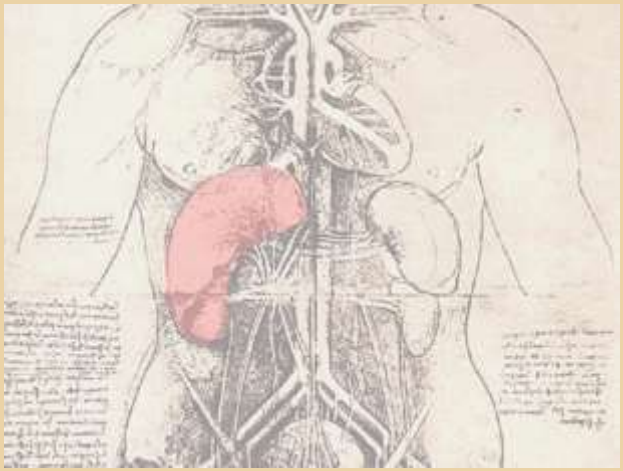

New biomarkers for drug-induced liver injury: First insights from clinical qualification

Joint Conference of European Human Pharmacological Societies, Nice, April 11-12, 2013

Michael Merz, Novartis Institutes for BioMedical Research

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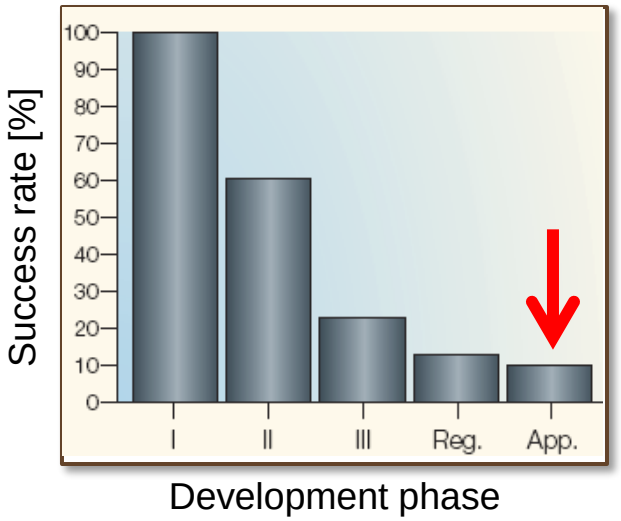
Outline

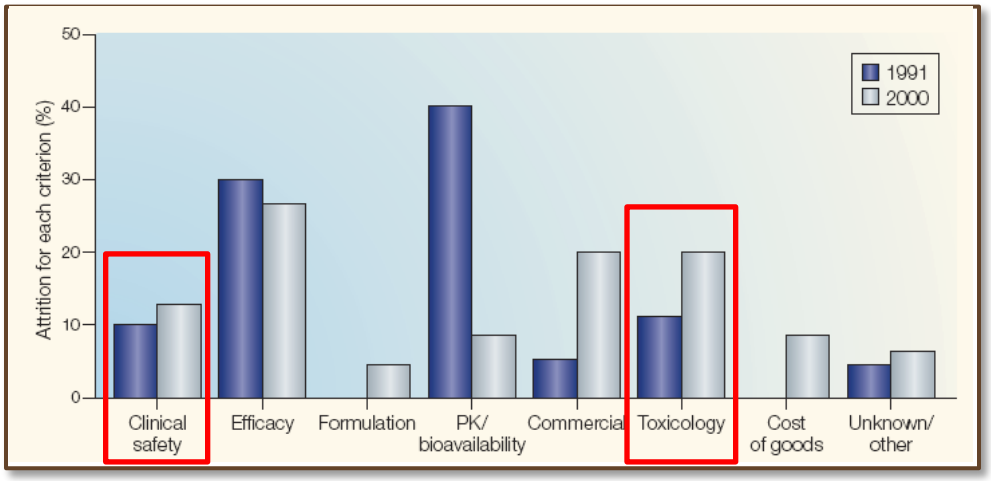
- DILI background
- Shortcomings of current liver safety biomarkers, requirements for advanced markers
- Biomarker qualification in the IMI SAFE-T consortium
- Initial findings for new liver safety biomarkers
- Biomarker discovery

Drug safety: room for improvement

Attrition in drug development



- Around 90% of compounds entering clinical development fail



- 30% of these failures are due to clinical safety and toxicology

Kola et al. (2004), Nat Rev Drug Discovery; 3: 711-15

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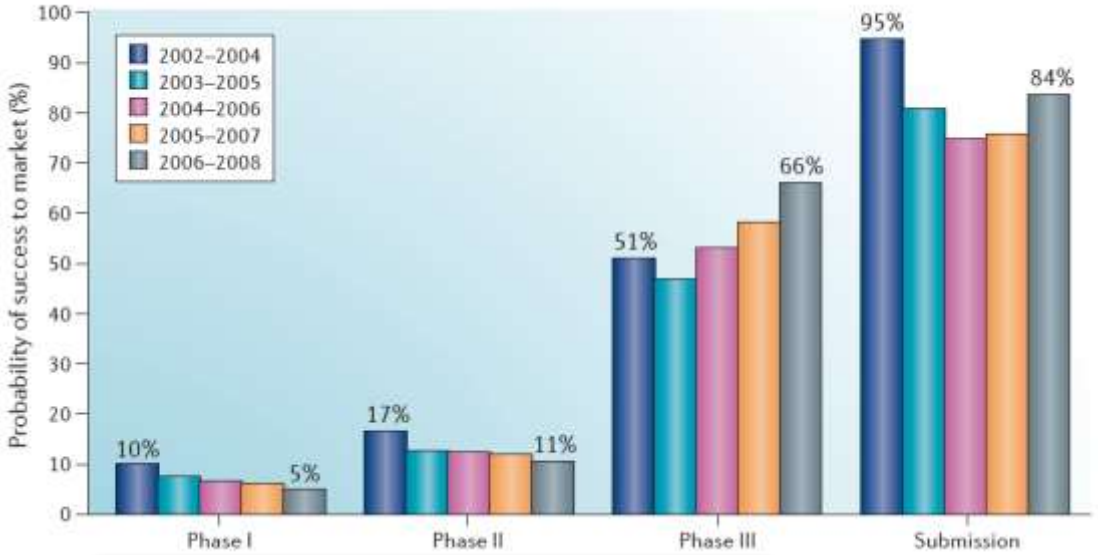
Attrition rate over time

A universal and eternal constant?

Nat Rev Drug Discovery 2012; 11(1): 17-18

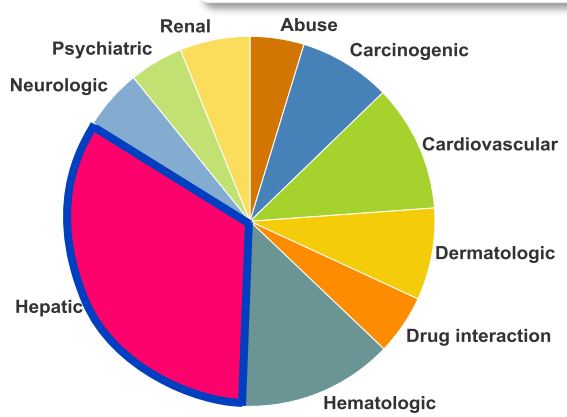


Probability of success by development phase

- Do we have to live with losing e.g. 1/3 of drugs in phase III and 1/6 during submission??

- For decades, drug-induced liver injury has been one of the key safety issues in drug development and post marketing

Reasons for withdrawals



Drug Info J 2001; 35:293

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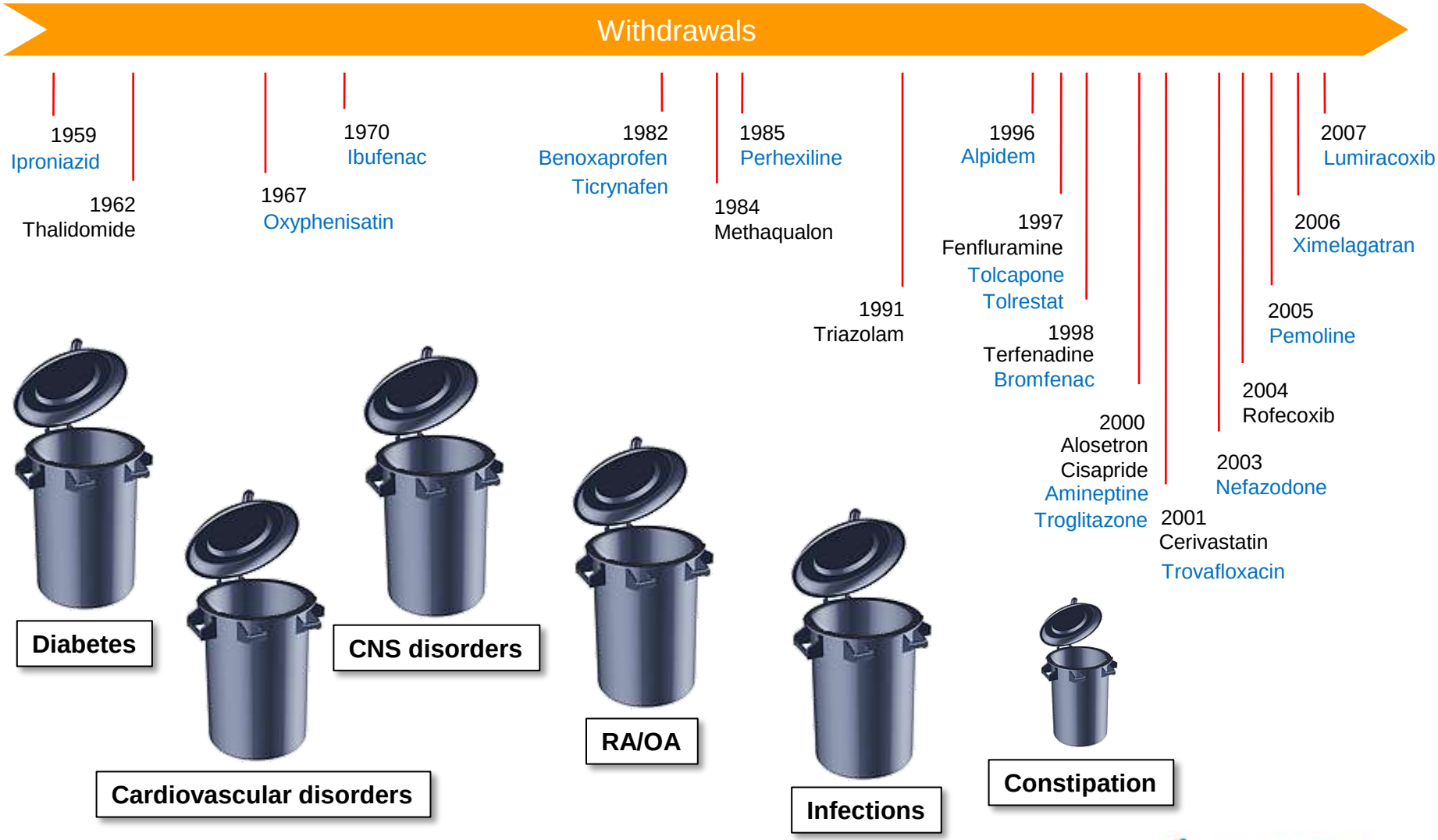
Withdrawals due to drug-induced liver injury (DILI)

