DILI AND THE IMI SAFE-T* CONSORTIUM: QUALIFICATION OF NEW TRANSLATIONAL SAFETY BIOMARKERS

*Safer And Faster Evidence-based Translation

http://www.imi-safe-t.eu

Drug-Induced Liver Injury Conference, March 23 - 24, 2011
Silver Spring, MD

Michael Merz[1], Ina Schuppe Koistinen[2]

[1]Novartis Institutes for Biomedical Research, Switzerland, [2] Global Safety Assessment, AstraZeneca R&D, Sweden

Outline

- SAFE-T objectives
- Structure and deliverables
- Biomarker qualification process
- Achievements
- Challenges
- Next steps

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

Biomarker attributes of interest

- Patient level
  - Lower injury threshold
  - Earlier time to onset
  - Larger extent of changes
  - Improved specificity
  - Better suited to monitor and predict clinical course
  - Better suited to assess causality
- Population level
  - Earlier and more specific 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

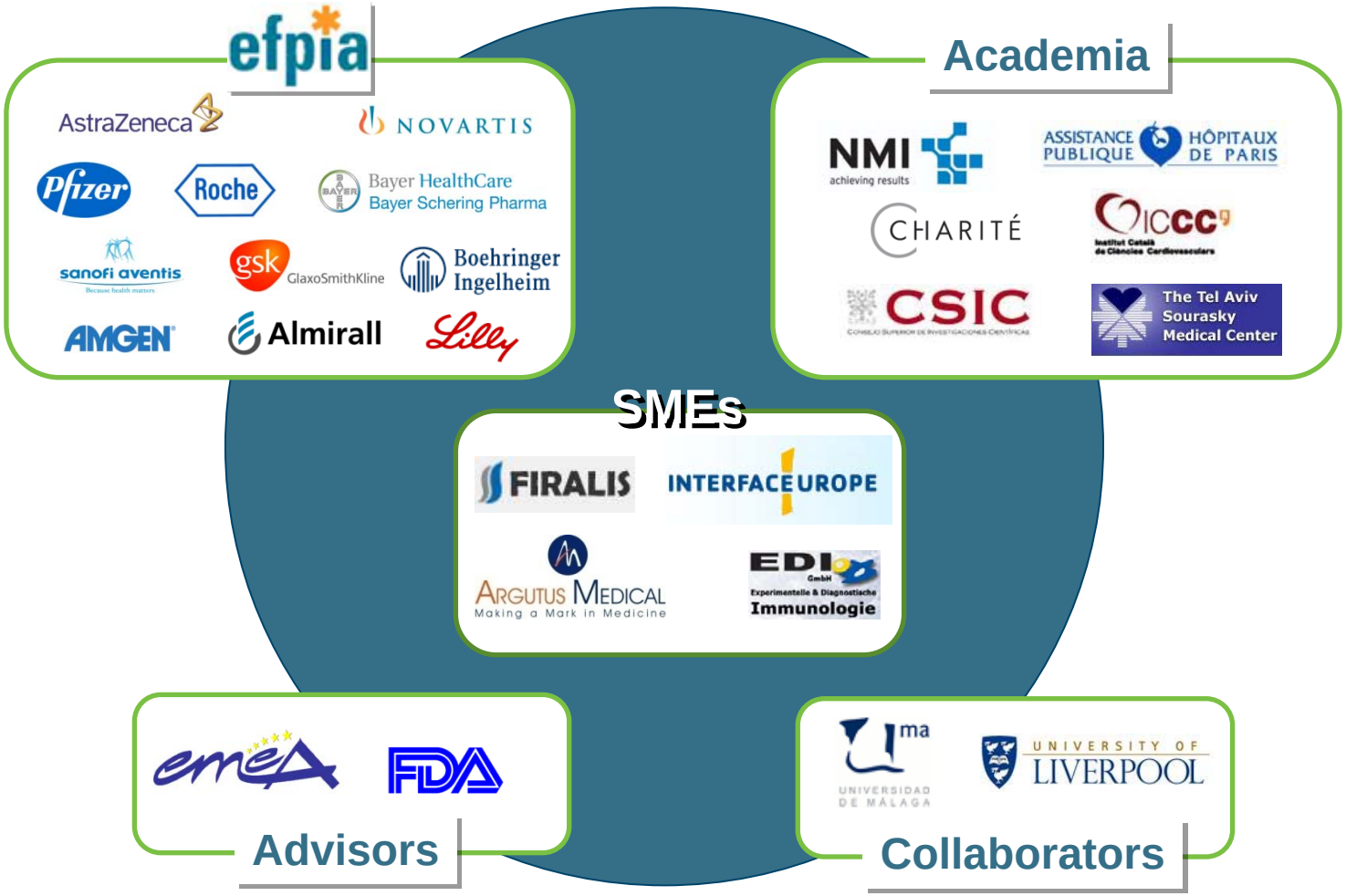
Key challenges for biomarker qualification

- 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
- Key target responses, i.e. specific adverse drug reactions, suitable and accessible for qualification are overall very rare

