IMI1 Final Project Report
Public Summary

Project Acronym: MIP-DILI

Project Title: Mechanism-Based Integrated Systems for the Prediction of Drug-Induced Liver Injury

Grant Agreement: 115336
Project Duration: 01/02/2012 - 31/03/2017

Executive summary

1.1. Project rationale and overall objectives of the project

The current test systems employed by the Pharmaceutical Industry are poorly predictive of Drug Induced Liver Injury (DILI); in particular idiosyncratic DILI cannot be predicted in animal systems. The Mechanism Based Integrated Systems for the Prediction of DILI (MIP-DILI) project seeks to address this situation by the development of innovative preclinical test systems which are both mechanism-based and of physiological, pharmacological and pathological relevance to DILI in humans. An iterative, tiered approach with respect to Test Compounds, test systems, bioanalysis and mathematical systems analysis has been adopted to evaluate existing models and develop new models that can provide validated test systems with respect to the prediction of specific forms of DILI and further elucidation of mechanisms that relate to idiosyncratic DILI. The approach encompasses completely characterised cell lines, well-defined and physiologically stable hepatocytes (potentially including hepatocytes from DILI sensitive patients derived from stem cell technologies), multi-cell type in vitro models and animal models. Triangulation of human, in vitro and animal data is providing a fundamental understanding of how drugs can harm the liver and how this relates to the idiosyncratic response. The objectives of MIP-DILI are:

1. to define the application and limitations of current and novel test systems and provide an improved panel of in vitro “best practice assays” for predicting DILI in the human population during drug development;
2. to explore and understand the relationship between in vitro assay signals and DILI in vivo, in preclinical test species and in man;
3. to develop and validate novel mathematical systems modelling approaches that integrate multiple preclinical data types to improve prediction of DILI in man;
4. to enhance shared understanding, between academia, pharma and regulatory agencies, of the value and limitations of new and existing approaches for DILI hazard identification and risk assessment.

1.2. Overall deliverables of the project

The MIP-DILI project consists of seven Work Packages (WP1-7) with the following deliverables:

1. WP1 is delivering the selection of Training and Test Compound sets, promising in vitro and preclinical in vivo models, and the management of data within MIP-DILI
2. WP2 is delivering the assessment of established and novel in vitro cell systems to determine how they compare physiologically with human liver, mechanistically by use of MIP-DILI Training Compounds and sensitivity/specificity at detecting DILI with Test Compounds.
3. WP3 is delivering preclinical in vivo model systems for determining the role of infectious inflammation in DILI and assessing the relationship between chemical stress and DILI.
4. WP4 is delivering an integrated bioanalytical capability to inform WP2, WP3 and WP5 of the physiological, drug metabolism and pharmacokinetic phenotype of the various test systems.
5. WP5 is delivering physiologically based mathematical models to give improved prediction of DILI, particularly the hepatic response to chemical stress, inflammation and regeneration.
6. WP6 is delivering MIP-DILI communication and dissemination activities.

7. WP7 is delivering the overall scientific and project management of MIP-DILI.

1.3. Summary of progress versus plan since last period

All scientific Work Packages in MIP-DILI have made good progress against the project plan in this reporting period.

WP1 continued gathering further data on the Test compounds, for general purposes (e.g. as input for the CDR), but also for HCA group and other working groups). Access to the CDR and the RDR will be maintained by Lhasa Limited for a period of 5 years after the project end date, to ensure that project participants are able to further use the data from contributors to continue their research. A major achievement concerning the set of Training compounds, i.e. selection procedure, the underlying mechanisms (both single and overlapping) and their utility as Training compound for human DILI has been a publication of Dragovic *et al.*, in Arch. of Toxicology (November 2016; DOI 10.1007/s00204-016-1845-1). The 4th and Final Workshop of MIP-DILI was organised in Paris in January 2017. The primary objectives were (i) to identify and validate an improved panel of best practice *in vitro* assays identifying hazard & risk assessment of human DILI, (ii) to present new learnings on the mechanistic insight on the Training and Test sets of compounds, and for nomination of selected compounds as reagents for use in *in vitro* and *in vivo* test systems, and (iii) to clearly identify the step changes in learning and delivery of a full scientific report as output.

