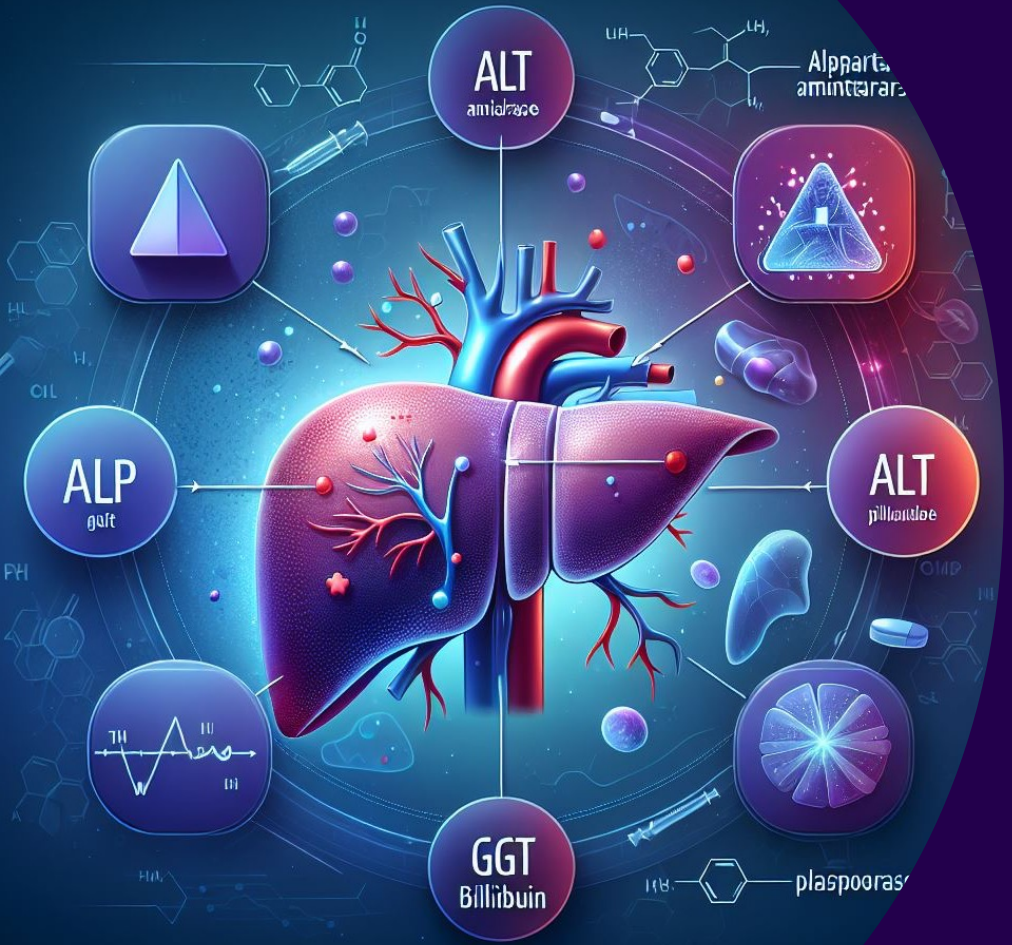

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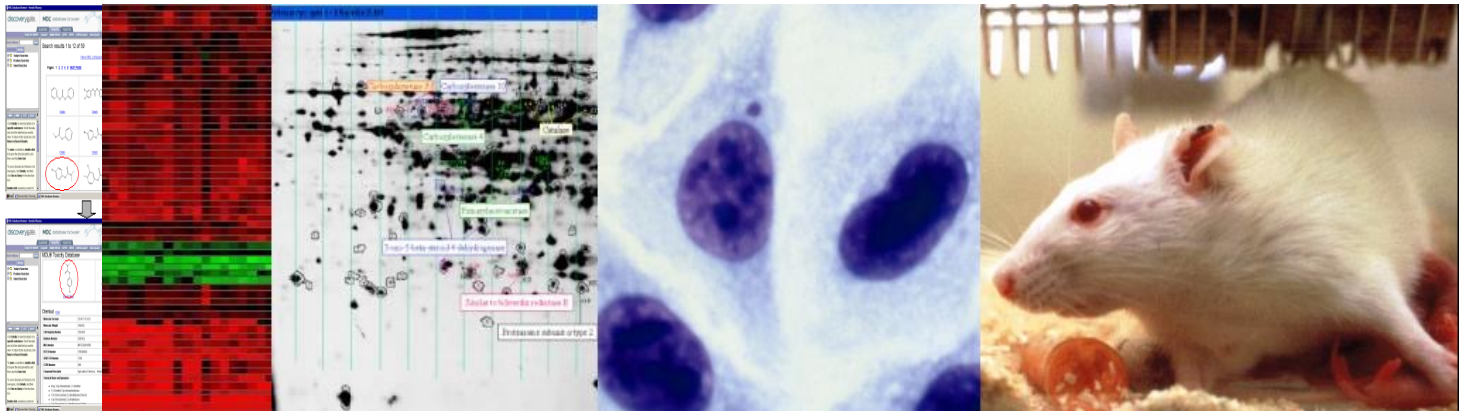
Interest and use of emerging safety biomarkers in drug development

EUFEMED Conference

May 23rd, 2025 – Mechelen Belgium

Philippe Detilleux, DVM, PhD, DACVP

Preclinical Safety Assessment



… using in silico, in vitro and in vivo models to avoid adverse effects in humans and obtain regulatory approval