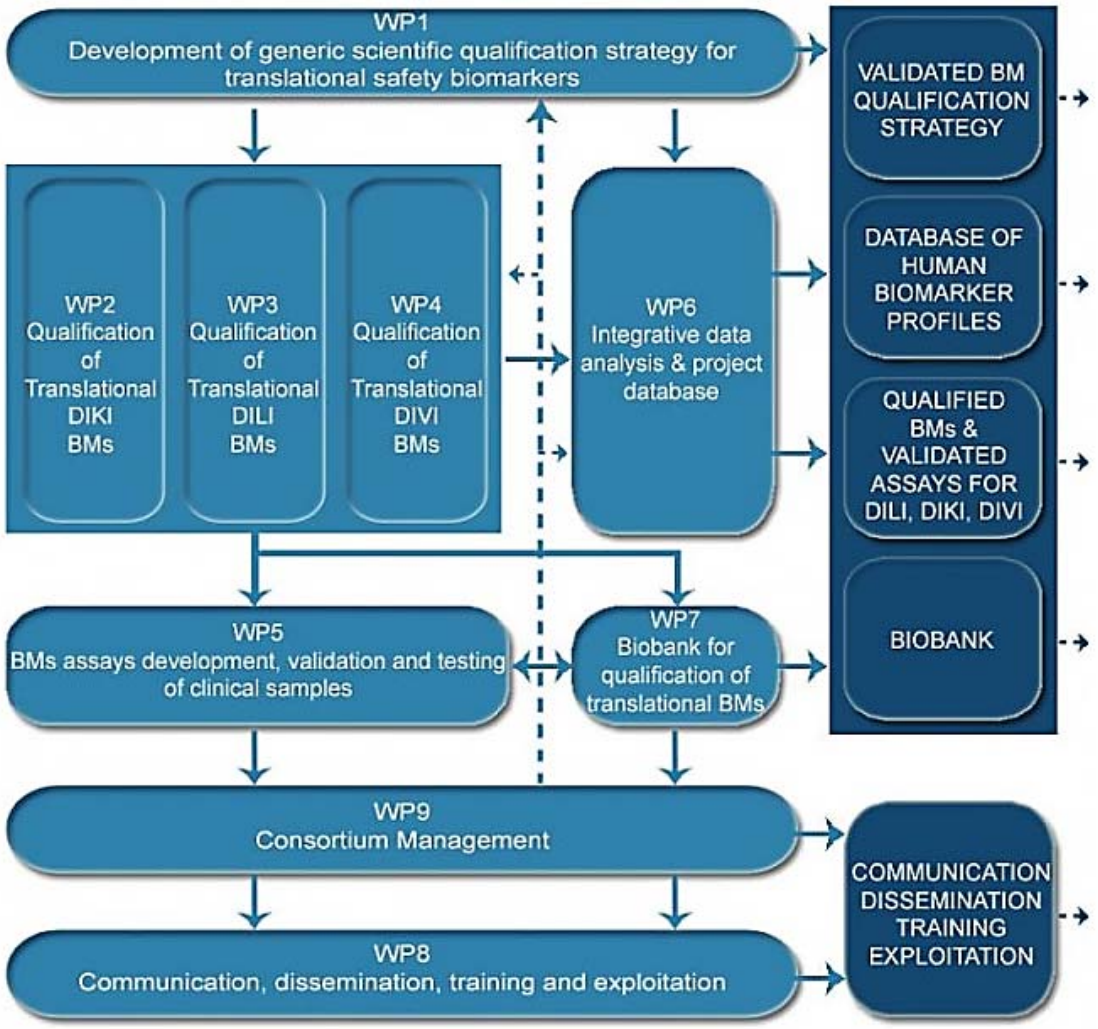
- ➢ Large sample sizes are required
- ➢ Multitude of patient populations need to be included

Qualification cannot be achieved by one company alone

SAFE-T participants



SAFE-T structure and deliverables



- Evidence-based decision making
- More reliable causality assessment
- Better mechanistic understanding
- Safer translation to clinical development
- Earlier and more specific signal detection
- Enhanced clinical monitoring

➢ **Improved patient safety**

➢ **Reduced attrition rates**

➢ **Accelerated and safer approval of innovative medicines**

Funding and timing

Financing

- IMI funding: 13.9 mio EUR
- EFPIA contribution, mainly in kind: 17.7 mio EUR
- Contribution academia/SME: 4.1 mio EUR

