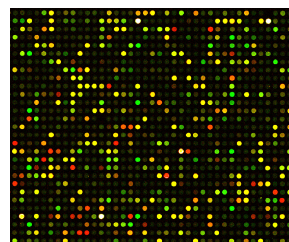


Outline

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- Omics



- Biomarker Qualification and Usefulness





Search for Biomarkers

- Employing genomics, metabolomics, proteomics
- Tissue, blood, urine
- Studying multiple drugs/chemicals



Acetaminophen Hepatotoxicity

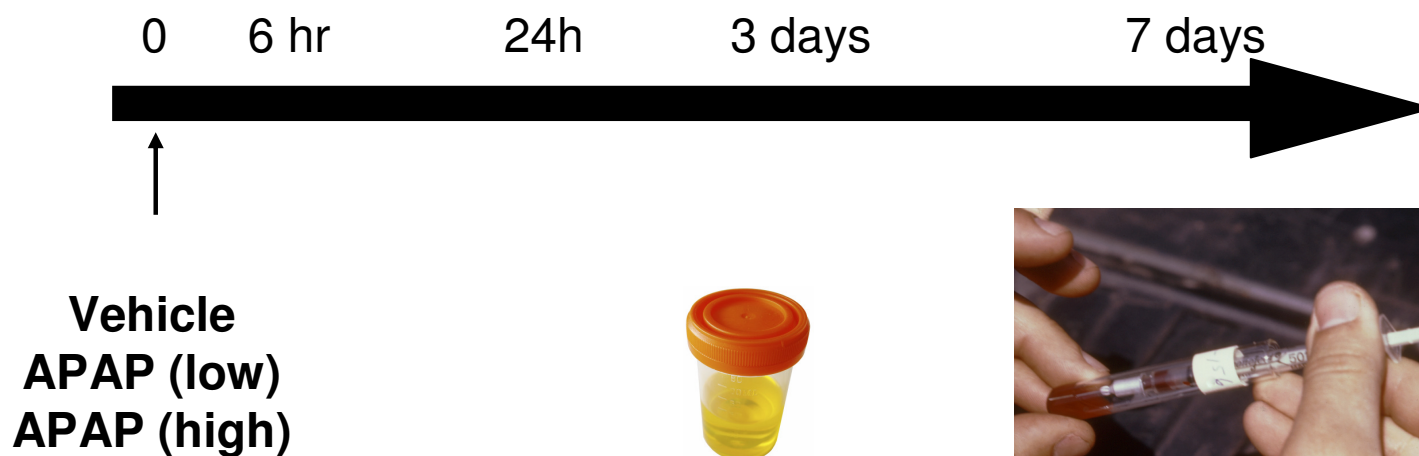
- Contained in many OTC drugs (e.g., Tylenol®, Nyquil®)
- Accounts for ~50% of acute liver failure (ALF) in the US
- N-acetylcysteine: good antidote if given within 12 hr
- 75% survival (9% transplants, 67% spontaneous remissions)
- Cannot predict who will recover without transplant
- Injury apparent by 1.3 days peaking at 3 days