Biomarker Definition

- **A characteristic that is objectively measured and evaluated as an indicator of normal biological processes, pathogenic processes, or pharmacological/ toxicological responses to a therapeutic intervention** *(NIH Biomarkers Definition Working Group Clin Pharmacol Ther 2001;69:89-95)*
  - ✓ Anatomic, physiologic, biochemical or molecular parameters associated with the presence and severity of specific physio-pathological processes
  - ✓ Detectable and measurable by a variety of methods including physical examination, laboratory assays, and medical imaging

- **Many different types of biomarkers**
  - ✓ Exposure
  - ✓ Efficacy
  - ✓ Safety (toxicity) - any analyte that can be quantified to indicate an adverse response to a test agent

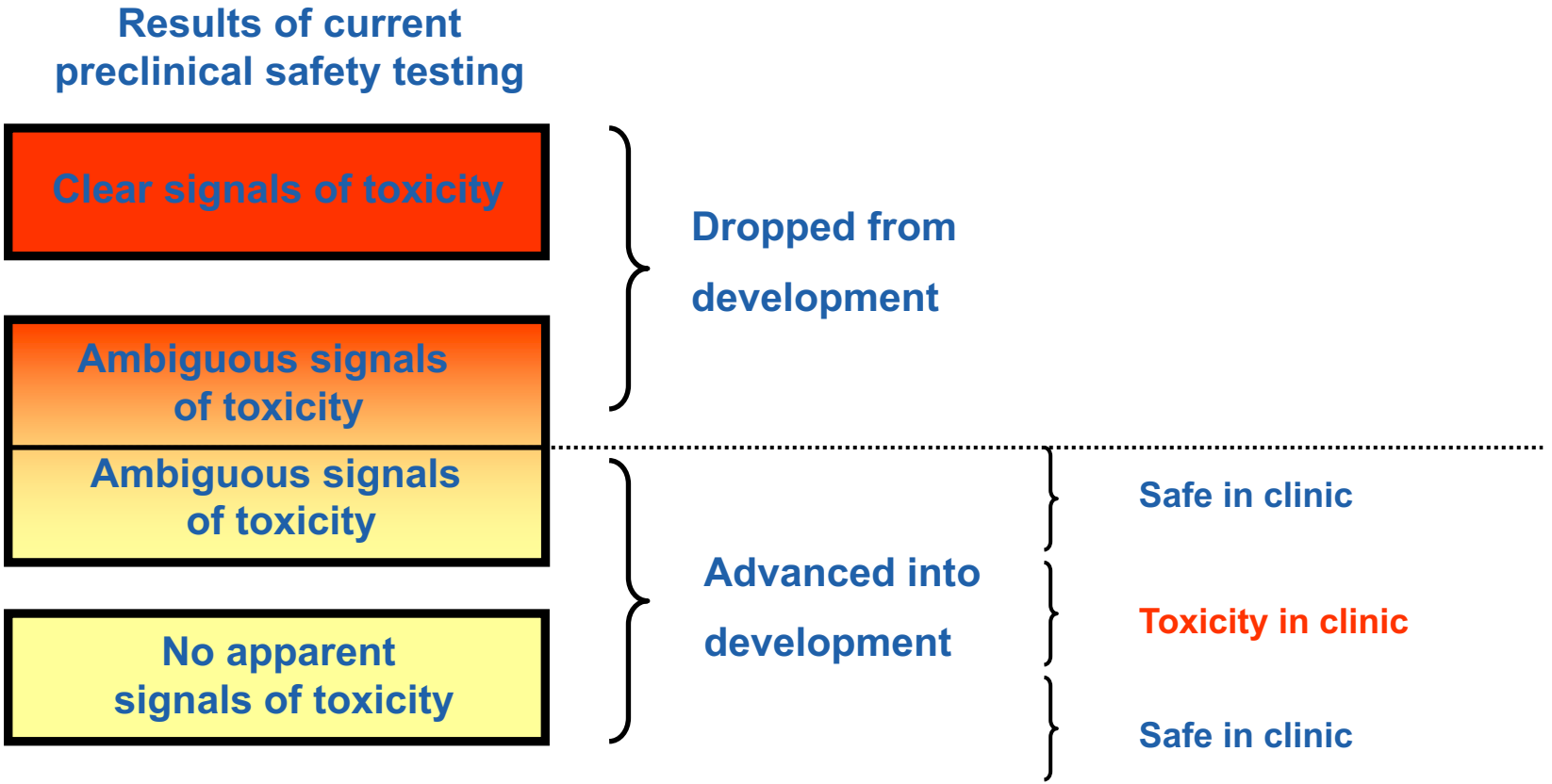
- **Examples of safety biomarkers**
  - ✓ Serum/plasma and urine analytes (urinary Kim-1, clusterin, □-GST for kidney; plasma troponin I for heart; plasma GLDH for liver…)
  - ✓ Hemodynamic or ECG parameters (BP, QT interval…)
  - ✓ Bioimaging technologies (MRI for brain, joints, bones…)

Characteristics of an ideal safety biomarker

A safety biomarker can broadly be defined as “a biomarker that is measured before or after an exposure to a medical product or environmental agent to indicate the likelihood, presence, or extent of toxicity as an adverse effect” (FDA, 2016)