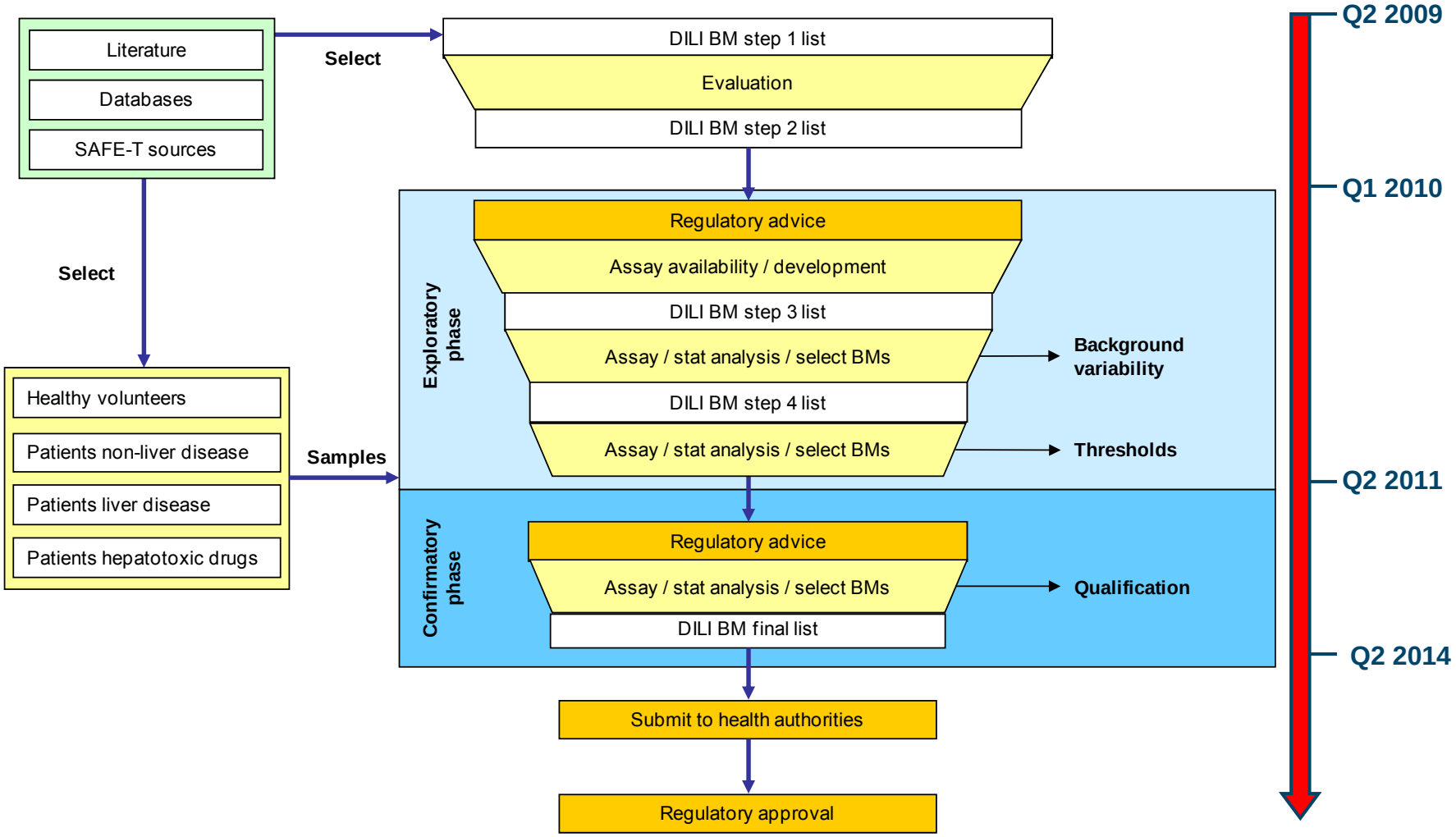
- Total project cost: 35.7 mio EUR

Timing:

- Starting date: June 15, 2009
- Duration: Five years

Biomarker qualification process

Elements and process flow



DILI biomarker candidates selected for qualification

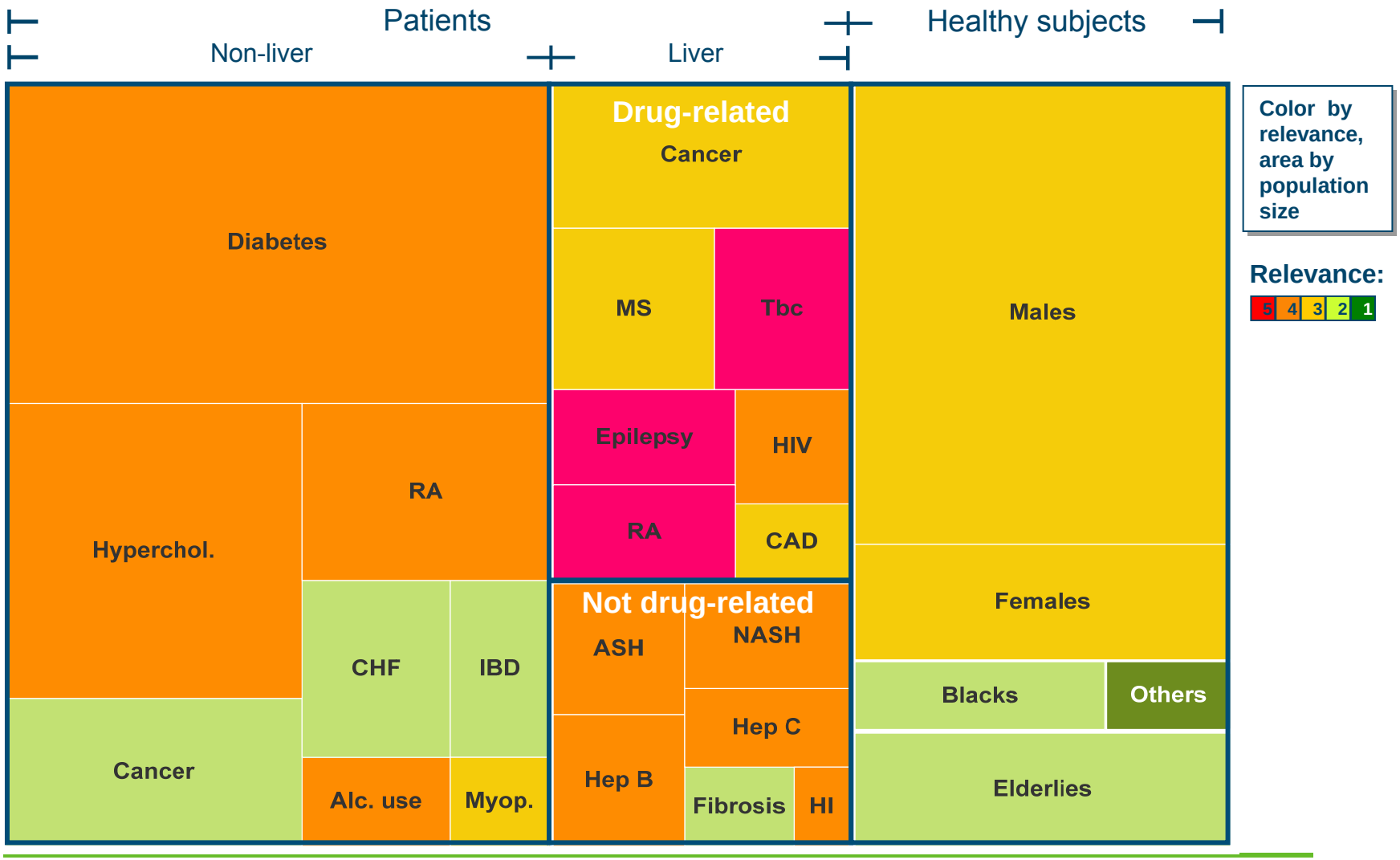
Serum or Plasma Marker	Assays		Liver specificity	Human data	Pathology
Albumin mRNA		RT-PCR ✓	highly specific	yes	hepatocellular damage
Microglobulin precursor (Ambp) mRNA		RT-PCR ✓	highly specific	yes	hepatocellular damage
Micro RNA 122		RT-PCR ✓	specific	yes	hepatocellular damage
Conjugated/unconjugated bile acids		LC-MS	highly specific	only in tissues	hepatocellular damage
High mobility group box 1 (HMGB1)	*	LC-MS ✓	not specific	yes	cholestasis
Cytokeratin 18 (KRT18)	*		not specific	yes	hepatocellular damage
Alpha fetoprotein (AFP)	✓		specific	yes	hepatocellular damage
Arginase 1	✓		highly specific	yes	hepatocellular damage
Colony stimulating factor receptor (CSF1R)	Immuno- ✓		not specific	yes	inflammation
F-protein (HPPD)	assays		highly specific	yes	hepatocellular damage
Glutathione S transferase alpha (GSTα)	LMX *		specific	yes	hepatocellular damage
Leukocyte cell-derived chemotaxin 2 (LECT2)	✓		not specific	yes	inflammation
ST6Gal 1	✓		specific	yes	inflammation
Osteopontin	✓		not specific	yes	inflammation
Ratio Paraoxonase (PON1) / Prothrombin	✓		not specific	yes	steatosis
Regucalcin (RGN)			not specific	yes	steatosis
ALT1/2		✓	specific	only in tissues	hepatocellular damage
Glutamate dehydrogenase (GLUD, GLDH)		Enzyme ✓	highly specific	yes	hepatocellular damage
Malat dehydrogenase (MDH)		activity ✓	specific	yes	hepatocellular damage
Purine nucleoside phosphorylase (PNP)		✓	specific	no	hepatocellular damage

