Qualitative and quantitative interpretation of omics data for chemical safety assessment

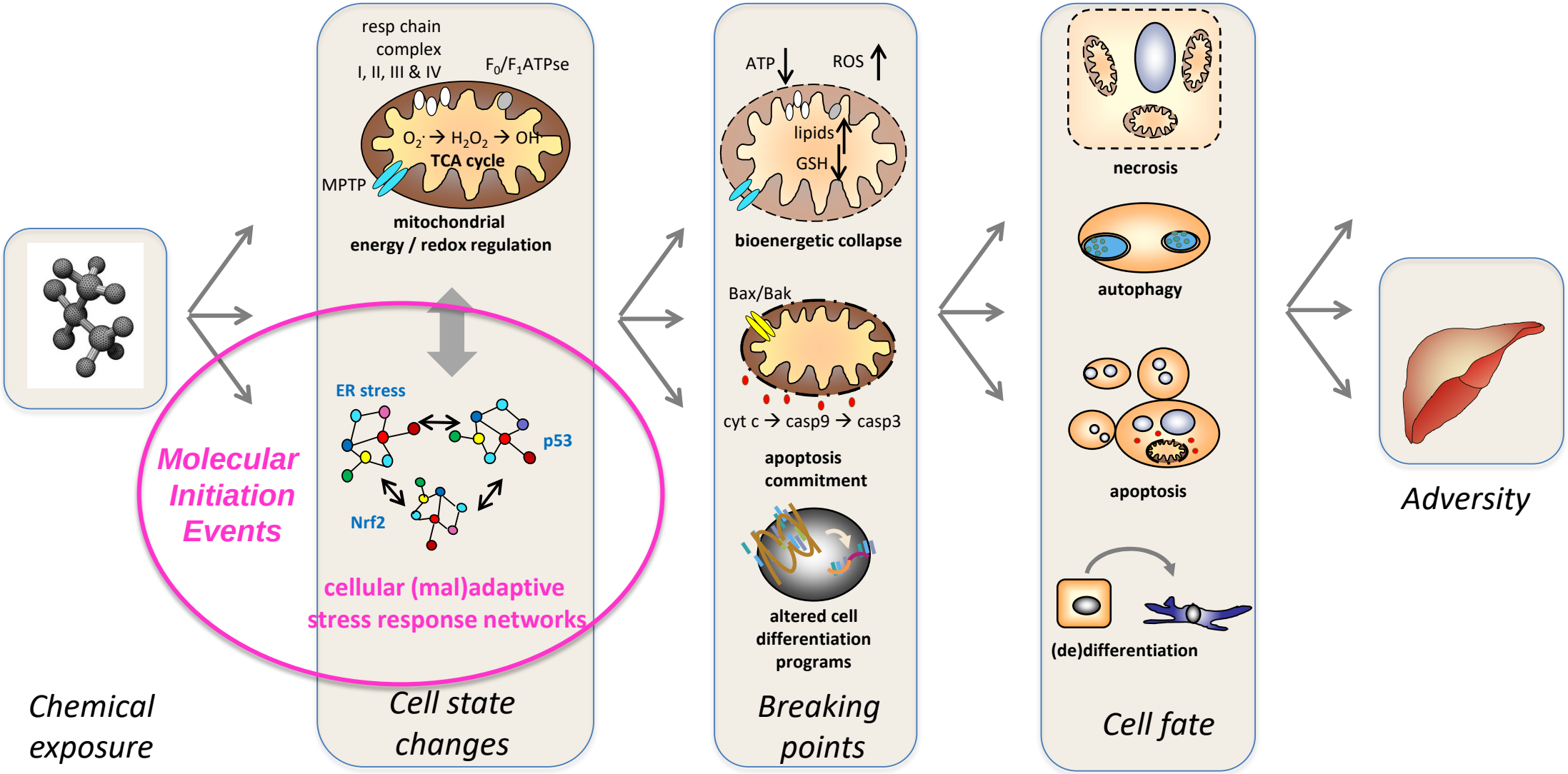
TXG-MAPr toolbox development in Horizon2020 RISK-HUNT3R and EFSA TXG-MAP projects

Bob van de Water (Leiden University, NL)

Universiteit Leiden

LACDR

From chemical exposure to adversity: Key Events and AOP frameworks and transcriptional changes.



Wink et al. Chem Res Toxicol. 2014

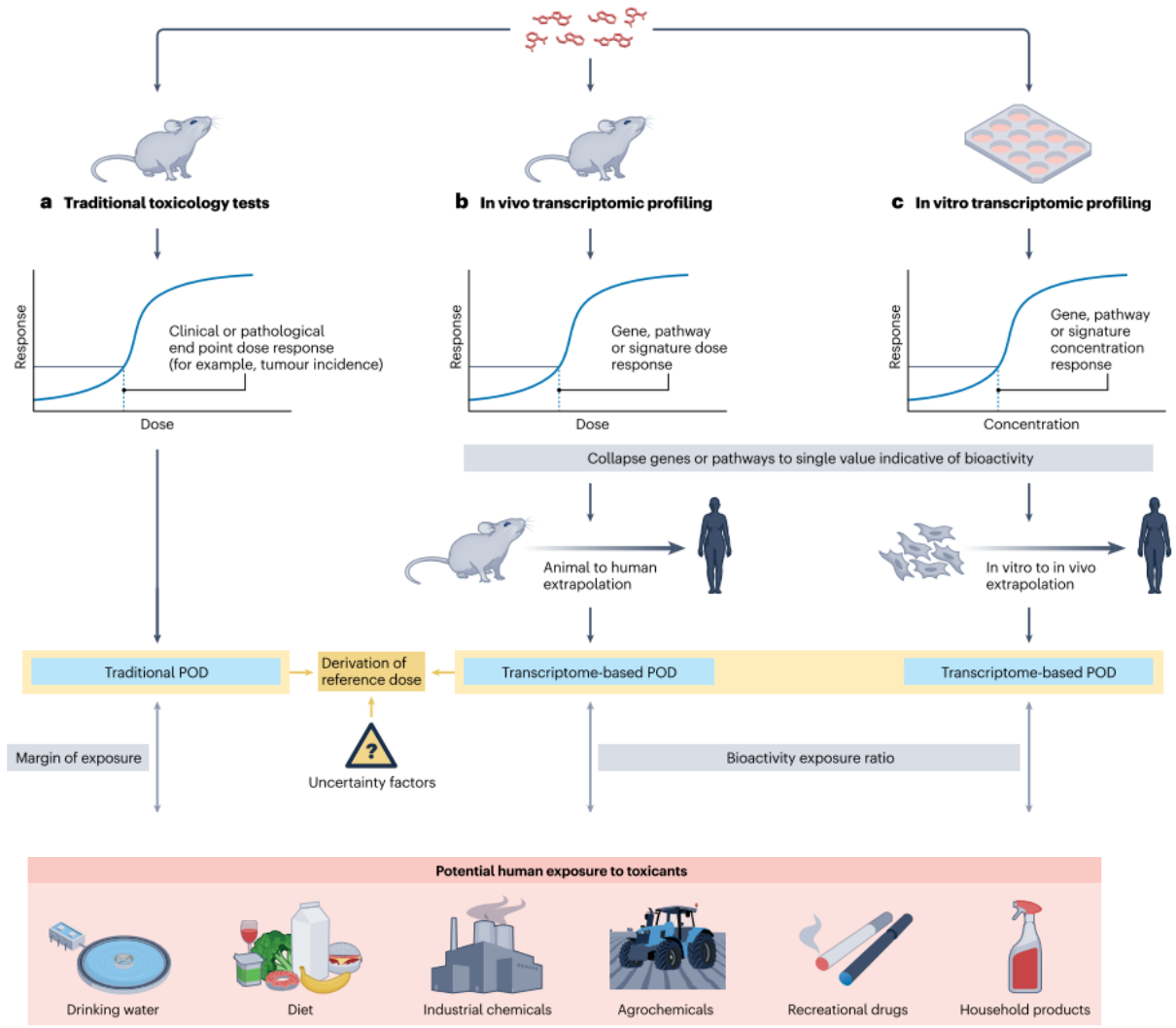
Toxicogenomics for drug and chemical safety assessment

- **omics technology** to measure molecular changes **in vitro and in vivo**
- mechanistic and quantitative information at scale

- Hazard identification
- Dose-response assessment

Why is this important?

urgent need to evaluate thousands of chemicals, while addressing societal pressures to adopt more humane testing practices



Meier et al. (2024)