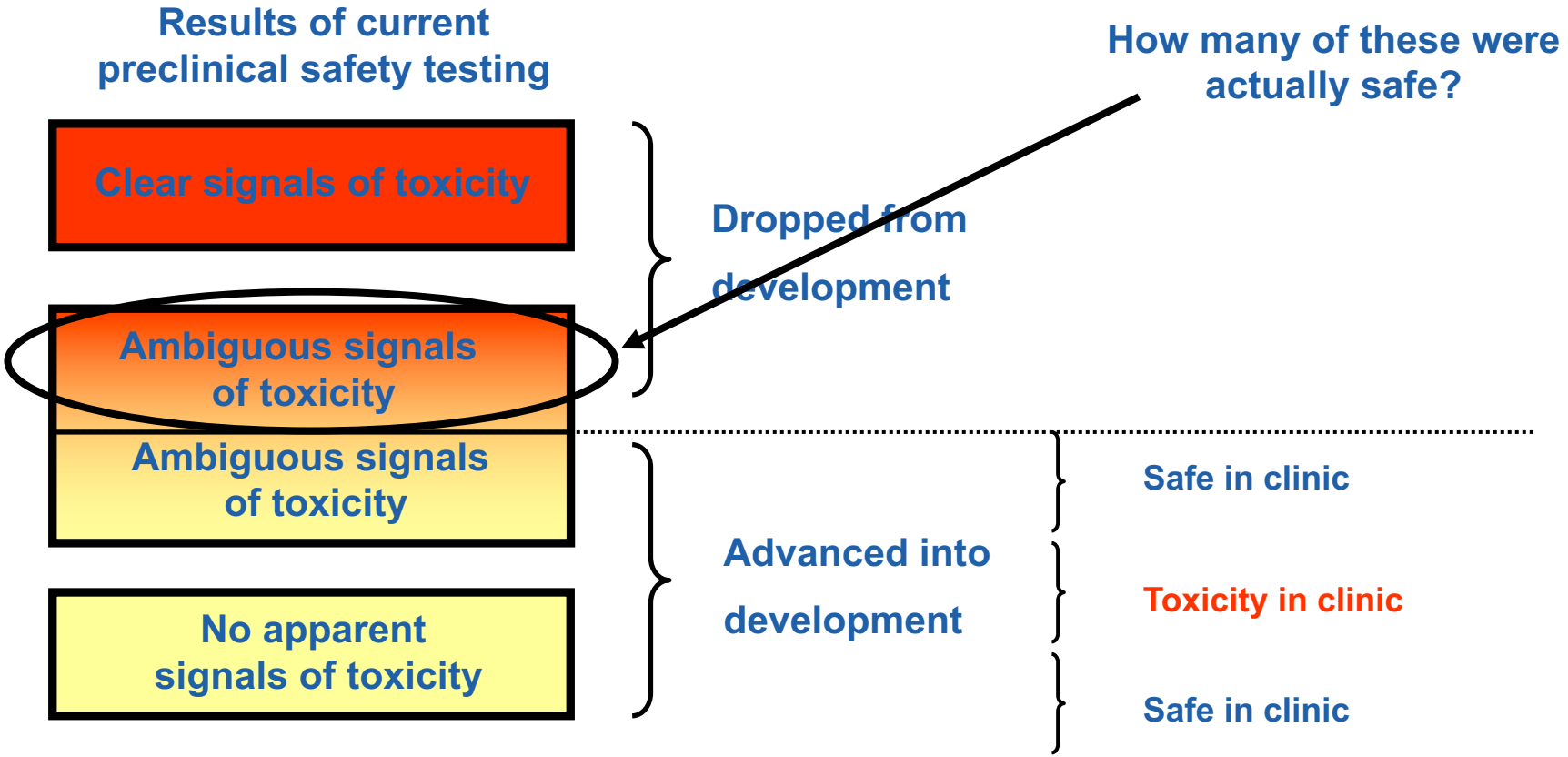
- **Specific**
  - ✓ Tissue and/or disease specific
- **Sensitive**
  - ✓ Detectable at low level of injury
  - ✓ Good correlation between biomarker level and degree of effect
- **Robust**
  - ✓ Rapid, simple and accurate analytical assay
- **Bridging**
  - ✓ Can be applied both in preclinical animal studies and in human clinical settings
- **Minimally invasive**
  - ✓ Available from easily accessible fluids

Safety biomarkers: the context



Based on "Drug development failures chart"
William B. Mattes, Critical Path Institute, Experience with a biomarker consortium – Fall 2007"