✓ SAFE-T has already developed an assay for singleplex measurement

* ELISA commercially available

DILI biomarker qualification:

The „population mosaic“



Currently planned clinical studies

- Multi-center study in patients with suspected drug-induced liver injury
- Single-center study in rheumatoid arthritis patients
- Single-center study in patients with acute lymphoblastic leukemia (ALL) or acute myeloid leukemia (AML) during anti-proliferative treatment
- Single-center study in patients receiving oxaliplatin based chemotherapy for advanced colorectal cancer
- Single-center study in colo-rectal cancer patients with liver metastases
- Single-center study in patients with chronic hepatitis C after liver transplantation
- Multi-center study in patients on antituberculosis treatment

SAFE-T achievements

- Generic qualification strategy defined
- Biomarker candidates prioritised, assay development ongoing
- Study protocols for prospective DILI studies submitted for IRB review
- Completed HV study to assess within and between subject variability (Sanofi Aventis), and secured access to HV samples (AstraZeneca)
- Set up central biobank for sample storage
- Initiated regulatory interactions via briefing meetings with EMA/FDA
- Established collaboration with Predictive Safety Testing Consortium (PSTC)

Key challenges for the consortium

	Gap/Challenge	How addressed?
Biomarker candidates	• Out of scope: o Genetic susceptibility markers o Preclinical assay validation o Preclinical biomarker discovery	• Covered by SAEC, DILIN, others • Close collaboration with PSTC
	• Lack of functional and susceptibility marker candidates	• Biomarker discovery based on human cases from SAFE-T clinical studies, using mass spec and protein antibody array analyses of plasma samples
Methodology	• Due to low DILI prevalence, any new marker will have a low PPV. o Improvement is mainly needed in specificity rather than sensitivity. o Added value of new markers may be primarily as part of panels	• Identify suitable marker panels • Use advanced statistical methods such as lasso regression and gradient boosted models
Logistics	• Access to DILI cases • Sampling requirements need to be aligned across different SAFE-T working groups • Sampling to be seamlessly integrated into standard clinical trial workflows	• Add two studies in high risk patients • Dedicated cross-work package team to ensure alignment • Provide standard protocol and ICF text sections • Simplify sample collection, processing, and shipment • Use samples available already

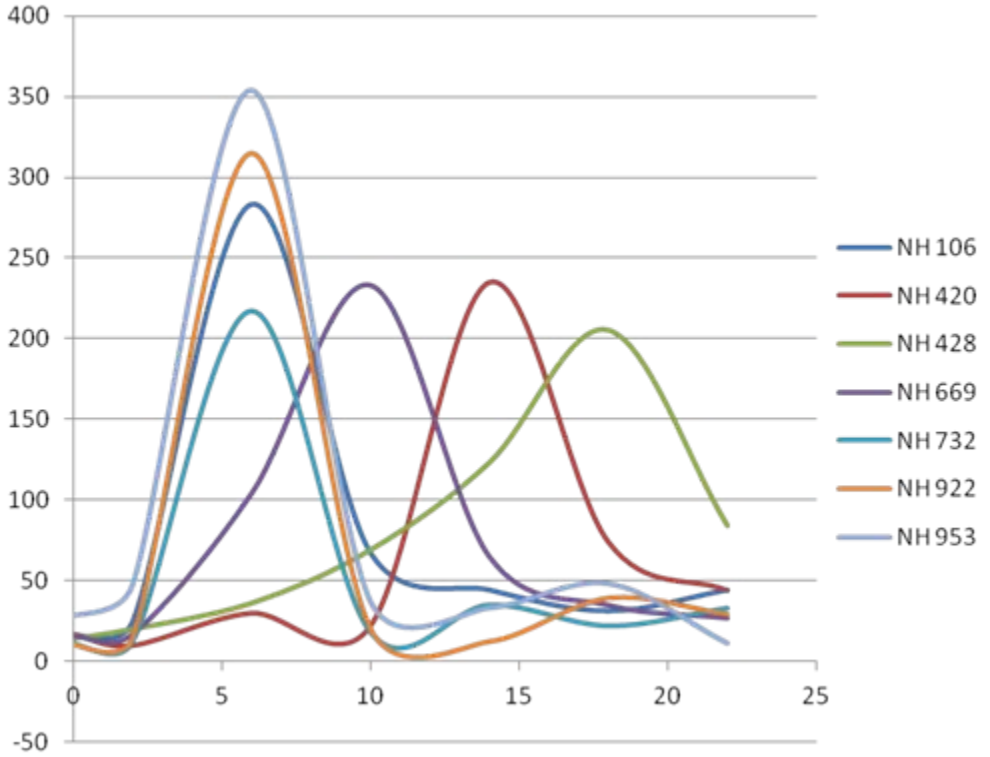
High risk patients I: nevirapine treatment, CDSS

- **Background**
  - o Nevirapine is cheap and therefore widely used in Africa
  - o The MRC Centre for Drug Safety Science (CDSS) at the University of Liverpool has a strong clinical network in Malawi
  - o Previous work has successfully recruited a 1000 HIV patient cohort to examine the mechanisms of nevirapine toxicity at both the genetic and biochemical level
  - o Key side effect of nevirapine is hypersensitivity (rash, SJS, DILI)
  - o Overall incidence of hypersensitivity reactions was 57/1117 (5.1%), 12.3% of which were DILI cases
- **Current treatment protocol**
  - o Continue on nevirapine to treat through reaction if at all possible
  - o DILI – only stop drug if patient develops jaundice
  - o LFTs are not routinely measured – no facilities, and expensive
  - o If transaminases rise, but patient is not jaundiced, the drug is continued