Reducing treatment options for key disease areas



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Drug-induced liver injury (DILI)

Key challenges

- DILI is the leading cause of acute liver failure in the United States
- In the post-approval setting, DILI is a leading cause of regulatory actions, including drug withdrawals, label changes and boxed warnings
- Across the industry, we regularly loose promising candidates due to DILI
  - A part of those may be false positives
- Of predominant concern are idiosyncratic, hepatocellular types of DILI
  - Non-dose dependent (?)
  - Not predictable (as yet)
  - High rate of liver failure, often fatal outcome
  - Rare
- A major issue is the lack of suitable markers allowing for
  - Early signal detection
  - Mechanistic assessment
  - Robust prediction of clinically relevant effects
  - Risk assessment in individual patients

Standard liver tests: ALT, AST, AP, γGT, bilirubin

Some shortcomings

- Inadequate sensitivity and specificity
- Limited predictive value, both from a translational and clinical outcome perspective
- Do not allow for differentiation between injury, upregulation, reduced clearance
- Half life of aminotransferases too long to allow for close monitoring and assessment of rapid changes in liver status
- Aminotransferase activities frequently confounded by e.g. effect of different diets and different levels of physical exercise
- Not supporting mechanistic understanding
- Focusing on liver only, not taking into account immune system involvement

☞**Clear need for alternative biomarkers of drug related liver injury.**

Specific liver test attributes of interest

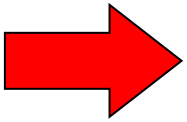
- Patient level
  - Lower injury threshold
  - Earlier time to onset
  - Larger extent of changes
  - Improved liver specificity
  - Better suited to monitor and predict clinical outcome
  - Better suited to assess causality
- Population level
  - Earlier and more specific liver signal detection in clinical development programs
  - Improved mechanistic insight
  - Superior in terms of identifying underlying pathology
  - Better suited to predict human risk from animal toxicity

Key challenges for biomarker qualification

- Substantial background variability in initial candidate markers
- Biomarker response varies across different populations
- Large initial number of biomarker candidates requires substantial sample volumes to be taken
- Cases with key target response, i.e. DILI, suitable and accessible for qualification, are overall very rare

➢ Large sample sizes are required

➢ Multitude of patient populations need to be included



Qualification cannot be achieved by one company alone

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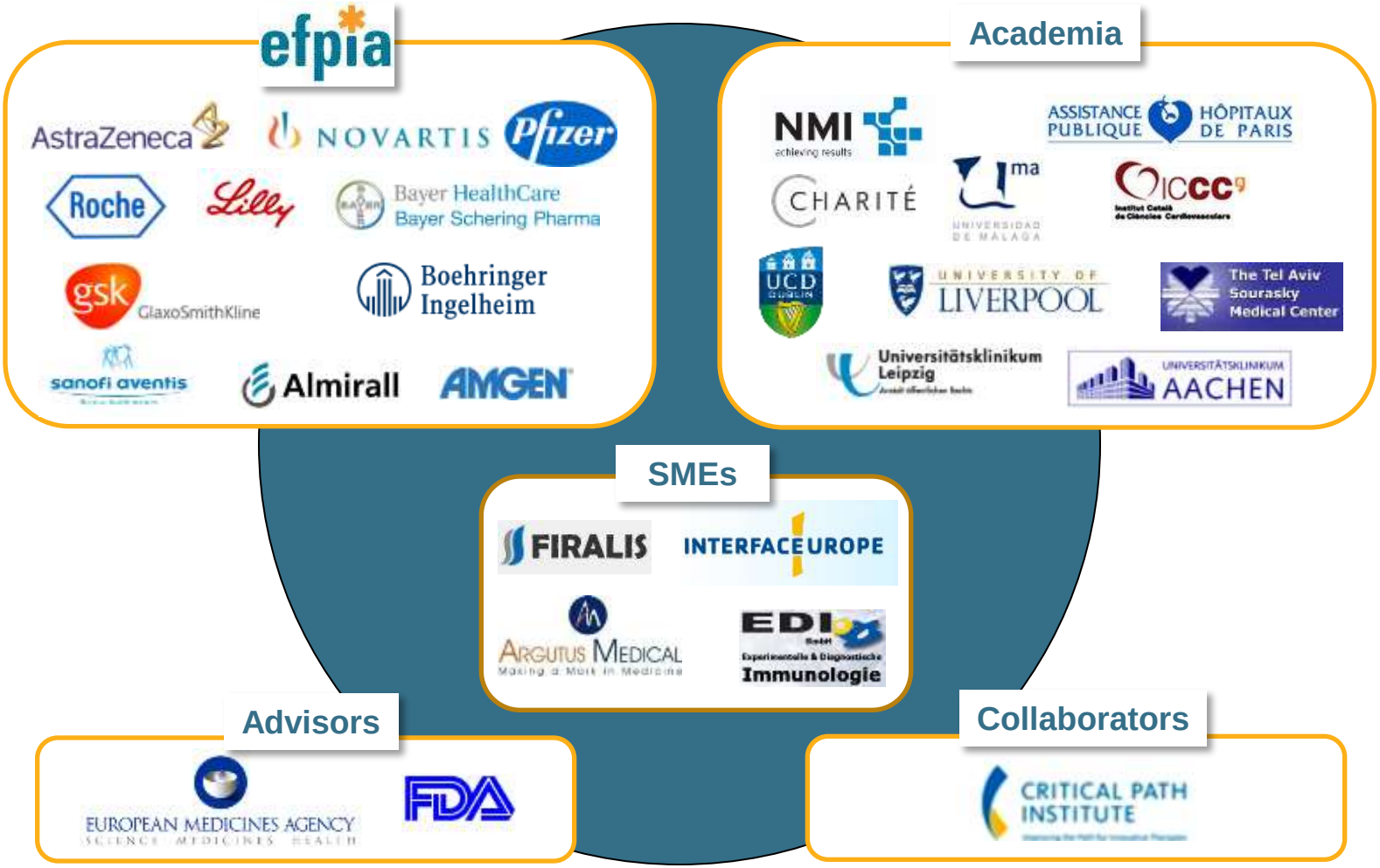
IMI SAFE-T Consortium

Objectives

- To evaluate utility of safety biomarkers for detecting, assessing, and monitoring **drug induced kidney, liver, and vascular injury** in humans
- To develop assays and devices for clinical application of safety biomarkers
- To compile enough evidence to qualify safety biomarkers for regulatory decision making in clinical drug development and in a translational context
- To gain evidence for how safety biomarkers may also be used in the diagnosis of diseases and in clinical practice