WP2 established a mitotoxicity working group evaluated the sensitivity and specificity of several mitotoxicity screening assays in order to produce a testing road-map specifically for mitotoxicity. The mitotox Ring Trial results show that the Glucose/Galactose assay and the Mitochondrial Membrane Potential assay performed best as short term assays and showed a relatively good predictivity (41-61 % sensitivity, 90 % specificity) towards drug-induced mitochondrial toxicity. A High-Content Analysis (HCA)-focused group selected a larger test set of compounds (69 DILI positive and 22 DILI negative drugs) and tested them in the *in vitro* assays that the various companies employ, and across different cell models and endpoints, using the same test concentrations to allow real comparisons. The data has been analyzed and typically shows sensitivity of ~50% and 90-100% specificity. The assays can be used in absence of a Cmax with 25-30% sensitivity and ~90% specificity but that sensitivity is boosted to 40-70% when accounting for Cmax, and that the optimal margin between *in vitro* toxicity and Cmax is 75-fold for this data set.

Comprehensive multi-omics characterization data of the 3D Primary Human Hepatocytes (PHH) spheroid model was provided. Whole proteome analyses revealed that PHH in 3D culture were similar to the liver in vivo from which the cells originated. PHH spheroids remained phenotypically stable and retained morphology, viability, and hepatocyte-specific functions for culture periods of at least 5 weeks. Using a combination of targeted and untargeted high-resolution mass spectrometry we showed that PHH spheroids retained overall metabolomic profiles during long-term culture. Furthermore, pharmacokinetic differences between donors were maintained. The spheroid ring trial aimed to i) compare the sensitivity of 3D spheroids to 2D sandwich cultures, the current alternative option for long-term in vitro exposures, ii) compare sensitivity of PHH to HepaRG in both 2D, iii) assess inter-laboratory reproducibility and robustness of the system as well as inter-donor variability, iv) distribute the spheroid protocol to different labs and share experience/expertise. Totally 24 persons from 9

different partners participated. In general hepatocytes from 3 different donors were used and the toxicities of 6 different compounds were assessed using ATP content measurements. It was concluded that the 3D spheroid system might be the best available High Throughput Screen (HTS) based in vitro system for monitoring drug induced hepatotoxicity and that PHH spheroids offer a robust and reproducible cell model that can be used to successfully identify hepatotoxic compounds. Good progress has been made to introduce EFPIA partners to this technology. The potential of these test systems to add value to DILI detection was acknowledged; indeed a majority of EFPIA members are interested to continue further work with this model and can see its application in R&D.

In vitro biorelevant models that can identify compounds causing Drug-Induced Cholestasis (DIC) by altering bile acid (BA) disposition was further evaluated by partners for its performance with several cholestatic drugs. Indeed, primary human hepatocytes in sandwich culture develop functional biliary canaliculi over time in culture and thus represent an *in vitro* model to investigate the effect of xenobiotics on toxicity caused by accumulating BAs subsequent to disturbed BA disposition, including those due to inhibition of export transporters. Using HepaRG cells in 2-D culture configuration the effects of > 30 compounds (the 14 training MIP-DILI drugs + some other compounds) were tested. The results from HepaRG were largely confirmed with primary human hepatocytes and were reproducible in Rennes and Servier labs.

A HepG2 Green Fluorescent Protein (GFP) reporter platform was developed to monitor the activation of generic adaptive stress response pathways following drug treatment. A disadvantage of HepG2 cells is their dedifferentiated status when cultured as monolayer, yet we established that when cultured our as 3D spheroids HepG2 cells have a more differentiated phenotype and can be cultured up to 4 weeks. Together the six HepG2 GFP stress response reporters 3D spheroids demonstrate an improved identification of DILI liability. The assessment of drug-induced activation of stress responses could be a useful tool to predict DILI liabilities. Until now the variability in the activation of these stress responses amongst individuals have not been fully mapped. It was found that the different individual donors was responsible for the highest variability in gene expression of all genes rather than the different treatment conditions. The transcriptome profile of the HepG2 reporter system showed little to no variation between reporters in basal conditions. Upon activation of the respective stress response pathways, some reporter lines were marked by distinct transcription profiles.