Slide by Munir Pirmohamed, MRC CDSS

Adaptation to nevirapine

- 7/1117 (0.6%) had abnormal TA and jaundice
- 1 (0.1%) patient died of liver failure
- 66 (5.9%) patients had abnormal ALT but no jaundice
  - o 7 (0.6%) grade III/IV
  - o 59 (5.3%) grade II



Slide by Munir Pirmohamed, MRC CDSS

New study in nevirapine treated HIV patients

- Implementation of a new 1000 patient nevirapine study
- All patients to provide pre-treatment sample (blood and urine)
- All patients to provide samples monthly out to 6 months post-treatment start
- Collect:
  - Matched samples (before and during treatment) of nevirapine-tolerant patients
  - Matched samples (before treatment, during acute DILI and post DILI) of patients that develop DILI but adapt
  - Matched samples (before treatment, DILI) of patients that have had to discontinue treatment

Slide by Munir Pirmohamed, MRC CDSS

High risk patients II: APAP overdose, CDSS

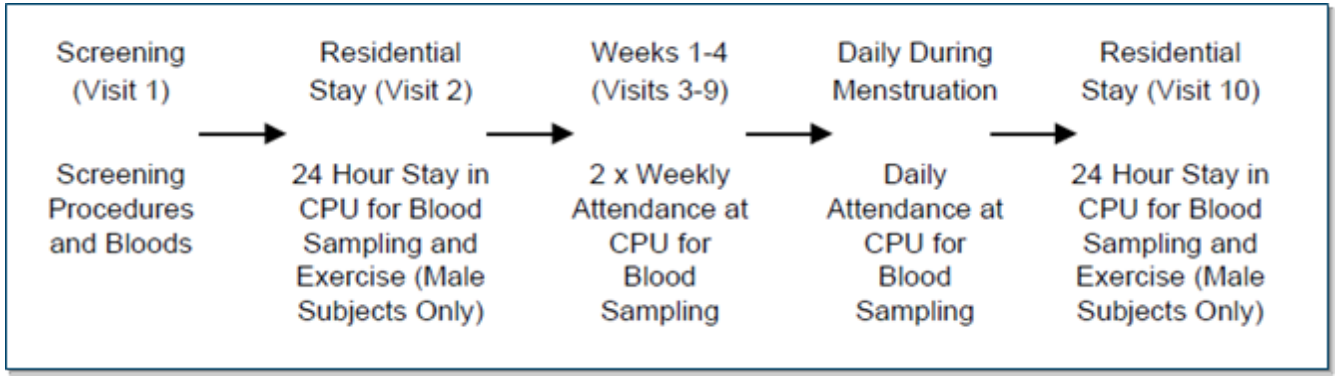
- Ethical approval available to collect serum, plasma and urine from APAP overdose patients
- n = 21 patients plus n = 21 healthy volunteers planned
- Sample collection (plasma, serum, urine), patients:
  - t1: Presentation or 4hrs post-overdose,
  - t2: 12 – 18 hrs after first sample (morning ward round),
  - t3 : Subsequent morning ward round
  - t4: 1 month post-overdose
  - t5 : 3 month post-overdose
- If patients remain hospitalised after t3, samples will be taken every 48 hours up to a maximum of 2 weeks post-presentation
- Sample collection (volunteers): daily for 3 days to collect plasma, serum and urine

Slide by Kevin Park, MRC CDSS

Using available samples: AZ HV study

Study design:

- 3x12 healthy volunteers, 12 males, 12 pre-menopausal females, 12 post-menopausal females
- Assess circadian and gender variation
- Daily sampling during menstrual cycle
- Male group with exercise to exhaustion
- **CK18** measured using Pevivas M65 and M30 assays



Slide by Ina Schuppe Koistinen, Astra Zeneca

Variability of cell death biomarkers

1º model

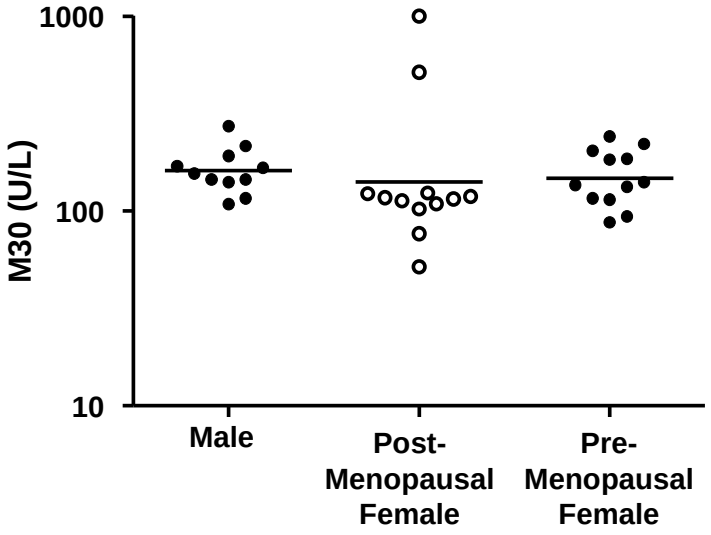
		Inter-Subject		Intra-Subject Inter-Day		Intra-Subject Intra-Day		Total	
Biomarker	N[a]	SD[b]	CV	SD[b]	CV	SD[b]	CV	SD[b]	CV
M30	33	0.55	59%	0.06	6%	0.11	11%	0.56	61%
M65	33	0.26	27%	0.07	7%	0.11	11%	0.29	30%
nDNA	33	0.54	58%	0.39	40%	0.74	85%	0.99	130%