Systematic reporting of toxicogenomics data

Regulatory Toxicology and Pharmacology 125 (2021) 105020

Contents lists available at ScienceDirect

Regulatory Toxicology and Pharmacology

journal homepage: www.elsevier.com/locate/yrtph

Commentary

Progress towards an OECD reporting framework for transcriptomics and metabolomics in regulatory toxicology

Joshua A. Harrill [a,1,*], Mark R. Viant [b,c,**,1], Carole L. Yauk [d,1,***], Magdalini Sachana [e], Timothy W. Gant [f], Scott S. Auerbach [g], Richard D. Beger [h], Mounir Bouhifd [i], Jason O'Brien [j], Lyle Burgoon [k], Florian Caiment [l], Donatella Carpi [m], Tao Chen [h], Brian N. Chorley [a], John Colbourne [b,c], Raffaella Corvi [m], Laurent Debrauwer [n,o], Claire O'Donovan [p], Timothy M

Regulatory Toxicology and Pharmacology 112 (2020) 104621

Contents lists available at ScienceDirect

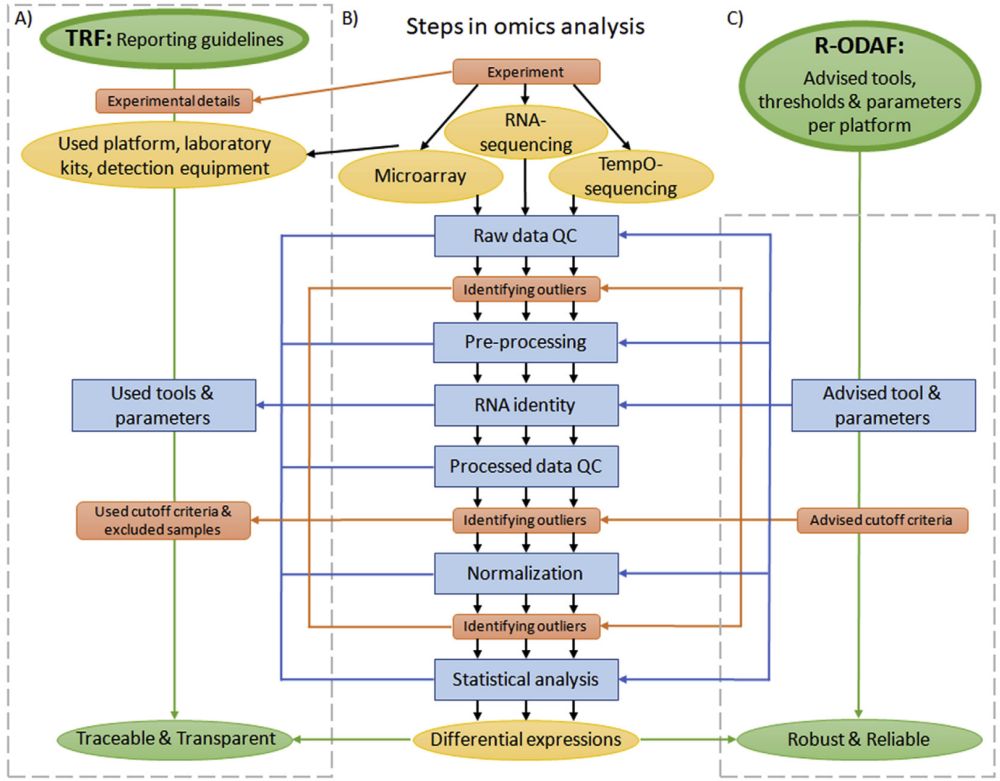
Regulatory Toxicology and Pharmacology

journal homepage: www.elsevier.com/locate/yrtph

Towards the development of an omics data analysis framework

Marcha Verheijen[a], Weida Tong[b], Leming Shi[c], Timothy W. Gant[d], Bruce Seligman[e], Florian Caiment[a,*]

[a] Maastricht University, Maastricht, the Netherlands
[b] National Center for Toxicological Research, U.S. Food and Drug Administration (FDA), Jefferson, AR, USA
[c] Fudan University (FU), Yangpu District, Shanghai, China
[d] Public Health England (PHE), Harwell Science and Innovation Campus, Oxfordshire, UK
[e] Biospyder, Tucson, USA

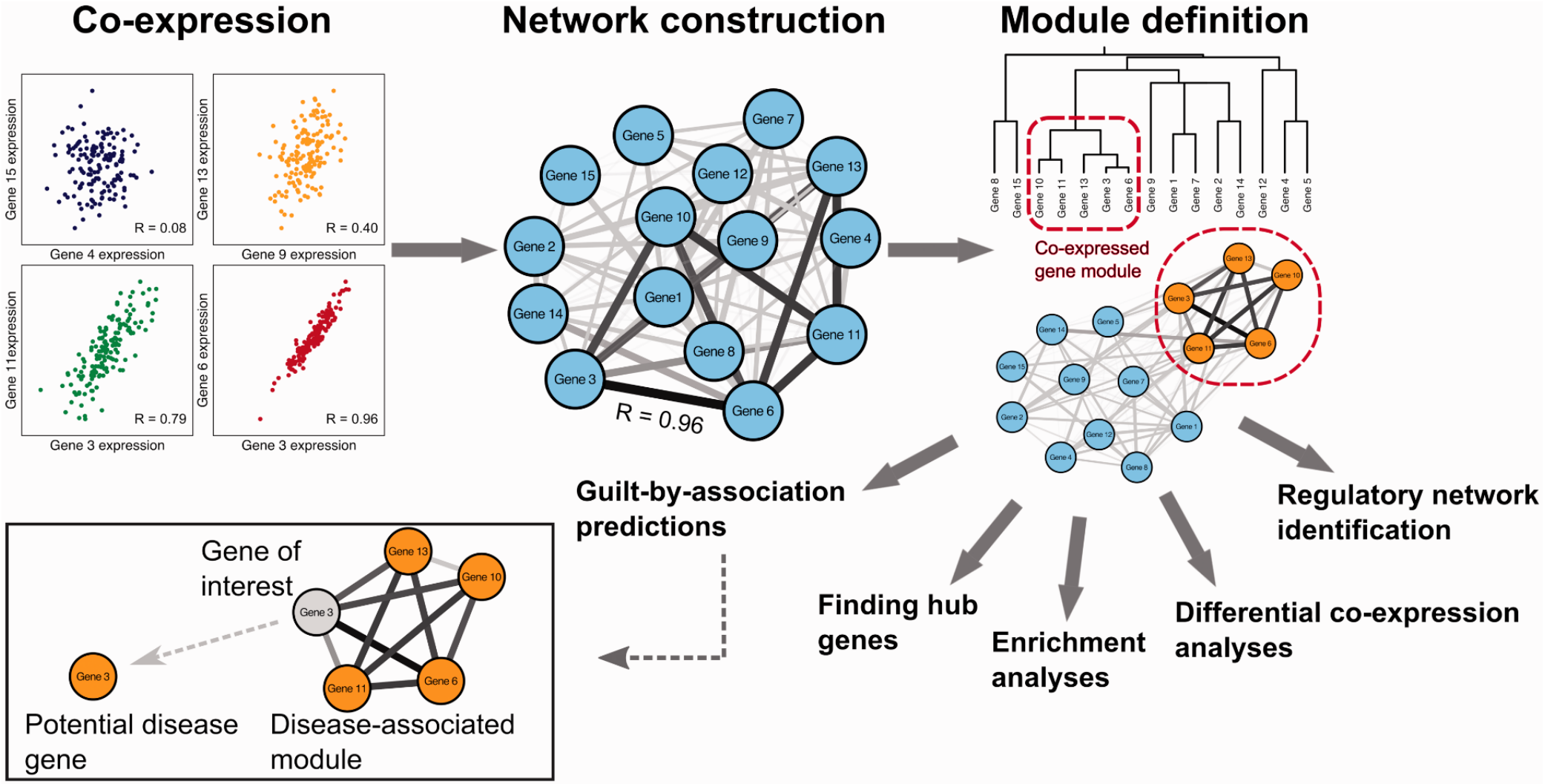


Current gaps:
toxicogenomics interpretation framework for implementation in risk assessment.

Transcriptomics interpretation strategies and regulatory confidence building.

- No broadly accepted **standard bioinformatics procedures** by regulators……
- …..hence, **variable differential expressed genes (DEGs) sets** depending on filtering steps.
- DEGs as input for mechanistic interpretation: **Differences per lab**?
- **Diversity of tools to derive pathways** modulation at p-value level: What does the p-value mean?
- **Redundancy in pathway annotation**: What is right or wrong?
- **Genes that are limited/not annotated** in biology: Association with toxicological programs?
- How to **facilitate the interpretation for the toxicologist/regulator**?
- How to determine relevant **transcriptomics-based BMC and PoDs** for NGRA?

