

How to handle human variability in risk assessment:

*A high throughput transcriptomics approach to
uncover toxicity pathway activation variability.*

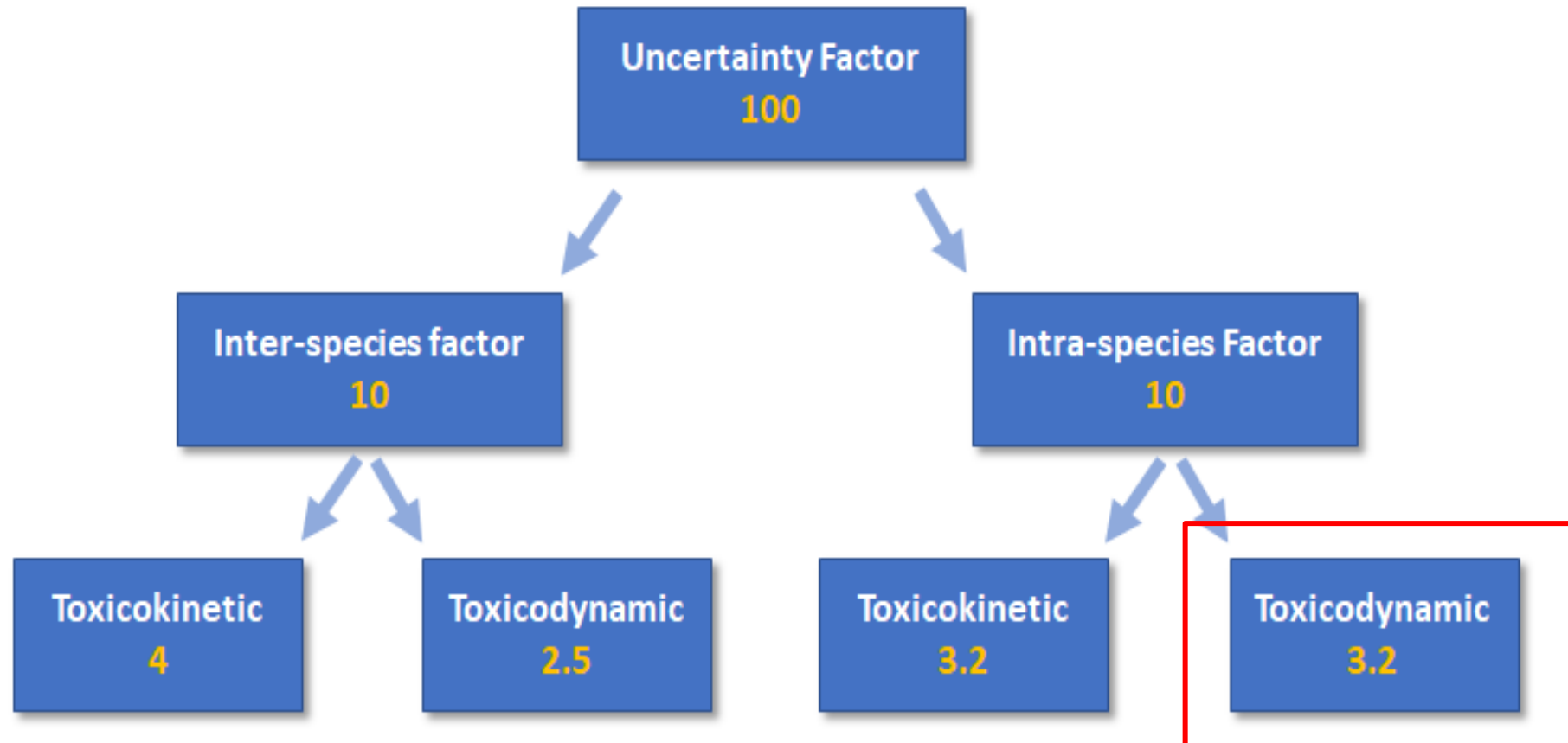
Bob van de Water



Universiteit
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LACDR
Leiden Academic Centre
for Drug Research

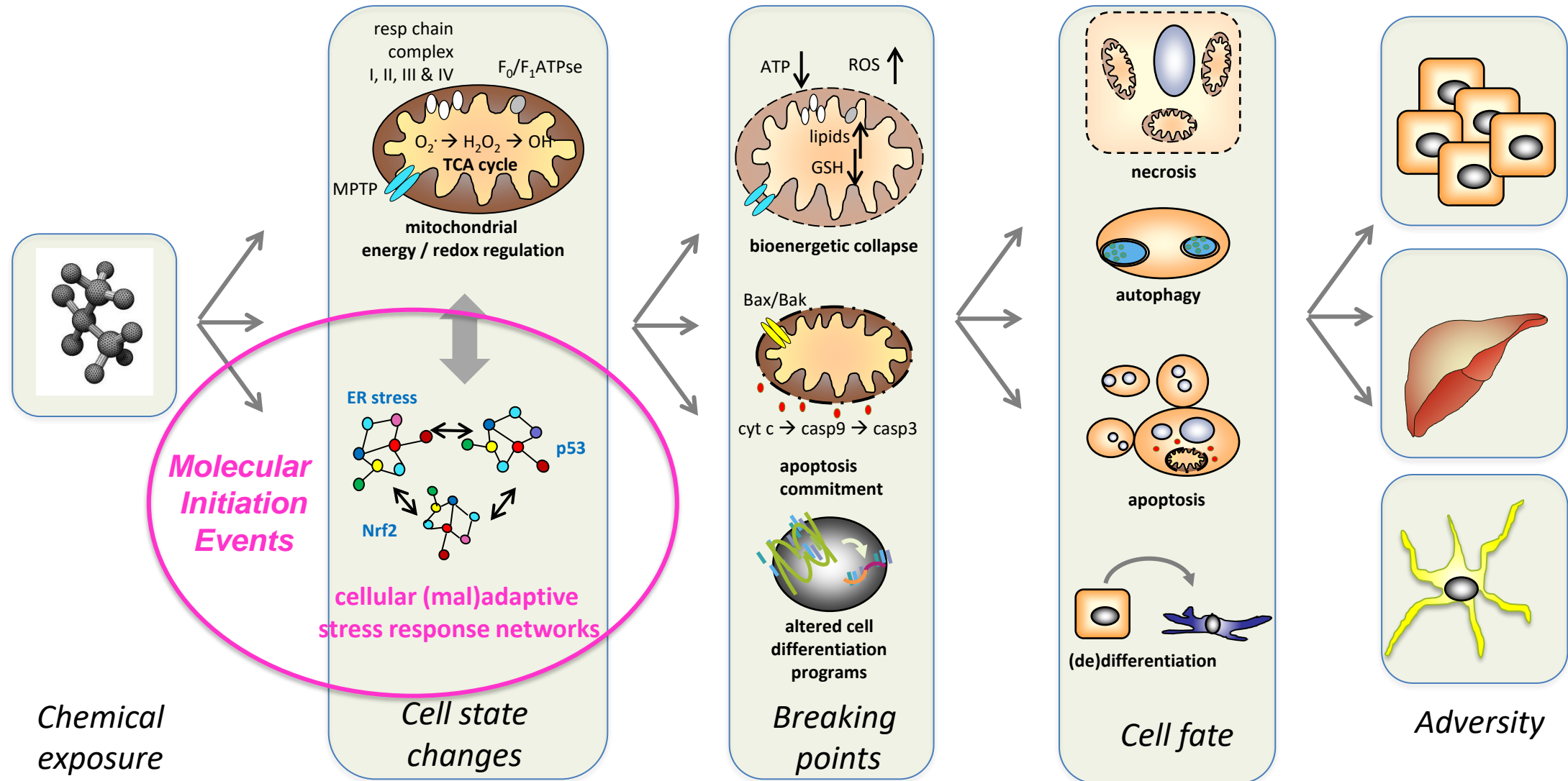
Current regulatory safety factors: Toxicokinetics & Toxicodynamics