Safety biomarkers: the context



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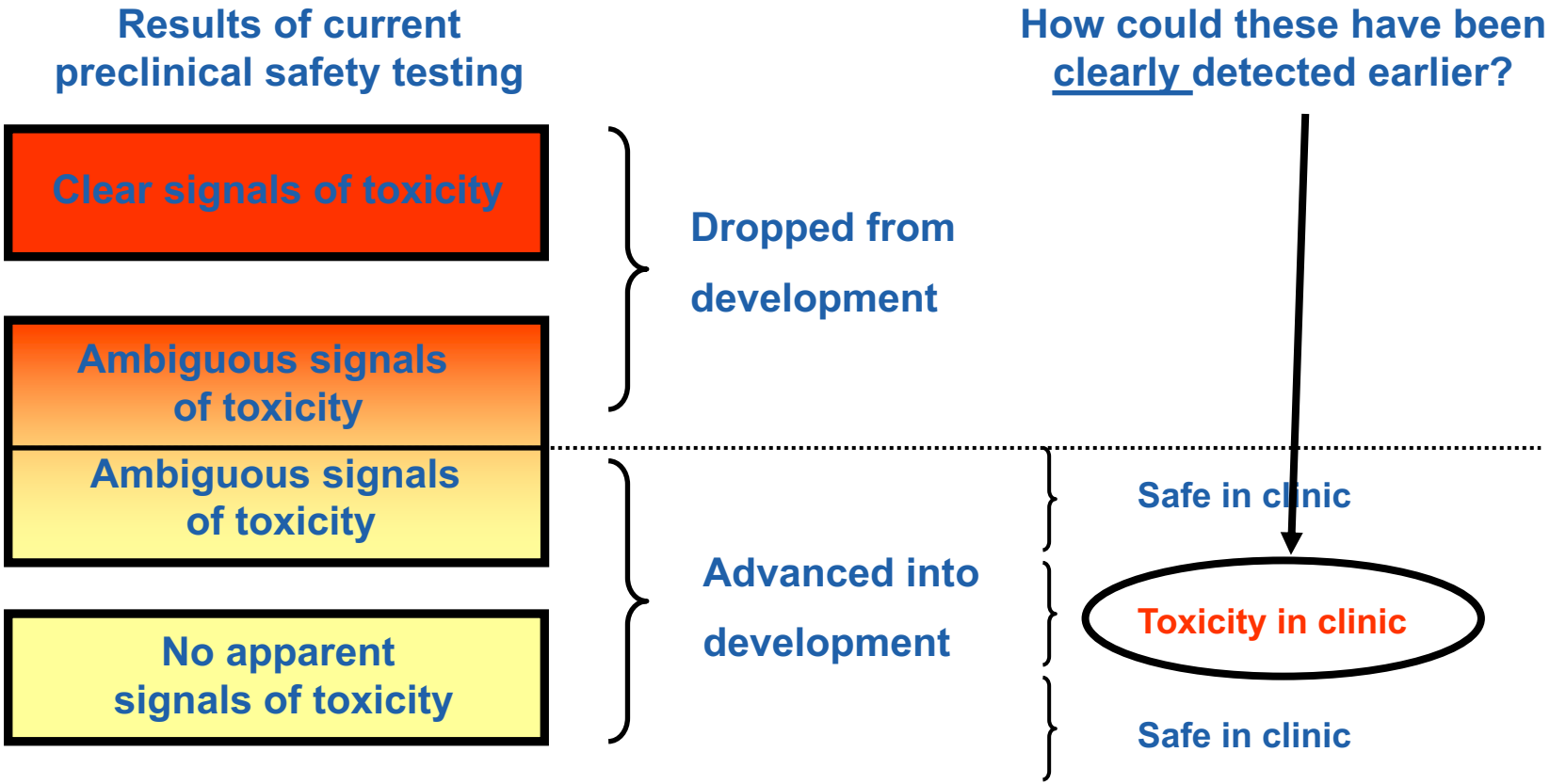
Safety biomarkers: the context



Based on "Drug development failures chart"
William B. Mattes, Critical Path Institute, Experience with a biomarker consortium – Fall 2007"

Safety biomarkers: the context

- **Most of the target organs critical for drug-induced injury have non-appropriate clinical monitoring**
  - Kidney
    - ✓ Current standards: serum creatinine (since 1904), BUN (since 1914)
    - ✓ Only increased when 50-60% of the kidney function is lost
  - Liver
    - ✓ Current standards: AST, ALT, bilirubin (ALAT: since 1954)
    - ✓ Not specific and do not predict who will recover and who will develop fulminant liver disease

- **Importance of identifying and developing appropriate SBMs to improve clinical drug safety monitoring**
  - More informed decision on future drug development
    - ✓ Go/No Go decision
    - ✓ Risk assessment
    - ✓ Risk mitigation
  - Sensitivity/specificity issues
    - False negatives:
      - Potential risk for study subjects
      - Future development costs wasted
    - False positives:
      - Potentially successful drugs lost

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Analytical validation and biological qualification

- **Analytical validation of assays**
  - Method validation is the formal process of assessing the suitability of an analytical assay and its measurement performance characteristics
    - ✓ Determination of analytical accuracy, precision, limits of quantification and stability
    - ✓ Determination of the range of conditions under which the assay will give reproducible and accurate data

- **Biological qualification**
  - Qualification is the evidentiary process of linking a biomarker with biological processes and/or clinical endpoints
    - ✓ Correlation with histopathology findings, which are used as the definitive comparator (reference standard)
    - ✓ Comparison with established tests used for the same purpose (diagnostic advantage)

Why do we need Regulatory endorsement of novel methods?

- **Progressing drugs through development and approval requires regulatory decision-making by agencies**
  - decisions that FDA/EMA may make concerning the dosing, safety, or effectiveness of a drug or biological product, during product development, NDA/MAA review, and post-approval (FDA, 2005).

- **"Qualification is a conclusion that within the stated context of use, the results of assessment with a DDT can be relied upon to have a specific interpretation and application in drug development and regulatory decision-making" (FDA, 2010)**

- **"Once a DDT is qualified for a specific context of use, industry can use the DDT for the qualified purpose during drug development, and CDER reviewers can be confident in applying the DDT for the qualified use without the need to reconfirm the DDT's utility" (FDA, 2010)**

DDT= Drug Development Tool (e.g. Biomarker)

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Consortia in the development and qualification of safety biomarkers

- **Role of Consortia:**
  - Provide a neutral collaborative environment for partnering, sharing, and leveraging the resources for biomarker development and qualification
  - Facilitate workshops, scientific discussions, gather input from scientific community, and to streamline advances in regulatory science
  - Provide an opportunity for scientific staff engagement to discuss current thinking on biomarkers and other regulatory science efforts.