Weighted Gene Correlation Network Analysis (WGCNA) for qualitative and quantitative toxicogenomics interpretation



Grocery stores and data sciences: concept of co-expression.



Gene network A



Gene network B



Gene network C

Glycolysis



Fatty acid metabolism



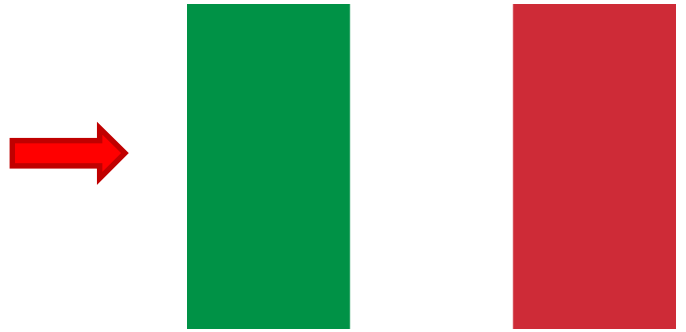
Regeneration



Grocery stores and data sciences: concept of co-expression.

Biological similarity for read across





Glycolysis

Fatty acid metabolism

Dose





Genes X, Y and Z



Genes X, Y and Z



Genes X, Y and Z

Toxicogenomics-MAP (TXG-MAPr) based on WGCNA

Weighted gene co-expression network analysis (WGCNA) based on all PHH TG-GATEs transcriptomic data

R-shiny framework for web-based interactive visualization

Module = network of co-expressed genes

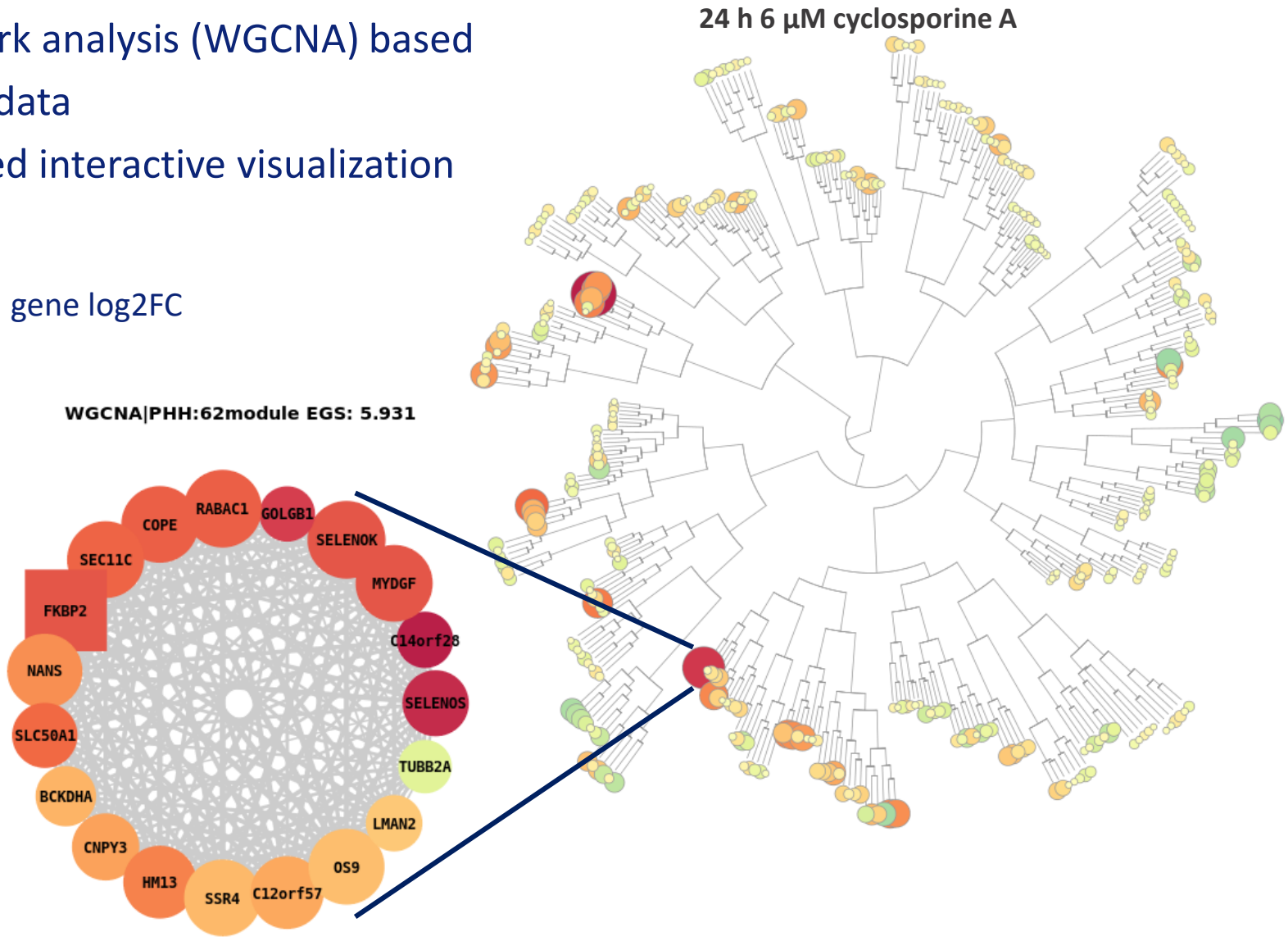
Eigengene score (EGs) = module score based on gene log2FC

TXG-MAP = Toxicogenomics-MAP

- Module responses (EGS) displayed in color and size
- Ward's hierarchical clustering of modules

Gene-set/module enrichment

- GO-terms
- Pathways
- Transcription factors
- Interacting proteins



EXPERIMENT:
ACETAMINOPHEN

EXPOSURE TYPE:
SINGLE

TIME:
24 hour

CONCENTRATION:
5000 uM

Absolute slider

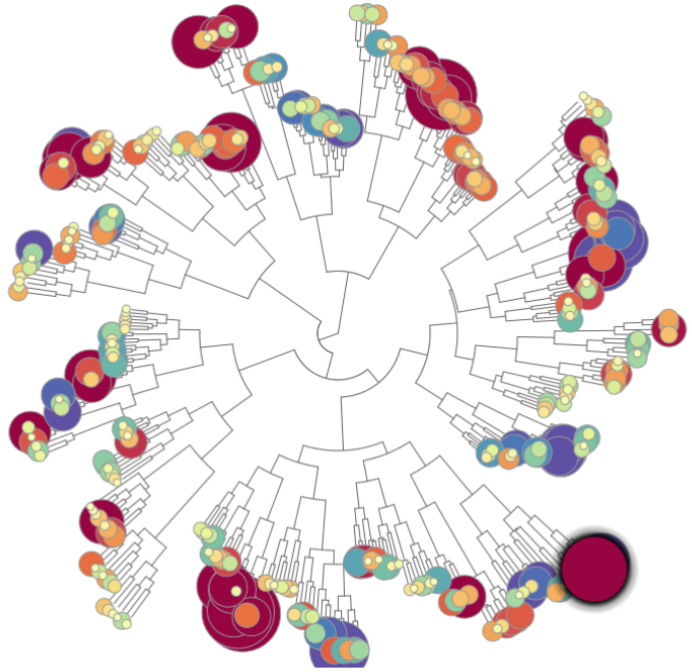
EIGENGENE SCORE:
-7.2 9.5

Module eigengenes

Show All entries Search:

module	module_size	eg_score	annotation
hPHH_103	13	9.45	
hPHH_194	9	8.95	
hPHH_366	5	8.84	
hPHH_144	11	7.94	Stress response, oxidative, NRF2
hPHH_119	12	7.91	Immune response, cytokine
hPHH_211	8	7.25	
hPHH_214	8	6.54	
hPHH_301	6	6.29	
hPHH_31	31	6.26	Metabolism, fatty acid
hPHH_37	27	6.11	DNA packaging
hPHH_199	9	5.53	
hPHH_266	7	5.52	Transcription
hPHH_318	6	5.43	Metabolism, ATP
hPHH_179	9	5.28	
hPHH_351	5	5.14	
hPHH_237	8	5.04	
hPHH_342	5	5.02	
hPHH_213	8	4.91	
hPHH_92	14	4.91	Immune response, chemotaxis