Acetaminophen (APAP) Study Design



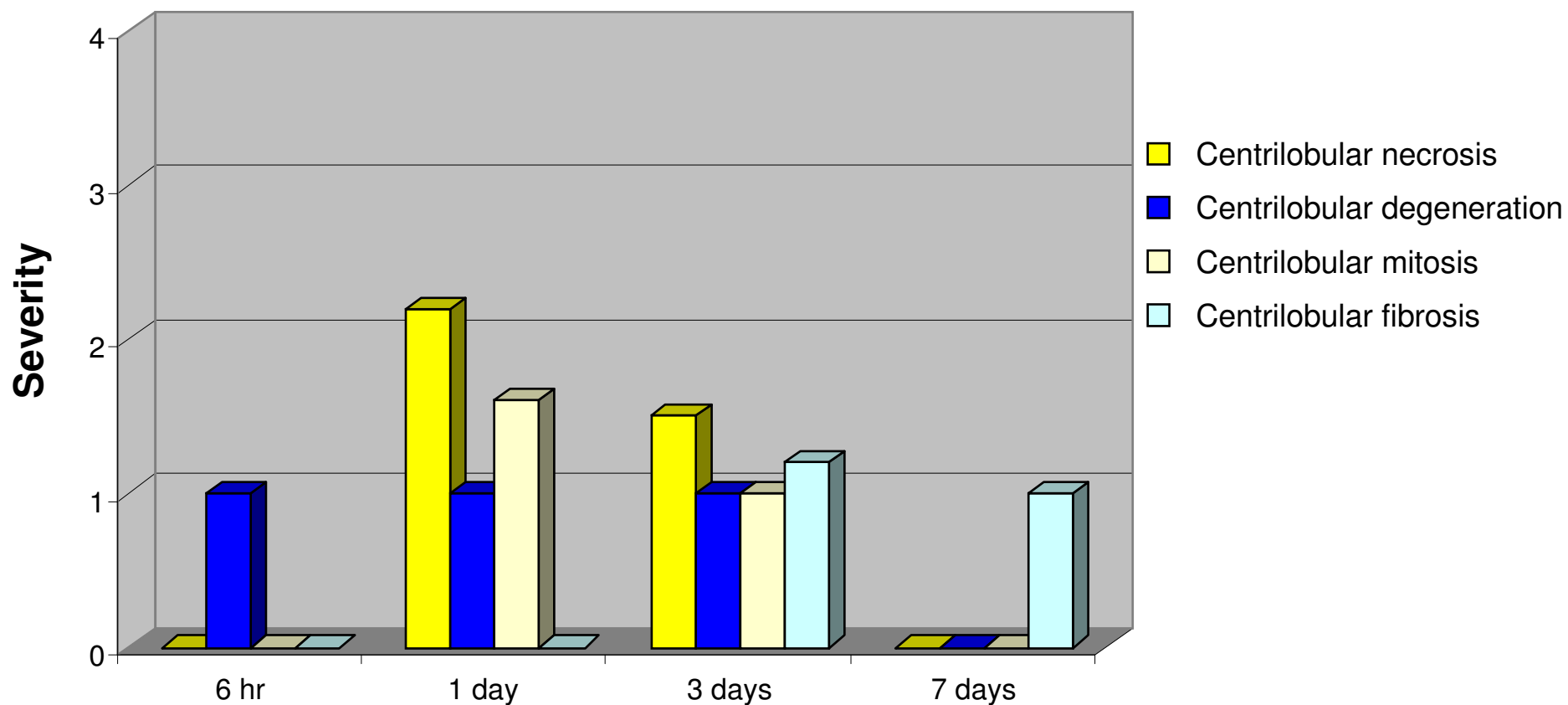
- **Male Sprague Dawley rats**
- **Route: Oral gavage**
- **Vehicle = 0.5% methylcellulose**