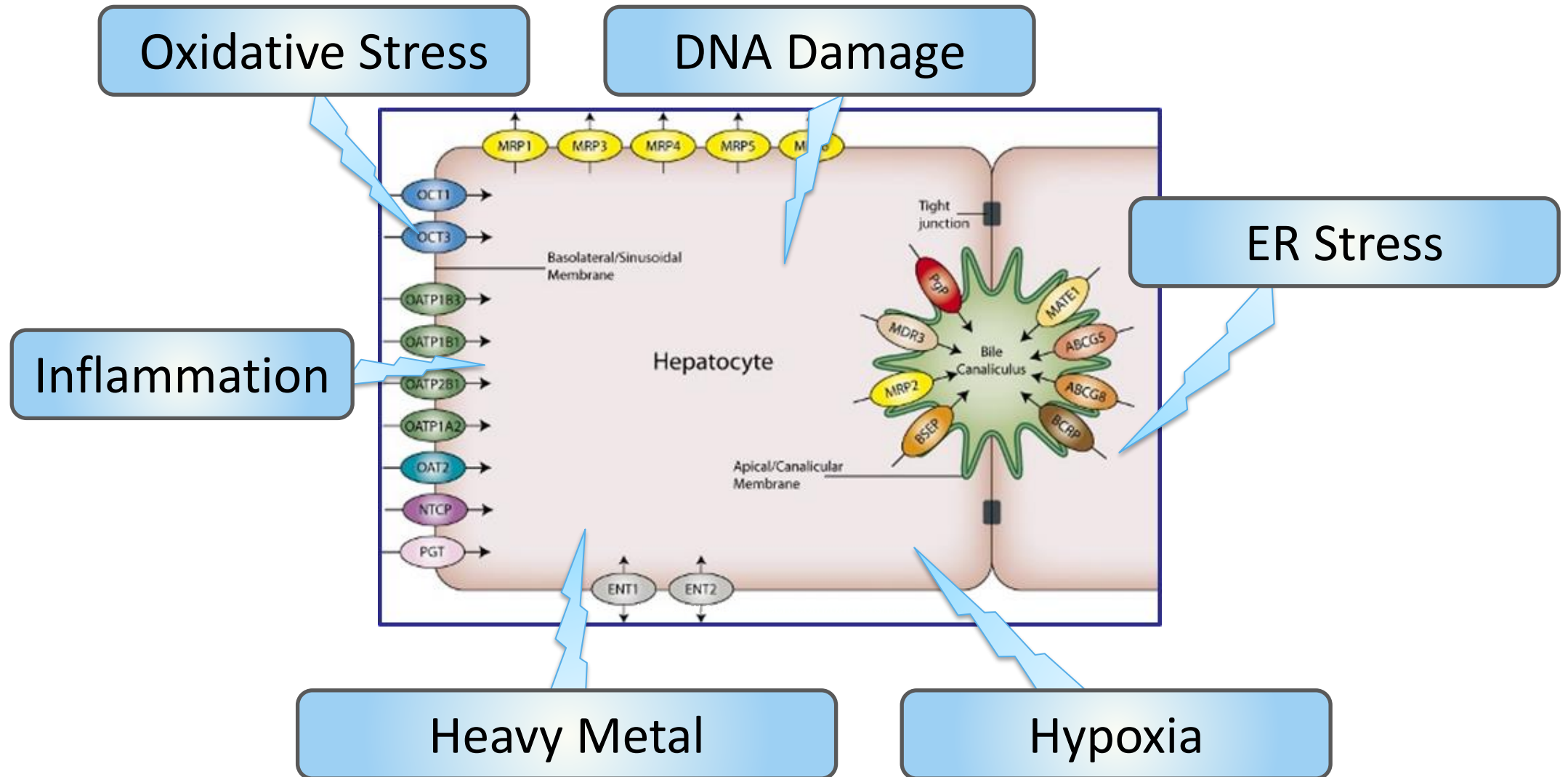
Interindividual variability in apical endpoints.

- individual high susceptibility to adverse drug reactions, e.g. drug-induced liver injury related to idiosyncratic drug toxicities, based on genetic polymorphisms (GWAS)
(Osanlou et al., 2018)
- iPSC-derived cardiomyocytes from up to 43 donors established the variability in cardiotoxic responses
(Blanchette et al., 2020; Burnett et al., 2021)
- a panel of lymphoblastic cell lines established from >1,086 donors have been applied to determine the cytotoxicity of more than 179 chemicals
(Abdo et al., 2015).

From chemical exposure to adversity: Key Events and AOP frameworks and cellular stress response.

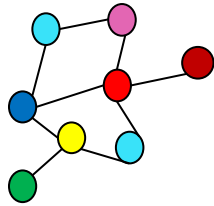


Cellular stress responses and molecular mechanisms of drug-induced liver injury (DILI)

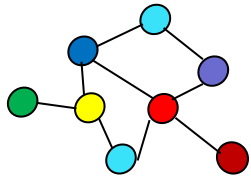


Pathways of toxicity: from adaptation to adversity

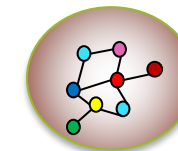
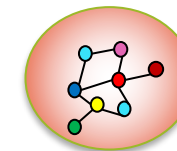
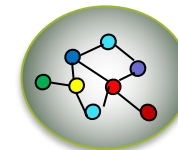
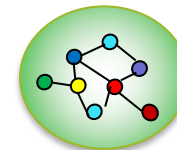
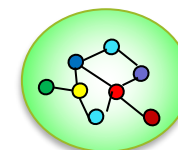
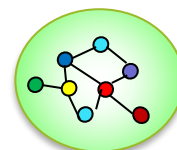
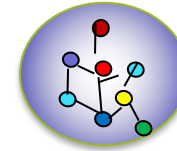
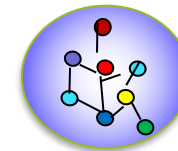
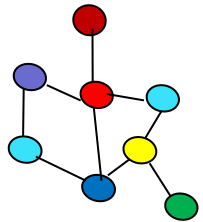
Unfolded protein response