SAFE-T participants



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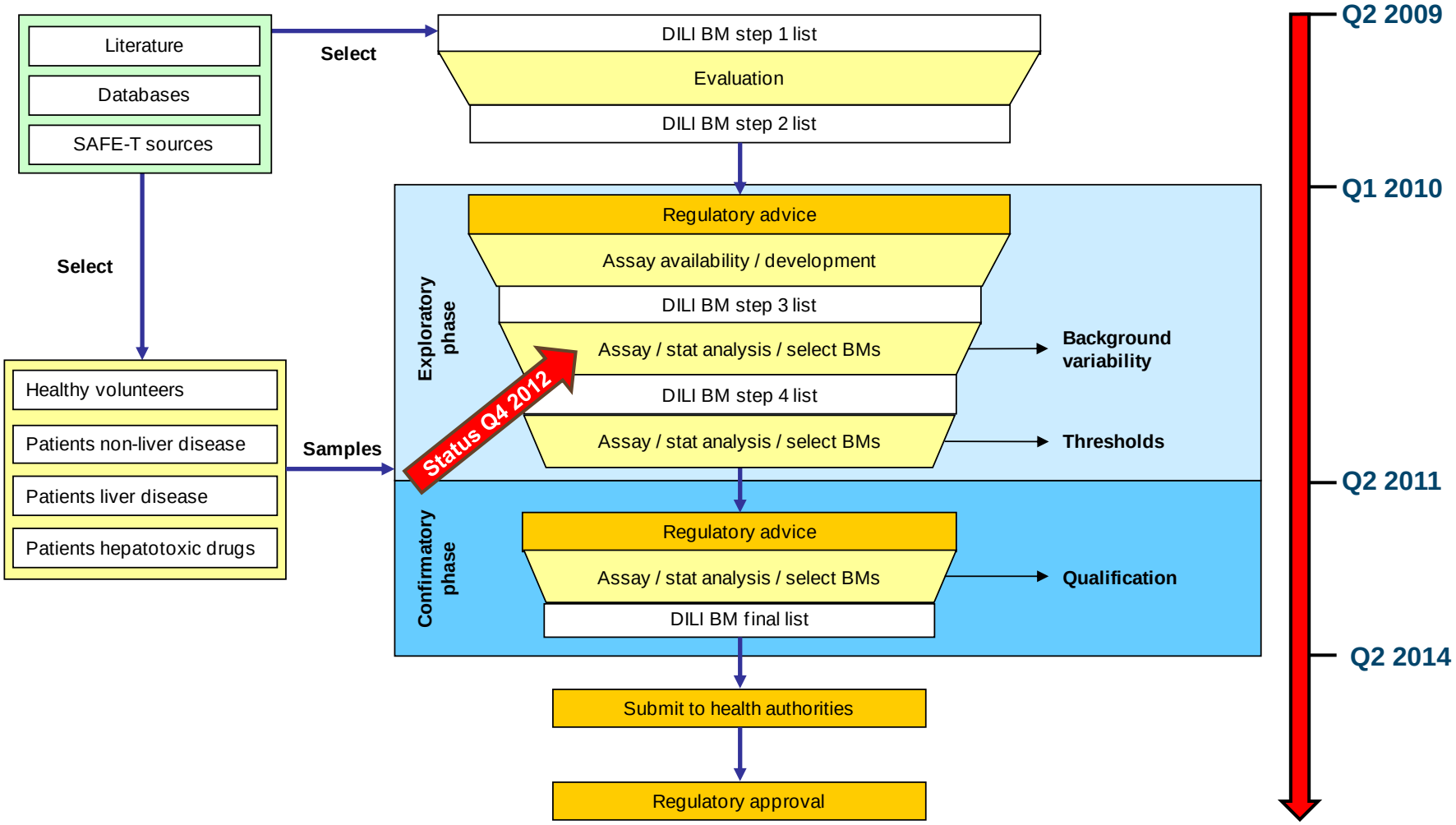
SAFE-T Biomarker qualification process

Elements and process flow



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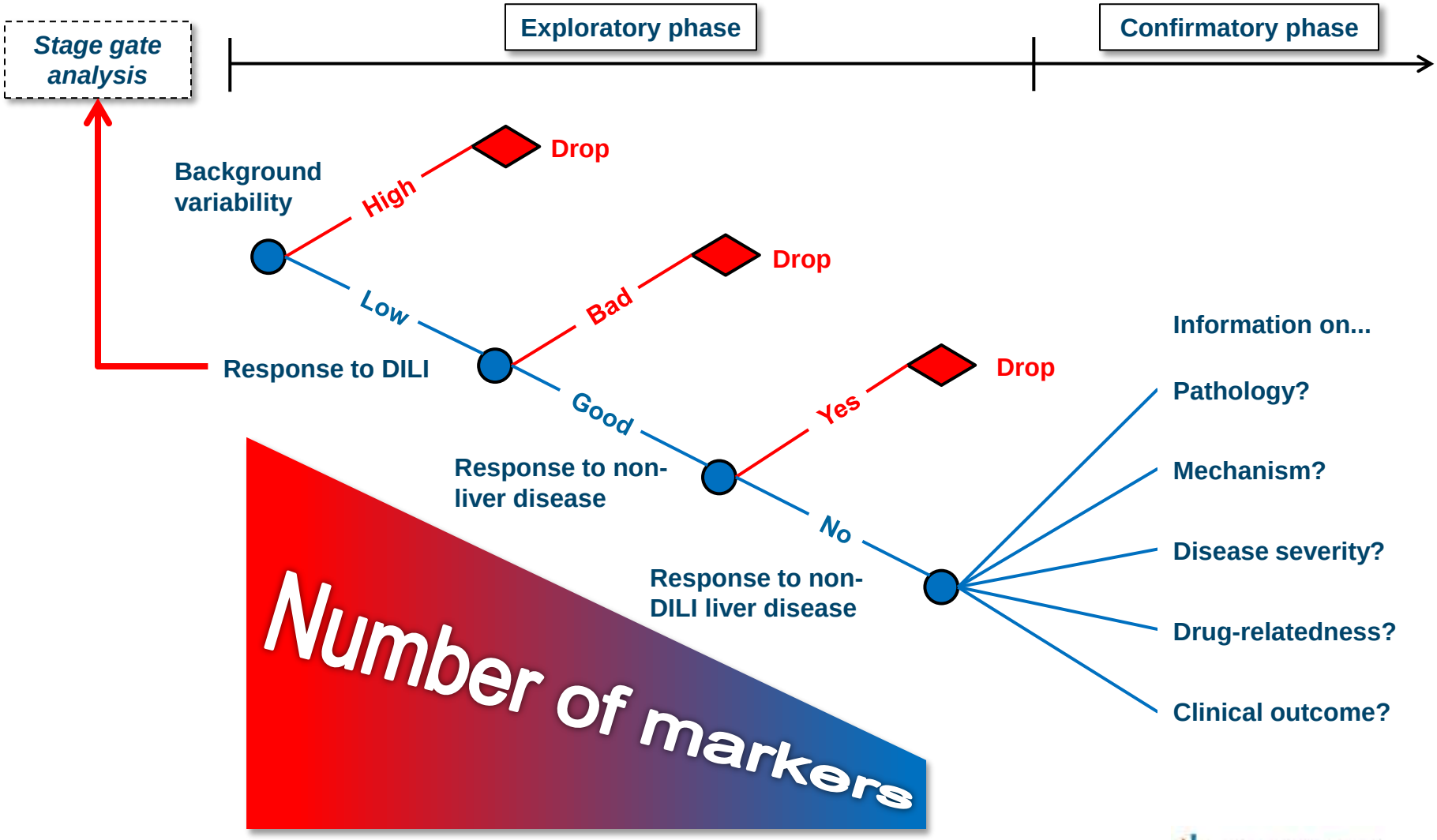
DILI biomarker selection process

Two (& a half) stage approach



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Ongoing prospective clinical studies

Populations and (some) key objectives

- Multi-center international study in patients with suspected drug-induced liver injury
  - Sensitivity, specificity, predictive value (outcome), association with standard markers, time profiles
- Single-center study in rheumatoid arthritis patients
  - Specificity, association with standard markers
- Single-center study in patients with acute or myeloid lymphoblastic leukemia on chemotherapy
  - Sensitivity, specificity, predictive value (outcome), association with standard markers, time profiles
- Multi-center study in patients after liver transplantation
  - Link to histopathology, association with fibrosis progression
- Multi-center study in patients on antituberculosis treatment
  - Differentiation of susceptible patients from adaptors and tolerators
- Multi-center Swiss study in patients with suspected drug-induced liver injury
  - Specificity, predictive value, association with genetic suspectibility markers
- Single-center study in nevirapine-treated HIV patients
  - Differentiation of susceptible patients from adaptors and tolerators
- Single-center study in acetaminophen-overdose patients
  - Predictive value (outcome), association with standard markers, time profiles, mechanistic understanding

Biomarker candidates

Initial selection

Candidate markers	Liver specificity	Pathology
microRNA 122	Highly specific	Hepatocellular injury
albumin mRNA	Highly specific	Hepatocellular injury
Microglobulin precursor (Ambp) mRNA	Highly specific	Hepatocellular injury
High mobility group (HMGB-1), hypo- and hyperacetylated forms	Not specific	Hepatocellular injury, inflammation
Cytokeratin 18, full length and caspase cleaved fragment	Not specific	Hepatocellular injury, necrosis vs apoptosis
Conj./unconj. bile acids	Highly specific	Cholestatic injury
Urocanic Acid	Not specific	Cholestatic injury, biliary hyperplasia
ALT1 & 2	Highly (ALT1)	Hepatocellular injury
Glutamate dehydrogenase (GLDH)	Specific	Hepatocellular injury
Purine nucleoside phosphorylase (PNP)	Specific	Hepatocellular injury
Malate dehydrogenase (MDH)	Not specific	Hepatocellular injury
Glutathione S-Transferase (GST-alpha)	Specific	Hepatocellular injury
F-protein (HPPD)	Highly specific	Hepatocellular regeneration
Arginase 1	Highly specific	Hepatocellular injury
alpha-fetoprotein	Specific	Hepatocellular injury
Regucalcin	Specific	Hepatocellular injury
alpha2,6-sialyltransferase (ST6gal)	Specific	Inflammation
Osteopontin	Not specific	Inflammation
Colony stimulating factor receptor (CSF1R)	Not specific	Inflammation
Paraoxonase 1 (PON1)/Prothrombin	Specific	Hepatocellular function
Leucocyte cell-derived chemotaxin2 (LECT2)	Not specific	Inflammation