Sacrifice time points

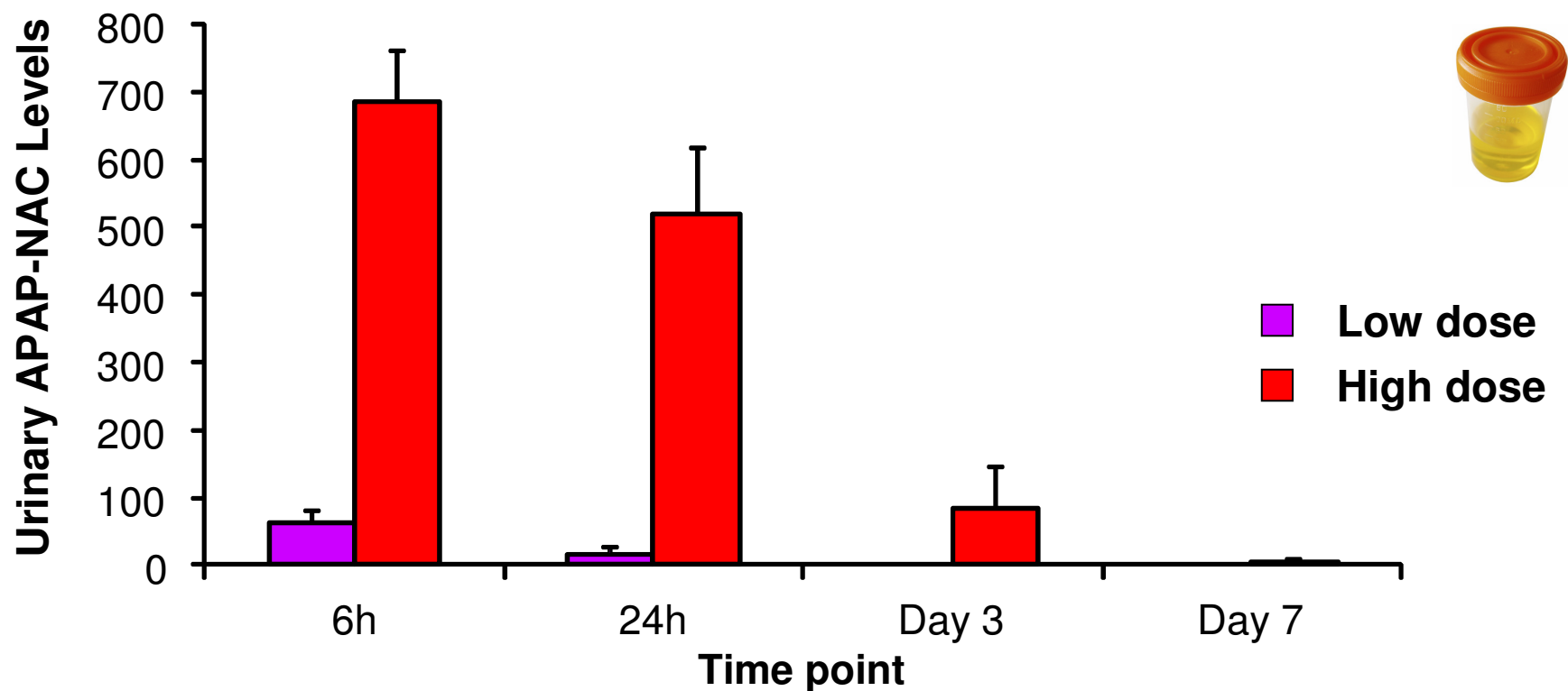




Histology of High Dose APAP



Metabolomic Biomarker of APAP Exposure

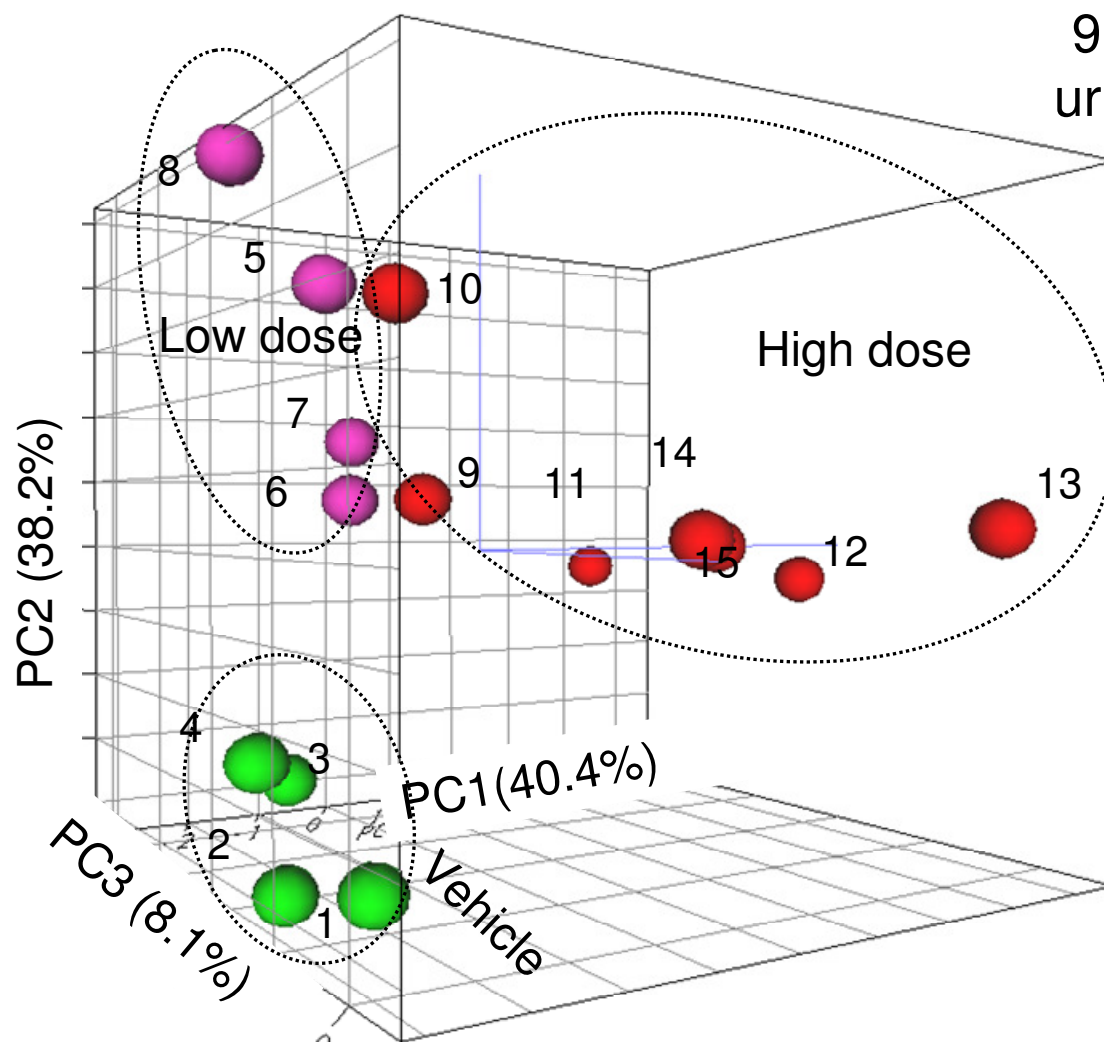


Urinary miRNA

- Can urinary levels of miRNA serve as non-invasive biomarkers of hepatotoxicity?
- miRNA
 - Small, ~22 nt long, non-coding RNAs
