SIGNATOPE

NMI
schafft Ergebnisse

TARGETED PROTEOMICS IN TOXICOLOGY

DR. OLIVER POETZ

CHALLENGES IN PUBLIC HEALTH PROTECTION IN THE 21ST CENTURY: NEW METHODS, OMICS AND NOVEL CONCEPTS IN TOXICOLOGY

BERLIN 16-NOV-2021

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CONTENT

- Targeted proteomics – Protein analysis methods

- Applications
  - *In vitro* platform for testing combinatorial effects
  - Drug induced kidney injury – targeted study
  - Drug induced vascular injury – hypothesis-driven
  - IMI - TransBioLine - Qualification of drug-induced organ injury bioimarkers

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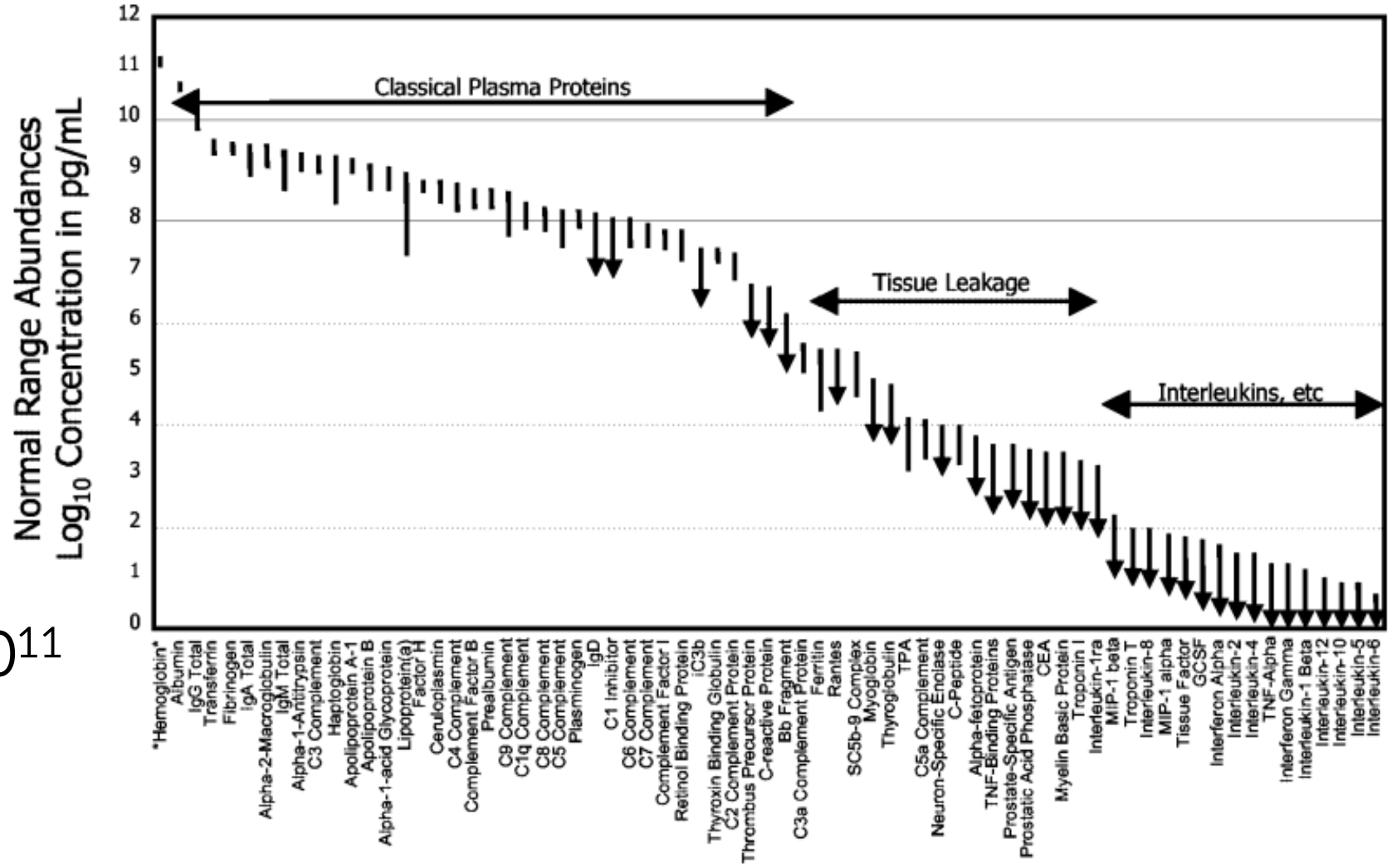
PROTEOMICS – THE CHALLENGE



Dynamic range 10^{11}

Anderson NL, Anderson NG.
The human plasma proteome: history, character, and diagnostic prospects. Mol Cell Proteomics. 2002 Nov;1(11)

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PROTEIN ANALYSIS METHODS

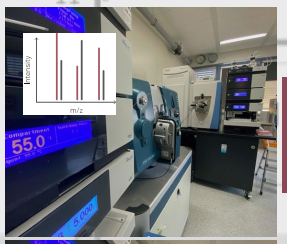
Sensitivity Multiplexing

Classical Plasma Proteins

Tissue Leakage

Interleukins, etc

Proteomics
(Mass spectrometry)



Range:
µM –nM*

100-
1000s

Targeted Proteomics

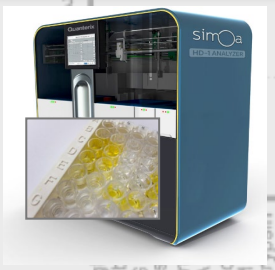
MS only

IA –LC MS/MS

Range:
µM – pM*

1-50

Sandwich
Immunoassays



Range:
down to aM

1-5

* Depends on matrix and starting volume

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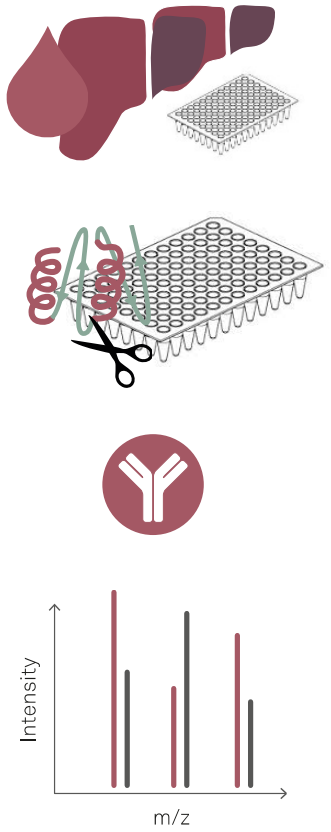
TECHNOLOGY – IMMUNOAFFINITY-LC-MS/MS (IA-LC-MS/MS)



- Sample
- Protein digest using trypsin down to peptides
- Add isotope-labelled peptide standards
- Enrich peptide standards and endogenous peptide derived from protein of interest using antibodies
- Analysis using multiplex targeted nLC-MS/MS

Quantification by ratio of endogenous peptide : internal isotope-labelled standards

Anderson, N. L. et al.. J Proteome Res (2004)
Jiang, J. et al., Proteomics Clin. Appl (2007)

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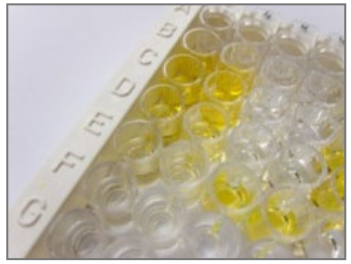
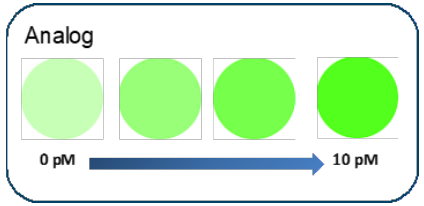
METHOD FOR TARGETED PROTEOMICS/ PROTEIN ANALYSIS

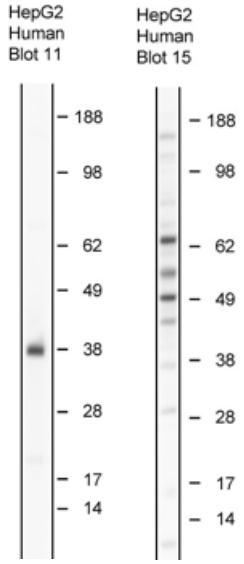
ELISA/Sandwich assays





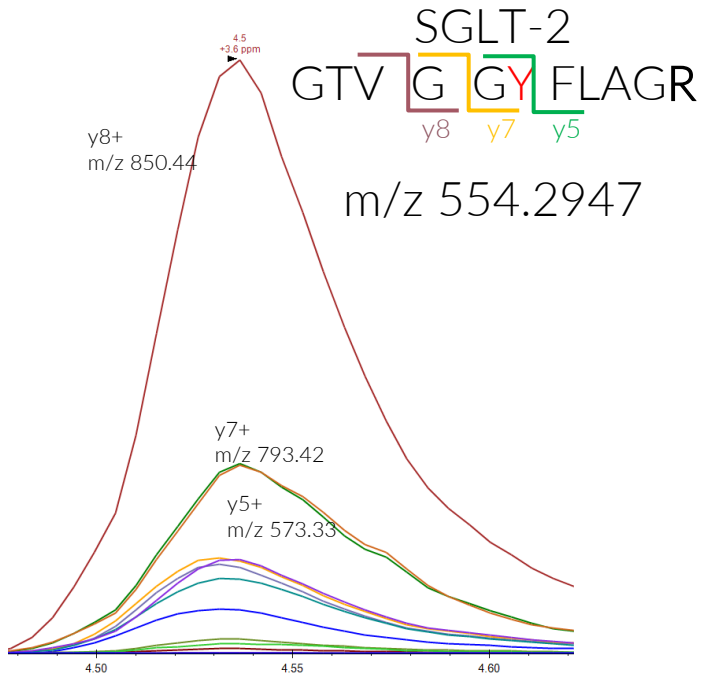
Specificity?
Membrane proteins?