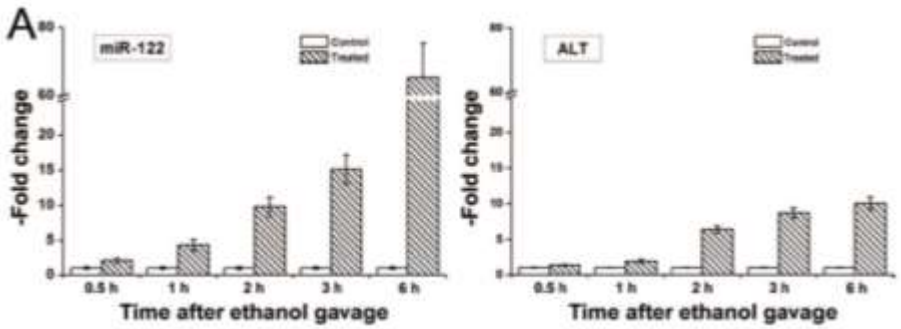
Blood-based microRNA biomarkers for DILI

Evidence from preclinical models

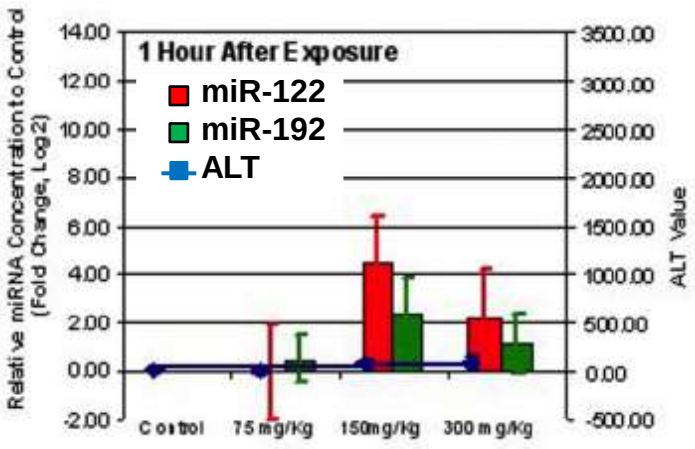
Courtesy Ina Schuppe Koistinen, Astra Zeneca

miR-122

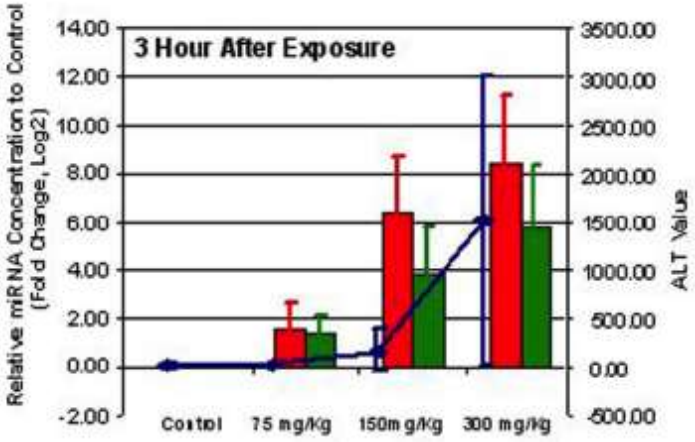
- Liver tissue specific
- Translatable to human
- Earlier detection than ALT; greater sensitivity; less variability...



Yi Zhang et al, Clin Chemistry, 2010



APAP/mouse



APAP/mouse

Wang et al, PNAS, 2009

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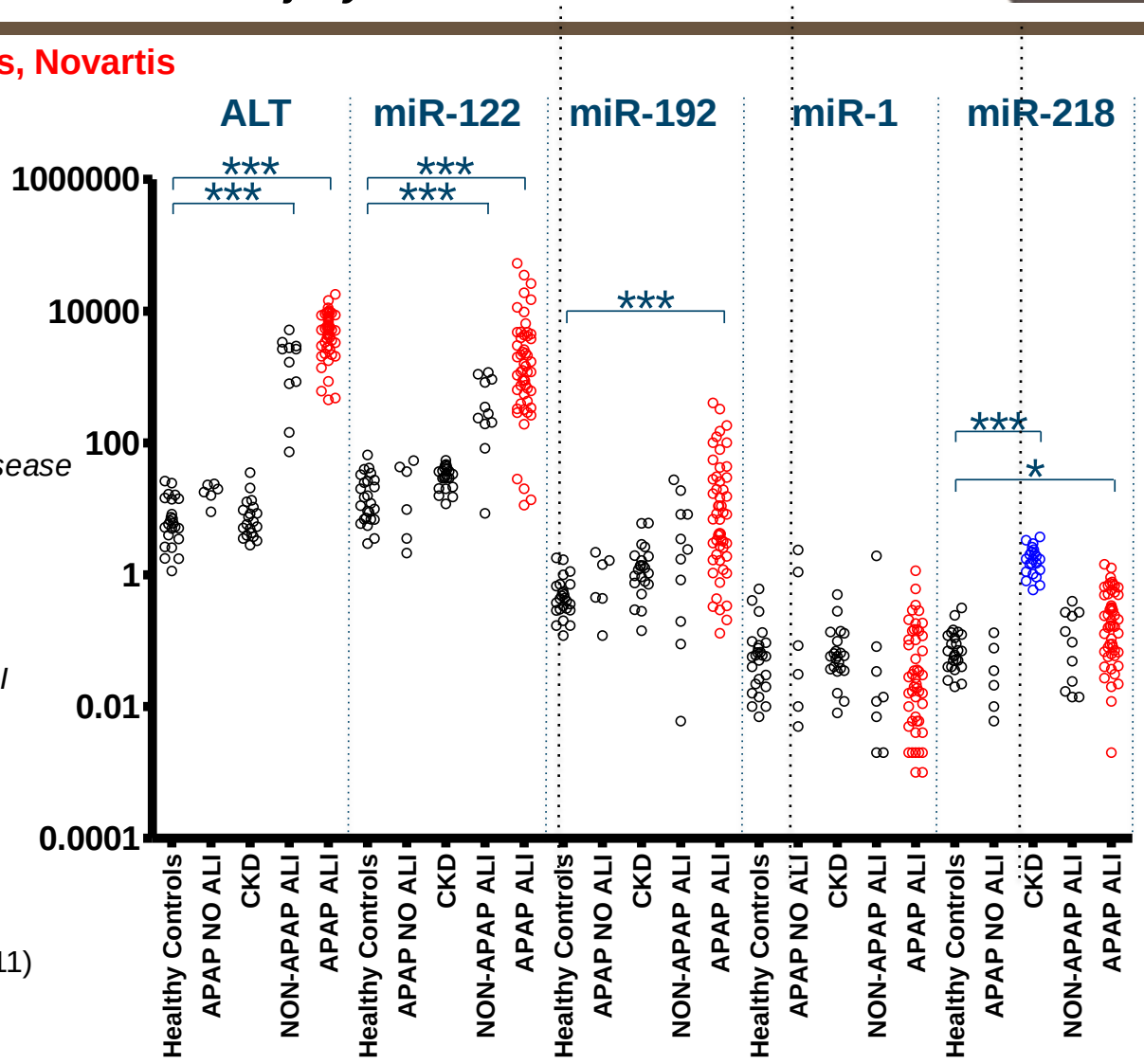
Serum microRNAs as human DILI biomarkers

Specificity of miR-122 for liver injury

Courtesy Jonathan Moggs, Novartis

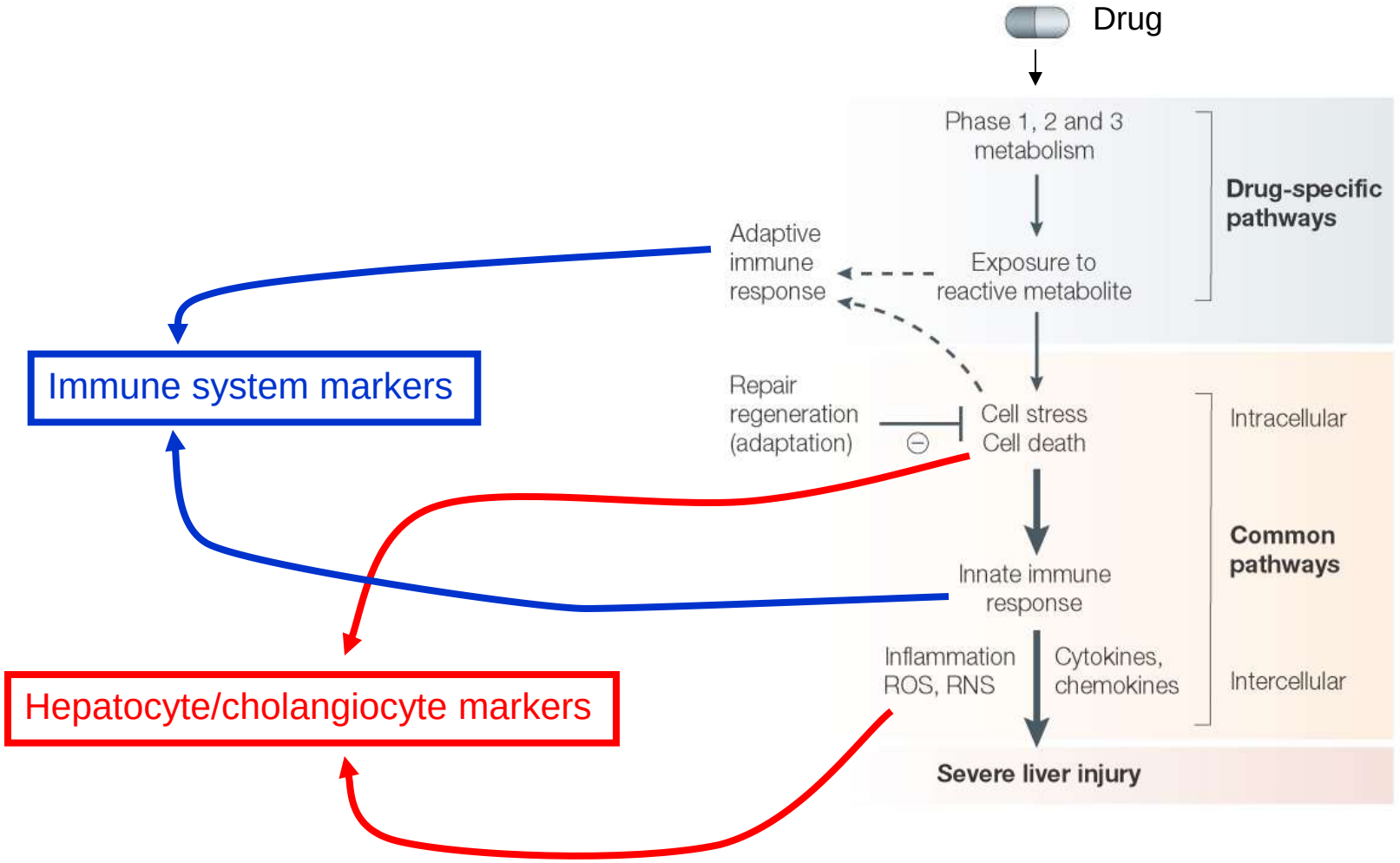


ALI: acute liver injury
CKD: chronic kidney disease
APAP: acetaminophen
non-APAP ALI:
- *autoimmune*
- *HBV*
- *HCV*
- *Clarithromycin DILI*