 - Actual biological targets and roles are still being worked out
 - Typically downregulate gene expression
 - Much more stable in extracellular fluids than mRNA
 - Tissue-specific expression has been shown for some miRNAs (e.g., miR-122 in liver)



miRNA: Can Differentiate Dose Effects



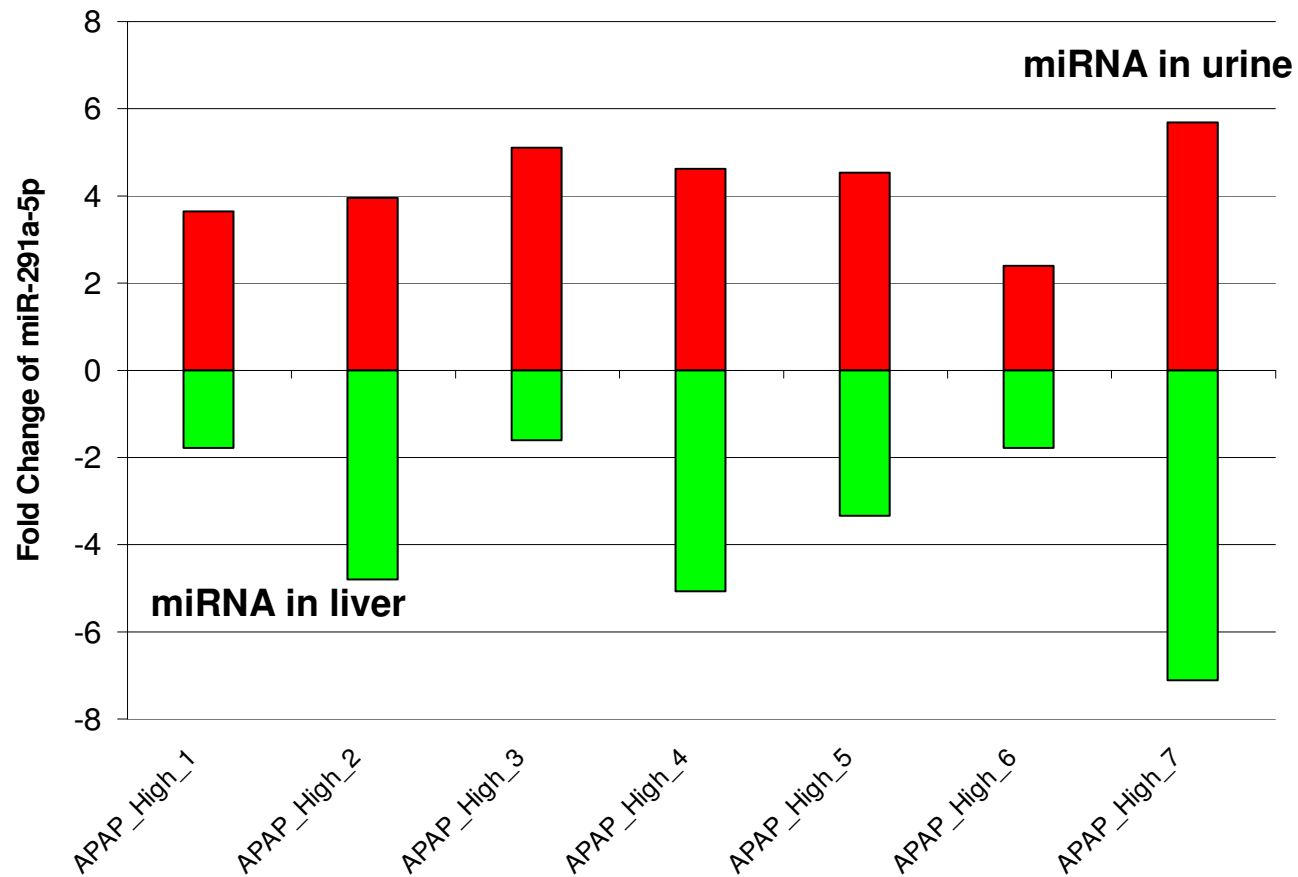
9 differentially affected urinary miRNAs at 24 hr