TXG-MAPr



TXG-MAPr Brush

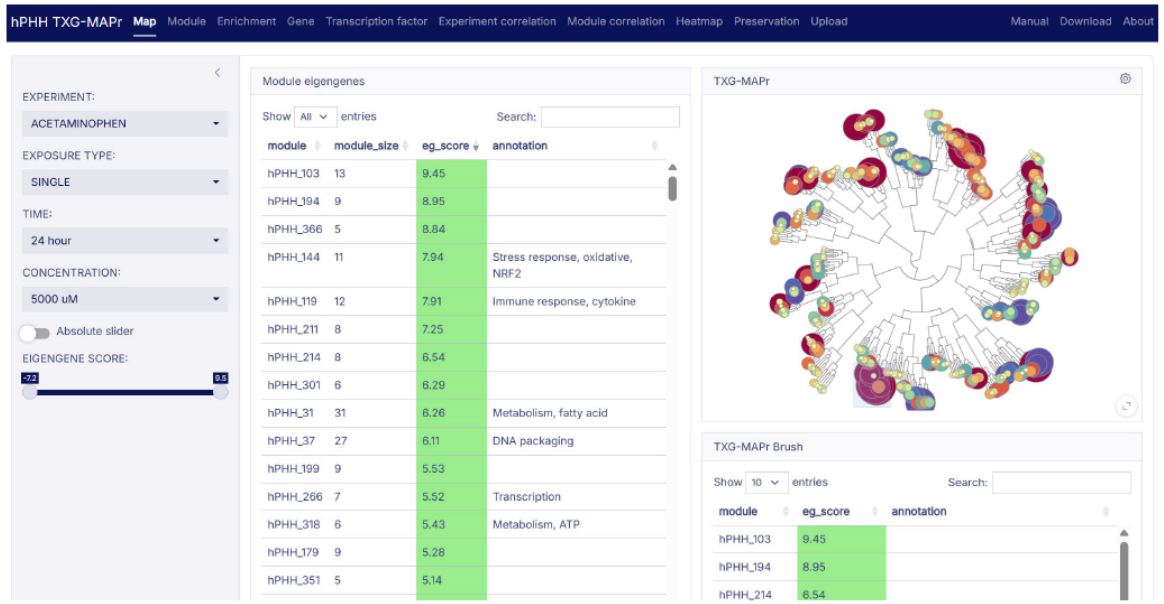
Show 10 entries Search:

module	eg_score	annotation
No data available		

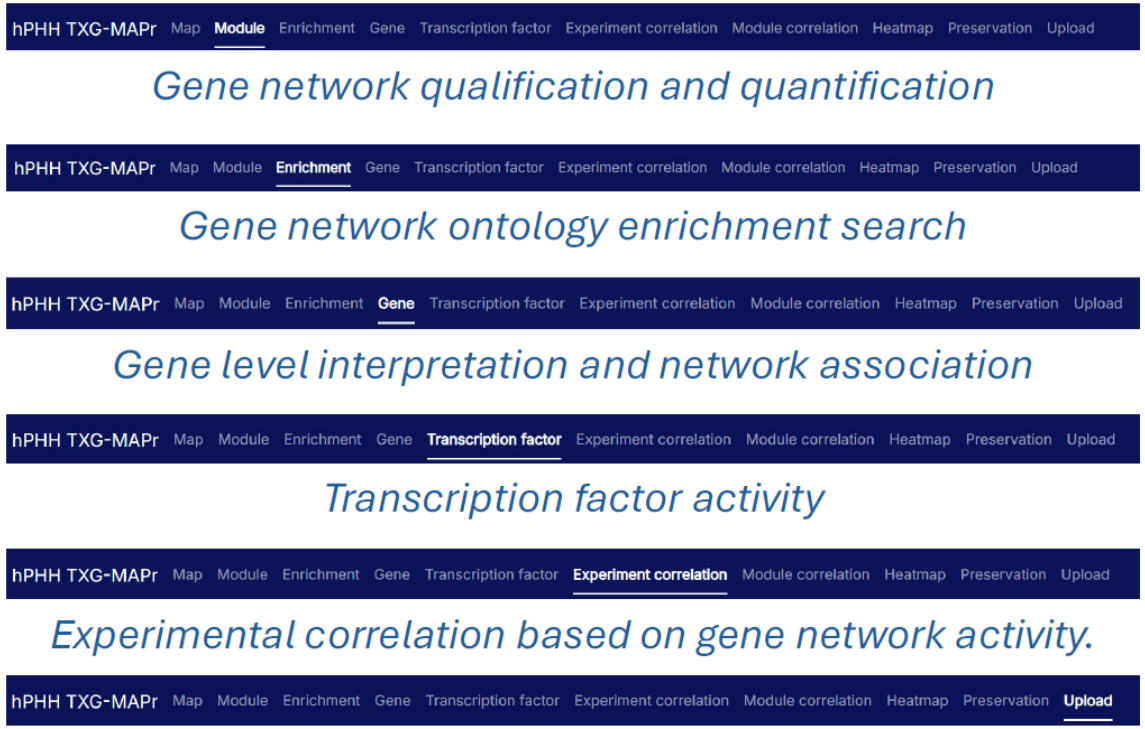
Callegaro et al. Arch Toxicol. 2021

The TXG-MAPr: a tool based on WGCNA

TXG-MAPr toxicogenomics interpretation toolbox



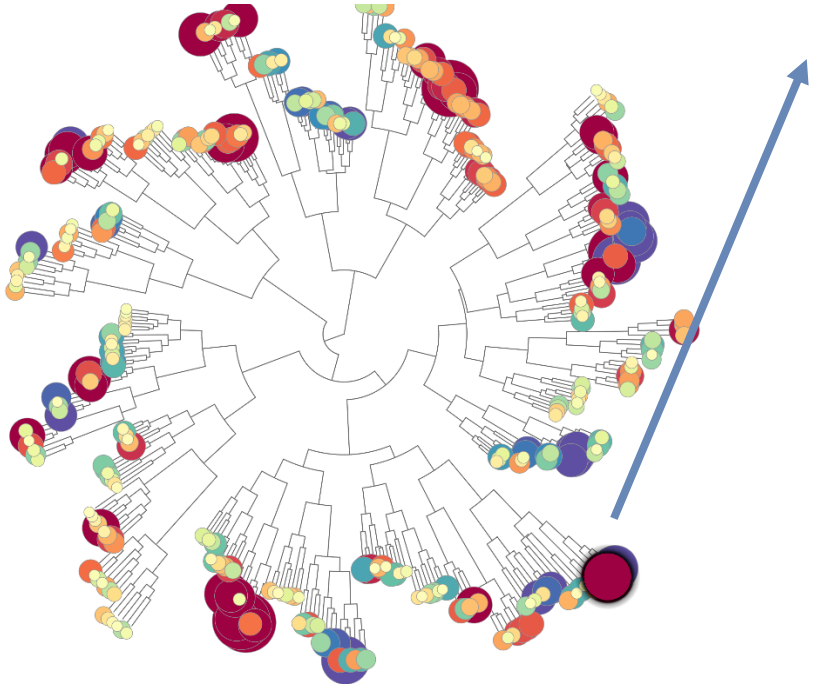
Interactive tabs for mechanistic insights



Universiteit Leiden

TXG-MAPr: mechanism of action

TXG-MAP: 5 mM APAP 24 hr PHH



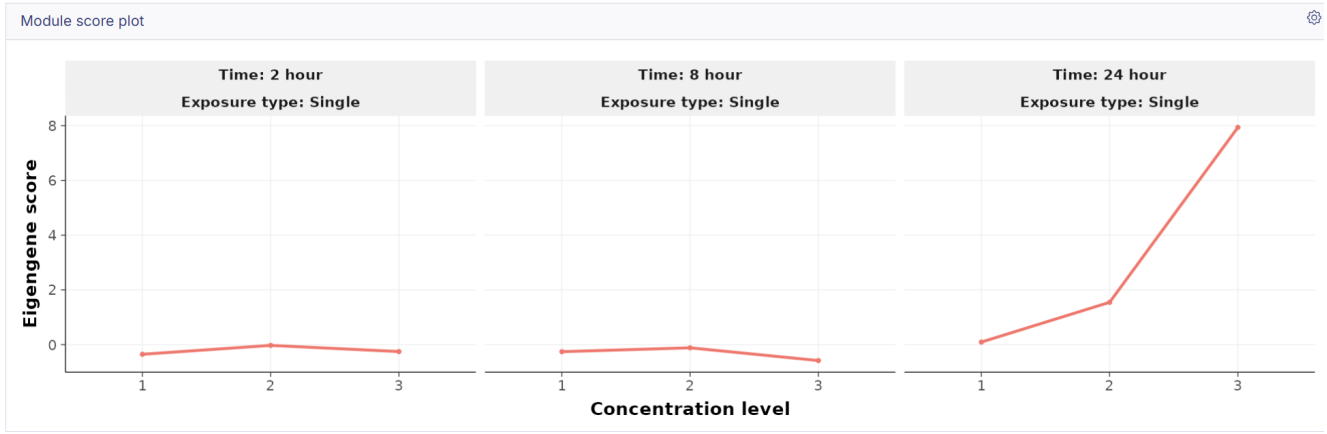
Gene co-expression (WGCNA) on TG-GATEs rat kidney data