Western Blot



Specificity?
membrane fractionation?

IA-LC-MS/MS



Specificity and
No membrane fractionation required

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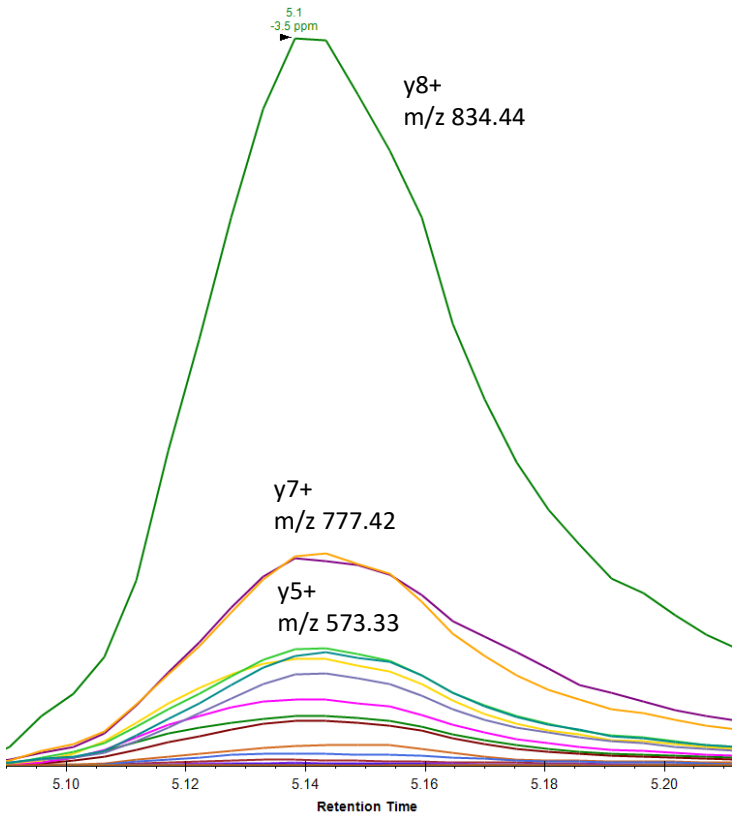
ISOFORMS IN THE MASS SPECTROMETER

SGLT-1

GTV G GF FLAG**R**

y8 y7 y5

m/z 546.2972

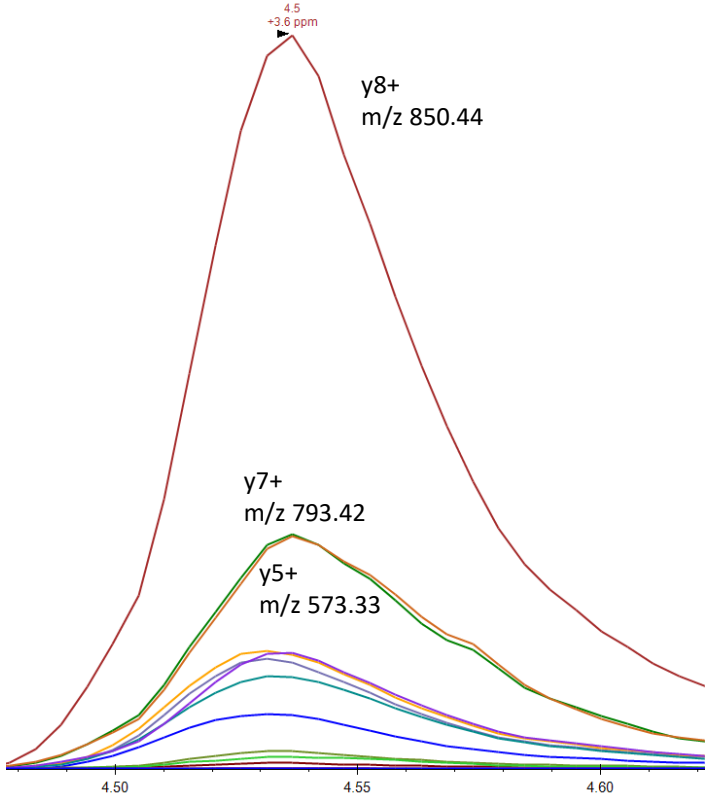


SGLT-2

GTV G GY FLAG**R**

y8 y7 y5

m/z 554.2947



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TECHNOLOGY –COMPARISON

	ELISA	Western Blot	Mass spec	IA-LC-MS/MS
Membrane protein	✗	✗	✓	✓
Speed	✓	✓	✗	✓
Specificity	✓	✓	✓	✓
Cross species	✗	✗	✓	✓
Robust	✓	✓	✗	✓
Multiplexing	20	1-5	100s-1000s	20
Sensitivity	high	high	low	medium
Dynamic range	10^3	10^1	10^4	10^4

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IN VITRO PLATFORM FOR TESTING COMBINATORIAL EFFECTS

BfR Bundesinstitut für Risikobewertung

Universität Bielefeld

NMI schafft Ergebnisse

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PROJECT COMBIOMICS II

- Two novel European Union regulations (Reg No 1107/2009; Reg No 528/2012) require the analysis of potential cumulative or synergistic effects of multiple pesticide and/or biocide exposure

- Develop protein and mRNA assay panels to assess the effects of single pesticides and their combinations in human HepaRG cells.