Starkey-Lewis et al., (2011)
Hepatology 54:1767

Standard liver tests: focus on liver only

Need to account for mechanistic background



Adapted with permission from Kaplowitz N, Nat Rev Drug Discov. 2005 Jun; 4(6): 489-99

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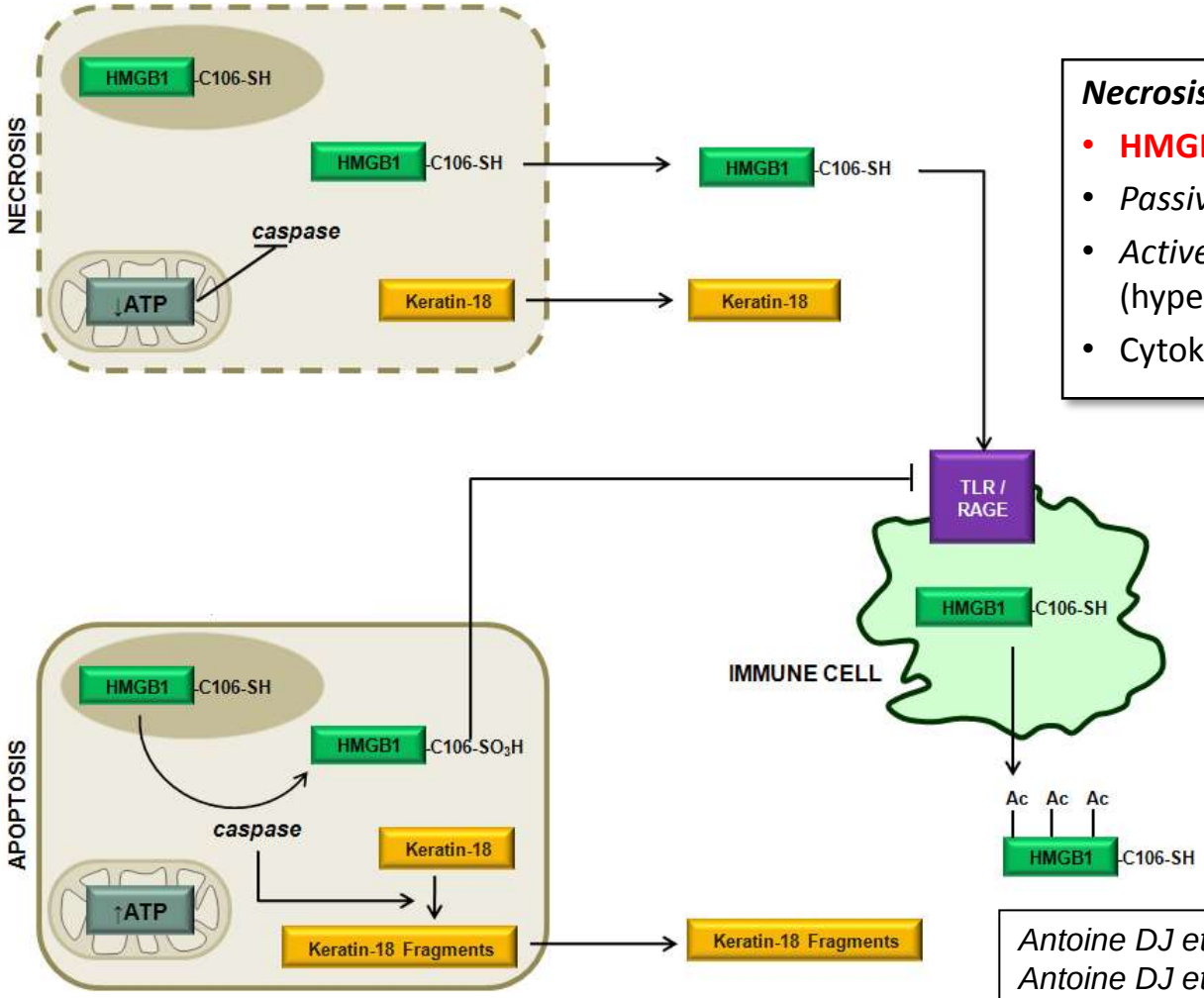
HMGB1 and Cytokeratin 18

Mechanism based biomarkers

UNIVERSITY OF LIVERPOOL

MRC Centre for Drug Safety Science

Slide courtesy Neil French, MRC CDSS



Necrosis and Inflammation:

- **HMGB1** – chromatin binding protein
- *Passive* release by necrotic cells
- *Active* release by activated immune cells (hyper-acetylated (Lys NLS))
- Cytokine activity (TLR/RAGE)

Apoptosis:

- **Keratin-18** – intermediate filament protein / structural integrity
- Is cleaved by caspases
- Fragment released into blood
- Full length K18 passively released during necrosis

Antoine DJ et al., 2010 Mol Med
Antoine DJ et al., 2009 Toxicol Sci

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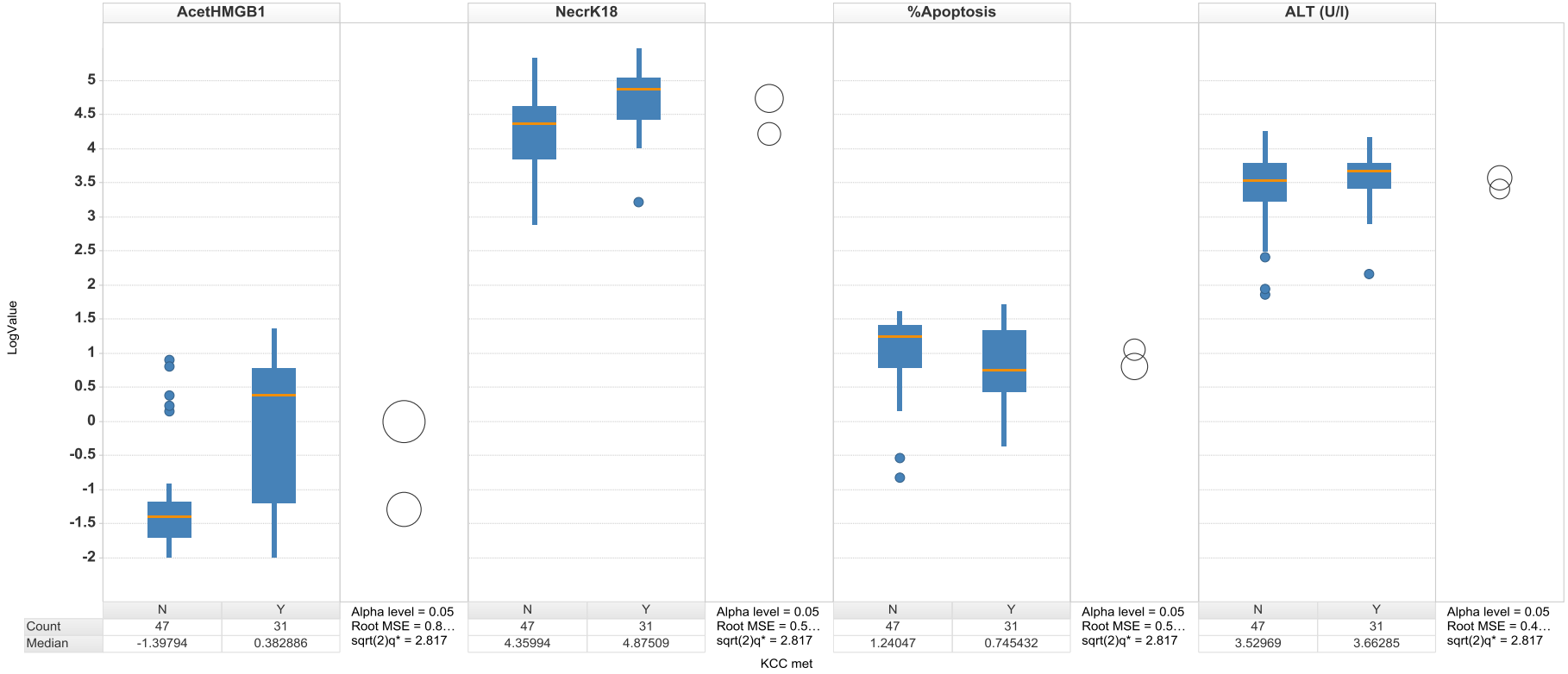
Patients post acetaminophen overdose

