Project full title:

Hepatic and Cardiac Toxicity Systems modelling

Project acronym:

HeCaTos

Collaborative project
HEALTH.2013.1.3.-1:
Modelling toxic response in case studies for predictive human safety assessment

FP7-HEALTH-2013-INNOVATION-1-602156-HeCaTos

Deliverable Report D14.2:

WP Planning and Contingency Reports

Work package 14

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Maastricht University (UM)

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Dissemination Level		
PU	Public	X
PP	Restricted to other programme participants (including the Commission Services)	
RE	Restricted to a group specified by the consortium (including the Commission Services)	
CO	Confidential, only for members of the consortium (including the Commission Services)	

Contributions to deliverable - Internal review procedure

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INTRODUCTION

HeCaToS aims at developing integrative *in silico* tools for predicting human liver and heart toxicity. The objective is to develop an integrated modeling framework, by combining advances in computational chemistry and systems toxicology, for modelling toxic perturbations in liver and heart across multiple scales. This framework will include vertical integrations of representations from drug(metabolite)-target interactions, through macromolecules/proteins, to (sub-)cellular functionalities and organ physiologies, and even the human whole-body level. In view of the importance of mitochondrial deregulations and of immunological dysfunctions associated with hepatic and cardiac drug-induced injuries, focus will be on these particular Adverse Outcome Pathways.

Models will be populated with data from innovative *in vitro* 3D liver and heart assays challenged with prototypical hepato- or cardiotoxicants; data will be generated by advanced molecular and functional analytical techniques retrieving information on key (sub-)cellular toxic evens. For validating perturbed AOPs *in vitro* in appropriate human investigations, case studies on patients with liver injuries or cardiomyopathies due to adverse drug effects, will be developed, and biopsies will be subjected to similar analyses. Existing ChEMBL and diXa data infrastructures will be advanced for data gathering, storing and integrated statistical analysis.

Model performance in toxicity prediction will be assessed by comparing *in silico* predictions with experimental results across a multitude of read-out parameters, which in turn will suggest additional experiments for further validating predictions. HeCaToS, organized as a private-public partnership, will generate major socioeconomic impact because it will develop better chemical safety tests leading to safer drugs, but also industrial chemicals, and cosmetics, thereby improving patient and consumer health, and sustaining EU's industrial competitiveness.

List of participants:

Participant no.	Participant organisation name	Country
1 (Coordinator)	Maastricht University (UM)	Netherlands
2	Hoffmann-La Roche (Roche)	Switzerland
3	InSphero	Switzerland
4	University Hospital La Fe (HULAFE)	Spain
5	ETH Zurich-Functional Genomics Center (ETH)	Switzerland
6	Imperial College London (ICL)	United Kingdom
7	LuxCel	Ireland
8	European Molecular Biology Laboratory-European Bioinformatics Institute (EMBL)	Germany
9	Genedata (GD)	Switzerland
10	Max Planck Society/Max Planck Institute for Molecular Genetics (MPIMG)	Germany
11	King's College London (KCL)	United Kingdom
12	Rheinisch-Westfaelische Technische Hochschule Aachen (RWTH)	Germany
13	MicroDiscovery (MD)	Germany
14	Optibrium Ltd (OPT)	United Kingdom

In a project aiming at forward-looking research several potential risks have to be considered.

For Overall Risk Management following agenda items will be discussed in Project Board meetings agendas:

- Identification of any risk on the project success;
- Evaluation the probability, cause, effect of the risks;
- Management of the risks: revision of relevance;
- Recovery plan: maximizing positive outcomes and minimizing negative outcomes of each risk.

The Project Management Team proactively manages these risks. It is felt that the level of risk foreseen is in line with the dimensions and objectives the HeCaToS Project, and that the composition of the consortium and the management structure provides the required flexibility to handle the reported risks.

The HeCaToS project is divided into the following interdependent major components (see Figure 1):

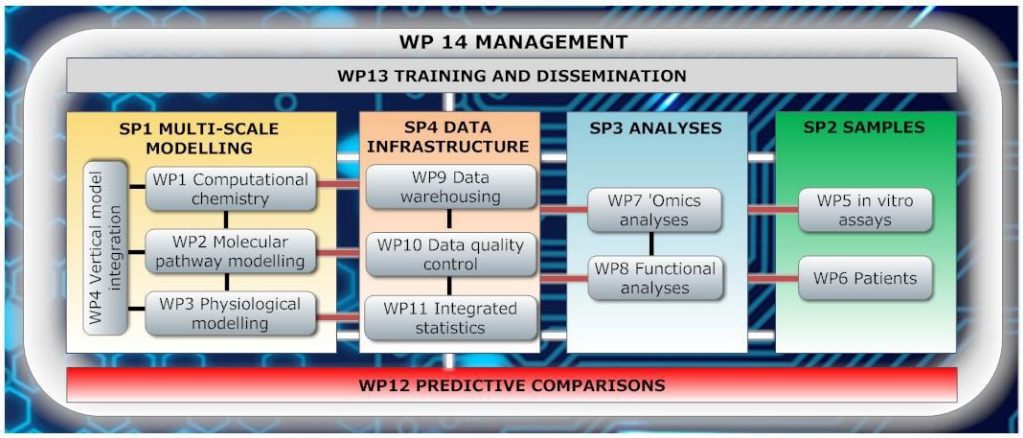

Figure 1: *Graphical presentation of the components showing their interdependencies*

