The Final EUTOXRISK Symposium 8

From *in vitro* to human. Translational strategies in EU-ToxRisk (WP9)
Anchoring of KE and AOPs to data from man and archived rodent tissues

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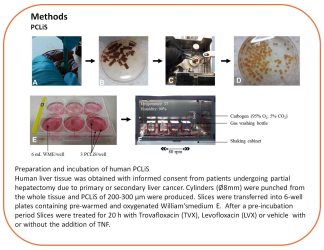
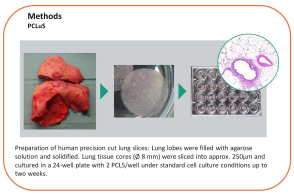
- WP9 work has provided anchorage of KEs in central AOPs used in EU-ToxRisk using a combination of data derived from complex human tissues ex vivo, data from human clinical exposures and from archived data from animal exposures.
- The work has illustrated the importance of genetically-derived variablity in KEs in determining dose-response relationships in adverse outcomes.
- The work has uncovered important information on the role of underlying human disease on AOPs.
- The strategy using this combination of approaches paves the way for further efforts to anchor KEs central to NAM-based testing strategies.

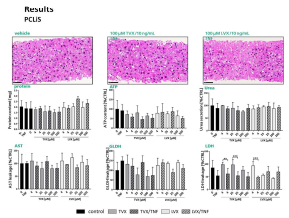
D9.1 KE activation in human precision cut organ slices

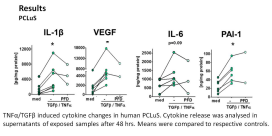
Anchorage of AOP KEs in intact human tissues

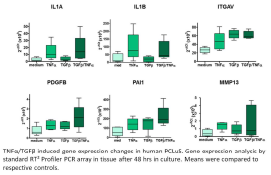
- Human tissue slices from liver and lung used to probe KEs in liver steatosis and lung fibrosis

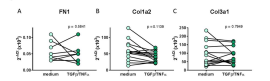












- PCLiS proved to be much more robust than 2D (HepG2) cell cultures in "capturing" KEs associated with steatosis.
- Early molecular KEs critical to respiratory fibrosis pathogenesis are detected with PCLuS.
- Important KE anchorage achieved between in vivo and in vitro systems.

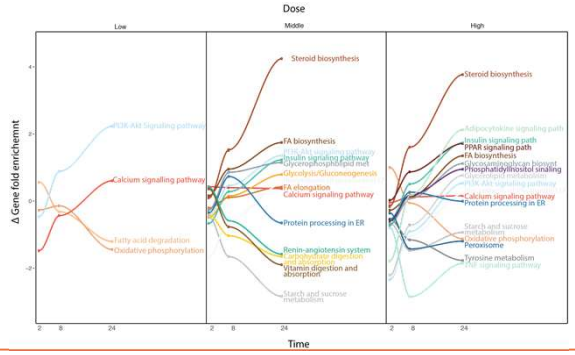
D9.2 Toxicity pathways in archived rodent material

Analysis of activation of KE and toxicity pathways obtained from transcriptomics analysis of archived rodent material from public data

- Collection of data from Array Express an GEO was realised. The outcomes from an internal developed pipeline is shown below:

ORGANISM	Total number	With multiple doses	With multiple time points	With multiple doses and multiple time points
Mus musculus	406	131	164	80
Rattus norvegicus	254	181	127	105
Homo sapiens	504	180	257	98

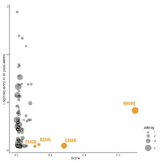
- Systematic pathway enrichment analysis was performed showing the variability of pathways as a function of time.
- Time series pathways analysis performed on a set of 28 DILI compounds suggested pathways impacted by DILI over time – More information on the analysis can be found in Aguayo-Orozco et al. (Front. Genet., 18 September 2018 | https://doi.org/10.3389/fgene.2018.00396)



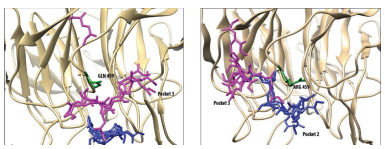
D9.3 Variation of KE and toxicity pathways in humans

Development and application of different bioinformatics methodologies to estimate the contribution of genetic variations in toxicity pathways

- Collection of 2574 genetic variations distributed over 912 genes and reported to have an impact on human variation in drug response.
- Docking protocol used to predict binding to variants: Ex KEAP-1.