One aim was to mimic DILI in a dish based on Induced Pluripotential Stem Cell (iPSC) derived hepatocytes co-cultured with T-cells isolated from the same donors and to address the immune system aspect of flucloxacillin induced DILI. Banks of six iPS donor lines were generated from healthy donors with known human leukocyte antigen (HLA) type. Three of the donors expressed HLA-B*57:01 shown to be linked to flucloxacillin induced DILI and three donors expressed random HLA types as controls. The new model system was assessed for its sensitivity to flucloxacillin. Data indicated that flucloxacillin-induced T-cell-mediated killing of iPS-derived hepatocyte-like cells can be mimicked in vitro. Flucloxacillin preferentially activated CD8+ T-cells from patients with DILI in an HLA-B*57:01-restricted manner. Amoxicillin, clavulanic acid and isoniazid activated CD4+ and/or CD8+ T-cells; however, the response was not restricted to a specific HLA allele. MIP-DILI diagnostic toolkit has been established to study DILI that develops in patients exposed to a range of structurally unrelated drugs. This approach has been developed at UoL and is currently being used on a case by case basis to explore the sensitivity and specificity of the diagnostic assays. These currently have utility to provide mechanistic

insight on drugs with potential immunological liabilities. However, the complexity of both the HLA locus (with more than 3000 alleles) and the assay that has been developed in MIP-DILI, means that predictive outputs are not currently feasible for utilization by EFPIA. UoL are continuing this work in collaboration with individual EFPIA partners.

WP3 was responsible for the development of *in vivo* models. The focus was on two particular concepts:

- The consideration of the role of inflammation, primarily infective (but also sterile), as an important factor in DILI as it occurs in patients
- The evaluation of chemical stress induced by drugs in liver cells, in particular biological perturbation observed under circumstances not associated with overt toxicity as assessed by standard histopathology and clinical chemistry

The first objective for this final reporting period was to further consolidate the infection model established in the previous period and initiate additional models to understand the role of the inflammatory system in idiosyncratic DILI. Further objectives included the assessment of up to four compounds in the infectious inflammation models with defined endpoints. These aims have been achieved and demonstrated that DILI occurs in a drug-specific fashion. Results confirm that DILI is a process caused through defined alterations in molecular signalling pathways that favour the induction of cell death upon TNF receptor signalling. In addition investigations into sterile inflammation as a contributor/initiating factor in DILI highlighted several markers/cell functions that might provide insight and add utility as predictive biomarkers with further investigation. The second objective was to act on the decision to end investment into the HOD reporter mouse model and progress the work on the Nrf2-Luc reporter mouse at the UoL. These studies could place chemical/oxidative stress responses detected *in vitro* into a whole body context, with consideration of pharmaco/toxicokinetics. This model has the potential to further refine our understanding of the association between drug metabolism, chemical stress and toxicity in mice, and provide a rationale for examining the value of Nrf2 as a marker of toxic stress in human systems. The key output from WP3 has been the enhanced understanding of some of the complex pathways involved in the initiation of DILI, and how the immune system can be intricately involved – possibly by numerous mechanisms. Throughout the studies undertaken a clear, common thread across all drugs causing DILI was not identified.

Differences in the phase I (cytochrome P450) and phase II (UDP-glucuronosyltransferase and sulfotransferase) metabolism of three MIP-DILI drugs (diclofenac, tolcapone and APAP), as well as the probe substrate 7-hydroxycoumarin (7-OH), was quantitatively compared in PHH in suspension and monolayer. To compare the metabolic potential of PHH (3 donors), HepaRG and HepG2, a large study has been performed to quantify P450 and Uridine Glucuronyl Transferase (UGT) activities in these cell systems using the methods developed (see above). Furthermore, clearance data was obtained for 13 MIP-DILI training compounds (amiodarone, paracetamol (APAP), bosentan, buspirone, diclofenac, nefazadone, perhexiline, entacapone, tolcapone, ximelagatran, metformin, troglitazone and pioglitazone). Both phase I and phase II activities in HepG2 were low. Phase II activities were overall comparable in PHH and HepaRG, albeit with substantial variation in individual UGT activities and between donors. the activities and/or protein levels of all major Cytochrome P450s (CYPs), UGTs, Sulphotransferases, glutathione transferases, quinone reductases (NQO1 and NQO2) were measured in liver fractions of 20 individuals provided by KalyCell (den Braver-Sewradj et al, manuscript in preparation). Absolute quantification of quinone reductases (NQO1, NQO2) and Glutathione-S-Transferases (GSTs; GSTA1, GSTA2, GSTM1, GSTM3 and GSTP1) in these liver fractions could be

achieved by using purified recombinant enzymes as references. The results showed large differences in expression levels, large differences in the balance of drug bioactivation and inactivation are anticipated, dependent on the drug and enzymes involved. In conclusion, for several MIP-DILI compounds significant variabilities in bioactivation and bioinactivation were predicted based on results from quantified levels of enzyme activities. Therefore, it is important to characterize the cell systems sufficiently to interpret toxicity. In this reporting period a similar proteomic study was also performed in support of the spheroid ring trial comparing 2D versus spheroid cryopreserved primary human hepatocytes. The experimental phase in all three studies have been completed at the end of March 2017 and data analysis is currently being performed which will support a number of additional publications.