Markers for inflammation, necrosis, and apoptosis

Based on Antoine DJ et al., 2012 J Hepat

Association with King's College Criteria for prognosis of acute liver failure



- Acetylated HMGB1 may be a prognostic DILI marker, indicating extent of inflammation
- Caspase cleaved cytokeratin 18 may have value as a prognostic DILI marker, indicating involvement of apoptosis as protective mechanism

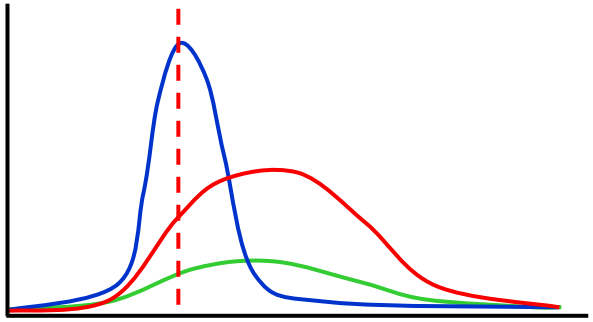
Diagnostic value of Glutathione S transferase α (αGST)

- Glutathione S transferase α (αGST):
  - Inducible phase II detoxification enzyme
  - Four isozymes of GST expressed in human and other mammals; αGST is a liver specific dimer expressed in human hepatocyte cytosol
  - High concentration in centrilobular cells: may be more sensitive than ALT and AST
  - Half life ~1 h in humans: may be useful for close monitoring
  - Low molecular weight: release into plasma may occur earlier than for ALT and AST

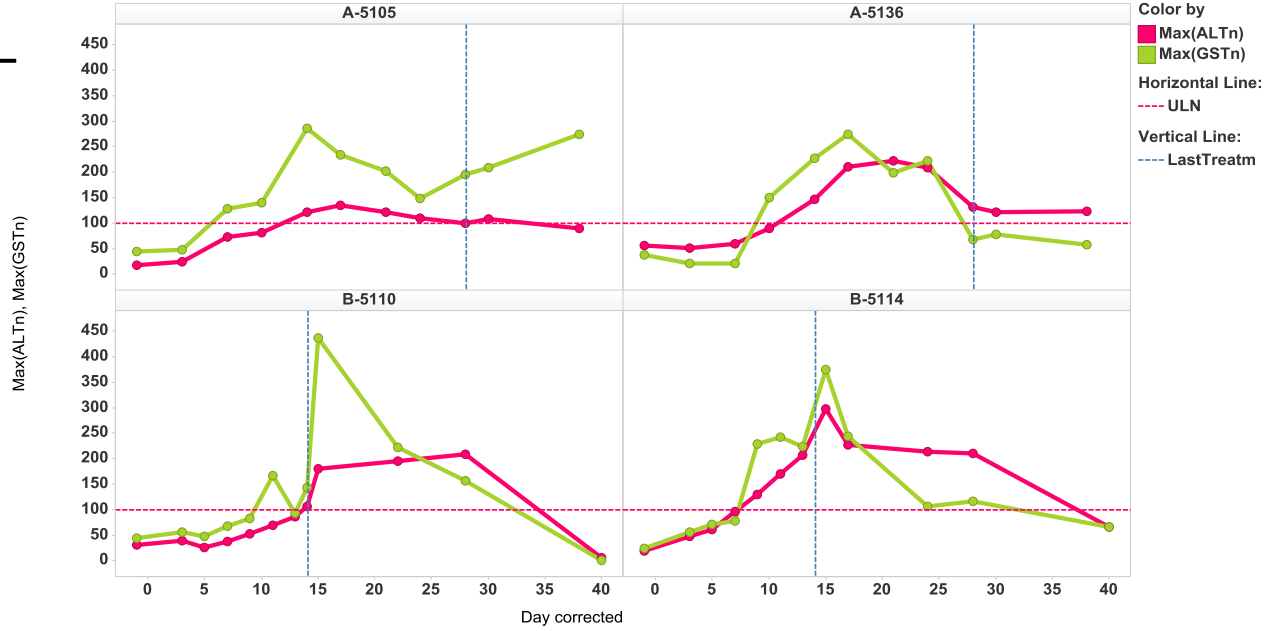
- Meta-analysis of four Novartis phase 1 studies using αGST for liver monitoring
  - 150 healthy subjects (108 m, 42 f), age 18 - 60, BMI 18 -32, duration 1-4 weeks
  - Key objectives:
    - Analyse correlation of αGST levels with age, BMI, and aminotransferases at baseline, and with aminotransferases during treatment (active drug and placebo)
    - Characterize time profiles of αGST as compared to ALT and AST
    - Explore to which extent αGST levels may be able to support causality assessment in case of elevated aminotransferases.

Diagnostic value of Glutathione S transferase α (αGST)

Preliminary results from Novartis meta-analysis



- Earlier onset and faster resolution?
- Helpful for causality assessment in a subset of cases?



- Time to onset of enzyme elevations may be marginally shorter with αGST
- In some patients, αGST returns to baseline faster , possibly supporting causality assessment

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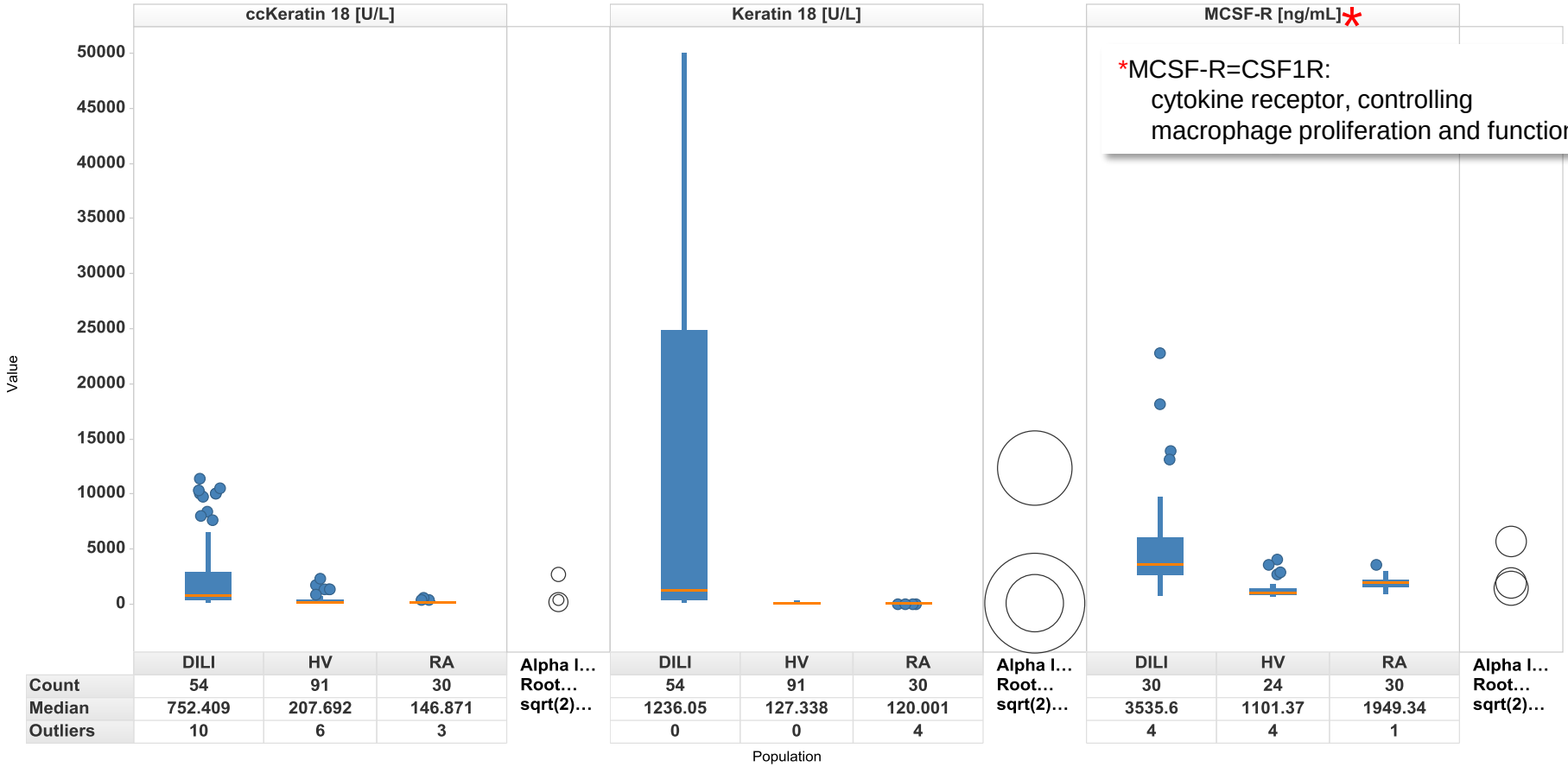
Initial, *preliminary* results of stage gate samples

CK18 full length and fragment, MCSF-R: DILI association



*MCSF-R=CSF1R:
cytokine receptor, controlling macrophage proliferation and function