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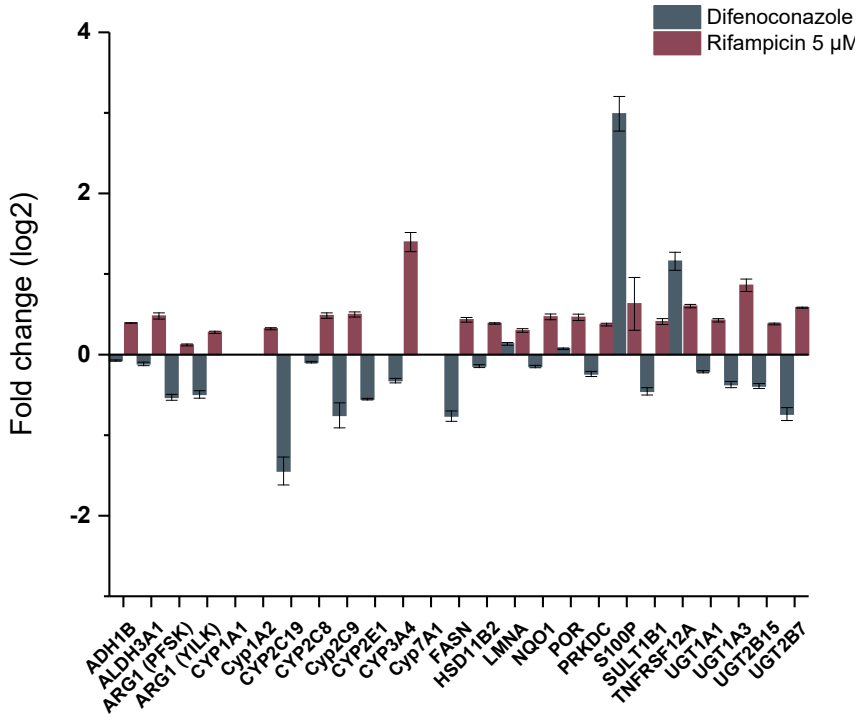
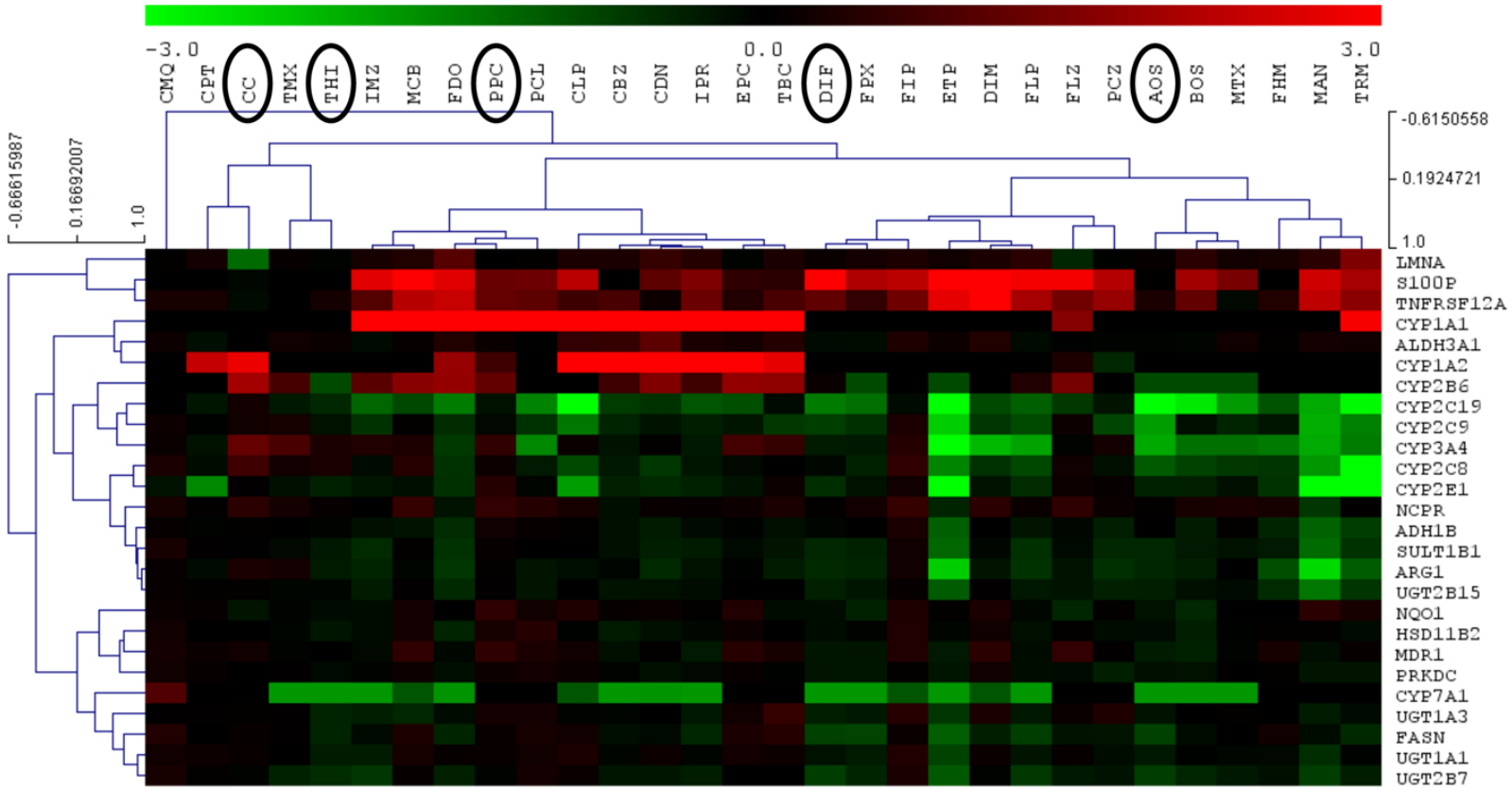
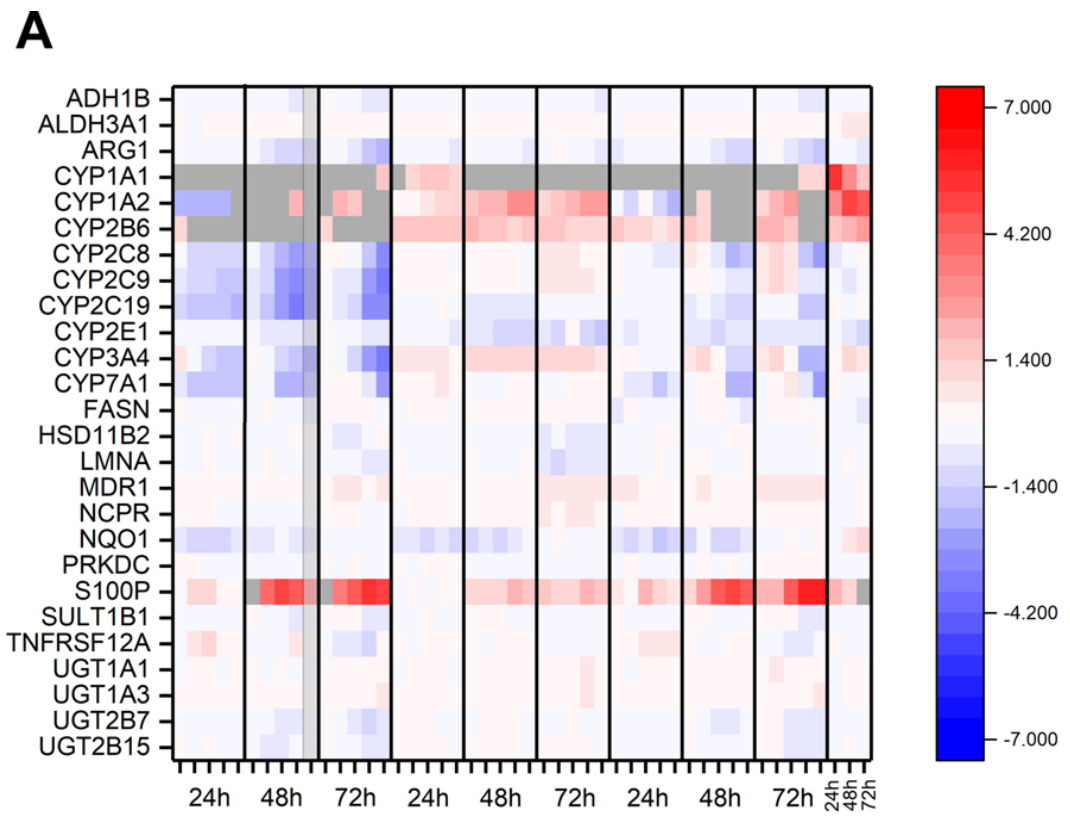
RESULTS INDIVIDUAL TREATMENT