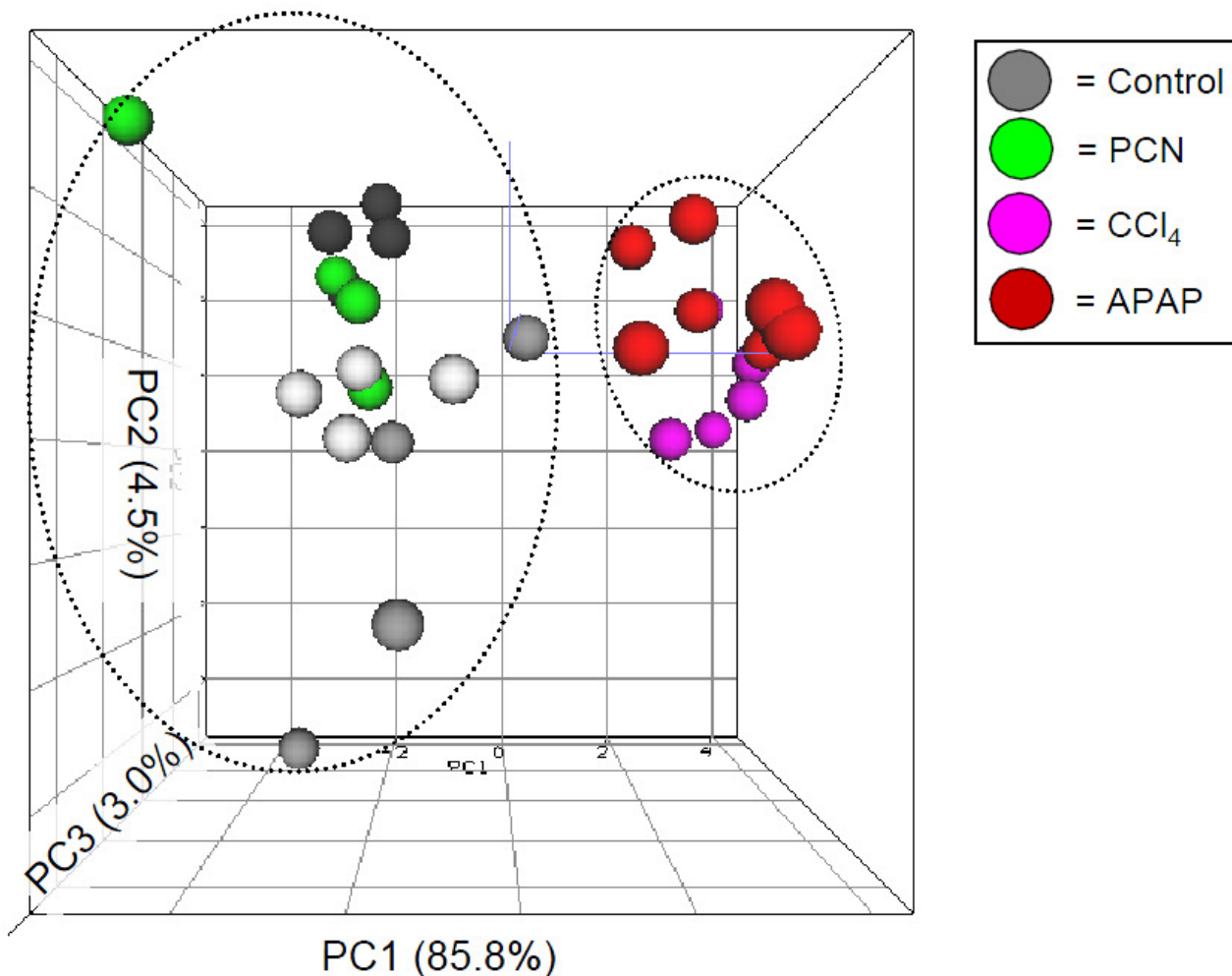
- Vehicle
- Low dose
- High dose

Yang et al., J Postgenom
Drug Biomark Develop
1:101. doi: 10.4172/2153-
0769.1000101

