of-Concept case studies to illustrate how toxicological knowledge can be capture *a priori* in the form of an Adverse Outcome Pathway (AOP), how in vitro and computational methods can be optimally combined to predict specific effects, and finally, how in practical terms 'new approach data' can be optimally generated and used for hazard and safety assessment purposes.

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W04-2
A High Throughput Microscopy Toxicity Pathway Reporter Platform for Chemical Safety Assessment

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Adaptive cellular stress responses are paramount in the healthy control of cell and tissue homeostasis after cell injury during hypoxia, oxidative stress or unanticipated side-effect of medications and other chemical exposures. We find that activation of adaptive stress responses occur well before the typical ultimate outcome of chemical cell injury. To increase our understanding of chemically-induced adaptive stress response pathway activation and its contribution to safety assessment we believe that a time-resolved, sensitive and multiplex readout of chemical-induced toxicological relevant cellular stress responses will be essential. For that purpose, we developed a platform containing a panel of distinct adaptive stress response reporter cell lines. These are used for automated high content live cell imaging and quantitative multi-parameter image analysis to elucidate critical adaptive stress response pathway activation that can contribute to human chemical safety assessment. To conserve the endogenous gene regulatory programs, we tag selected reporter target genes with GFP using BAC-transgenomics approaches. Here we demonstrate the functionality of individual BAC-GFP toxicity pathway reporter HepG2 cell lines to their respective specific model compounds. The application of these reporters in high throughput chemical safety assessment in relation to drug-induced liver injury will be discussed in further detail. Moreover the use of these reporters in differentiated 3D HepG2 spheroids for repeated dose toxicity is demonstrated. We anticipate that a phenotypic adaptive stress response profiling platform will allow a high throughput and time-resolved classification of chemical-induced stress responses assisting in a mechanism-based, rapid and quantitative safety assessment of chemicals. This work is part of the MIP-DILI project supported by the Innovative Medicines Initiative (grant agreement n° 115336), and the FP7 SEURAT-1 DETECTIVE project (grant agreement 266838).

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W04-3
Functional intravital imaging of hepatotoxicity: Comparing intact livers to 3D in vitro systems

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With the advent of functional multi-photon imaging and fluorescent reporter techniques it has become possible to directly observe toxicity in living organs with subcellular (250 nm) resolution. We adapted this intravital technique to analyze acute and long-term toxicity in livers of mice and compared the observations to those obtained by hepatocyte 3D in vitro systems. Administration of hepatotoxic compounds, e.g. APAP and CCl4, generated a typical sequence of events which can be seen in time-lapse videos: compromised mitochondrial metabolism followed by bile acids entering the hepatocytes from the canalicular side, leading to irreversible hepatocyte death. Next, the damaged tissue is infiltrated by neutrophils, macrophages and stellate cells, which may enlarge the extent of initial tissue damage. Later the original tissue architecture may be restored, whereby proliferating hepatocytes use sinusoidal endothelial cells (LSEC) as 'guide rails'. As soon as endothelial cells are destroyed, perfect regeneration switches into a 'scar regeneration mode', which finally leads to fibrosis and cirrhosis. The currently available 3D in vitro systems and bioreactors show APAP or CCl4 induced loss of mitochondrial activity and compromised bile canalicular integrity in a similar way as observed in vivo. However, major limitations are that they do not establish functional sinusoids and the complex interplay of LSEC, stellate cells and immune cells in vivo is not recapitulated. Having these limitations in mind we nevertheless tried to establish a predictive in vitro system focusing only on biomarkers and cell functions that reflect the in vivo situation to a qualitatively and quantitatively acceptable degree and for which the link to adverse in vivo effects is sufficiently understood. Using 21 hepatotoxic and non-hepatotoxic compounds with known human pharmacokinetics a differentiation between both classes was possible and hepatotoxic in vivo blood concentrations could be predicted within an error margin of approximately one order of magnitude. These systems may be helpful in prioritization of compounds and give an orientation about the margin of safety.

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W04-4
A 3D liver co-culture system for evaluating drug-induced adverse outcome pathways leading to fibrosis

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Chronic liver diseases lead to liver fibrosis and subsequent cirrhosis of the liver. Hepatic Stellate Cells (HSCs) have been identified as key regulators in several adverse outcome pathways (AOPs) leading to fibrosis. In the quiescent state, HSCs store vitamin A and have a balanced ECM production, but upon (chronic) injury