- **Three main consortia between pharmaceutical companies, academic, regulatory advisors for qualification of new safety biomarkers**
  - Critical Path Institute (CPI) Predictive Safety Testing Consortium (PSTC)
    - ✓ Both preclinical & clinical safety biomarkers
  - Health and Environmental Sciences Institute (HESI)
    - ✓ Preclinical SBM
  - Innovative Medicines Initiative (IMI)
    - ✓ Clinical SBM

CRITICAL PATH INSTITUTE

efpia European Federation of Pharmaceutical Industries and Associations

imi innovative medicines initiative

SAFE-T

Trans Bio Line

ihi innovative health initiative

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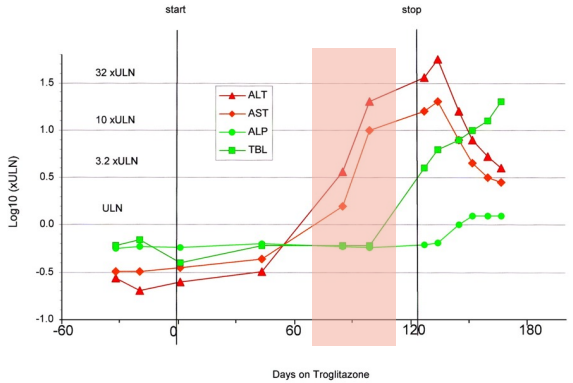
Another Challenge

- **Translation from preclinical to clinical**
  - Species specificities for certain safety biomarkers
  - Lack of analytical tools for rodent and/or non-rodent species
  - Needed:
    - ✓ Non immunologically based assays (e.g. LC/MS-based protein assays)
    - ✓ New types of biomarkers (e.g. micro RNAs)

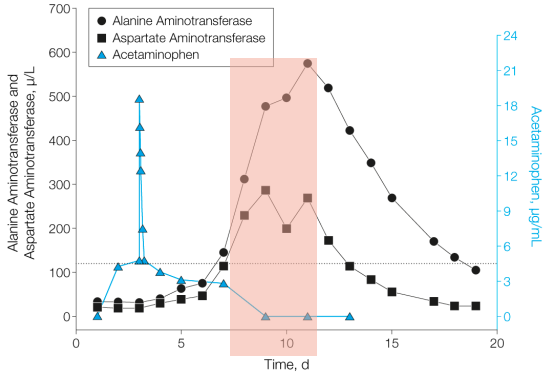
- **Two examples**
  - SAFE-T: Assay development and analytical validation (fit-for-purpose) for 2 renal safety biomarker candidates for which no immunoassays are available
    - ✓ Aquaporin-2 (for collecting duct injury)
      - LC/MS method developed using selected petide *(Journal of Chromatography A, 1301 (2013) 122-130)*
      - Range : 0.500 ng/mL to 50.0 ng/mL (0.250 ng/mL detectable)
    - ✓ Podocin (for glomerular injury)
      - LC/MS method developed using a tryptic peptide (*Journal of Pharmaceutical and Biomedical Analysis 94 (2014) 84-91)*
      - Analytical range from 0.500 to 50.0 ng/mL
  - HESI: Micro RNAs in rodents and Non-Human Primates

The DILI Challenge

Two clinical outcomes with similar aminotransferases



Paul Watkins, Tox Path 2005



Paul Watkins, JAMA 2006

<u>Severe drug induced liver injury</u>

- Increased aminotransferases
- Bilirubin indicates severe state (appears late)
- Preclinical studies usually do normally not progress to this stage, but histopathology can confirm liver injury at earlier timepoints

<u>Transient/adaptive aminotransferases</u>

- No bilirubin increase
- Aminotransferases return to normal
- Similar situation are encountered preclinically
- Preclinical studies can confirm lack of injury with histopathology assessment

The two red areas look identical until the bilirubin distinguishes between them …. then it's often too late (histopathology is often not available nor reliable)

The DILI Challenge

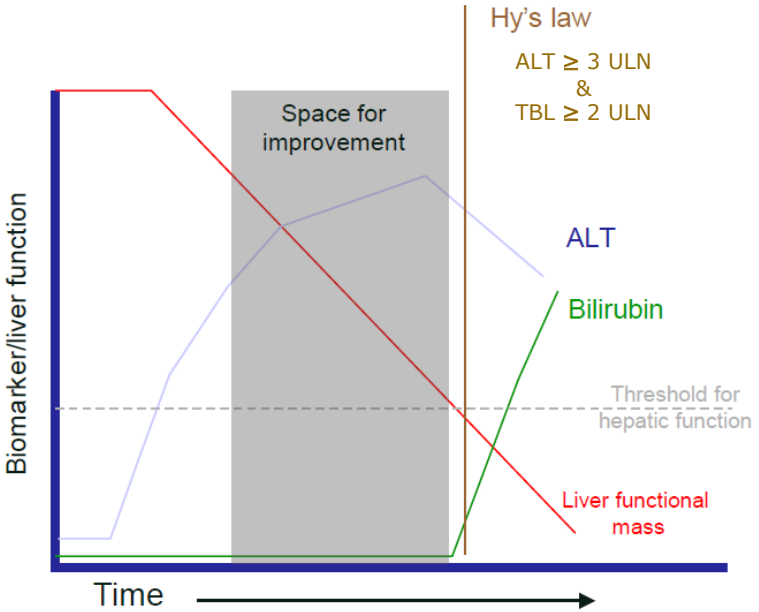
Better Hy's Law

Current status

- Conventional biomarker-based DILI diagnostic paradigm detects liver injury only after substantial (sometimes irreversible) damage has occurred
  - ALT is sensitive enough but not specific enough
  - Bilirubin is not sensitive but specific enough

Gaps

- Sensitive and specific biomarkers that detect DILI before substantial or irreversible damage has occured
- Biomarkers with better prognostic value (transient vs progressive increase/damage)
- Translational biomarkers (improve DILI risk assessment in preclinical species)
- Early indentification of individuals susceptible to idiosyncratic DILI