Comparison of miRNA in Liver and Urine

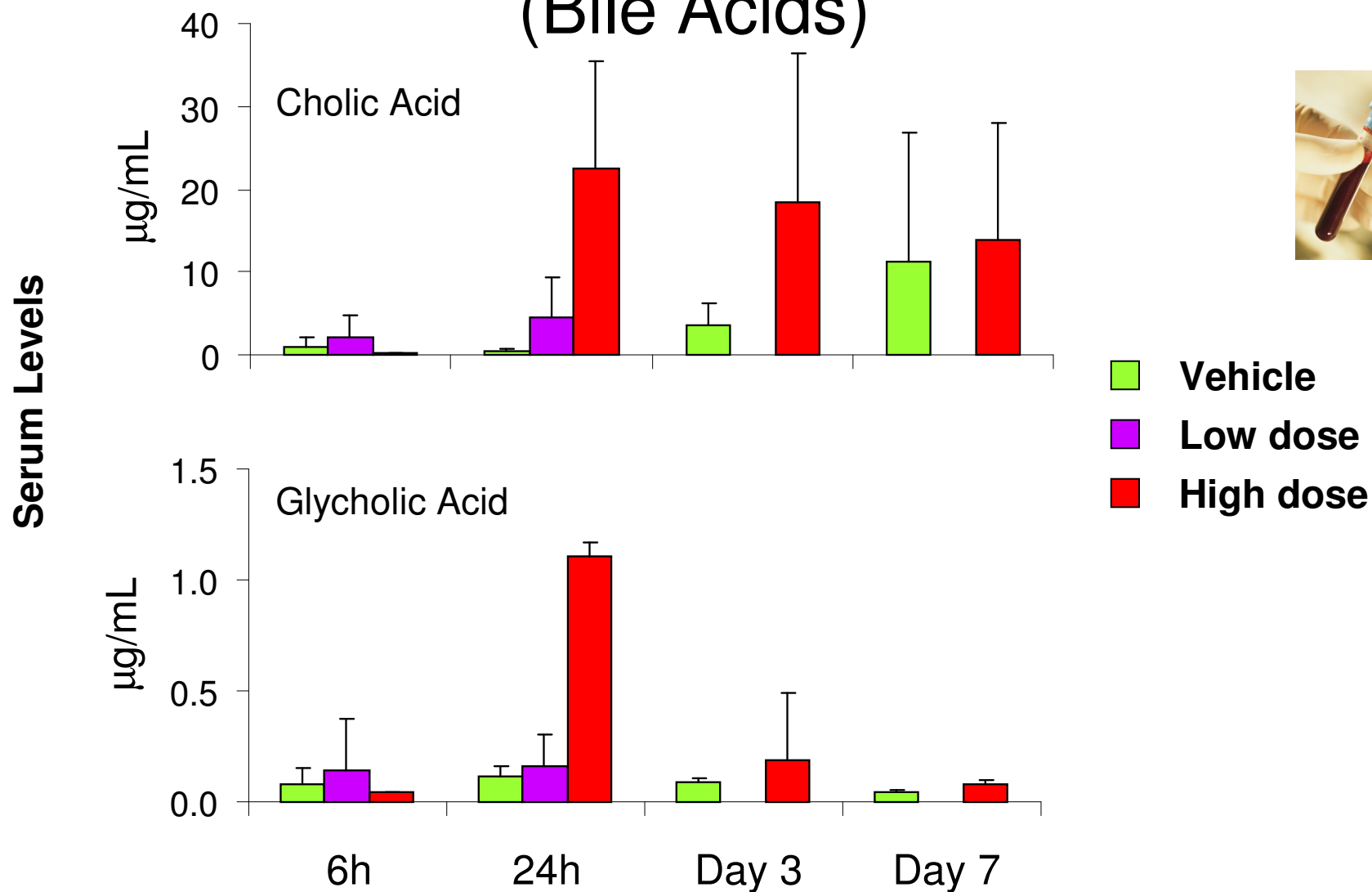


PCA of 10 Urine miRNAs



Visualization of data presented in Yang et al., Toxicol Sci 125: 335–344, 2012

Metabolomic Biomarkers of APAP Toxicity (Bile Acids)





Summary

- Potential metabolic biomarkers of toxicity and recovery can be identified
- Levels of 10 urinary miRNAs were altered by treatment with APAP and CCl₄ but not penicillin or vehicle
 - Additional studies need to be performed to determine how universal these biomarkers might be of liver injury
- Translational nature under investigation
 - Studies underway to look at miRNA and metabolite levels in urine from humans that have overdosed with APAP

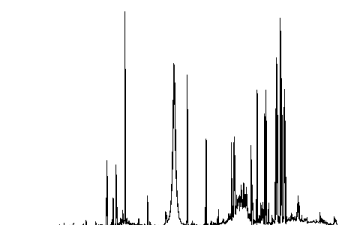
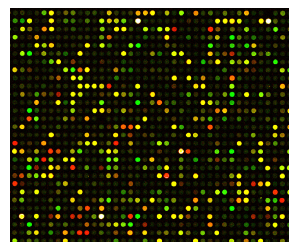