The aim of WP5 "Systems Analysis" was to develop mathematical models describing the response of the liver to inflammatory and proliferative stimuli and use these models to assess the impact of the stimuli and other perturbations, like viral infections, on drug induced hepatotoxic responses. The objectives of WP5 were to develop novel tools for the identification of intervention points of DILI compounds to improve predictions of DILI. Dynamic pathway model structures were successfully developed for HGF, IL-6 and TNFalpha induced signal transduction. The parameters of these dynamic pathway models were calibrated based on the time and dose resolved experimental data and the model predictions were validated by additional experiments. Combinations of different immunological mechanisms caused by virus infection and compound treatment lead to an increased death in mice. DILI-compounds such as ximelagatran induced in combination with e.g. LCMV virus a shift of the hepatotoxic threshold in mice. This work was performed in close collaboration with WP3. These results show that hepatotoxicity is decided on multiple scales and that this complexity requires an integrative mathematical modelling approach to disentangle the different impacts. By integrating both the impact of the inflammatory and proliferative signalling pathways and the perturbations caused by drug exposure, our data-based dynamic pathway models provide innovative tools for the prediction of consequences to a cellular systems and therefore contribute to the prediction of DILI. The established mathematical model, validated by the use of these two prototypical cholestatic drugs and the integration of bile canalicular dynamics, provides an important development for the further study of human hepatobiliary function and quantitative prediction of bile acid transport in the human hepatocyte (Kaschek et al., under review).

1.4. Significant achievements since last report

New findings have been made in the field of drug-induced cholestasis. Rather successful outcomes have been seen using 2Dsw cultures for 3 days where specific identification of drugs with cholestatic action can be analysed. Furthermore, similar results are obtained in 3D spheroid cultures cultivated for 2 weeks, and in addition the long term stable spheroid system can be used for analyses of mechanisms involved in drug induced cholestasis. A completely novel method for determination of drug induced cholestasis has been identified where measurement of bile canaliculi deformation (constriction or dilatation) and impairment of the Rhokinase (ROCK)/Myosin light chain kinase (MLCK) activity can be used with high specificity and specificity as evident from experiments using 19 different cholestatic drugs. The spheroid ring trial has been successful in transferring the spheroid protocol to

multiple labs as well as allowing groups to share their experiences and difficulties. The data generated at multiple sites is largely in agreement suggesting robust and reproducible outcomes in both culture formats. 3D spheroids were in general more sensitive than 2Dsw which however perform very well for detection of cholestatic compounds. It was also found that species differences in hepatotoxicity can be monitored in the 3D spheroid system and that 3D HepG2 models based on reporter constructs for monitoring drug toxicity mechanisms are useful. Following the hypothesis that DILI is the integrative effect of alterations tipping the balance from regenerative processes in the liver towards cell death, dynamic pathway models were established for TNFalpha-induced NFkappaB signalling (Oppelt et al.), IL-6-induced Signal Transducer Activator of Transcription (STAT3-signalling (Sobotta et al.; Jünger et al.) and HGF-induced proliferative responses (Vlasov et al.). First steps towards model integration were taken, thereby describing both pro-inflammatory and regenerative signalling in the liver and providing means for an integrative analysis. Cell population and single cell studies performed in HepG2 cells and primary mouse as well as human hepatocytes, showed early effects of DILI compounds on different signalling pathways, which affect cellular decisions such as proliferation and survival. Tools for the prediction of DILI were established by characterizing changes in the activation kinetics of the pathways and thereby identifying vulnerable nodes in the network. In the future, based on these developments targeted measurements in combination with our calibrated mathematical models can be used to identify DILI compounds. Insights into the mechanisms of alterations in pro-inflammatory and proliferative signalling upon drug exposure were gained. To understand different stress responses upon drug exposure, an Nrf2 stress response model was established. The model was capable of describing time-resolved data generated by microscopy and immunoblotting. A significant role of autophagy was identified. The predictive power of the model was confirmed and different responses of drugs were traced back to few changes in the reaction rates, thereby elucidating the mechanism of oxidative stress responses upon DILI compound exposure (Hiemstra et al.). Furthermore, human hepatobiliary function can be studied with our established Ordinary Differential Equation (ODE) based dynamic model that describes bile acid uptake, basolateral and canalicular export in human cells. The established mathematical model was validated by two prototypical cholestatic drugs and enables a quantitative prediction of bile acid transport in human hepatocytes. This provides an important development for future studies of human hepatobiliary function (Kaschek et al.).