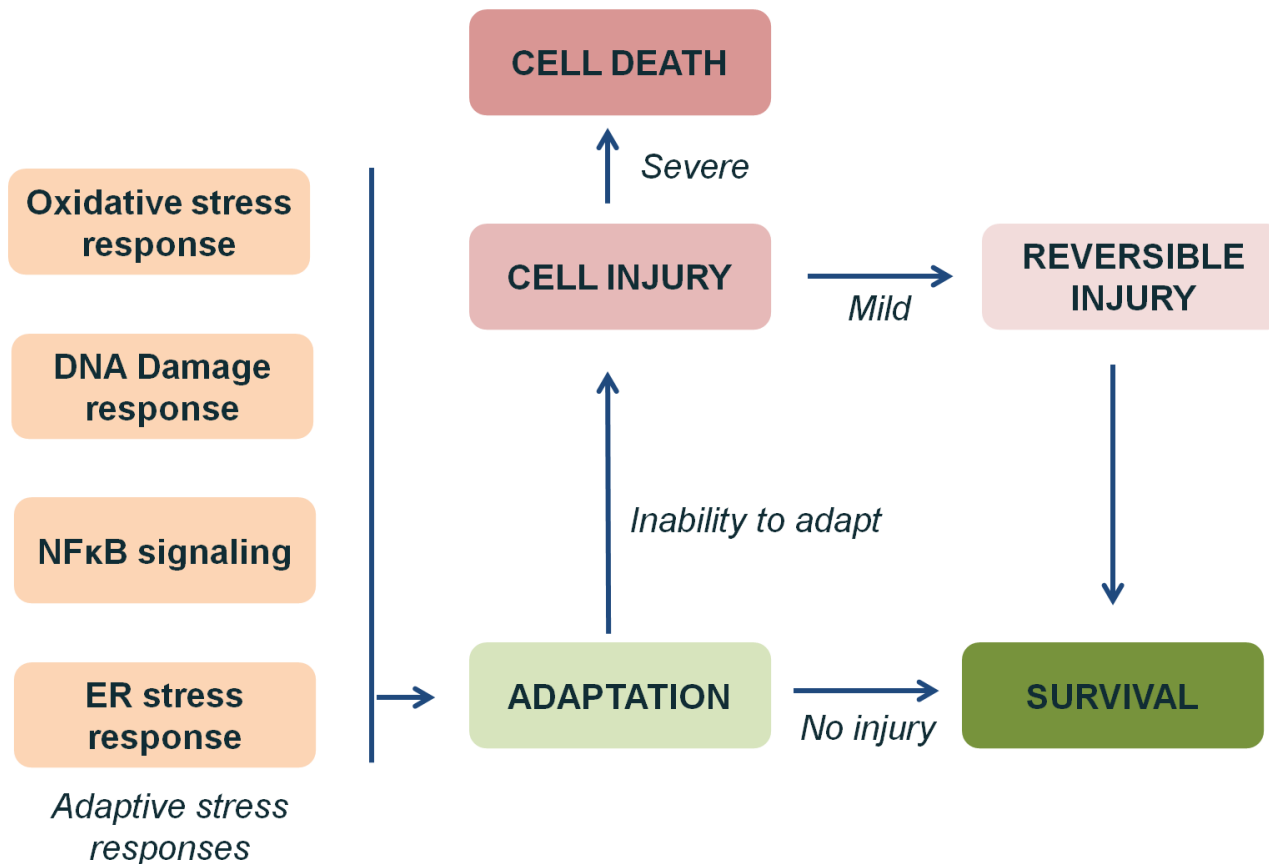
Oxidative stress



Etc.

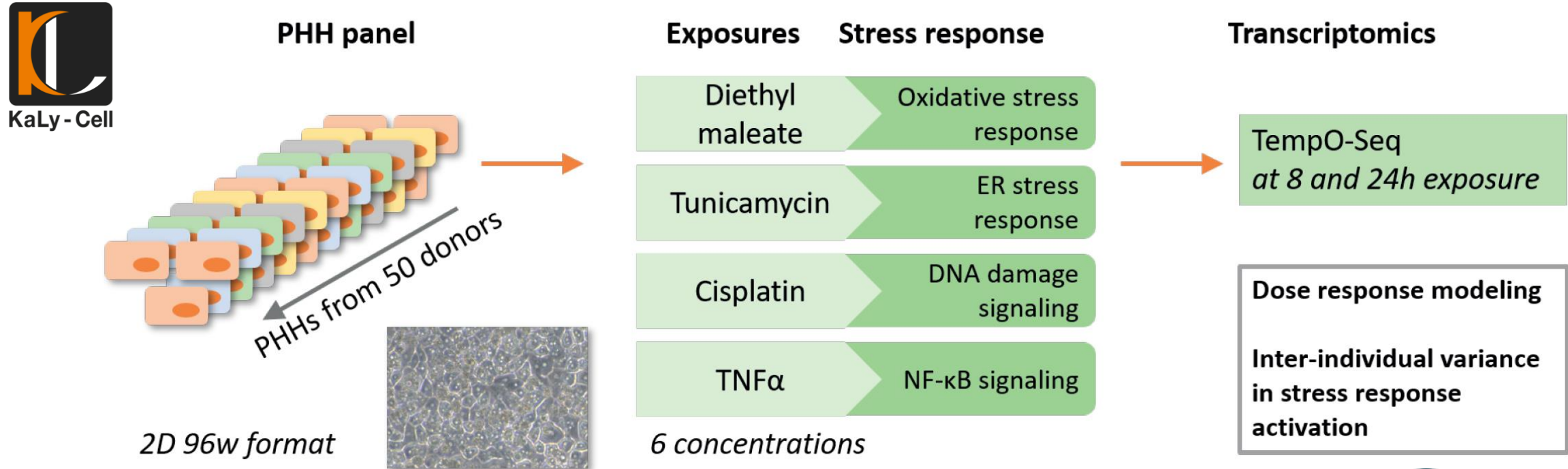


Human population variability in adaptive stress response activation?



- What is the population variability of cellular stress response pathway activation?
- Are sensitive individuals equally sensitive for different stress pathways?
- Is the variability reflecting disease status of individuals?
- Can we model the cellular stress response variability?

Variability in adaptive stress response activation in human primary hepatocytes: study design.

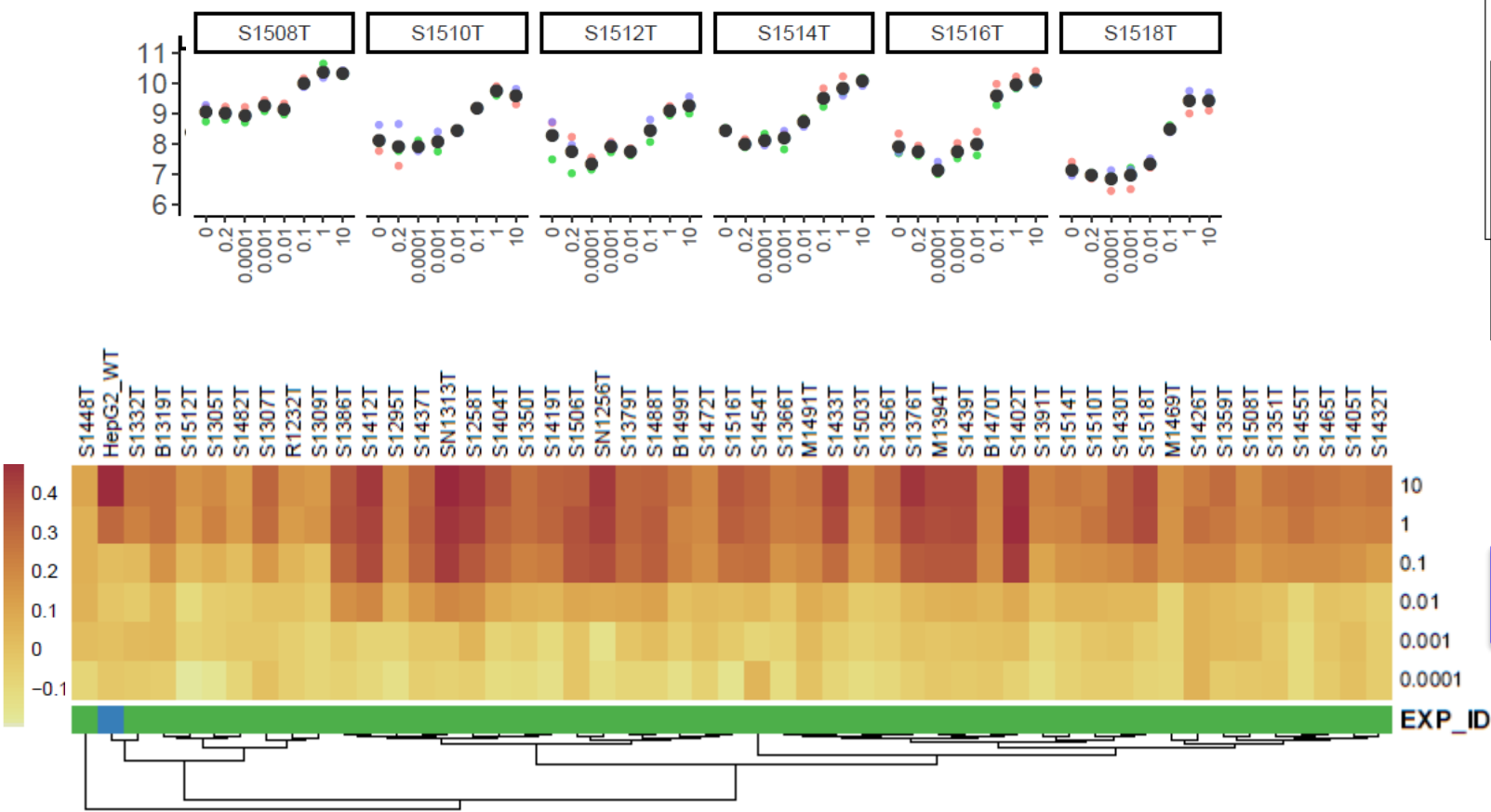


- Targeted TempO-Seq using S1500+ gene panel (~3,300+ genes)
- ~8,000 samples
- BMDExpress 2.0