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- Biomarker Qualification and Usefulness





Regulatory Qualification

- Qualification = fit for purpose
- Validation = testing platform characteristics
- Regulatory qualification
 - Rigorous, contextual qualification by FDA-EMA
 - Leads to their use
 - e.g., KIM-1 for renal toxicity in rats
- Non-regulatory qualification
 - Can be used but uptake slow (convincing colleagues, etc.)
 - Performance characteristics not fully established
 - e.g., use of KIM-1 for clinical decision-making

CDER/FDA Biomarker Qualification

- Case by case
 - Within a specific IND/NDA/BLA/Labeling Update
 - For a specific drug
 - Driven by a specific drug development need
- Accepted over extended period of time
 - As scientific experience accumulates in varied uses
 - Usually very extended time-frame
 - Evidence collection haphazard, not cohesively directed



Biomarkers as Drug Development Tools

DDT Type	Name	Submitter	Qualification Date	Link to Supporting Information
Biomarker	Seven Biomarkers of Drug-Induced Nephrotoxicity in Rats	Predictive Safety and Testing Consortium (PSTC), Nephrotoxicity Working Group (NWG)	4/14/2008	Predictive Safety Testing Consortium (PDF - 163KB)
Biomarker	Nonclinical Qualification of Urinary Biomarkers of Nephrotoxicity	International Life Sciences Institute (ILSI)/ Health and Environmental Sciences Institute (HESI), Nephrotoxicity Working Group	9/22/2010	HESI Nephrotoxicity Qualification (PDF - 234KB)
Biomarker	Nonclinical Qualification of Circulating Cardiac Troponins T and I as Biomarkers of Cardiac Morphologic Damage	PJ O'Brien, WJ Reagan, MJ York and MC Jacobsen	2/23/2012	Biomarker Qualification Decision (PDF - 144KB)



Usefulness

Characteristics of Biomarkers-I

- Cross species translation not critical but preferred
- What is the setting in which it will be used?
 - Clinical: non-invasive (e.g., body fluids)
 - Preclinical: tissue and body fluids available
- What is the “gold standard” to which it will be compared?
 - Preclinical may be histology, clinical may be serum biomarker
 - How to determine if it is translational if different comparisons used?



Characteristics of Biomarkers-II

- Regulatory qualification
 - Rigorous, contextual qualification by FDA-EMA
 - Leads to their use
 - e.g., KIM-1 for renal toxicity in rats
- Non-regulatory qualification
 - Can be used but uptake slow (convincing colleagues, etc.)
 - Performance characteristics not fully established
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Assessment of Biomarkers



Training Set

Normal vs. Adverse Event

Curated Population



Validation Set

Normal vs. Adverse Event

Less Curated
Population



Use of Final Model



Open access

Patients with
concomitant
diseases,
medications,
etc.



MAQC Efforts

- International consortium headed by Drs. Shi and Tong at NCTR
- Includes EPA, pharmaceutical companies, platform providers, academics and all regulatory centers at FDA
- MAQC-I: improved understanding of QC and basic analysis methods
- MAQC-III: looking at next generation sequencing



MAQC-II (2007-2010)

- Which modeling factors are important?
- Why does an approach succeed or fail when faced with new data?
- 202 participants from 98 organizations
 - 36 data analysis teams/protocols
- 6 data sets (13 endpoints); 3 human diseases, 3 animal toxicology endpoints
- Independent peer review
- Blinded statistical validation
- 12 papers in Nature Biotechnology and The Pharmacogenomics Journal, August 2010
- Supplement in October 2010(www.nature.com/focus/maq2/)



How Are Pharmacogenomic Biomarkers Being Used?