Ward's hierarchical clustering

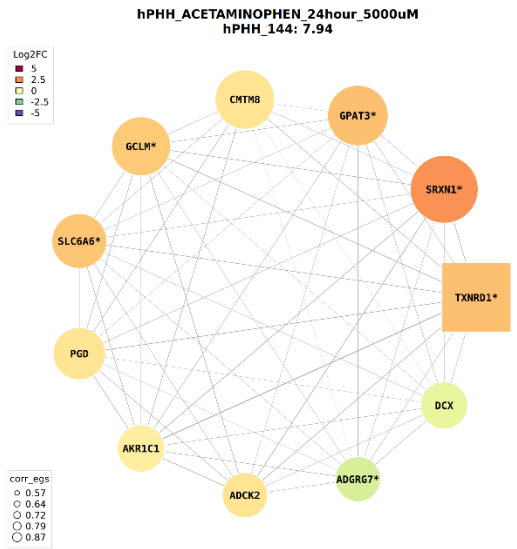
Callegaro et al. Arch Toxicol. 2021

Module eigengene score (EGs) response

hPHH:144



hPHH:144 Module composition



Module enrichment

Show 10 entries Search:

module	source	term	overlap	pvalue	padj	genes
hPHH_144	CPDB:Wikipathways	NRF2 pathway	4/142	9.40e-7	0.00000564	SLC6A6; TXNRD1; PGD; GCLM
hPHH_144	CPDB:Wikipathways	Nuclear Receptors Meta-Pathway	4/316	0.0000226	0.0000678	PGD; SLC6A6; TXNRD1; GCLM
hPHH_144	CPDB:Wikipathways	Photodynamic therapy-induced NFE2L2 (NRF2) survival signaling	2/23	0.0000841	0.000168	SRXN1; GCLM
hPHH_144	CPDB:KEGG	Glutathione metabolism - Homo sapiens (human)	2/54	0.000471	0.000706	GCLM; PGD
hPHH_144	STRING:InterPro	NADP-dependent oxidoreductase domain	2/NA	0.0000437	0.00130	GCLM; AKR1C1
hPHH_144	STRING:InterPro	NADP-dependent oxidoreductase domain superfamily	2/NA	0.0000437	0.00130	GCLM; AKR1C1
hPHH_144	CPDB:Reactome	Metabolism	5/1966	0.00302	0.00362	GPAT3; TXNRD1; AKR1C1; GCLM; PGD

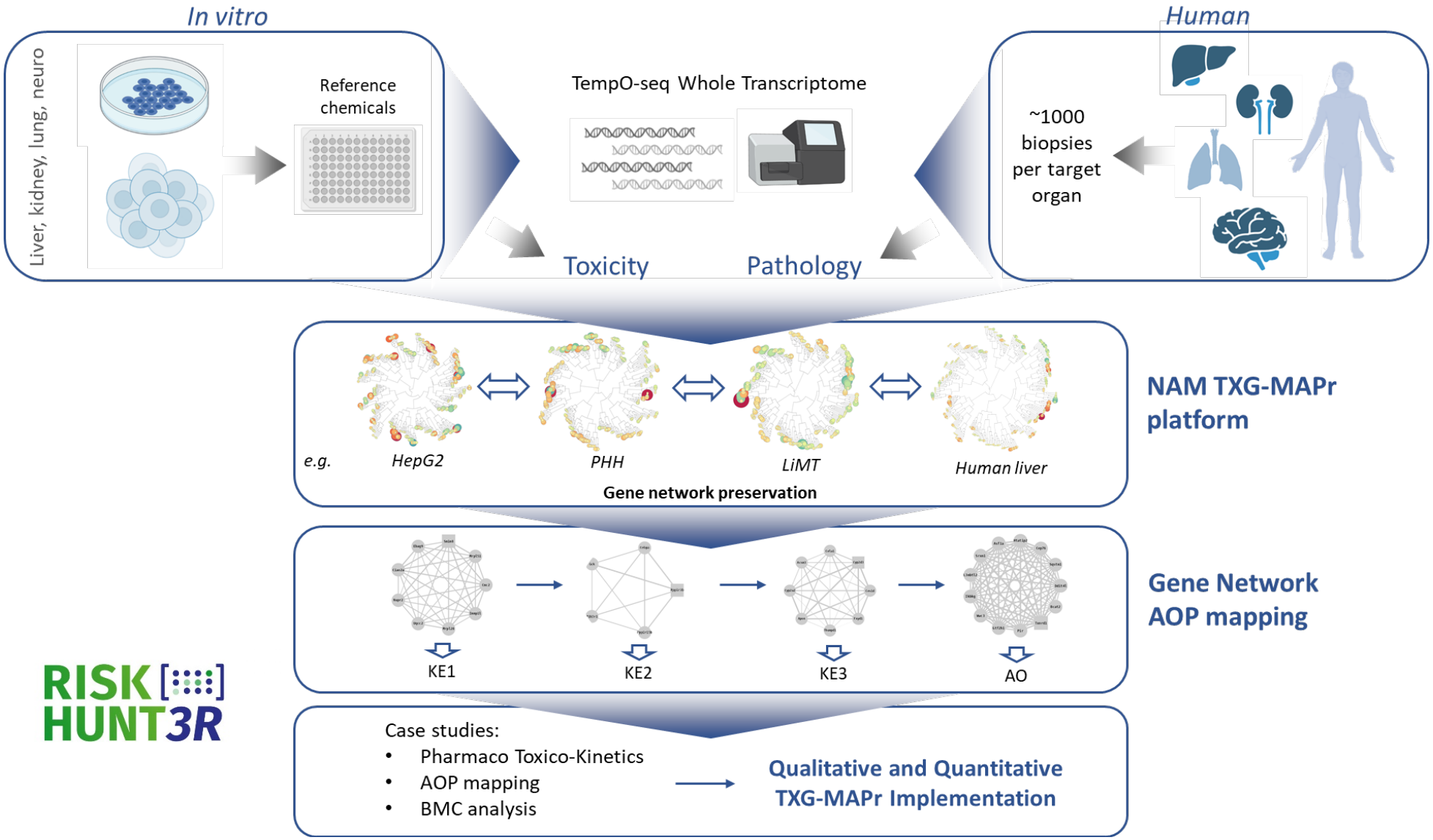
Showing 1 to 10 of 10 entries Previous 1 Next

Module transcription factor enrichment

Show 5 entries Search:

module	gene_symbol	pvalue	n_genes	genes	confidence
hPHH_144	E2F6	0.00294	2	SRXN1; TXNRD1	A_B_C
hPHH_144	NFE2L2	0.0000732	3	SRXN1; TXNRD1; GCLM	A_B_C
hPHH_144	BACH1	0.00000138	5	SRXN1; TXNRD1; SLC6A6; GCLM; PGD	A_B_C_D
hPHH_144	BACH2	0.000813	3	TXNRD1; SLC6A6; PGD	A_B_C_D
hPHH_144	BCL3	0.0000402	4	SRXN1; TXNRD1; SLC6A6; PGD	A_B_C_D

What is the preservation of biological transcriptional programs between different target organ test systems and human in vivo?