Contingency planning within the HeCaToS project aims to prepare an organizational tool to respond well to possible risks and its potential impact in the day-to-day operation of the HeCaToS project. Such planning is a management tool, to respond effectively to three basic questions:

1. What is going to happen?
2. What are we going to do about it?
3. What can we do ahead of time to get prepared?

As the HECaToS has a number of partners across Europe steps have been taken to address the major risk areas:

- The project has world-class researchers and technologists in a well-balanced consortium;
- Multidisciplinary natures of the consortium. Effort has been redistributed between partners on the consortium in order to maximize the collaboration and minimize the risk of lack of integrating the results produced;
- The project has a management structure appropriate to a project of this size and complexity;
- There are a number of critical relationships between tasks and deliverables in the project. Project progress will be closely monitored and reported formally to the Commission at the end of each year. There will also be more frequent informal contacts between the coordinator and the Commission, as required by either party. In this way any problems will be identified at an early stage and remedial action will be taken and reported to the Commission.

RESULTS

SP1 Multi-scale Modelling - Contingency planning component

This sub-project aims to create discrete and continuous mathematical models of different granularities for heart and liver toxicity by integrating relevant components and reactions. It consists of the following Workpackages:

- **WP1 Computational chemistry** will develop novel models for interactions with macromolecules based on quantum and molecular mechanics, specific descriptors for atoms, fragments and molecules, and will link SAR, physicochemical and ADMET information from ChEMBL to the underlying mechanistic models, thereby applying GPU-AMD and sequence analysis methodology to enable in silico testing of chemical/target (protein/ DNA/membrane) interactions.

- **WP2 Molecular pathway modeling** will identify pivotal AOPs for liver and heart toxicity by network analyses, combining different levels of cellular information, leading to specific molecular and physiological components.

- **WP3 Physiological modelling** will advance current physiological liver and heart models for creating interfaces with molecular pathway models and computational chemistry models

- **WP4 Vertical model integration** will integrate different these sub-modules delivered by Task Forces 1.1-1.3, describing functional endpoints at different scales of biological organisation.

Table 1. Main risks and associated contingency plans of Multi-scale Modelling Component

Risk	Likelihood	Impact	Risk score	Contingency
SP1: Some computational modelling formalisms could be difficult or even impossible to merge	Mid	low	Mid	Continuously perform proof of concept studies for model integration across different scales
WP1: No clear correlation will appear between QM-calculated reactivity of metabolites and observed toxicity	Mid	Low	Low	Focus on empirical models of biological activities of metabolites to predict potential for metabolic bioactivation
WP1: Insufficient data available on Mitochondrial tox targets to permit Naïve Bayes models	Mid	Mid	Mid	Investigate alternative modelling strategy or endpoints
WP2: Insufficient data available to perform ODE based dynamical modeling of adverse outcome pathways	Mid	Mid	Mid	Resort to static statistical modeling approaches losing resolution and specificity

WP2: Adverse outcome pathways are strongly entangled and mathematical modeling becomes very complicated	Mid	Mid	Mid	Focus on aspects with clearly defined dependencies losing completeness of description
WP3: Insufficient data to constrain calcium dynamics, electrophysiology, contraction or mitochondria in human myocytes	Mid	Mid	Mid	Use existing non -species/ temperature/pathology specific models with corresponding caveats of relevance
WP3: Insufficient data to constrain calcium dynamics, electrophysiology, contraction or mitochondria in cardiac spheroids	Mid	Mid	Mid	Use existing non -species/ temperature/cell type specific models with corresponding caveats of relevance
WP3: Insufficient data to constrain model of human heart	high	low	low	Use existing local data sets on heart failure patients as a demonstrative framework
WP3: No clear link identified between protein dysfunction and cellular dysfunction	low	high	mid	Identify new tests that confirm model predictions and identify parameters that would best explain cellular dysfunction that will need to be measured
WP4: The model simulations will be highly sensitive to parameter values which are difficult to accurately extract from available data	high	low	low	- Run simulation sweeps in parallel to identify the system level perturbations which will provide data from which parameters can be extracted more exactly; - Where necessary existing data from experimental programs will be used to further test and constrain the parameter identification process

SP2 Samples - Contingency planning component

This sub-project will provide samples from experimental and patient studies to inform models in Sub-project 1. It consists of the following Workpackages:

- **WP5 *in vitro* 3D assays** will optimize 3D heart and liver spheroids allowing monitoring of affected cell type, subcellular compartment and type of response over time, and will challenge these assays by a range of prototypical liver and heart toxicants also studied in patients in WP6.