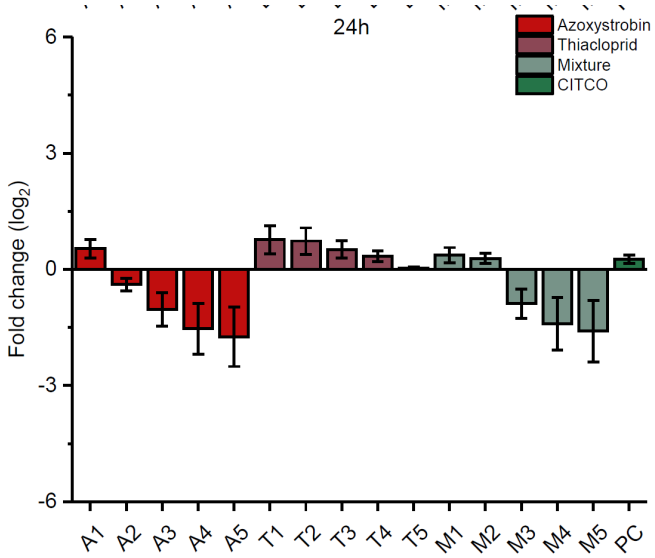


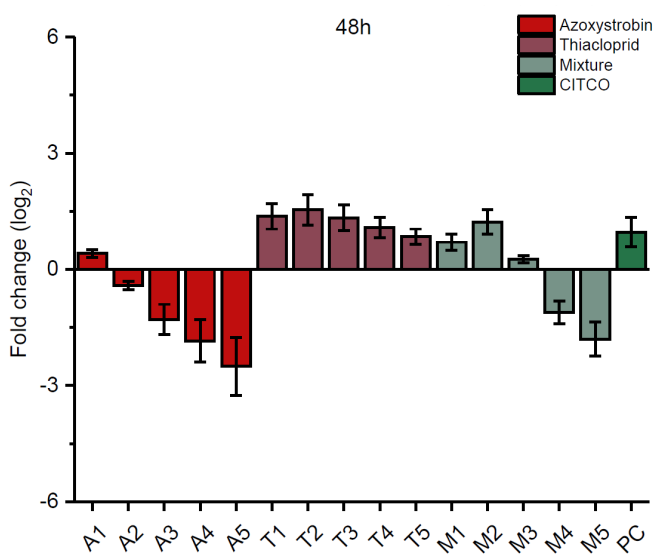


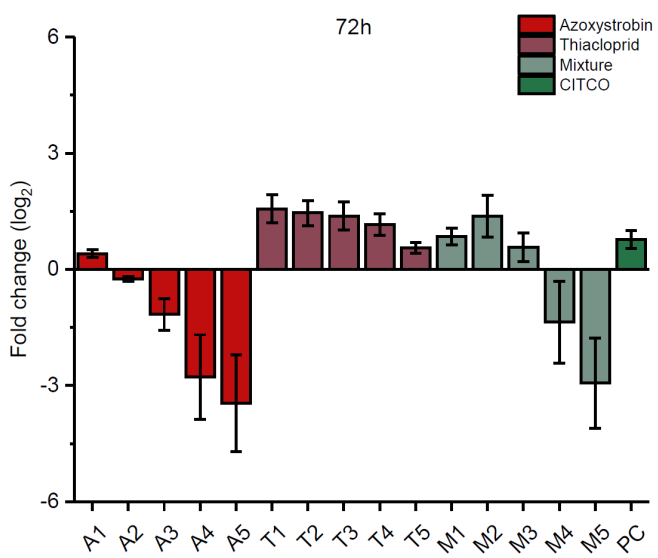
RESULTS COMBINATORIAL TREATMENT



RESULTS COMBINATORIAL TREATMENT CYP3A4







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SUMMARY

- Multiplex assays for the quantification of 33 different analytes developed.
- 30 substances were characterized individually in terms of potency to change the abundance of 33 proteins and mRNAs.
- Based on these results, combinations have been selected which should have synergistic, additive, or antagonistic effects.
- Combinatorial linear model was used identify synergistic, additive or antagonistic effects of the mixtures.

- Treatment using pesticide mixtures revealed combinatorial effects depending on the analyzed compound, compound concentration, target protein and time.

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DRUG-INDUCED KIDNEY INJURY

Tobramycin study in beagles 

janssen
PHARMACEUTICAL COMPANIES OF Johnson&Johnson

PSTC
CRITICAL PATH INSTITUTE

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HISTORY - DRUG-INDUCED KIDNEY INJURY BIOMARKER

- KIM-1 J Biol Chem. **1998** Feb 13;273(7):4135-42.
- In 2006 the Predictive Safety Testing Consortium (PSTC) was formed, 190 scientists from industry and government scientists
- 23 potential urinary protein biomarkers tested in 33 animal studies
- June 2007 and January 2008 data presentation to FDA, April **2008** had accepted that these biomarkers outperformed the current standards (Biomarkers on a roll, Nature Biotechnology volume 28, page 431 (**2010**)
- Jan **2018**

DDTBMQ000014

August 15, 2018

John-Michael Sauer, PhD
Critical Path Institute
Predictive Safety Testing Consortium (PSTC)
1730 E. River Road, Tucson, AZ 85718

> 20 years to get sensitive safety biomarkers for DIKI

Re: Biomarker Qualification Determination

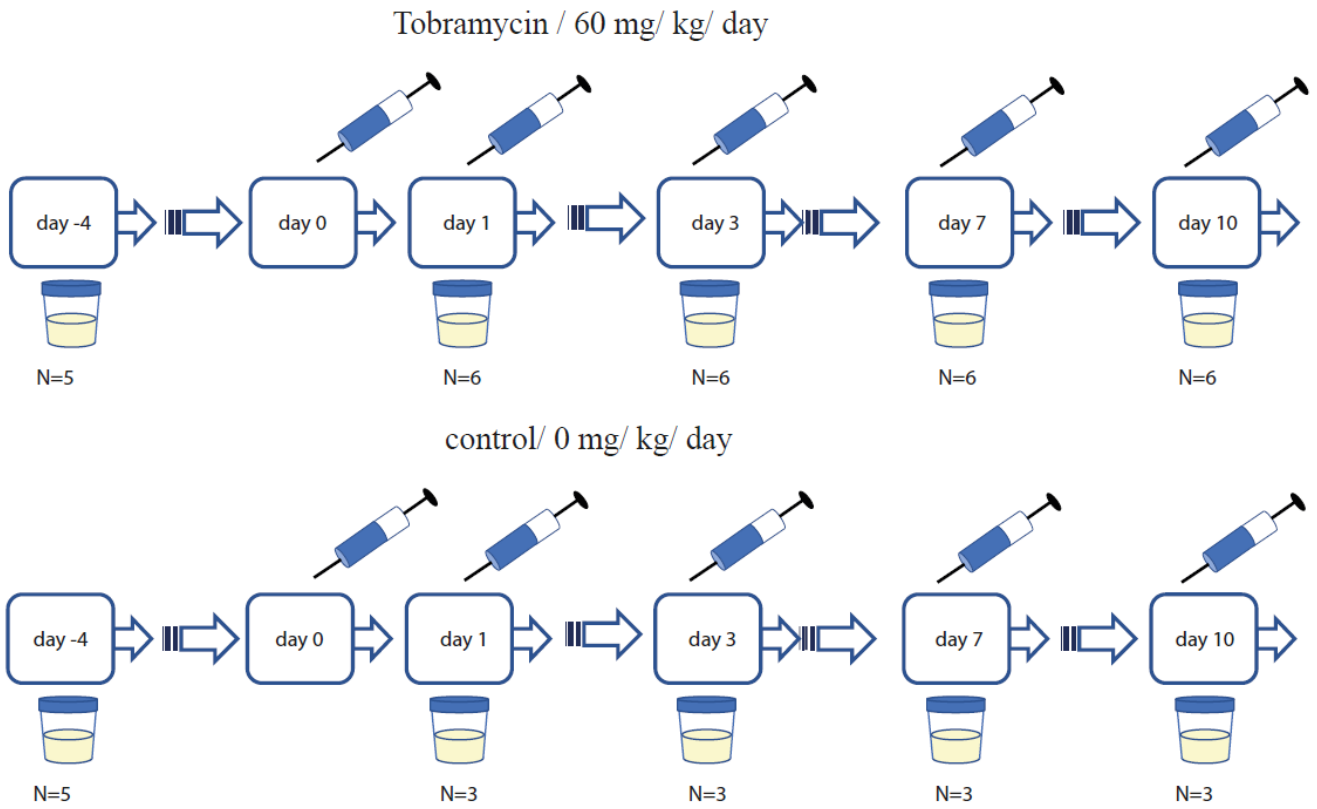
Dear Dr. Sauer:

Please refer to your Full Qualification Package for biomarker qualification DDTBMQ000014 dated and fully completed January 22, 2018, and reviewed under the legacy qualification process prior to establishment of the section 507 process of the Federal Food, Drug, and Cosmetic Act (FD&C).

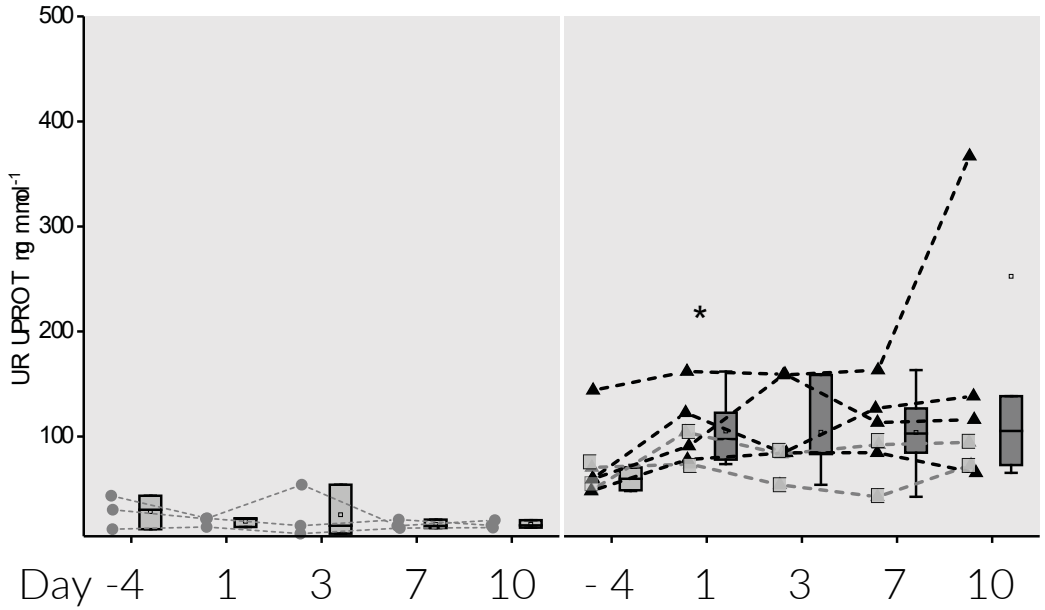
The Biomarker Qualification Program (BQP) has completed its review of your submission and is qualifying the following biomarker for the listed context of use (COU):