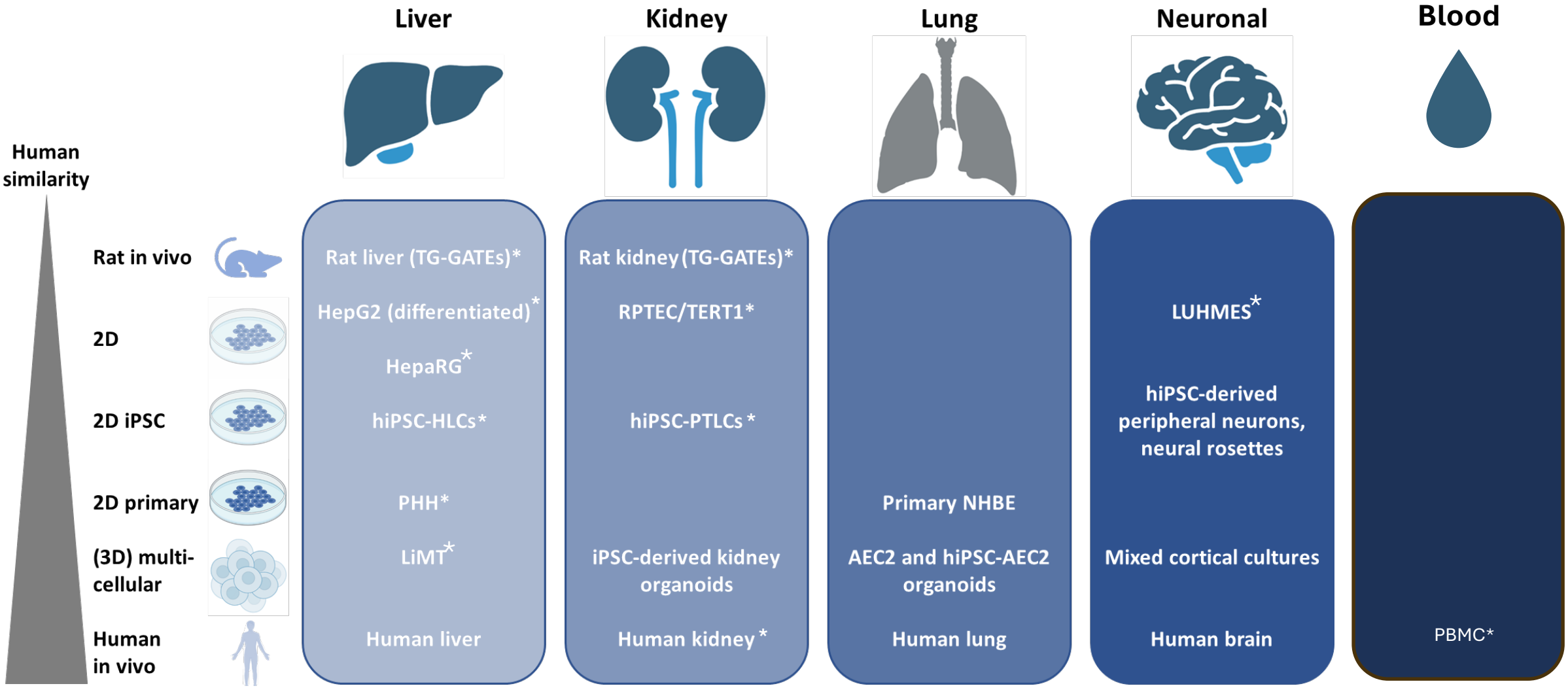


RISK HUNT3R

TXG-MAP project

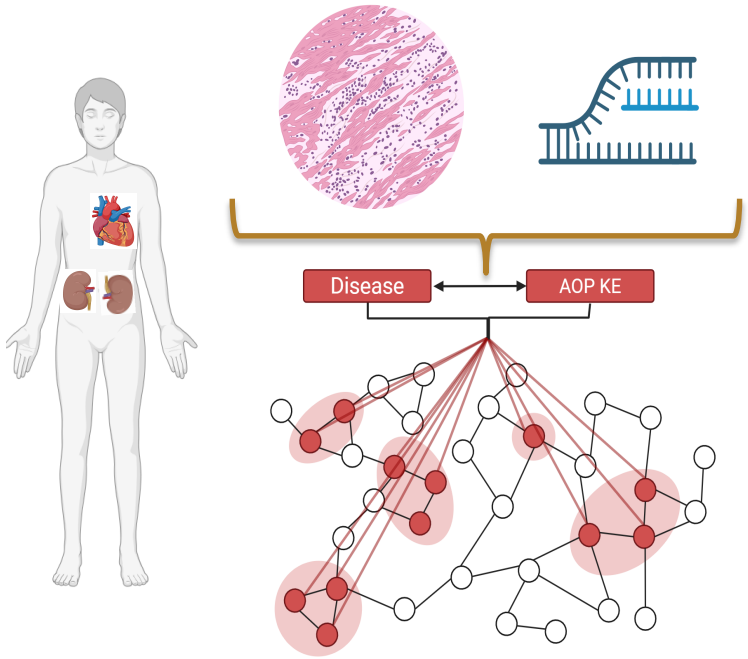
efsa EUROPEAN FOOD SAFETY AUTHORITY

The TXG-MAPr tools platform

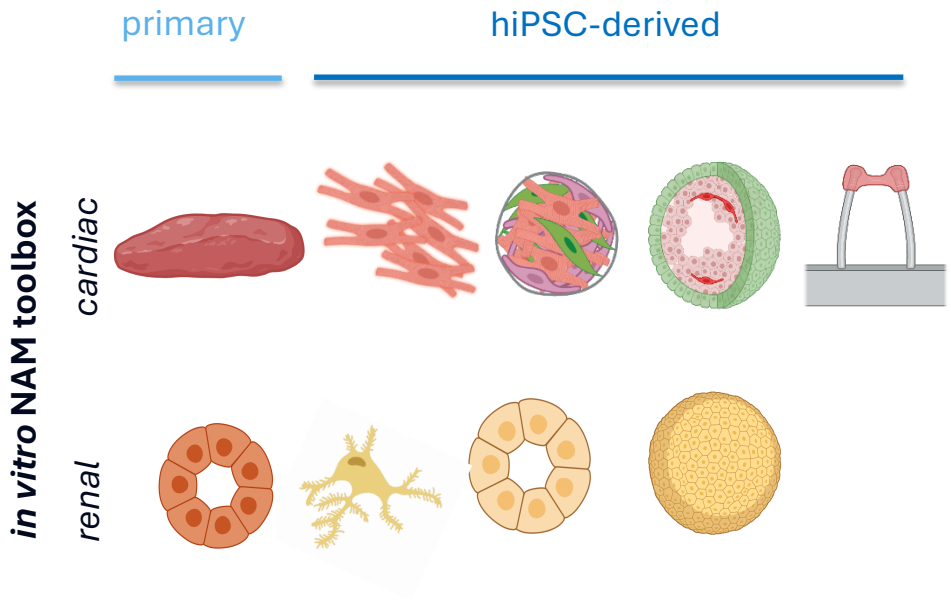


*** TXG-MAPr tools already established**

Toxicogenomics interpretation framework and human (patho)physiology context

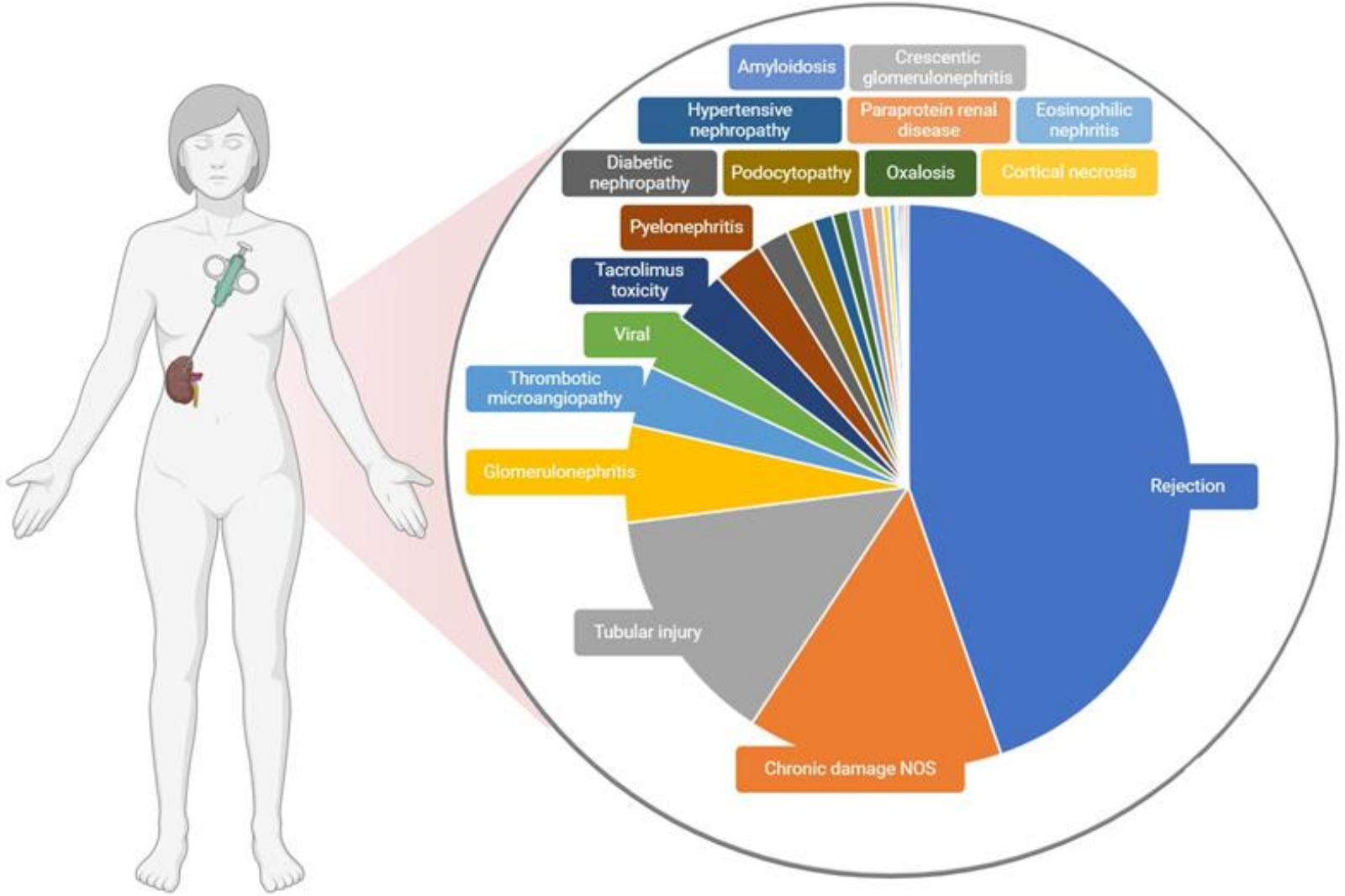






The human kidney TXG-MAPr

- >1400 kidney biopsy samples
- Diversity of renal pathologies
- Pathology BAM scoring
- TempO-Seq of all biopsies
- WGCNA analysis
- TXG-MAPr building