Current and emerging liver biomarkers

From: CLINICAL PHARMACOLOGY & THERAPEUTICS I VOLUME 107 NUMBER 2 1 FEBRUARY 2020

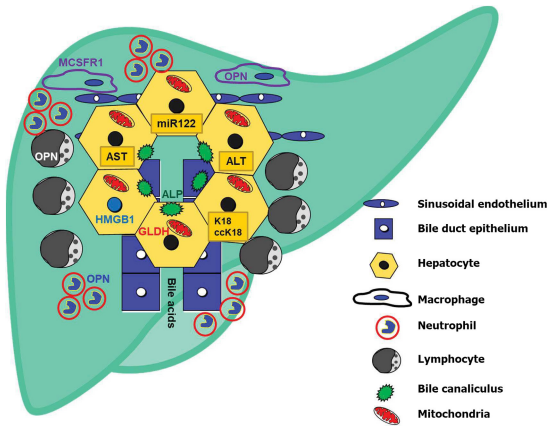


Table 1 Current serum liver biomarkers used in clinical practice

Biomarker	Tissue specificity	Cellular localization	Liver damage detected
Alanine aminotransferase (ALT)	Multiple tissues	Cytoplasmic	Hepatocellular injury
Aspartate aminotransferase (AST)	Multiple tissues	Cytoplasmic & mitochondrial	Hepatocellular injury
Total bilirubin (TBIL)	Liver	N/A	Cholestasis & hepatobiliary injury, hepatocellular injury in association with ALT/AST and a measure of liver function
Alkaline phosphatase (ALP)	Multiple tissues	Cell membrane	Cholestasis & hepatobiliary injury
Gamma-glutamyl transferase (GGT)	Kidney > Liver, Pancreas	Cell membrane	Cholestasis & hepatobiliary injury

N/A, not applicable.

Table 2 Association of potential DILI biomarkers with context of use and mechanisms of liver injury

Context of Use (COU)	Potential biomarker	Mechanism	Population variability	Assay	References
Mechanistic diagnostic to supplement ALT	GLDH	Necrosis	Low interindividual and intraindividual variability; not age or sex dependent	Enzyme activity assay	58,60–64
	HMGB1, total	Necrosis	High intraindividual variability	ELISA; commercially available	58,60,65–67
	ccK18	Apoptosis	Low interindividual and intraindividual variability	ELISA; commercially available	60,62,68,69
	MCSFR1	Immune-mediated	Low interindividual and intraindividual variability	Immunoassay	58,60,70,71
	Total and individual bile acids	Biliary injury/ dysfunction	High interindividual and intraindividual variability	LC MS; routinely available	58,72,73
Predict severe outcome after DILI diagnosis	OPN	Hepatic inflammation and necrosis	Low interindividual and intraindividual variability	ELISA or immunoassay; not routinely available	58,60,74–76
	K18	Necrosis/ Apoptosis	Low interindividual and intraindividual variability	ELISA; commercially available	60,62,68,69
	MCSFR1		See details above		
Early diagnostic before ALT elevations	GLDH		See details above		
	HMGB1, total				
	K18				
	ccK18				

ALT, alanine aminotransferase; ccK18, caspase-cleaved cytokeratin 18; DILI, drug-induced liver injury; GLDH, glutamate dehydrogenase; HMGB1, high mobility group box protein 1; K18, total cytokeratin 18; MCSFR1, macrophage colony stimulating factor receptor 1; OPN, osteopontin.

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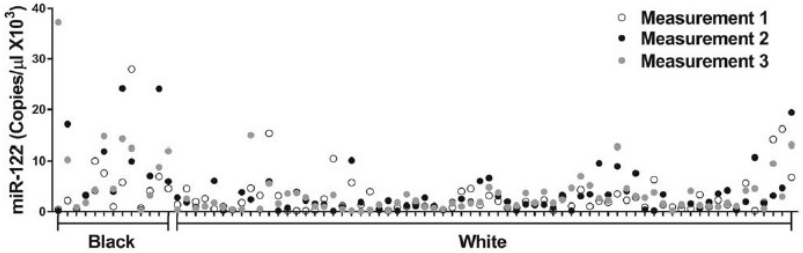
Drug-Induced Liver Injury: An International Collaborative Effort (PSTC, Safe-T and DILIN)

Study Design

Samples: Serum or plasma from multiple cohorts

- Healthy volunteers (n=192 and n=81)
- Subjects who took potentially hepatotoxic drugs (n=55 and n=92)
- DILI patients (n=98, n=28, and n=143)

Assays: 14 promising DILI biomarkers (incl. GLDH and miR-122)



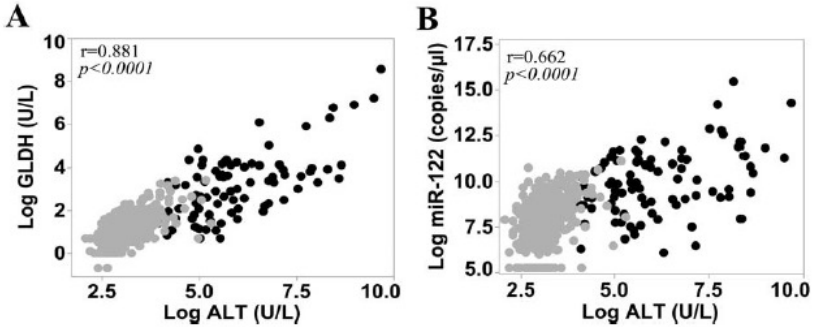
Results

miR-122

- High intra- and inter-individual variability
- Not only **passive release** from damaged cells but can be **actively released** in vesicles in response to stress
- Complex not standardized assay
- Deprioritized by PSTC in favor of GLDH

GLDH

- Best correlation with ALT (the gold standard)
- Liver specific (not released following muscle injury like ALT)
- Faster clearance and thus better indicator of recovery