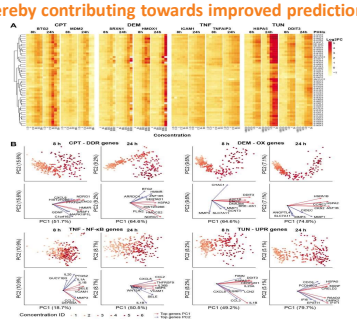
Prediction of the SNV impact on chemical bioactivity in KEAP1. The x axis corresponds to the predicted score from the LDA model. The y axis is the frequency of the SNV observed in the global population. The SNP R459Q on the right has the highest score.



The docking of dimethyl fumarate (in magenta) on the WT (left) and mutated (right) KEAP1 show a different binding mode.

Transcriptomic mapping of the inter-individual variability of cellular stress response activation in primary human hepatocytes

- Variability in stress response activation across the human population could be established, thereby contributing towards improved prediction of DILI.

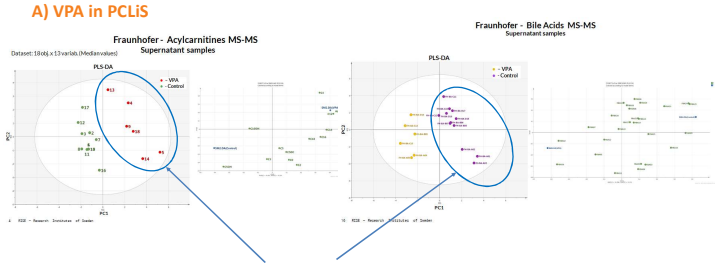


Inter-individual variability in concentration-dependent stress response activation. Hierarchical clustering of log2 fold change gene expression of key genes for each evaluated stress response pathway of panel of PHHs exposed to reference compounds. B) Principle component analysis (PCA) based on log2 fold changes of stress responsive genes of panel of PHHs exposed to DEM, TUN, CPT and TNFα. Top 5 genes mostly determining P. Niemeijer M et al 2021. BioXiv **doi:** https://doi.org/10.1101/2021.08.26.457742

D9.4 Toxicity pathway activation in humans

Anchorage of KEs in liver steatosis in biomarker analysis of bile acids, acylcarnitines, steroids, cytokines and lipids from VPA-treated PCLiS and plasma from epileptic children on VPA therapy

A) VPA in PCLiS

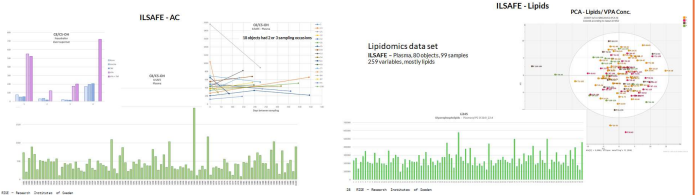


- Panels of specific acylcarnitines and bile acids were altered in response to VPA treatment.

B) VPA in children

Wide distribution in all subjects of one of the acylcarnitines shown to increase in PCLiS in response to VPA. No trend for change over multiple sampling points.

Wide distribution of specific lipids in all subjects. Similarly wide distributions in steroids and bile acids.



- No specific biomarker anchorage between PCLiS and clinical samples, possibly due to differences in exposure (toxicology vs pharmacology).
- Need to define boundaries of "normal levels" of biomakers in children using comparative untreated cohorts.

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www.eu-toxrisk.eu/