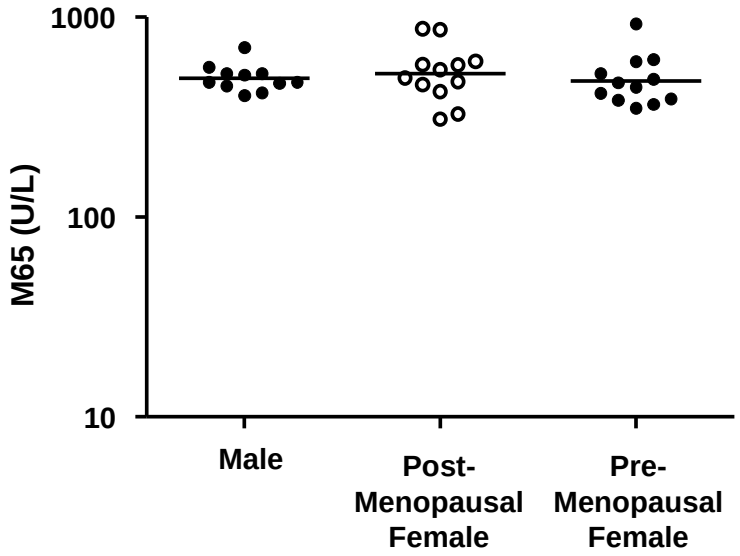
2º model using means from 24 hour periods

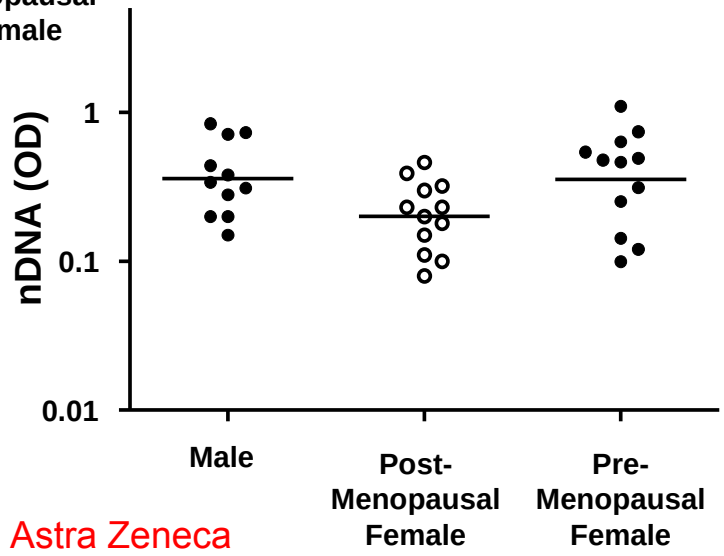
		Inter-Subject		Intra-Subject Inter-Day		Total	
Biomarker	N[a]	SD[b]	CV	SD[b]	CV	SD[b]	CV
M30	33	0.54	58%	0.08	8%	0.55	59%
M65	33	0.26	26%	0.09	9%	0.28	28%
nDNA	33	0.58	63%	0.45	48%	0.73	84%