- Significant associations with DILI

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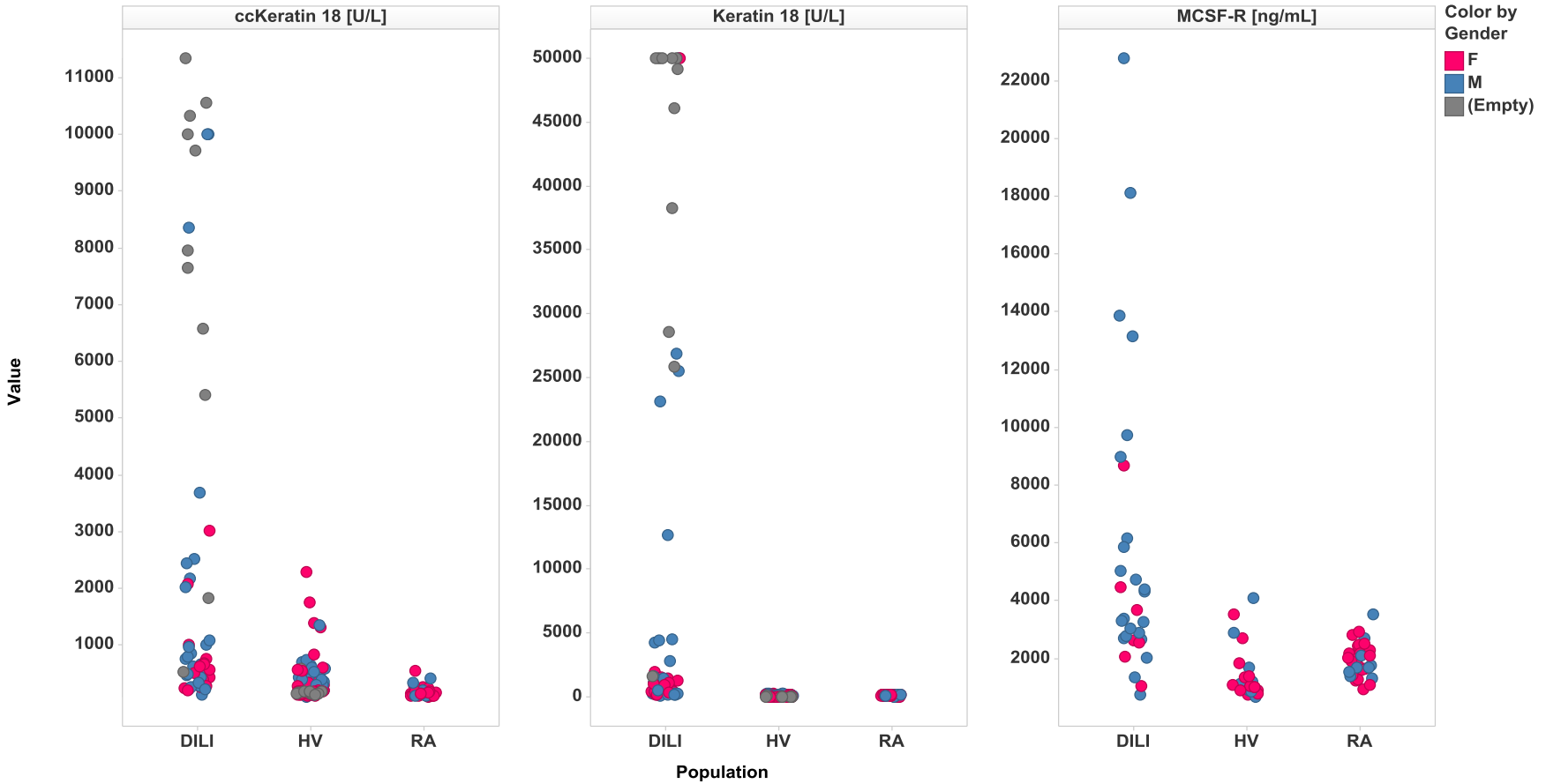
Initial, *preliminary* results of stage gate samples

CK18 full length and fragment, MCSF-R: DILI (and gender?) association



- MCSF-R: association with DILI *and* gender?

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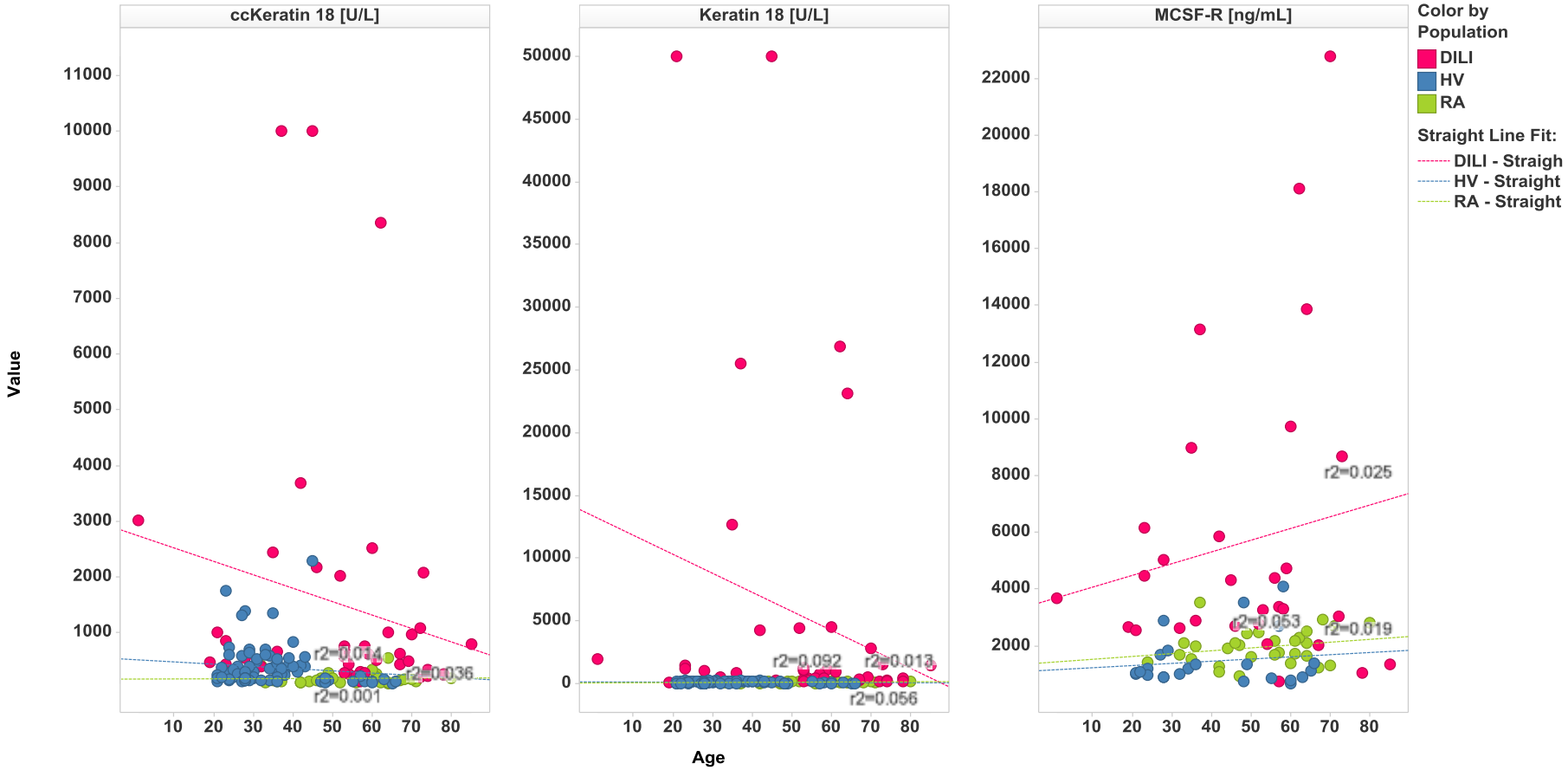
Initial, *preliminary* results of stage gate samples

CK18 full length and fragement, MCSF-R: absence of age dependency



- No associations with age

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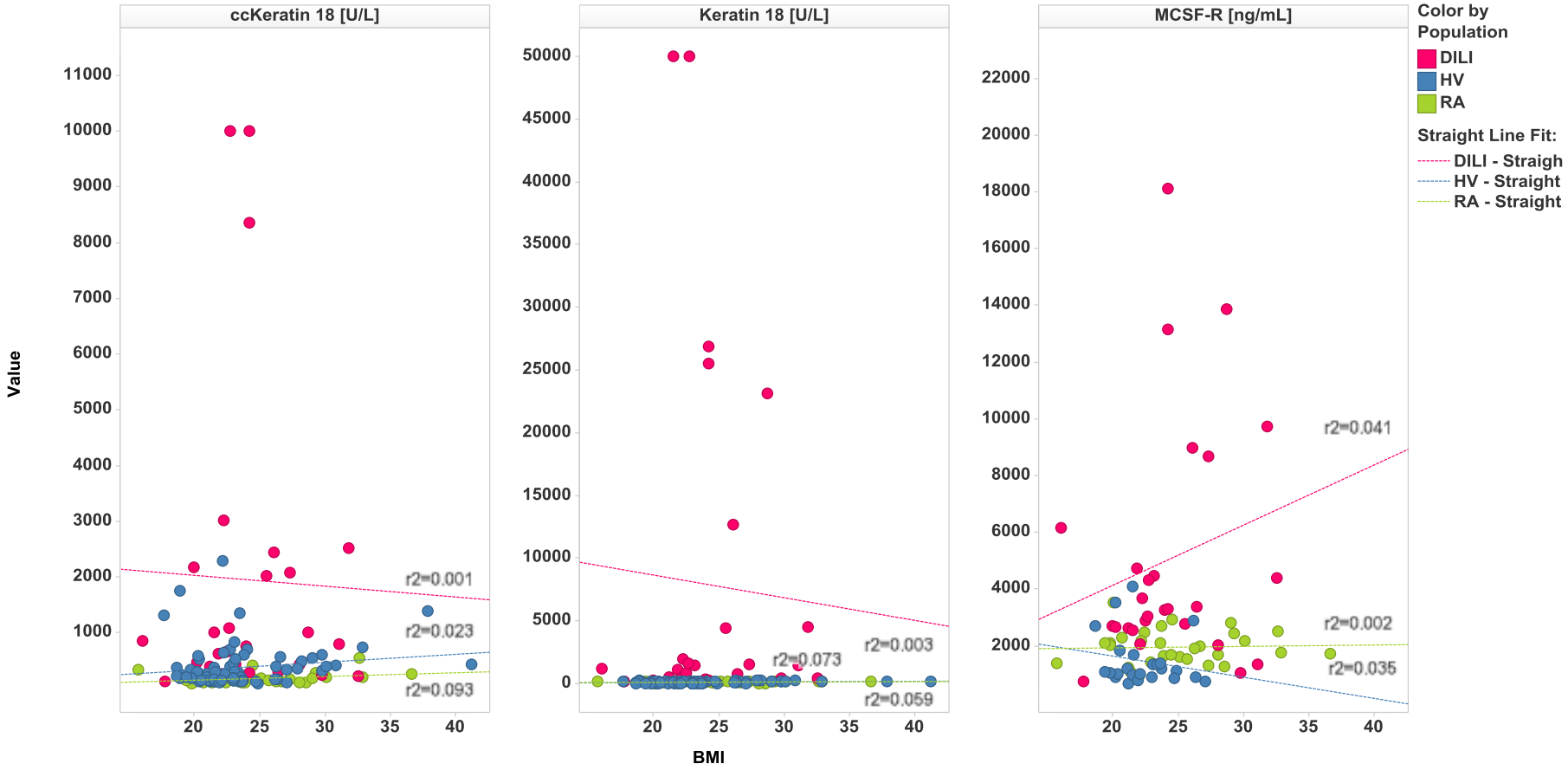
Initial, *preliminary* results of stage gate samples