Types of Genetic/Genomic Biomarkers

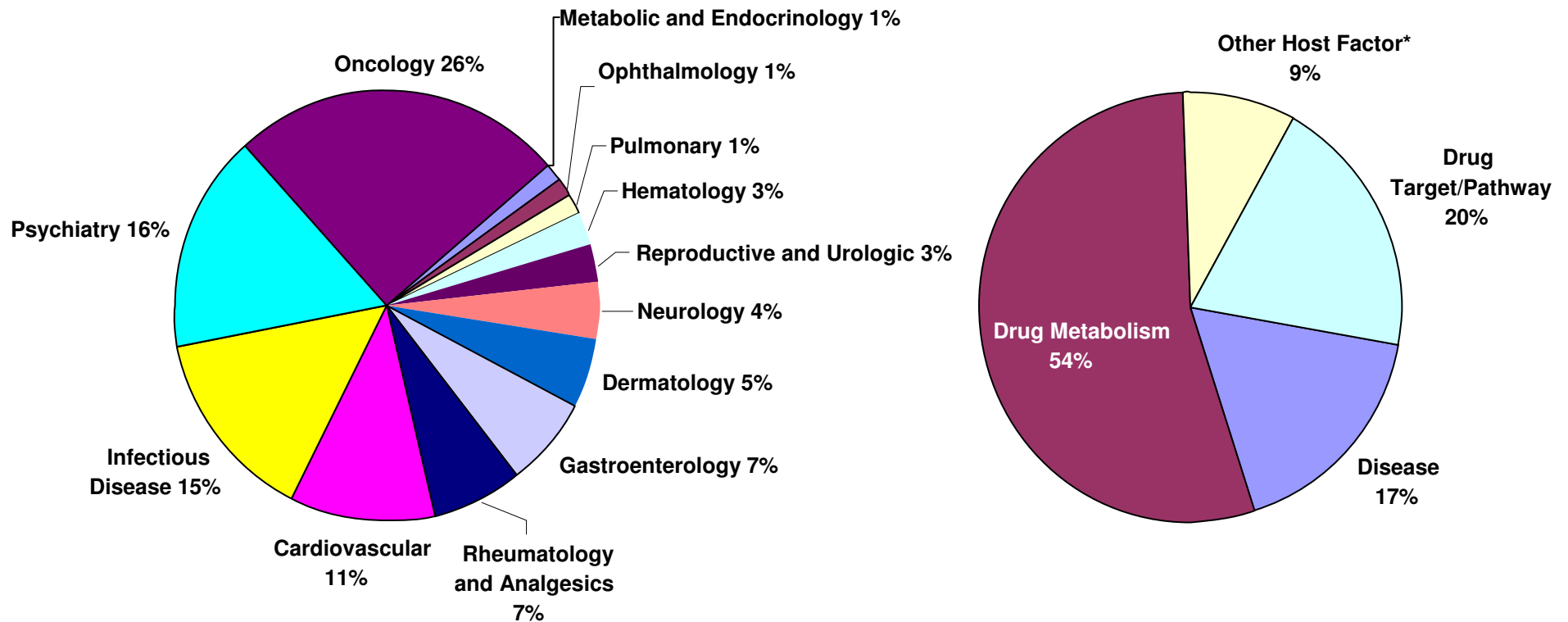
- Predictive
 - HLA-B*5701 and Abacavir (Ziagen®)
- Diagnostic
 - AlloMap®
- Prognostic
 - MammaPrint®

Gene Signatures Not Reproducible and/or Harmful?

- Researchers at Duke University “identified” gene signatures of drug sensitivity in NCI60 cell line panel; started clinical trials using these to identify treatment-specific regimens for cancer patients
 - But.....
- “When these cell line-based predictors were applied to patient data, there was no significant correlation between observed response and predicted response either for individual drug predictors or combined predictions;” “...patients at risk.”

Michiels et al., *Lancet* 365:488-492, 2005; Baggerly and Coombes, *Annals of Applied Statistics* 3:1309-1334, 2009; Liedtke et al, *Breast Cancer Res Treat* 121:301-309, 2010

PGx in Drug Labels



Cancer, psychiatric, and infectious disease therapeutics make up more than half of the drugs with PGx labeling

Most PGx labeling is related to drug metabolism



If You Build It....

- Flucloxacillin-induced DILI
 - 8.5 cases/100,000 patients
 - Strong association with HLA-B*57:01
 - 7% Caucasian carry this gene
 - But, of 833 positive, 1 develops DILI
 - 83,800 negatives, 1 develops DILI
 - 13,500 individuals need to be screened to prevent 1 case
 - Would deprive good therapy to thousands of individuals and not cost effective
 - Unlikely to be used



If You Build It....

- Ximelagatran-induced DILI
 - 7900 cases/100,000 patients, drug withdrawn
 - Strong association with HLA-DRB1*07:01
 - 20-24 individuals need to be screened to prevent 1 case



Summary, Part I

- Need better biomarkers in animals and humans
- Hopefully translational in nature
- Omics offers much promise
- Biomarkers need to be statistically validated and biologically qualified in a rigorous manner



Summary, Part II

- FDA recognized PGx as a key tool for therapeutic individualization over a decade ago
 - Early initiatives focused on scientific exchange (e.g., VXDS) have now evolved into integrative aspects of therapeutic product evaluation
- Pro-active incorporation of PGx principles in drug development is now the main focus
- Policies, guidance, and infrastructure continue to evolve with experience



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