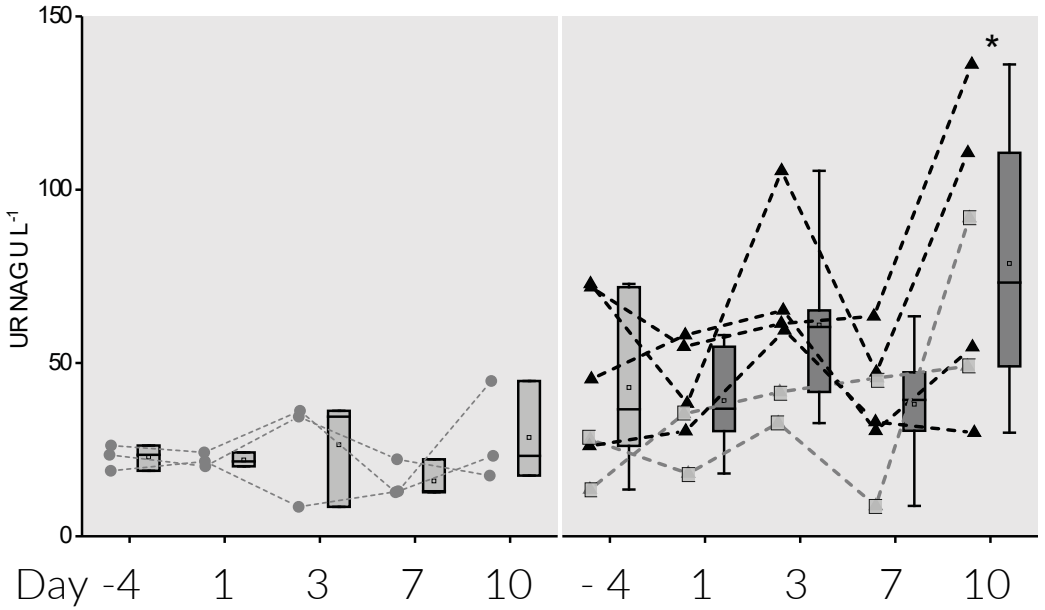
Biomarker: biomarker panel interpreted via a Composite Measure (CM) of the following six urinary biomarkers: Clusterin (CLU), Cystatin-C (CysC), Kidney Injury Molecule-1 (KIM-1), N-acetyl-beta-D-glucosaminidase (NAG), Neutrophil Gelatinase-Associated Lipocalin (NGAL), and Osteopontin (OPN)

DRUG-INDUCED KIDNEY INJURY - TOBRAMYCIN STUDY IN CANINES



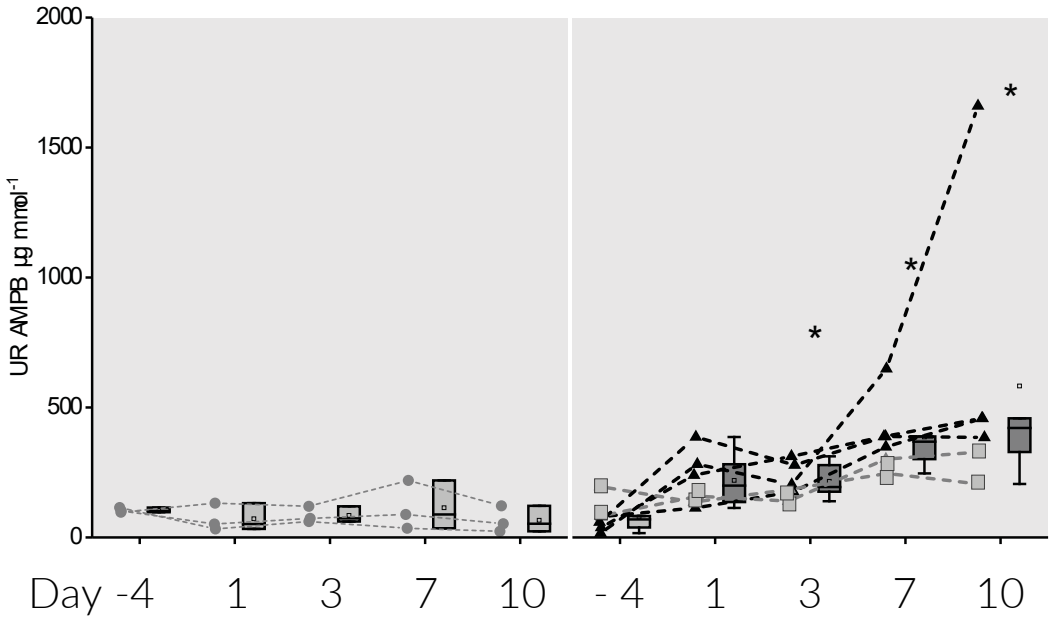
TOBRAMYCIN STUDY IN BEAGLES –TARGETED PROTEOMICS

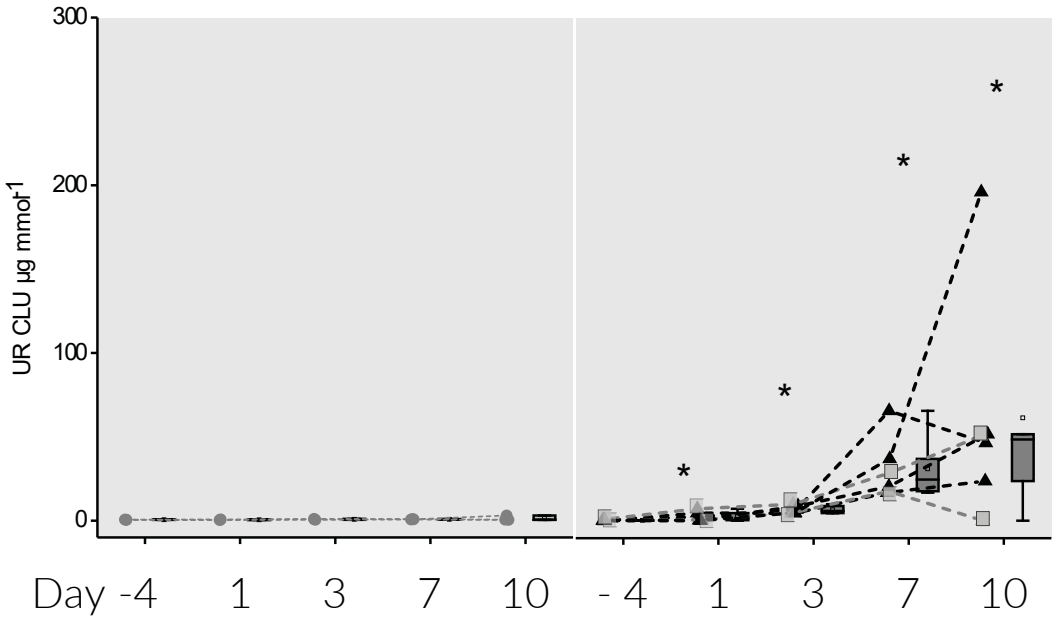




Vehicle control
Grade 1 & 2
Grade 3

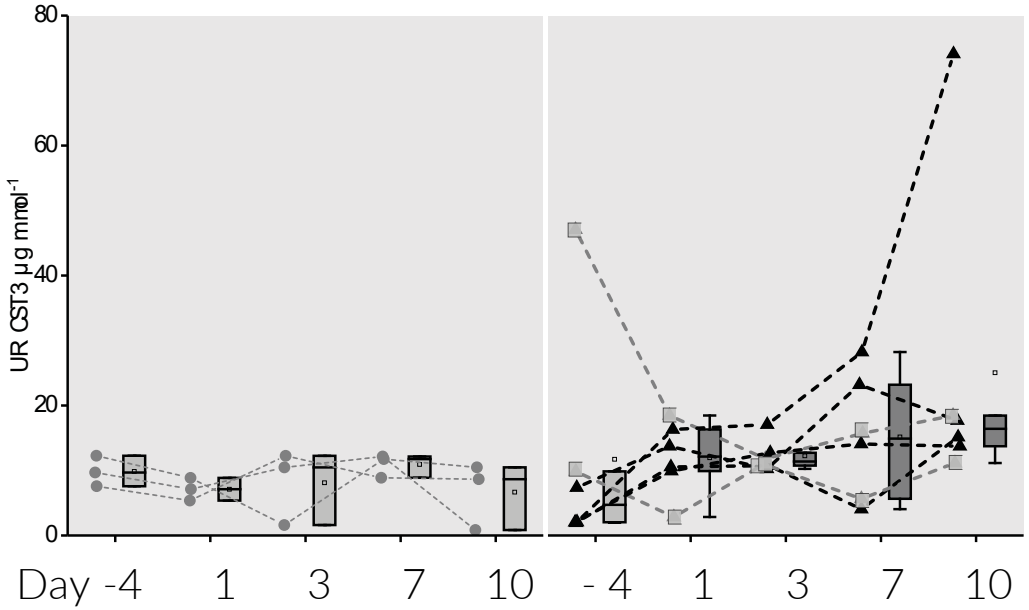
TOBRAMYCIN STUDY IN BEAGLES –TARGETED PROTEOMICS