1.5. Scientific and technical results/foregrounds of the project

Throughout the life-time of the project, several large ring trials have greatly facilitated collaborative working and integrated teamwork between EFPIA and Academia members. The output of these efforts has led to adoption of standardised protocols for a number of *in vitro* test systems. These have been underpinned by *a priori* selection of a common set of Training and Test compounds for use by members of the consortium. The introduction of Tier II spheroids for evaluation by the consortium provided dissemination of technical training and know-how on the preparation, and use of spheroids by EFPIA. Similarly, mitochondrial and drug metabolism ring-trials, and High-Content Analysis Working groups have led to the adoption and sharing of practices. During Workshop IV (January 2017), the output of work was presented along with Decision Trees on how and when improved and novel tests systems can be applied in Drug Discovery Research. These Decision trees offer a guide on which of the test systems and sequence of Test Systems can be practically employed from Hit-2-Lead, Lead Optimization and Pre-clinical Candidate selection. Notable complexities of immunogenicity of drugs prevent use of tests in *de novo* drug discovery of candidate drugs, but rather application for use in highly focused investigative studies where potential evidence of immunogenicity during clinical research or post marketing authorisation has been identified. Out-facing presentations on new results to the scientific community have facilitated dissemination.

1.6. Potential impact and main dissemination activities and exploitation of results

DILI is a leading cause of failed development of new candidate drugs due to toxicity, withdrawal of approved drugs from the market, and of restricted drug usage arising from cautionary labelling. Financial costs of hepatotoxicity are enormous for the pharmaceutical industry, when not only phenomena such as market withdrawal and lawsuits from affected patients are born in mind. The costs of discontinuation of the development process, approval delays and restrictions on initially foreseen indications must also be considered. This, together with the adverse impact of DILI on human health, constitutes a powerful reason to select drug candidates for development that have reduced propensity to cause DILI.

The current *in vitro* test systems used by pharmaceutical industry are poorly predictive of toxicological potential *in vivo*, in preclinical tests species and in man. Therefore, the joint undertaking among all stakeholders of the successful validation of *in vitro* test systems developed within the MIP-DILI will enable industry to:

- predict, as early as possible, whether an individual compound is likely to cause DILI in humans
- reduce the likelihood that drug development will fail due to DILI, and thereby increase the rate of success of drug development and increase industrial productivity
- Reduce the incidence of human ill health arising from DILI in clinical trials

Publications arising from the MIP-DILI consortium pertaining to recommended assays, positioning, methods, endpoints, cell types, statistical analysis and interpretation of hepatic safety information will be publically available on the MIP-DILI website when it is relaunched by UoL.

a. Improved health of European patients

As a frequent side effect of many drugs, DILI constitutes a significant threat to patient health and has an enormous economic impact on health care expenditures.

Severe DILI leading to liver failure, transplantation or death is a rare event, which typically is unpredictable and whose pathogenesis is poorly understood. Nevertheless, DILI represents an important health problem. Although the incidence of idiosyncratic DILI caused by **individual** approved drugs given at therapeutic doses is relatively low and estimated at 1 per 10 000 to 1 per 100 000 treated patients, many hundreds of licensed drugs may cause severe DILI in man. Therefore the cumulative DILI burden posed by all approved drugs is substantial. It has been estimated that every seventh case of acute hepatic failure is due to an adverse drug reaction and that DILI has become the leading cause for liver transplantation.