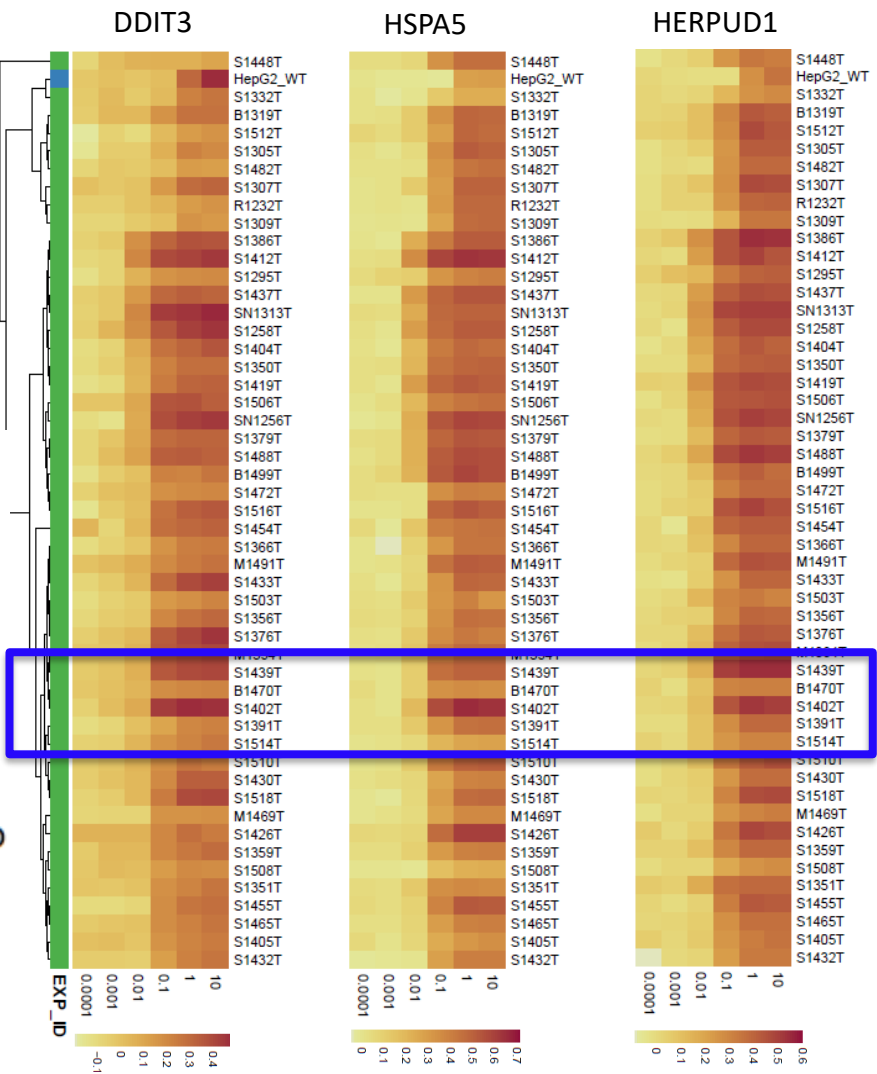


Different PHH donors have specific offset of UPR activation

CHOP/DDIT3



ER stress response - Tunicamycin



Human population variability in cellular stress response activation: *BMD-Express-based BMC for individual genes.*

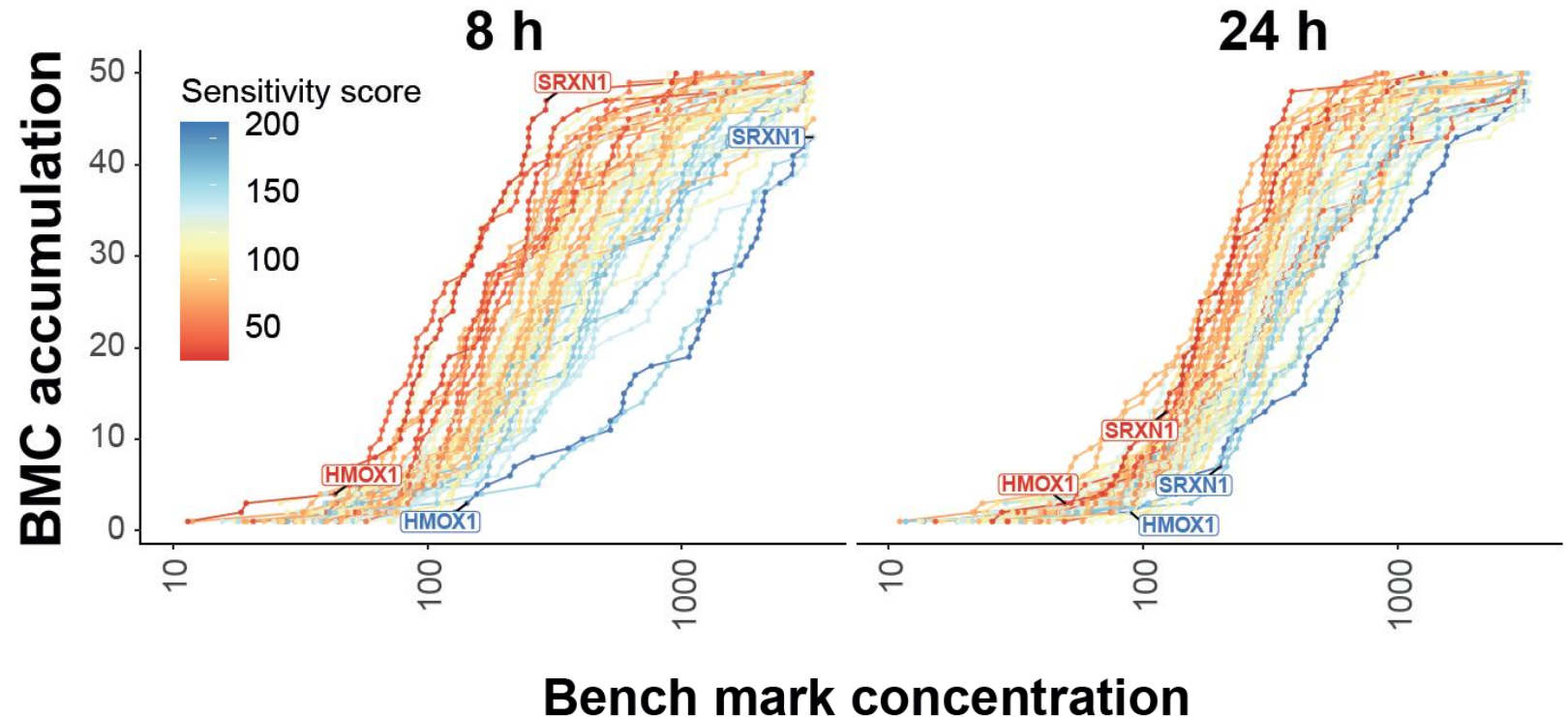
Diethylmaleate – Oxidative stress response

BMC determination of:

- Each gene
- Each compound
- All 50 PHHs

Distribution of BMCs of OSR-related genes defined as:

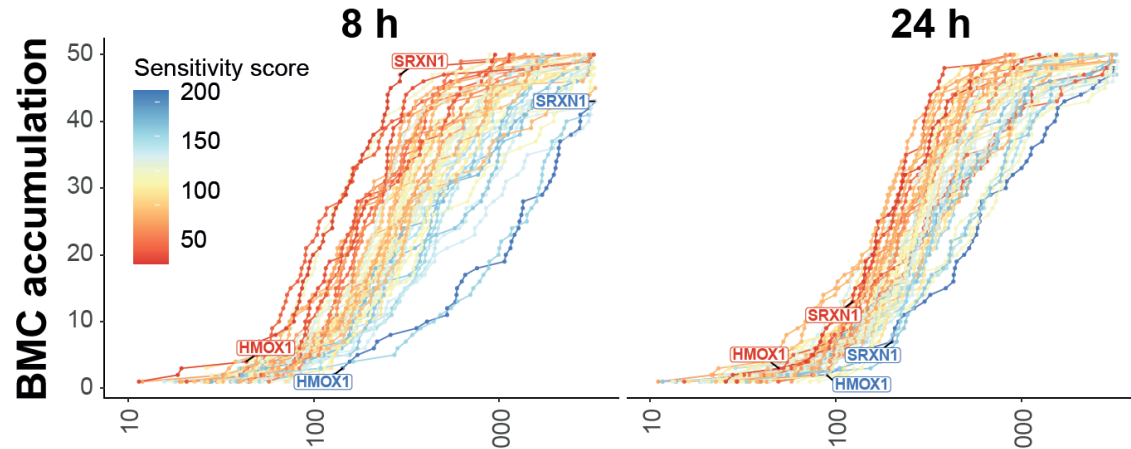
- Top 50 upregulated genes



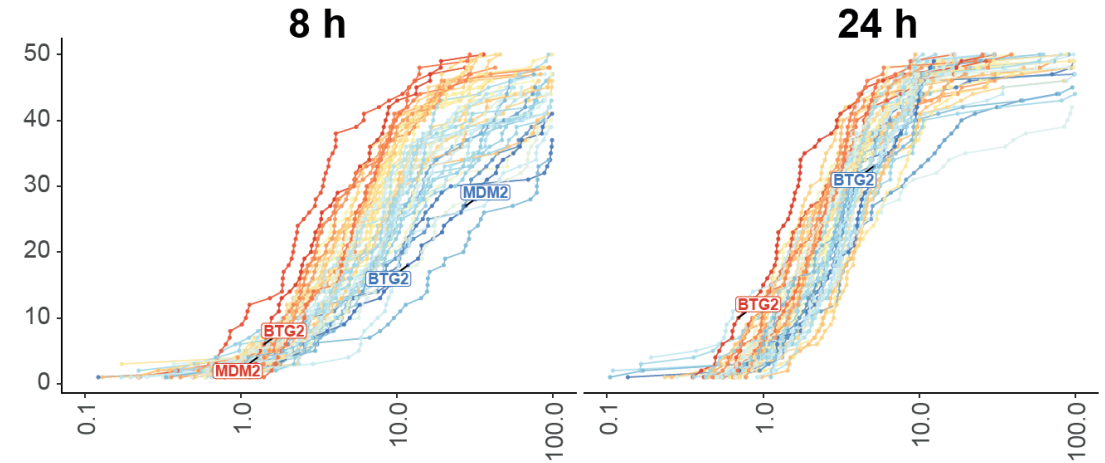
BMD
Express2.0

Human population variability in cellular stress response activation: *BMD-Express-based BMC for individual genes.*

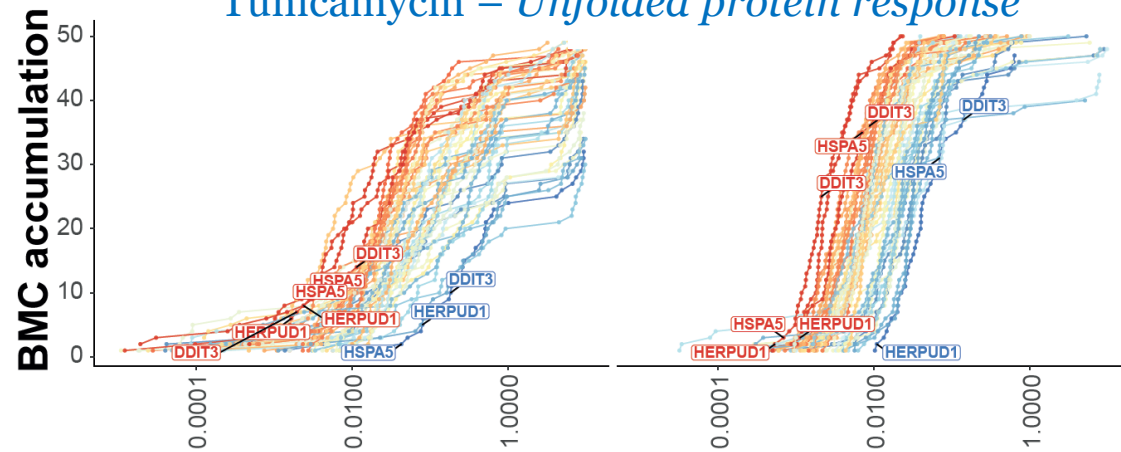
Diethylmaleate – Oxidative stress response