Slide by Ina Schuppe Koistinen, Astra Zeneca

Effect of gender and reproductive status on cell death biomarkers

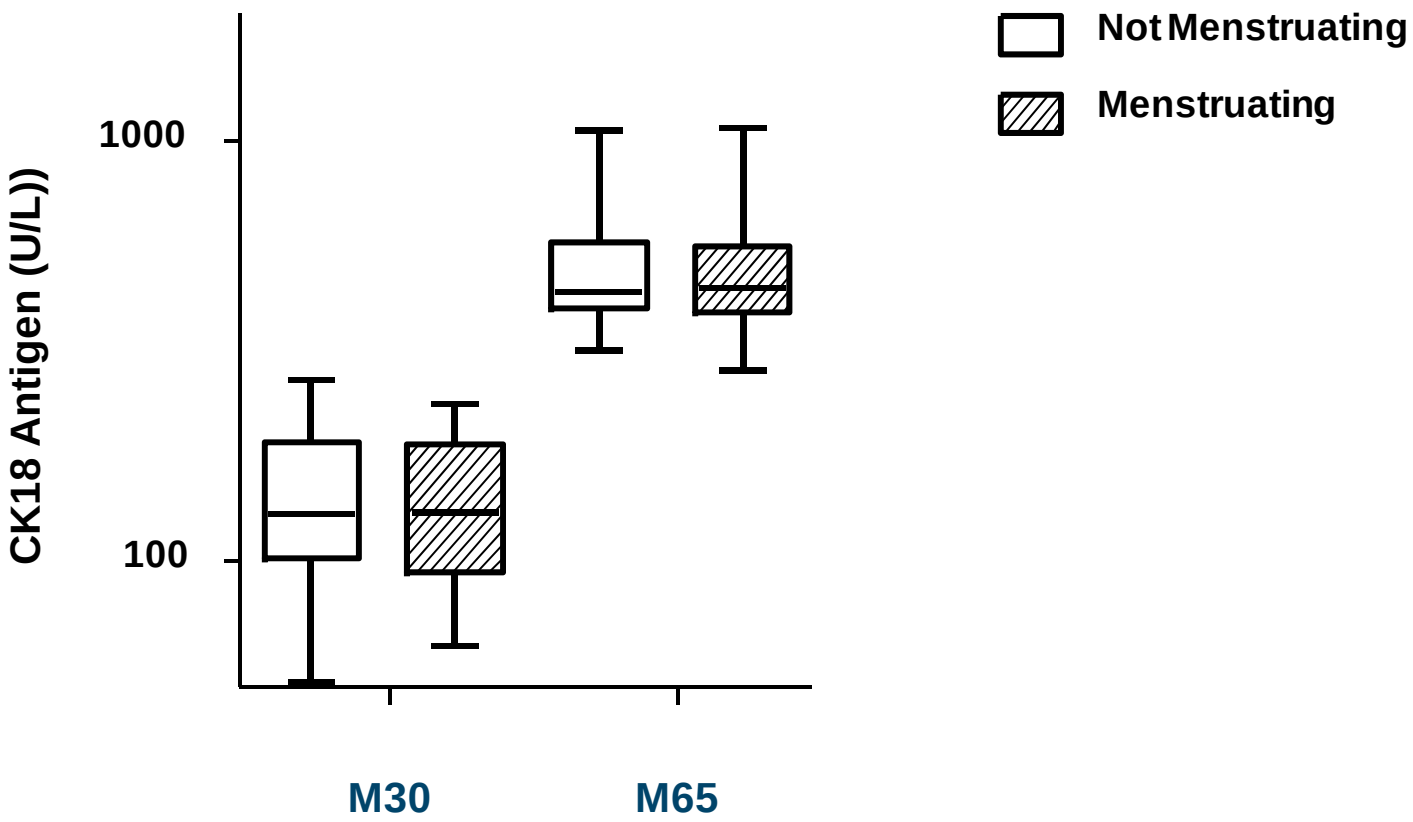






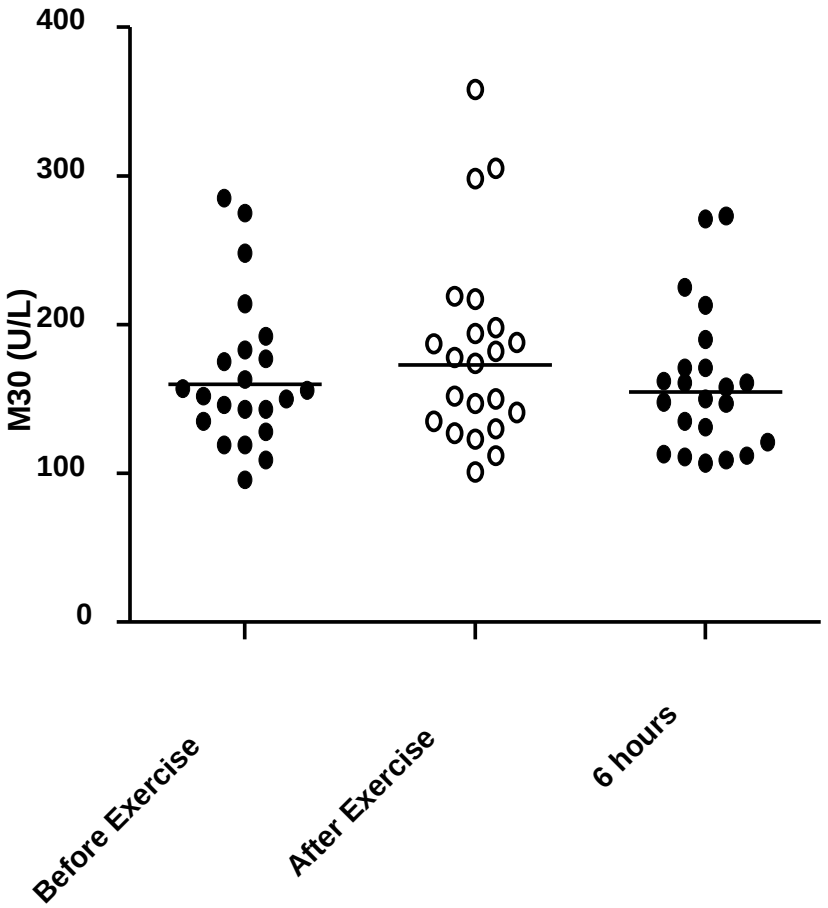
Slide by Ina Schuppe Koistinen, Astra Zeneca

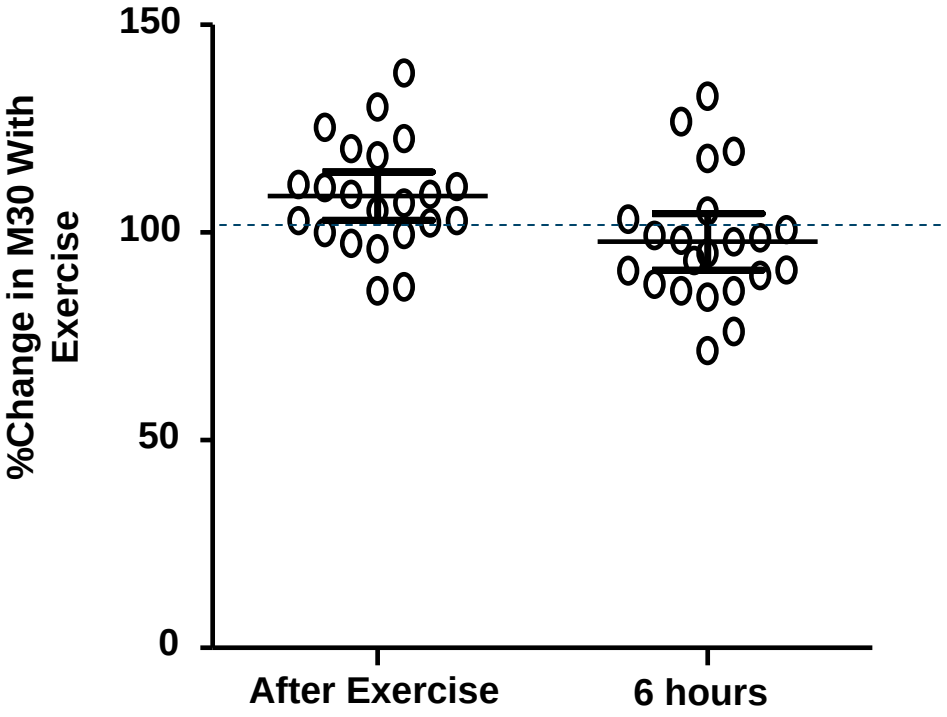
Changes in cell death biomarkers with menstruation



Slide by Ina Schuppe Koistinen, Astra Zeneca

Effect of exercise on M30





Slide by Ina Schuppe Koistinen, Astra Zeneca

Summary

- Low intra subject variability of CK18 (M30 and M60 assays)
- No circadian rhythm of CK18
- No major effect of gender
- No significant effect of menstruation
- A brief period of exercise led to brief raises in CK18
  - No biochemical evidence of muscle damage
  - Subjects need to restrain from exercise for at least 4 hours before samples are taken