MIP-DILI has and is in the process of publishing and communicating our findings of successful implementation of a new predictive DILI test cascade based on the MIP-DILI project results. Upon Pharma adopting these recommendations this can therefore be expected to improve the health of European patients, once safer drugs have been developed and licensed. Indeed, there are numerous examples of the Pharma partners within MIP-DILI having already adopted many of the recommended assays.

b. Improved DILI management

To date, standard non-clinical toxicity studies are the cornerstone of prevention of hepatotoxicity in humans, although their predictive power for all hepatotoxic liabilities in man is unsatisfactory. In particular, it is evident that these studies are insufficient to enable reliable prediction of whether or not an individual candidate drug may cause DILI in the human population. They also do not provide mechanistic information, which may be of crucial value in determining clinical outcome (i.e. whether of or not liver failure might occur).

The MIP-DILI project has generated results that enhance:

- Accurate prediction of propensity to cause DILI, which is required to enable early deselection of high risk compounds
- Understanding of molecular and biological processes that contribute to DILI, and may in due course aid in prediction of at risk patients (i.e. personalised healthcare) and in design and evaluation of novel susceptibility biomarkers

c. New multidisciplinary development tools, new development paradigms in innovative pharmaceutical science.

Substantial scientific progress has been made over the course of the last >30 years in understanding the molecular and biological mechanisms by which DILI may occur, in animal and in man. Although our current knowledge remains incomplete, much has been learned and many useful new *in vitro* and *in vivo* model systems have been developed.

However, prior to MIP-DILI, there was no consensus within industry or the scientific community on whether any of these new approaches could enable selection of drugs with reduced propensity to cause DILI, if they were to be applied during drug discovery to aid compound selection. There was also no consensus on the approach that should be used to evaluate models for this such use, or on the key gaps in scientific knowledge and experimental capability that need to be tackled before an “ideal”

predictive DILI test cascade can be devised which will underpin innovative pharmaceutical science. MIP-DILI has provided a framework that will enable this challenge to be tackled. It is not considered likely that technology, which will be evaluated and developed, will fully solve the problem. However, the MIP-DILI project represented an essential first step, without which it will not be feasible to devise an appropriate new development paradigm.

1.7. Lessons learned and further opportunities for research

The IMI-EU initiative provided a framework by which to bring the Pharmaceutical Industry and Academia together to work pre-competitively. Through an over-arching and pre-competitive agreement, the questions industry faced could be openly shared and strategies devised to answer these questions. Within MIP DILI, Public Private Partnership provided a platform for industry to work towards greater alignment on the use of Test systems for the prediction and prevention of Drug-Induced Liver Injury (DILI) and optimization of their use.

Refinement on the use of existing and development of novel Test Systems was a key feature of the project. Importantly, much greater alignment on the use of terminology describing mechanisms of human DILI was achieved. Moreover, simple 2D screens whilst commonly described as poorly predictive of human DILI nevertheless have a broader utility for detecting cell-health as a first tier screen. These commonly applied simple high-throughput Test Systems by industry form common entry points for a structured three-tiered approach in the detection of chemical safety hazards that is defined by the MIP DILI Roadmap.

The MIP DILI Roadmap for the hazard identification and risk assessment of new chemical entities has enabled optimization of 2D and 3D (spheroids) and knowledge on how to deploy these test systems for improved and optimal use by industry. These improvements have been underpinned through the physiological and pharmacological characterisation of available model systems demonstrating opportunities to create a greater understanding of the mechanisms of DILI and chemical safety hazards at relatively low costs compared to alternatives such as Microphysiological Systems (MPS).

Consensus by the MIP DILI consortium acknowledges immune mediated human DILI cannot at this time be predicted *ab initio,* because of the dominance of human rather than chemical variables in idiosyncratic DILI. Nevertheless, a continuation of research in this field is strongly recommended. The consortium has also identified extra-hepatocellular signalling as being a major factor in human DILI. The role of extrahepatic signalling in liver injuries requires further knowledge on the science and technologies. Future work on bile duct hyperplasia and cholangiopathies is also strongly encouraged since translation of signals detected *in vivo* pre-clinically are not readily translated to clinic where mechanisms still remains poor understood and *in vitro* cell models are not available at this time.

Non-scientific learnings from MIP DILI point towards operational alignment and continuity of support by the Project Management office. Short-comings within MIP DILI placed excessively high demands on the Managing Entity and Deputy Coordinator throughout the life-time of the project. For this reason, our experience necessities a consideration of processes under the IMI-JU framework to facilitate mechanisms for the timely removal and replacement of entities that provide project critical functions to ensure optimal output on the overarching aims & objectives of a project.