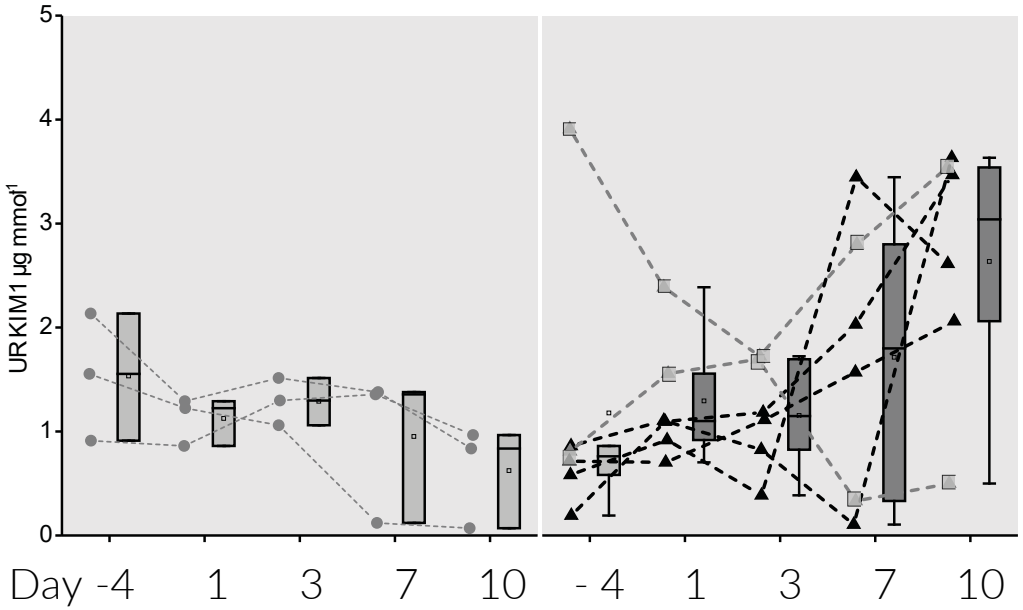


Vehicle control
Grade 1 & 2
Grade 3

TOBRAMYCIN STUDY IN BEAGLES –TARGETED PROTEOMICS



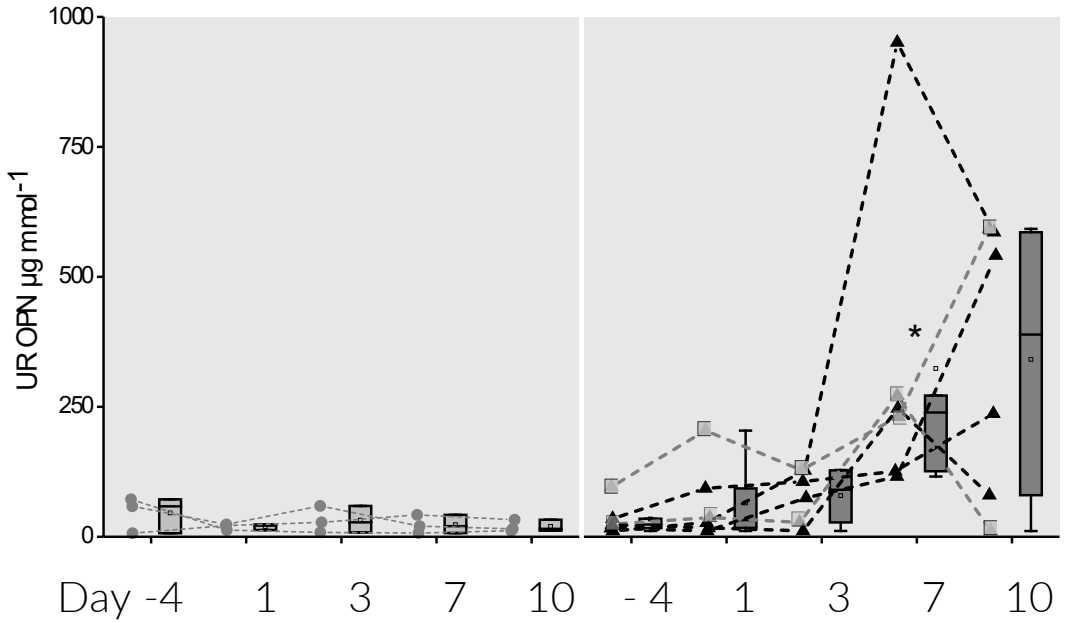
Control Tobramycin

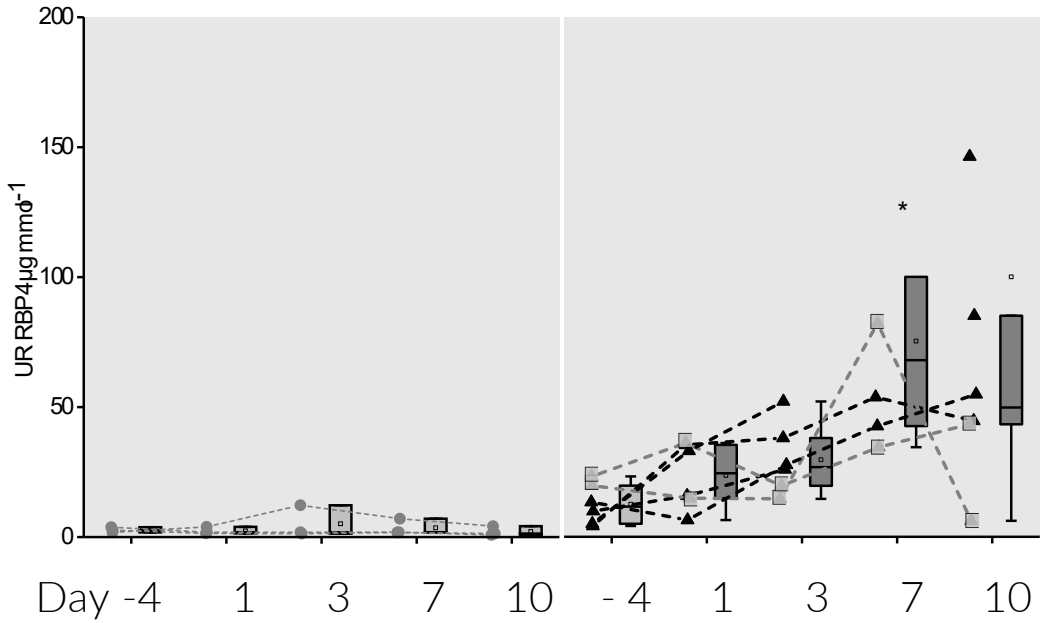


Control Tobramycin

Vehicle control
Grade 1 & 2
Grade 3

TOBRAMYCIN STUDY IN BEAGLES –TARGETED PROTEOMICS





Vehicle control
Grade 1 & 2
Grade 3

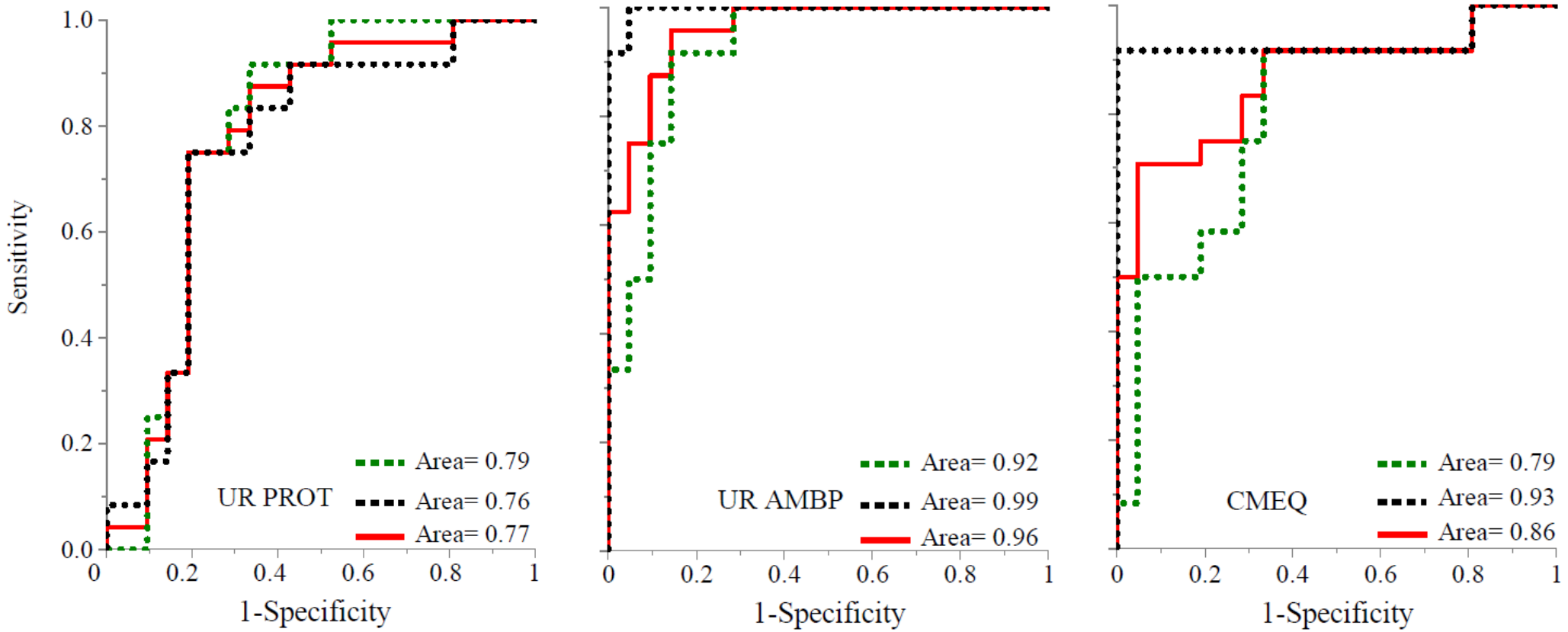
TOBRAMYCIN STUDY IN BEAGLES –TARGETED PROTEOMICS

UR NAG, UR OPN, UR RBP4, UR CST3, UR CLU, UR AMBP and UR KIM-1

$$CM_{it}^{EQ} = \exp \sum_{j=1}^{7} \frac{1}{7} \log(FC_{ijt})$$

w_j: the weight for the biomarker j = 1
FC_{ijt}:fold change between the controls and treated subjects i for biomarker j at time t

DRUG-INDUCED KIDNEY INJURY –TARGETED PROTEOMICS



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janssen PHARMACEUTICAL COMPANIES OF Johnson&Johnson

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SUMMARY

- Tobramycin nephrotoxicity was characterized by increases in Ur NAG and Ur GGT and microscopic kidney proximal tubular injury in male beagle dogs (J&J Group)
- 86 proteins significantly changed (q-value < 0,05 & FC> 2) in kidneys from treated dogs, as observed by global proteomic approach (non-targeted)
- Significant increases in urinary biomarkers CLUS, OPN, AMBP, RBP4 and KIM-1 were noted in tobramycin-treated dogs vs controls, as measured by targeted proteomics
- KIM1 does perform poorly compared to rats and humans