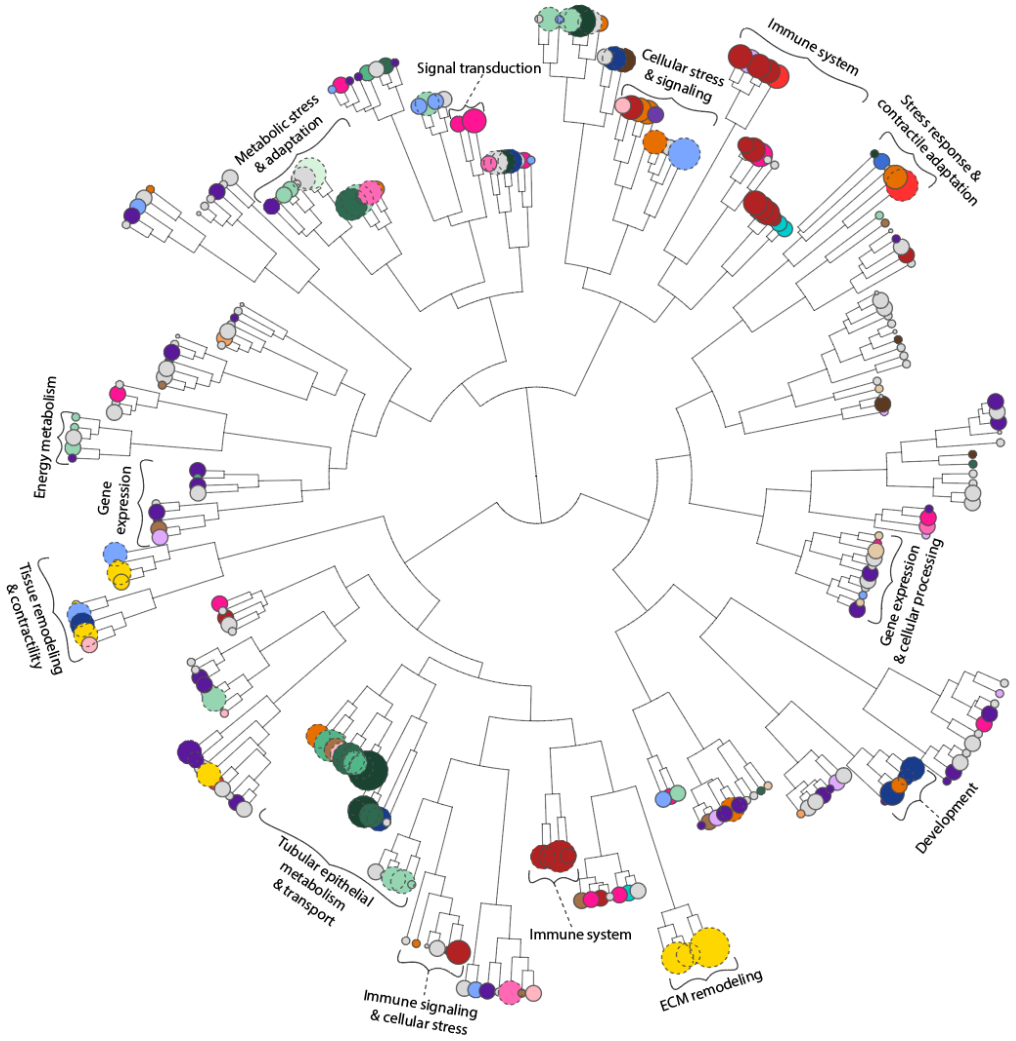


Bruns et al. *in preparation*

The human kidney TXG-MAPr

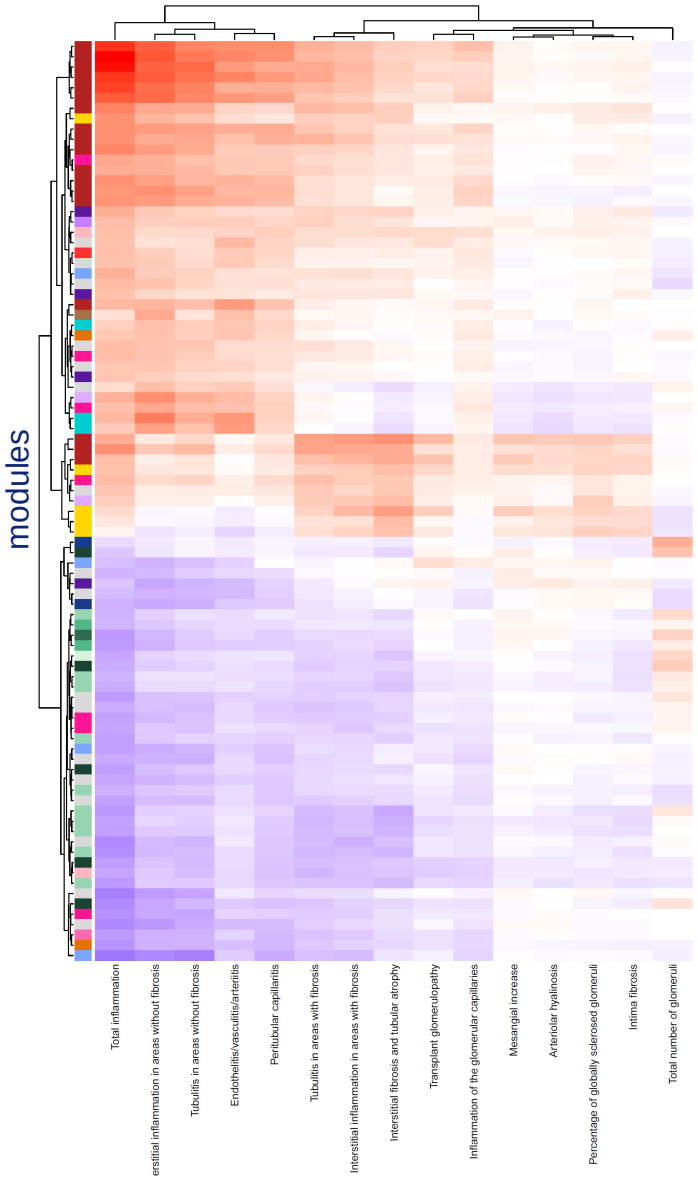
Annotation

- Cell−cell communication
- Cell cycle
- Cellular stress response
- Cytoskeleton signalling
- Development
- DNA damage & repair
- Energy metabolism
- Epigenetics
- Extracellular matrix organizatic
- Gene expression
- Immune system
- Inflammatory stress response
- Lipid metabolism
- Metabolism
- Receptor signalling
- Signal transduction
- Transport of small molecules
- Unannotated
- Vesicle transport
- Xenobiotic metabolism