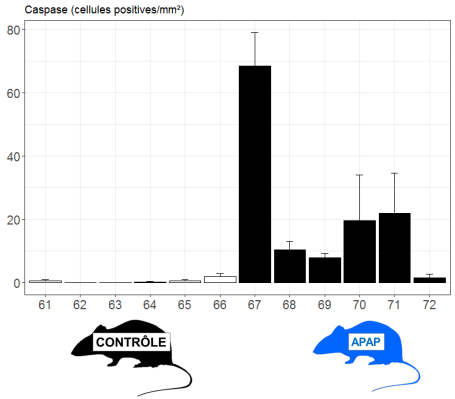


From: R. Church et al. Hepatology Vol 69 (2) 760-773 (2019)

miR-122: a liver specific µRNA in rats

Study Design

Test system: SD male Rats
Duration: 24 hours
Animals/sex/group: 6
Route: oral (gavage) single dose
Dose levels: 0, and 1500 mg/kg/day (acetaminophen)
Clinical pathology: hematology, coagulation and clinical chemistry 24 hrs post dose
Anatomic pathology on a selection of organs/tissues
IHC on liver: activated caspase 3

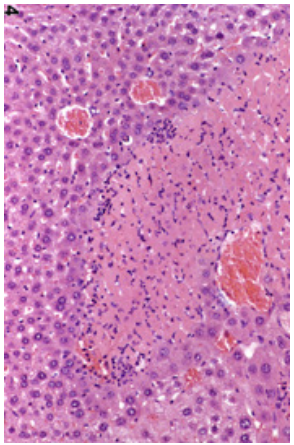


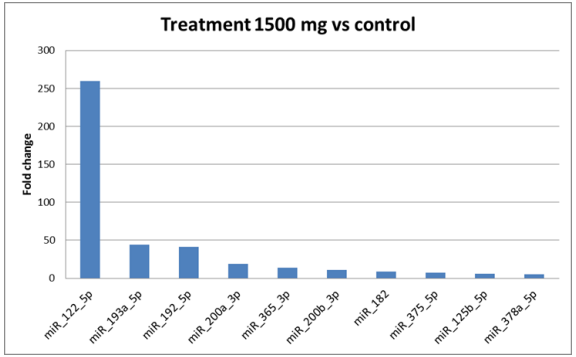
	miRNA	Treatment 1500 mg vs control (Fold Change)
	miR_122_5p	260
2	miR_193a_5p	44
3	miR_192_5p	41
4	miR_200a_3p	19
5	miR_365_3p	14
6	miR_200b_3p	11
7	miR_182	9
8	miR_375_5p	7
9	miR_125b_5p	6
10	miR_378a_5p	51

Parameter	Control animals			Treated animals		
	Mean	SD	n	Mean	SD	n
AST	67	4,6	6	1922*	3004,6	6
ALT	35,8	27,4	6	518,8*	754,2	6
ALP	144,7	33,5	6	203,3 NS	59,4	6
GLDH	6,2	0,28	2	207,65 *	175,96	6
Bile acids	22,763	15,764	6	75,975*	24,257	6
T chol	1,157	0,198	6	1,160 NS	0,31	6
Trig	0,55	0,183	6	0,68 NS	0,21	6
Urea	4,935	0,675	6	5,528 NS	0,642	6
Creat	23,83	2,14	6	21*	2,19	6

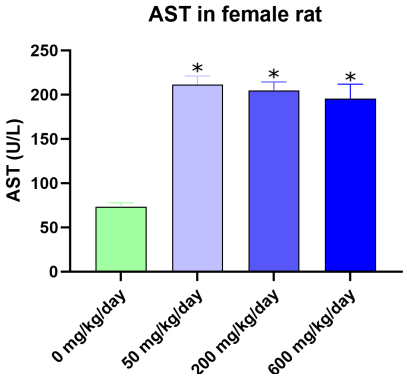
* = significant trend (p<=0.05) through indicated dose level

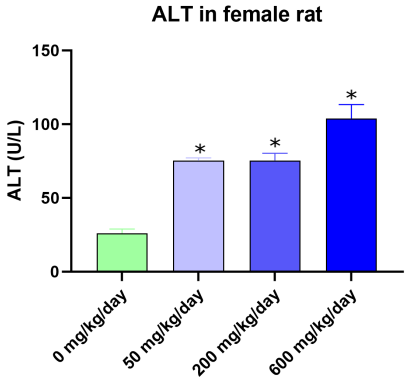
NS = no significant trend through indicated dose level



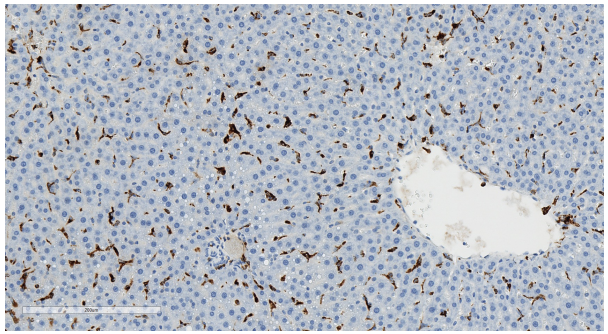


Case Study: Cpd A

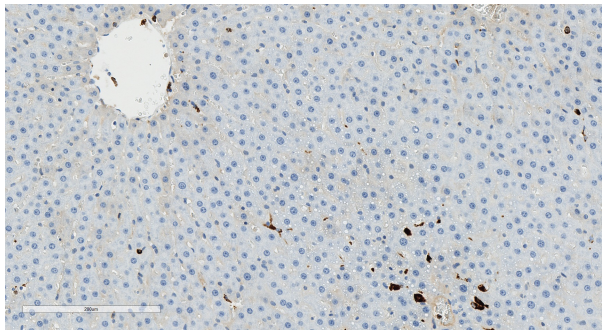




All graphs showing mean +/- sem
* p<0.05, statistics Jonckeere



Female No. 21, control: liver, IHC CD68 x20



Female No. 36, 600 mg/kg/day: liver, IHC CD68 x20

Study Design

Duration: 4 weeks
Animals/sex/group: 5
Route: oral (gavage) QD
Dose levels: 0, 50, 200 and 600 mg/kg/day
Clinical pathology: hematology, coagulation and clinical chemistry on Day 29 **Anatomic pathology** on a selection of organs/tissues
IHC on liver: Ki67, CD68