- DIKI Marker currently tested in combination with 2D invitro chips (Nortisbio)

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DIKI BIOMARKER

Origin	Biomarker	human	canine	rat
			Validation status	
Tubular	KIM1	ongoing	Full	Full
Tubular	AMPB	ongoing	Full	Full
Tubular	OPN	ongoing	-	Full
Tubular	CST3	ongoing	Full	Full
Tubular	CLUS	ongoing	Full	Full
Tubular	RBP4	ongoing	Full	-

Partial validation: accuracy & precision (in six replicates N=3), dynamic range, parallelism, linearity, selectivity and short-term sample stability.

Full validation experiments comprise additionally: accuracy & precision (in six replicates N=6), long-term sample stability, interference.

In Collaboration with PSTC CRITICAL PATH INSTITUTE

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DRUG-INDUCED VASCULAR INJURY

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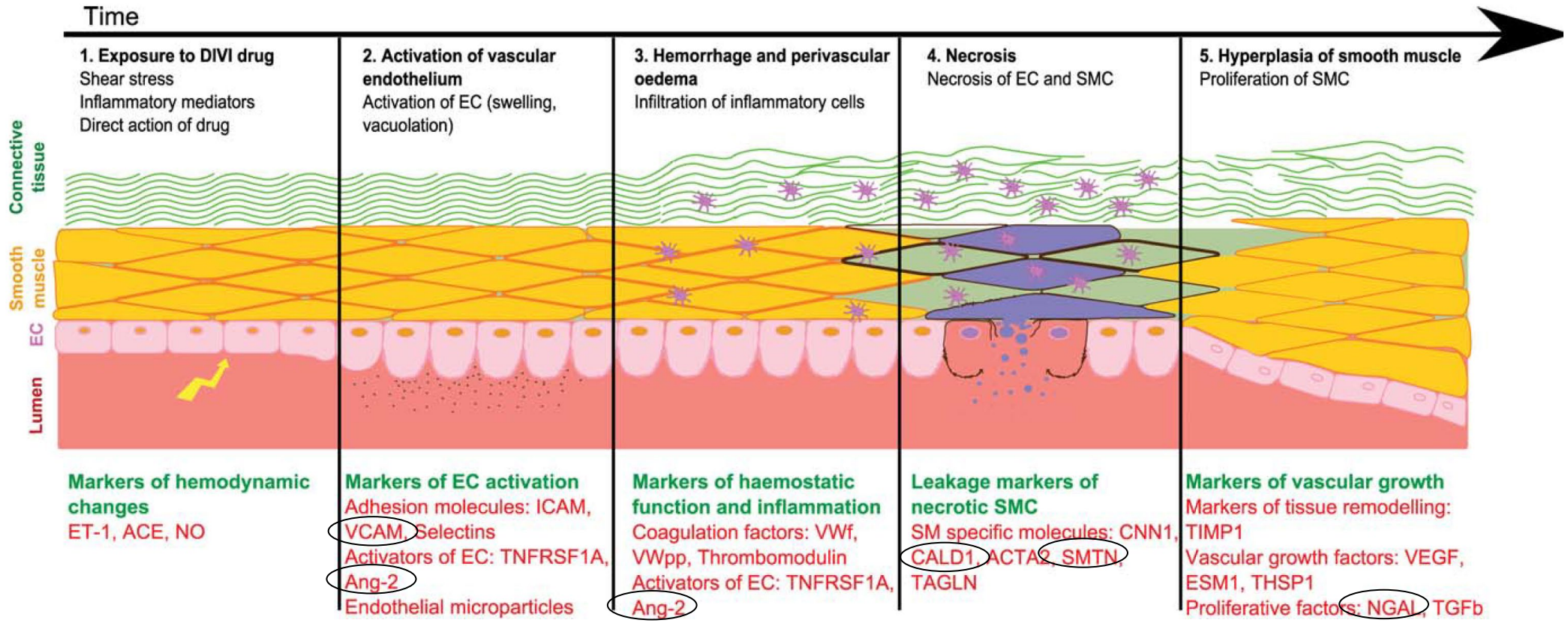
POTENTIAL DIVI BIOMARKER



Bendjama, K., *et al.*(2014) Translation strategy for the qualification of drug-induced vascular injury biomarkers. *Toxicol Pathol* **42**, 658-671

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DISEASE MODEL FOR DIVI – GRANULOMATOSIS WITH POLYANGIITIS

- Center for Interdisciplinary Clinical Immunology, Rheumatology and Autoimmune Diseases (IDNIRA), Tübingen
- 41 samples from patients with ANCA associated vasculitis that affects small- and medium-size vessels
- 18 HV samples HV samples

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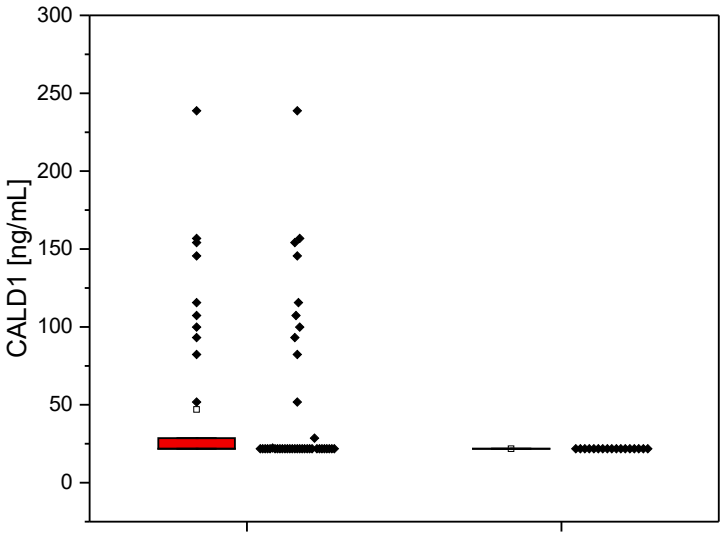
Universitätsklinikum Tübingen