pathology associations

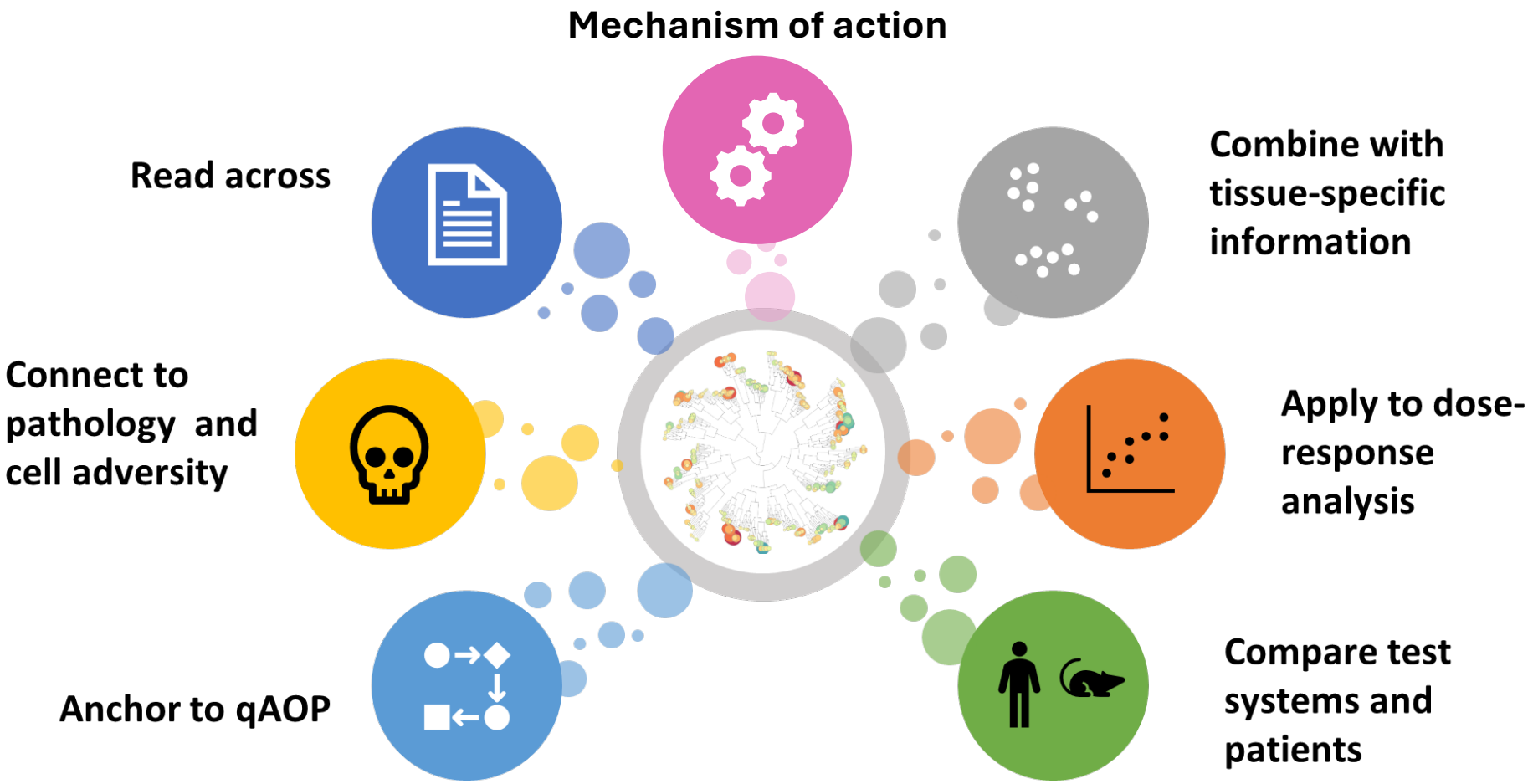
modules



Bruns et al. *in preparation*

TXG-MAPr applications

Applied in safety (hazard) assessment of drugs, chemicals and cosmetics



Publications

2021
Callegaro et al. Archives of Toxicology, 95(12), 3745–3775

2022
Vrijenhoek et al. ALTEX, 39(2), 207–220

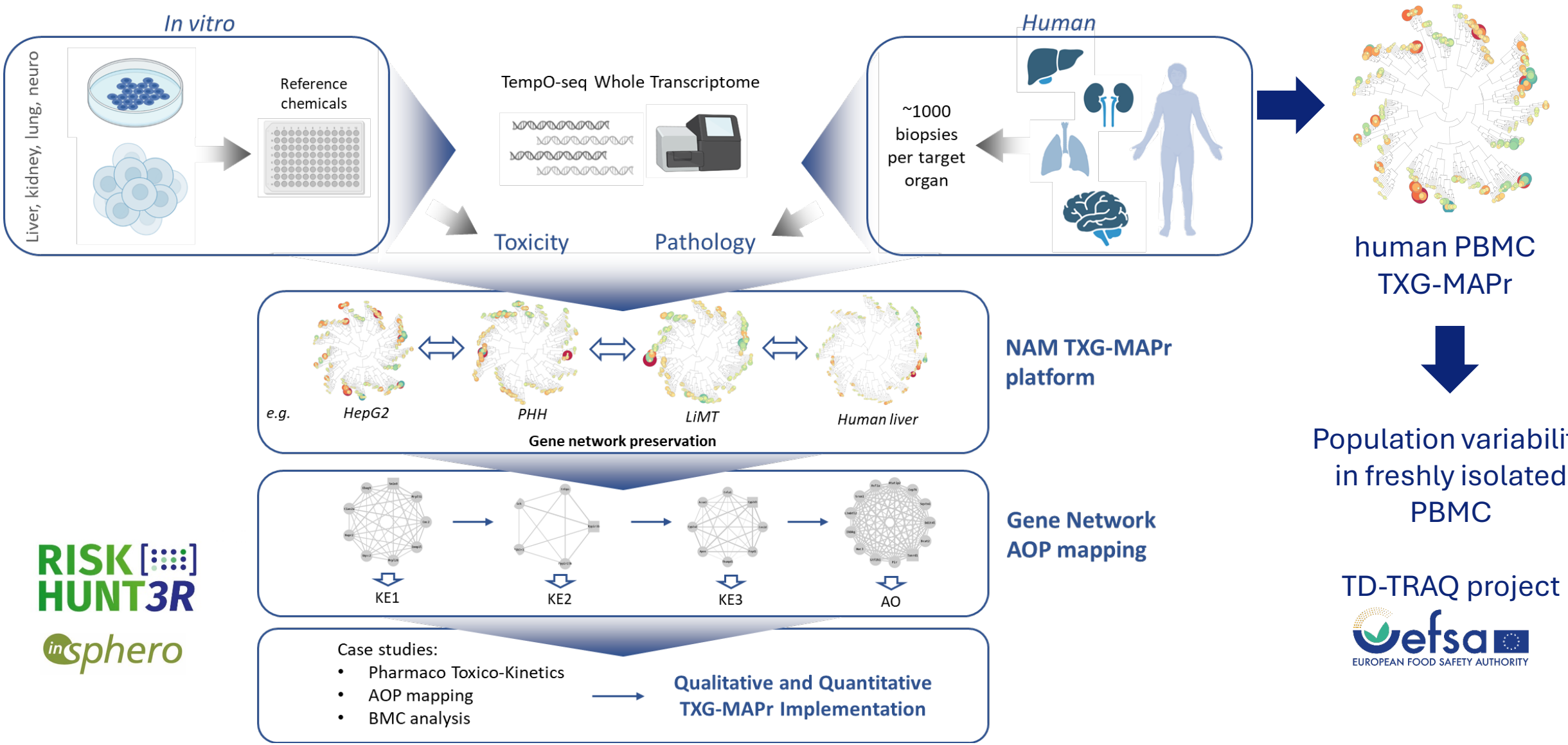
2023
Callegaro et al. iScience, 26(3), 106094

2024
Kunnen et al. ALTEX, 41(2), 213–232
Vlasveld et al. Liver International, 44(3), 760–775
Wijaya et al. Toxicol Sci, 198(1), 14–30

2025
Wijaya et al. Cell Biology and Toxicology, 41(1):49
Kunnen et al. iScience, 28(7):112978
van Kessel et al. Toxicol Sci, 209(1): kfaf159
Bruns et al. Brief Bioinform, 26(4): bbaf421

2026
Danilyuk TY, et al. Toxicol Sci, 209(8): kfag088
Bruns et al. Toxicol Sci, 209(9): kfag115
van Kessel et al. Archives of Toxicology, *in press*
van Kessel et al. Toxicol Sci, *submitted*

What is the preservation of biological transcriptional programs between different target organ test systems and human in vivo?



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Gerhard Burger
James Stevens

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RISK HUNT3R

ASPIS

EUTOXRISK

Cosmetics Europe the personal care association

PARC

transQST

eTRANSAFE

efsa EUROPEAN FOOD SAFETY AUTHORITY