Results

- Centrilobular hepatocellular hypertrophy (CYP450 induction)
- No evidence of hepatocellular injury (H&E)
- Increased liver enzyme (AST, ALT)
- Küpffer cell depletion (ICH for CD28, IBA1)
- No increase in miR-122

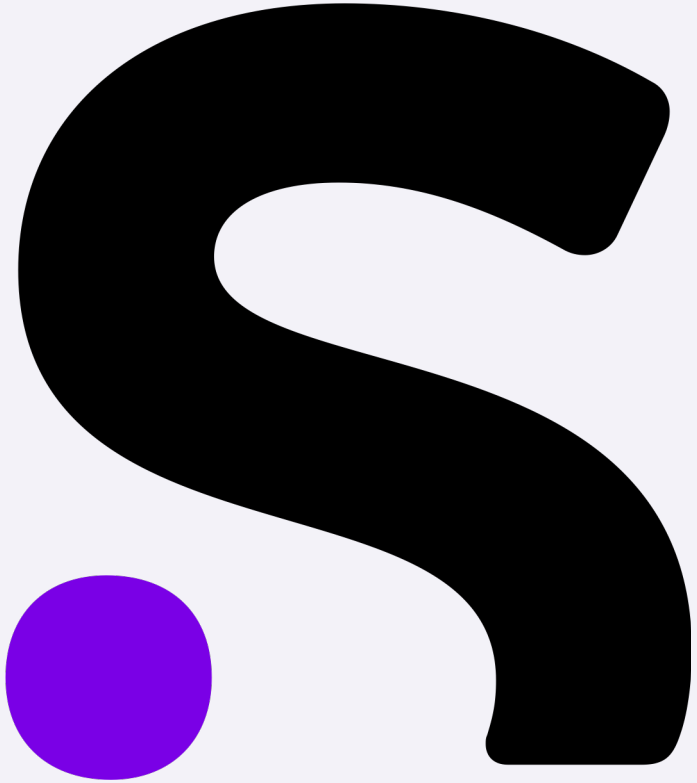
Mechanism of Action

- Cpd A deplete Küpffer cells in the liver (and cells of macrophage lineage elsewhere)
- Critical roles of Küpffer cells in innate immunity, clearance of senescent or malformed red blood cells and in **homeostasis of soluble bloodstream constituents,** such as growth factors, hormones, and serum enzymes
- Increases in liver enzymes may be related to decreased clearance

Conclusions

- **Important needs for development of new safety biomarkers**
  - Improved preclinical toxicity profile characterization
  - Improved clinical safety monitoring
- **This requires identification of promising biomarkers and formal analytical validation/biological qualification processes**
  - Preclinical development
  - Clinical development
- **Critical role played by collaborative consortia and precompetitive research in this field**
- **Regulatory acceptance of new biomarkers key to successful use**

Thank you ☺



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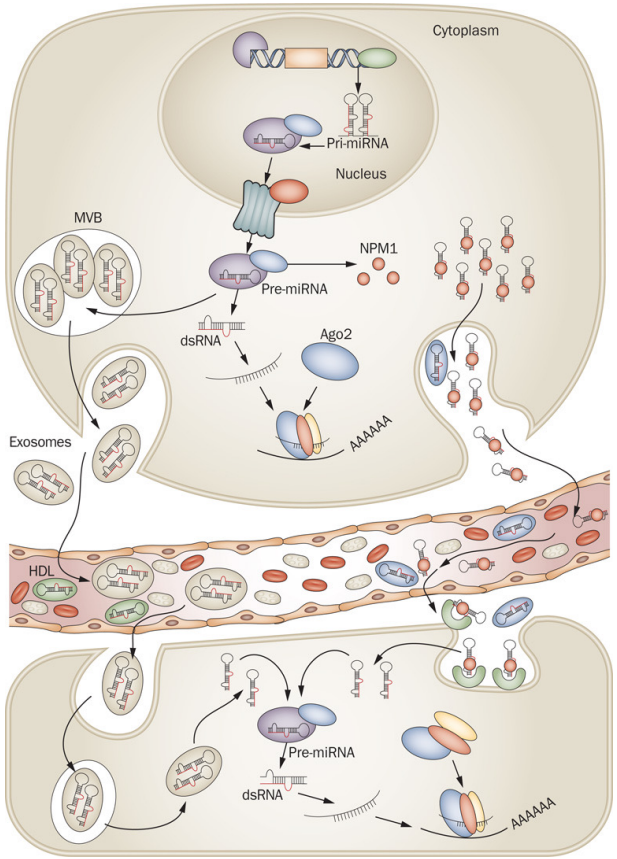
Circulating µRNA as novel biomarkers



- MicroRNAs are transcribed by RNA polymerase II from intergenic or from intronic sequences of coding genes as long precursor transcripts
- Mature ~22 nt long ss miRNAs exert their regulatory action by binding mRNAs leading to translational repression or degradation of target transcripts
- Furthermore, mature miRNAs can be loaded into microvesicles, exosomes or protein complexes and can be released from e.g. liver as circulating miRNAs into the blood stream
- miRNAs are often tissue-specific and therefore cell-free miRNAs can be viewed as a reflection of organ health