CK18 full length and fragement, MCSF-R: absence of BMI dependency



- No associations with BMI

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Parallel to *qualification*: DILI biomarker *discovery*

Why?

- Biomarker candidates do not cover all objectives of SAFE-T DILI WP
  - Lack of susceptibility markers
  - Lack of sensitive functional markers, some pathologies poorly represented
  - Most markers identified in pre-clinical models

How?

- Based on human DILI cases from SAFE-T clinical studies
- Characteristic changes in serum proteome and metabolome expected
  - Mass spec and protein antibody array analyses of plasma samples ongoing

Watkins Study: early predictive DILI markers

Study design and initial output

Courtesy Ina Schuppe Koistinen, AstraZeneca

Healthy men and women (18-55 years) were treated with 4g acetaminophen/day for 7 days

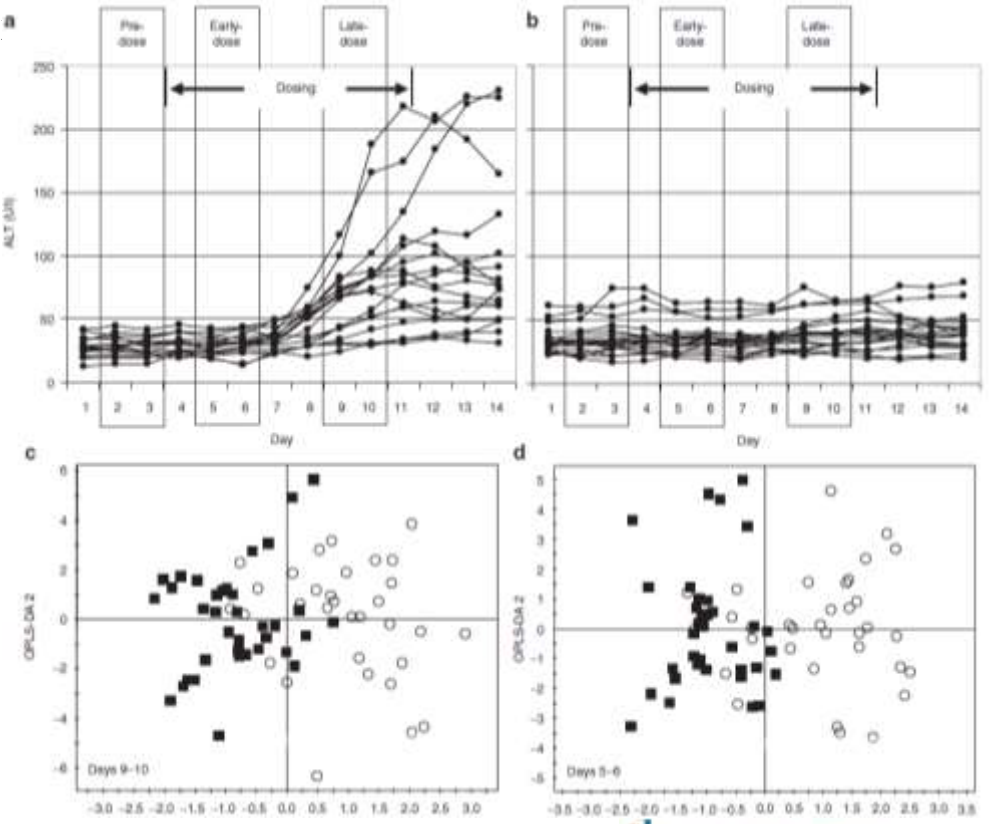
17 subjects: **responders (ALT >2.0 x baseline level)**

15 subjects : intermediate responders (ALT 1.5-2.0 x baseline level)

18 subjects: **non-responders (ALT <1.5**

Results

- Urine metabolite profiles prior or at start of treatment not predictive of DILI
- Urine profiles at day 5-6 (prior to raised ALT) could distinguish responders from non-responders
- Predictive metabolites include APAP and endogenous metabolites



Winnike JH, Li Z, Wright FA, Macdonald JM, O'Connell TM, Watkins PB. Use of pharmaco-metabonomics for early prediction of acetaminophen-induced hepatotoxicity in humans. Clin Pharmacol Ther. 2010;88(1):45-51

O'Connell TM, Watkins PB. The application of metabonomics to predict drug-induced liver injury. Translational Medicine, 2010, 88(3): 394-99

Watkins Study: early predictive DILI markers

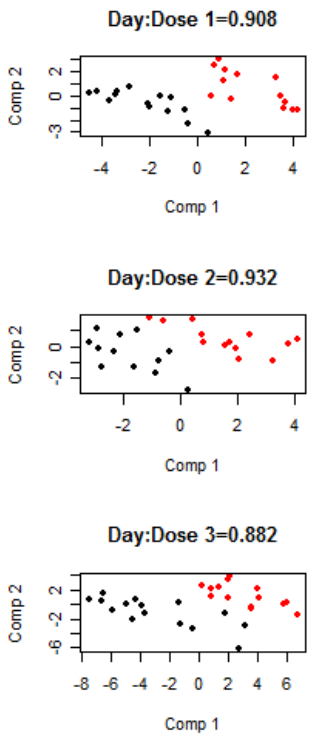
Additional benefit of serum proteomics and metabolomics

AstraZeneca

Courtesy Ina Schuppe Koistinen, AstraZeneca

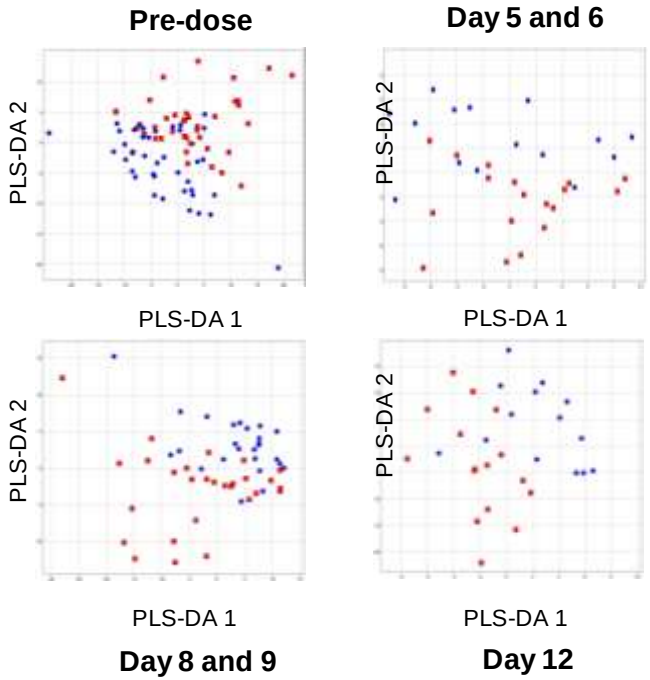
- LC-MS based profiling for compound and endogenous metabolites
- Suspension bead protein array (antibody based)

Metabolomics



- Endogenous metabolites and protein profiles identified, that:
  - Predict ALT elevations at baseline (susceptibility markers)
  - Predict ALT elevations early during treatment
- Pathways involved
  - Pyruvate and glutamate metabolism at baseline
  - Cell death pathways activated at the time of ALT elevations
  - Overlap with ximelagatran candidate biomarkers

Proteomics



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Conclusions

- Advanced safety biomarkers for prediction, detection, and assessment of drug-induced liver injury (DILI) are urgently needed.
- Due to the low incidence of DILI, large sample sizes across a range of different populations are required for clinical qualification of new markers.
- Large scale public private partnerships involving industry, academia, small to medium sized enterprises, and regulators such as the IMI SAFE-T consortium may be the most efficient way to successful biomarker qualification.
- A list of promising DILI biomarker candidates has been selected by the SAFE-T consortium for clinical qualification.
- Preliminary data on a subset of markers offer first insights into potential predictive and diagnostic value.
- Regulatory approval of new DILI biomarkers with defined contexts of use is expected by 2015.

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