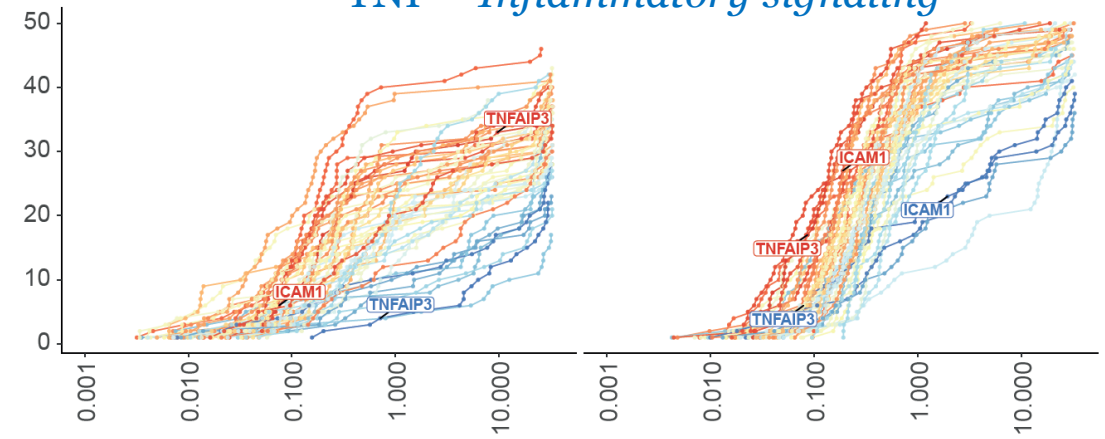
Cisplatin – DNA damage response



Tunicamycin – Unfolded protein response



TNF – Inflammatory signaling

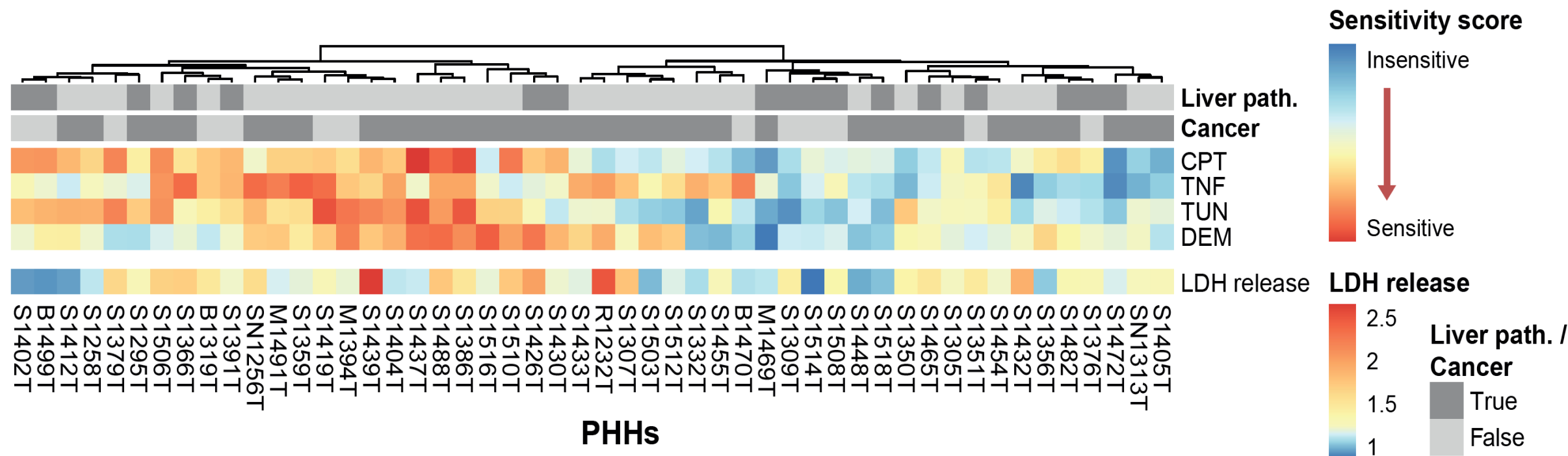


Bench mark concentration

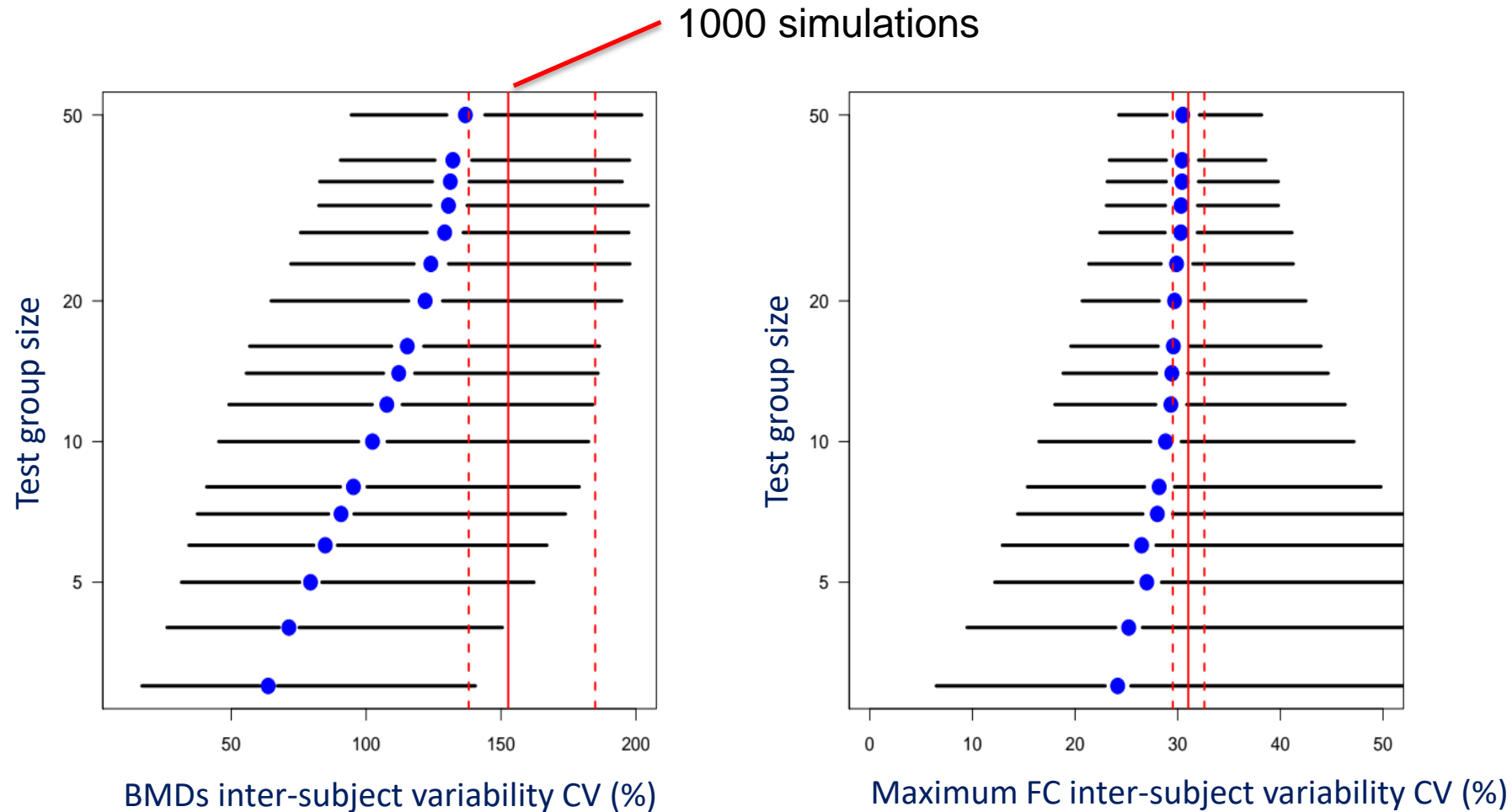
Bench mark concentration

Niemeijer et al. 2023 EHP in press

Different PHH donors have specific offset of cellular stress responses.



Simulated distributions of BMD and FC inter-subject variability of UPR activation by tunicamycin



Niemeijer et al. 2023 EHP in press

Human population Toxico-Dynamic Variability Factor (TDVF) for cellular stress response activation in 50 PHH

		Median BMC ± SD (min-max)	FC min-max Median BMC	TDVF _{0.01} Exp data	TDVF _{0.01} Model*	SD TDVF _{0.01} Model*
8 h	TUN	0.534 ± 1.320 (0.009 - 7.768)	864.1	6.545	6.317	0.582
	DEM	352.486 ± 243.643 (111.726 - 1405.220)	12.6	2.517	1.828	0.066
	CPT	9.488 ± 5.867 (2.640-35.447)	13.4	2.786	3.241	0.202
	TNF	12.447 ± 13.310 (0.157 - 40.633)	258.8	26.524	5.315	0.423
24 h	TUN	0.023 ± 0.061 (0.002 - 0.447)	223.5	4.950	4.811	0.386
	DEM	315.597 ± 108.883 (176.656 - 623.096)	3.5	1.701	1.624	0.047
	CPT	3.083 ± 1.022 (1.471 - 6.401)	4.4	1.847	2.184	0.090
	TNF	0.926 ± 2.252 (0.137 - 15.263)	111.4	2.516	2.556	0.128

Niemeijer et al. 2023 EHP in press

Conclusions & Discussion

- Interindividual variability on toxicodynamics toxicity pathway dependent
 - Variability can be greater than TDVF of 3
-
- Variability in PHH similar for different target organs?
 - Variability due to procedure of PHH isolation and cryopreservation?
 - How about freshly isolated cells?
 - Further underpinning of TD safety factors for regulatory implementation

EFSA: TD-TRAQ project



Characterisation of human variability in toxicodynamics: towards the development of quantitative Adverse Outcome Pathways (qAOPs)