Acknowledgements

Alastair Greystoke CRUK-AZ Clinical Fellow for Biomarker Development

Study Delivery: Gail Harris, Tracey Randall, Kirsty Mackay **Statistics:** Martin Jenkins, Helen Mann, Athula Herath

Cognizant: Nazneen Solkar **Sample Logging:** Matthew Lancashire

CPU: Debbie Vinsun, Pascal De Feyter, Dilly Goonetilleke, Helen Redding, Raj Chetty, Emeline Ramos

Management: Andrew Hughes, Glen Clack, Caroline Dive

Slide by Ina Schuppe Koistinen, Astra Zeneca

SAFE-T: next steps

- Set up consortium database
- Initiate prospective studies
- Include sampling into standard clinical trials
- Finalize agreement with PSTC

Acknowledgements

(Incomplete) SAFE-T participant list, team leaders

Neus Prats	Almirall	**Katja Matheis**	Boehringer Ingelheim	**Andrew Nicholls**	GSK	**Steve Hall**	Pfizer
Eric Massana	Almirall	**Christine Rentzsch**	Boehringer Ingelheim	**Elaine A. Irving**	GSK	**Stefan Sultana**	Pfizer
David Sciberras	Amgen	**Arno Kalkuhl**	Boehringer Ingelheim	**Fiona J McClure**	GSK	**Michael Lawton**	Pfizer
James Matcham	Amgen	**Ulf Neumann**	Aachen Hospital	**Theo Dare**	GSK	**Silvia Guionaud**	Pfizer
Patrice Cacoub	AP-HP	**Volker Schmitz**	Charité Hospital	**Landry Cochard**	Interface Europe	**Denise Robinson-Gravatt**	Pfizer
Thierry Poynard	AP-HP (GHPS)	**Eckart Schott**	Charité Hospital	**Marc Loher**	Interface Europe	**Bernard Souberbielle**	Pfizer
Mona Munteanu	AP-HP (GHPS)	**Ralph Schindler**	Charité Hospital	**Piret Noukas**	Interface Europe	**Jim Dykens**	Pfizer
Joe Keenan	ARGUTUS	**Thomas Berg**	Leipzig University	**Nicole Schneiderhan-Marra**	NMI	**Peter Colman**	Pfizer
Barry Hayes	ARGUTUS	**Florian van Bömmel**	Leipzig University	**Jens Göpfert**	NMI	**Geoff Johnston**	Pfizer
Mark Pinches	AstraZeneca	**Lina Badimon**	CSIC-ICCC	**Stefanie Rimmele**	NMI	**Andrew Berridge**	Pfizer
Ina Schuppe Koistinen	AstraZeneca	**Teresa Padro**	CSIC-ICCC	**Hannes Planatscher**	NMI	**Jacky Vonderscher**	Roche
Håkan Andersson	AstraZeneca	**Xavier Sánchez-Vallve**	CSIC-ICCC	**Frank Dieterle**	Novartis	**Lucette Doessegger**	Roche
Sally Price	AstraZeneca	**Thomas Joos**	EDI	**Peter Hoffmann**	Novartis	**Joachim Eberle**	Roche
Jesper Hedberg	AstraZeneca	**Jean-Marc Vidal**	EMA	**Dietrich Rothenbacher**	Novartis	**Christoph Wandel**	Roche
Björn Glinghammar	AstraZeneca	**Hüseyin Firat**	Firalis	**Ursula Knauf**	Novartis	**Rodolfo Gasser**	Roche
Jenny McKay	AstraZeneca	**Kaïdre Bendjama**	Firalis	**John Prince**	Novartis	**Nadir Arber**	SMC-Tel-Aviv
Axel Kretschmer	Bayer Schering	**Peter Thomann**	Firalis	**Jeffrey Donohue**	Novartis	**Bernd Stowasser**	Sanofi Aventis
Thomas Krahn	Bayer Schering	**Béatrice Molac**	Firalis	**David Laurie**	Novartis	**Isabelle Clavier**	Sanofi Aventis
Heidrun Ellinger-Ziegelbauer	Bayer Schering	**Fuat Firat**	Firalis	**Marie Anne Valentin**	Novartis	**Magali Guffroy**	Sanofi Aventis
Matthias Gottwald	Bayer Schering	**John Haselden**	GSK	**Philip Bentley**	Novartis	**Joachim Tillner**	Sanofi Aventis

Backups

Establishing extremal dependence

- If ALT is high and liver-related, we expect a novel biomarker to also be elevated
  - ALT and the biomarker are said to exhibit *extremal dependence*
  - Suggests quantifying extremal dependence and discarding biomarkers without strong extremal dependence on ALT
- How to establish extremal dependence?
  - Correlation in the bulk of the data does NOT imply correlation in the extremes
    - E.g. if X and Y are normally distributed, they are *independent* in the extremes unless corr(X, Y) = 1
  - Instead, use measures that specifically measure extremal dependence:
    - Coefficient of tail-dependence χ (chi): $\chi = P(X > u \mid Y > u)$ for a large quantile u (see Coles et al, 1999)
    - Multivariate conditional Spearman's ρ: $\rho_{MCS} = corr(X, Y \mid Y > u)$ (Schmid & Schmidt, 2007)

Identifying predictors of liver injury

- Many biomarkers, therefore many possible relationships to DILI
- Traditional approaches
  - Take each biomarker in turn
    - Can't find panels of biomarkers
  - Use stepwise regression methods
    - Well known to be deeply flawed
- Preferred approaches
  - The lasso (a.k.a. L1-penalized regression)
  - Gradient boosted models
  - Each can be used to choose a model with multiple biomarkers, using cross-validation to obtain unbiased estimate of model performance
  - See Hastie et al, 2009, for more detail

References

- S. Coles, J. E. Heffernan and J. Tawn, *Dependence measures for extreme value analyses, Extremes*, 4, 339 – 365, 1999
- T. Hastie, R. Tibshirani and J. Friedman, *The Elements of Statistical Learning (Second Edition)*, Springer, 2010
- F. Schmid and R. Schmidt, *Multivariate conditional versions of Spearman's Rho and related measures of tail dependence*, The Journal of Multivariate Analysis, 98, 1123 – 1140, 2007