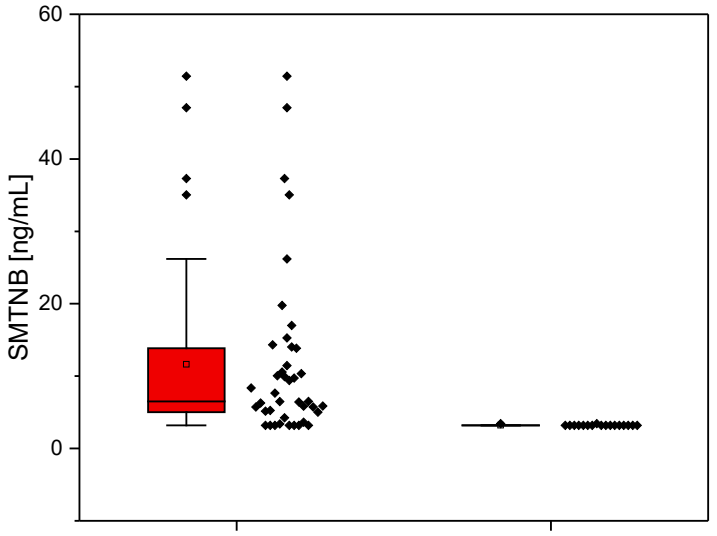
POTENTIAL DIVI BIOMARKER – SMOOTH MUSCLE PROTEINS

CALD1



P = 4.43E-03
LLOQ = 43.7 ng mL^{-1}

SMTNB



P = 4.20E-05
LLOQ = 6.4 ng mL^{-1}

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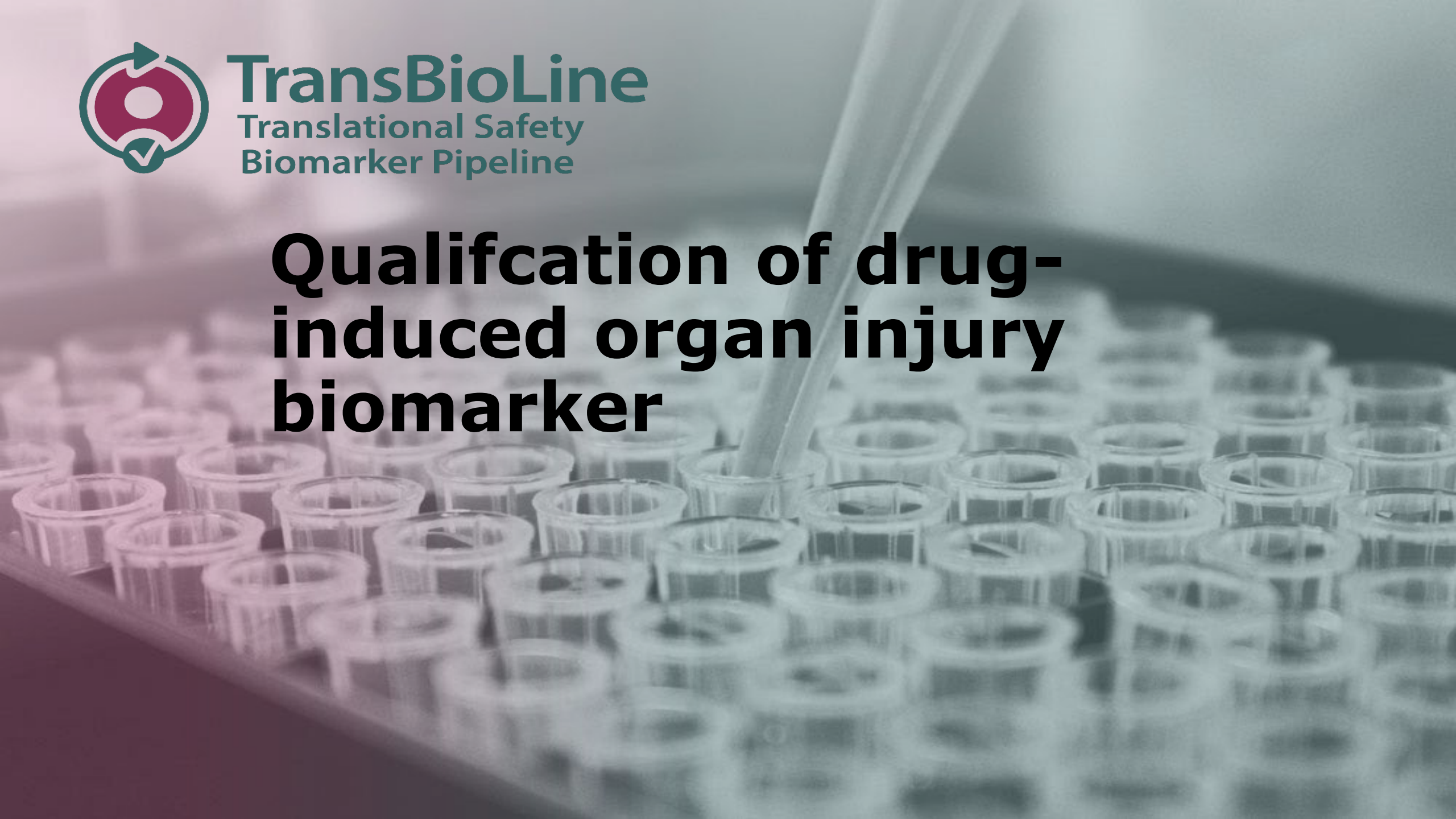
SUMMARY

- First SMTNB – specific protein assay
- 6 plex human assay developed and validated according FDA guideline
- Results of DIVI Biomarkers in human GPA-samples promising
- Analysis of other models in TransBioLine ongoing
- 6 plex rat assay developed for exploratory purposes

TransBioLine
Translational Safety
Biomarker Pipeline

Qualifcation of drug-induced organ injury biomarker



Objectives

- **Develop and validate assays for new safety biomarkers, suitable for application in drug development and clinical practice.**

- **Regulatory qualification of new safety biomarkers**
  - Generate exploratory and confirmatory data enabling regulatory qualification of new safety biomarkers in defined Contexts of Use (CoU) for application in drug development

- **Facilitate application of the new markers in clinical practice**
  - Establish robust biomarker datasets across key patient populations to enhance diagnosis and prognosis of disease

- **Establish liquid biopsies as new diagnostic tool**
  - Implement profiles of circulating microRNAs as tissue- and mechanism- specific diagnostic tools, supported by suitable bioinformatics systems, to facilitate in-depth mechanistic understanding of drug side- effects and disease.

- **Facilitate sustainability of consortium efforts, as well as of predecessor consortium IMI SAFE-T's achievements**
  - Establish and maintain a European expert and knowledge network for biomarker qualification, allowing for continuous feed-in and qualification of new biomarker candidates, as well as access to comprehensive safety biomarker data.
  - Utilize subsets of samples at SAFE-T's biobank, build on data maintained on the consortium's tranSMART database.

Consortium Overview

TransBioLine

efpia

innovative medicines initiative

Major Focus: Development of exploratory and confirmatory datasets for regulatory biomarker qualification

Project Leader: Shashi Ramaiah, Pfizer
Project Coordinator: Sophia Samodelov, UZH

Funding period: 01-Feb-2019 to 31-Jan-2024
Total partners: 29
EFPIA companies: 7
Work packages: 11
Years: 5 (2019-24)
Total Budget: € 28 Million
IMI contribution: € 14 Million (in cash)
EFPIA contribution: € 14 Million (in kind)

Biomarker Pipeline

New Biomarker	Source of BM Literature and/ or Discovery Phase	Learning Phase (Exploratory Phase)	Confirmatory Phase
WP1 DIKI, 11 proteins*§	Discovery & Literature analysis		Clinical studies
WP2 DILI, 6 proteins*$	Supported by Safe-T data		Clinical studies
WP3 DIPI, 4 proteins#§	Literature analysis		Clinical studies
WP4 DIVI, 15 proteins*§	Supported by PSTC & Safe-T data		Clinical studies
WP5 DINI, 5 proteins*§	Literature analysis		Clinical studies

*** Accepted to FDA´s Biomarker qualification progam**
§ QA received from EMA
 # applied to FDA´s Biomarker qualification progam
$ EMA Request for Qualification Advice

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TEAM & COLABORATORS & FUNDING

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Oliver Pötz
Hannes Planatscher
Helen Hammer
Wael Naboulsi
Cornelia Sommersdorf
Andreas Steinhilber
Viktoria Anselm
Tobias Meisinger
Anja Tausch
Hanna Haag
Sabrina Fenchel
Sonja Burger

NMI schafft Ergebnisse

Oliver Pötz
Thomas Joos

Universitätsklinikum Tübingen

Bundesministerium für Bildung und Forschung
FKZ: 031B0395

GO-Bio

NMI SPIN-OFF

TransBioLine

High-Tech Gründerfonds

imi innovative medicines initiative

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