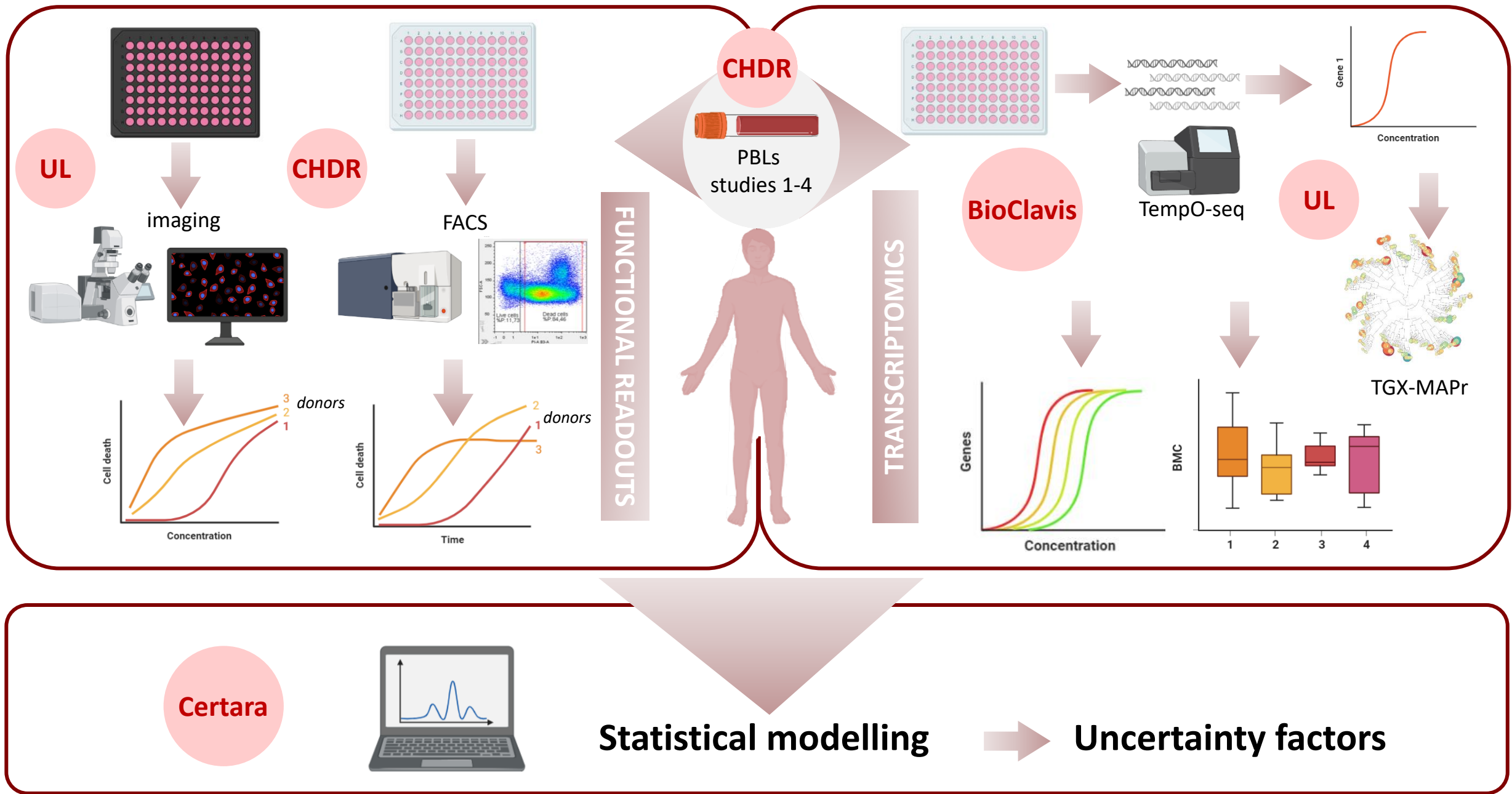
ToxicoDynamic analysis based on high-throughput TRanscriptomics for AOP-driven human population variance Quantification

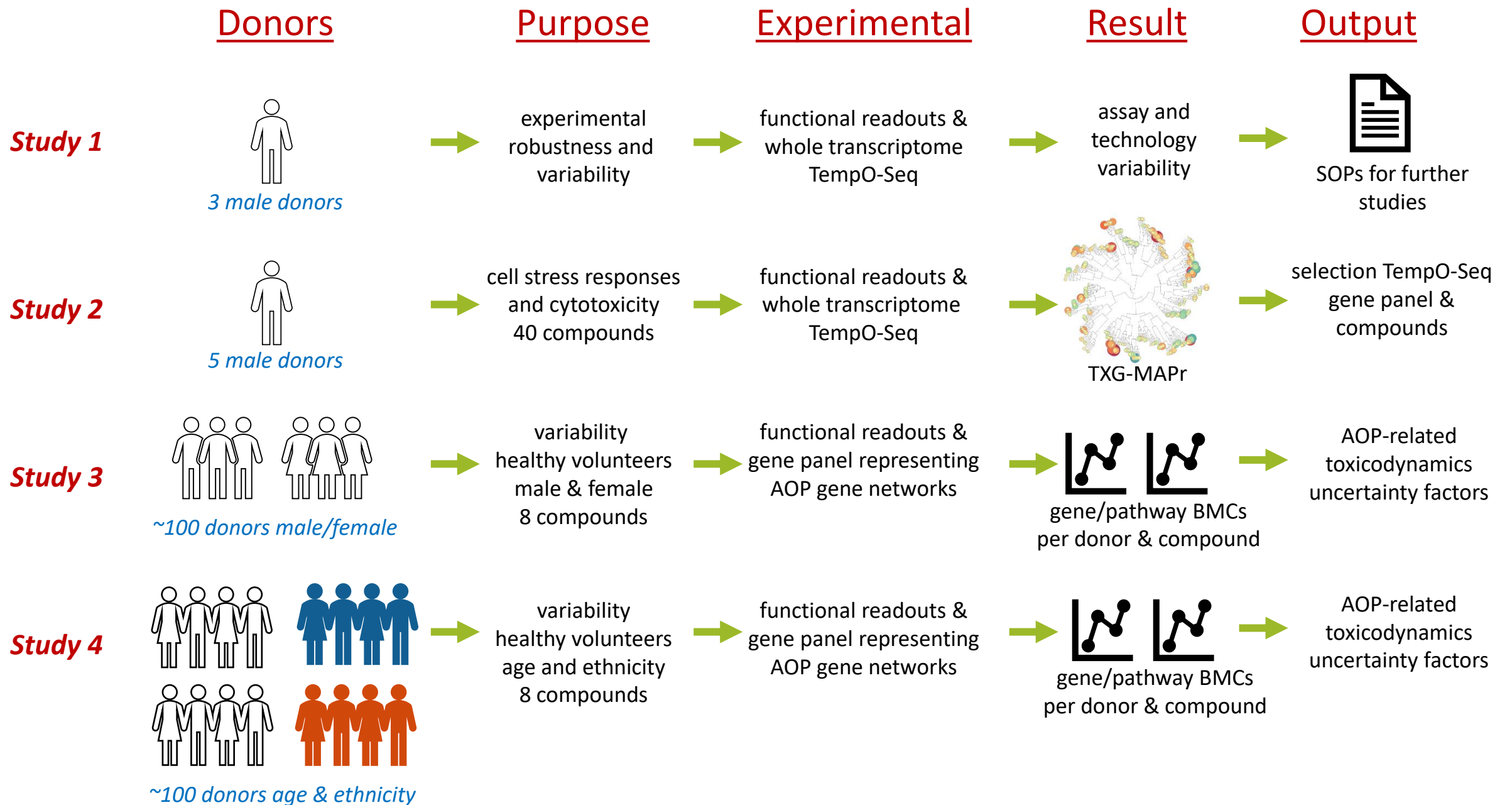
Project funding:

1.6 million Euro – 4 years

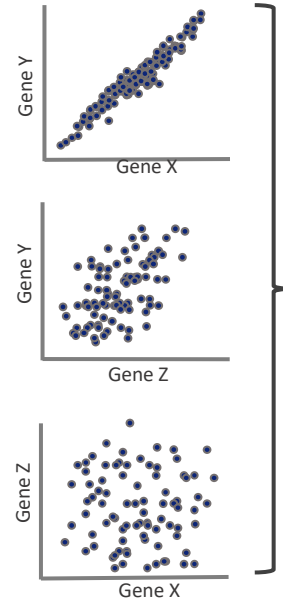
Partners:

Leiden University (coordination), Centre for Human Drug Research, Certara, Danish Technical University, BioClavis



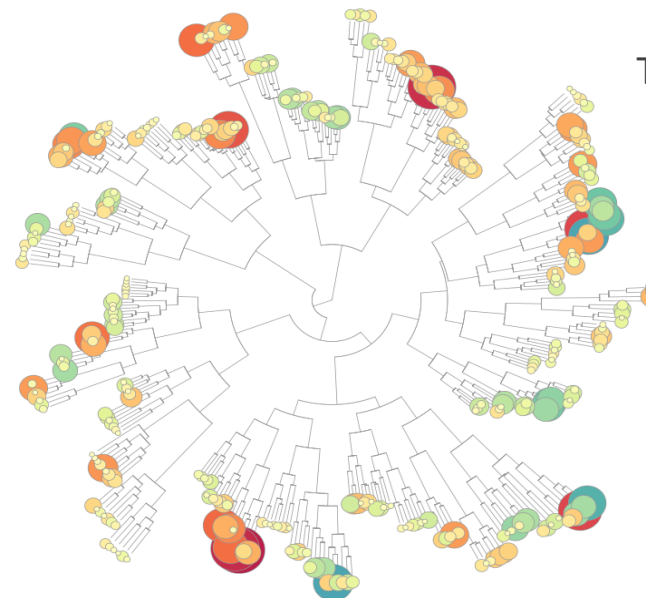


1. ~40 compounds and conc response
WT TempO-Seq transcriptomics (study 2)



Gene co-expression analysis

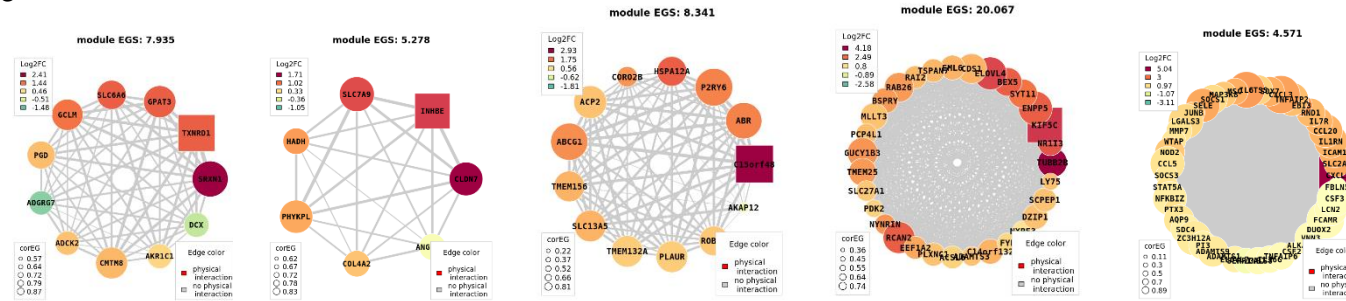
2. Establish a
PBMC TXG-MAPr
gene
co-expression
networks



Toxicogenomics map
(TXG-MAPr)

Study 2

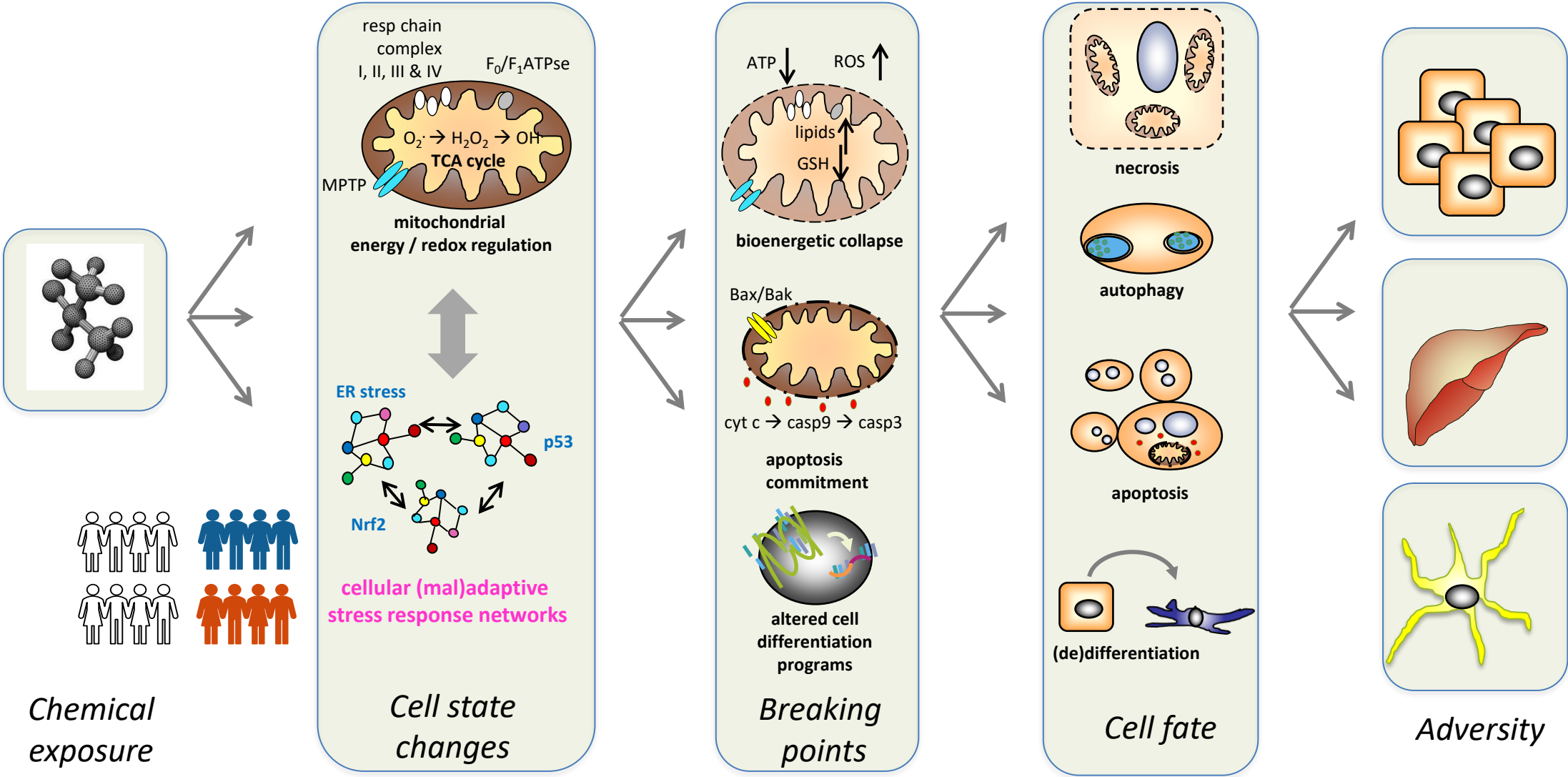
Selection of stressor
specific gene networks



3. Define PBMC modules representing cellular stress response pathways relevant to cytotoxic responses and AOP key events.

4. Establish targeted TempO-Seq panel for high throughput screening of population variability for the project

Towards understanding the interindividual variability of toxicity pathways



Acknowledgement



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Matthijs Moerland (CHDR)

Frederic Bois (Certara)

Susanne Hougaard (DTU)

Joel McComb (BioClavis)

Bob van de Water



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HUNT3R**



[:::] EUTOXRISK



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