- **WP6 Patients** will generate liver or heart samples from well-selected drugtreated patients. For better exploring disease-related pathways, patients with relevant liver pathologies or cardio-myopathies will also be sample.

Table 2. Main risks and associated contingency plans Samples Component

Risk	Likelihood	Impact	Risk score	Contingency
WP5: *In vitro* treatment leads to unexpected effects, ie toxicity & loss of sample; *In vitro* sample yield may be very small	Mid	Mid	Mid	- Define solid treatment scheme with backup replicates; perform test runs for sample logistics; - Clearly define upfront on feasibility of planned experiments and sample preparation
WP6: Insufficient inclusion of patients treated with hepato- or cardiotoxic agents, developing a toxic phenotype	Mid	Mid	Mid	Collaborate with other university centres or large regional centres, or seek for international collaboration. Include sufficient patients at baseline, before getting treatment, taking into account a prevalence of 1-5 percent
WP6: Patients with toxic cardiomyopathy will not survive the follow-up	High	Mid	Mid	- Get blood samples at baseline and at short term follow up and include 120 patients to get long term follow-up in at least 40 surviving patients; - Get cardiac biopsies in patients with late cardiotoxicity (getting cardiac samples at baseline is ethically impossible). - Focus on late cardiotoxicity

SP3 Analyses - Contingency planning component

This sub-project will analyse *in vitro* and patient liver and heart samples delivered by WP2**.** It consists of the following Workpackages:

- ***WP7 'Omics analyses*** will obtain compartmentalized information on toxic metabolites and their macromolecular targets, by investigating through deep sequencing methods nuclear RNA, cytoplasmatic mono- and polyribopolysomal RNA, subcellular microRNA/mRNA complexes, mitochondrial RNA, and the epigenome of mitochondrial DNA; by quantitatively exploring the (phospho-)proteome at the global level, as well as of subcellular events by multiple LC-MS technologies; and by analysing intracellular distribution of 13C-labeled substrates (fluxomics), by GC-MS/NMR. Verification of critical gene functions will be performed by siRNA knockdown methodology.

- ***WP8 Functional analyses*** will focus on innovative analyses of mitochondrial and immunological hepatoxic and cardiotoxic endpoints, e.g. oxygen consumption, glucolytic flux, ROS formation analysis by ESR, inflammatory mediators such as IL-b and TNF (by i.e. multiplex assays, inflammosome arrays, etc), and cardiomyocyte QT prolongation using the xCELLigence platform.

Table 3. Main risks and associated contingency plans Analyses Component

Risk	Likelihood	Impact	Risk score	Contingency
WP7: GC-MS analysis will lack sensitivity for metabolomics/-fluxomic analysis of cellular models	Mid	Low	Low	Use Thermo benchtop orbitrap or Waters Synapt-G2 technology, coupled to LC
WP7: Individual sample amount is not sufficient for quant. proteomics analysis, particularly for phospho-peptide-enrichment of spheroid cultures	Mid	Low	Low	- Pooling of multiple samples to increase the amount of starting material; - Development of experimental protocols and data analysis methods to maximize sensitivity of applied approach; - Experimental determination of minimum input requirements via test materials
WP7: Individual DNA sample amount is not sufficient for MeDIP-seq analysis	Mid	High	Mid	- Pooling of multiple samples to increase the amount of starting material; switch from spheroid cultures to human tissue or *in vitro* assays; - Establish protocols for low DNA concentration experiments (e.g. Taiwo et al., Nature Protocols 7, 617–636, 2012)
WP8: Not all functional assay types intended for use will prove compatible with spheroid cultures or specific cell types used	Low	Med	Med	- Use an alternative model or cell type of equal biological relevance (maybe 2D fro cardio models; particularly for Xcellegence measurements); - Develop and determine maximum performance during protocol development; - Deploy contingency if/where required
WP8: Variability in cells or culture result in variability in metabolic activity of model	Low	Low	Low	- All data will be normalized to untreated controls; - Assess consistency during protocol development
WP8: Transfer of samples from source to test lab compromises sample activity	Low	Low	Low	- Temporarily transfer testing to facility where samples are being generated; - Assess sample viability post transfer during protocol development

SP4 Data Infrastructure - Contingency planning component

Sub-project 4: Data infrastructure will capture, assess quality, store and analyse these raw data and meta data. It consists of the following Workpackages:

- **WP9 Data warehousing** will capture processed data, by exploiting the data infrastructure of the diXa project, using the ISA-Tab compliant data entry for the deposition of metadata.

- **WP10 Data quality control** will perform quality control of 'omics data and its meta data, generated by Sub-project 3, for which partner Genedata will apply its range of relevant software tools.

- **WP11 Integrated statistics** will integrate the 'omics data with functional data into AOPs using sophisticated cross-'omics computational methods, pathway finding tools and relevant integrative analysis tools methods such as linear models and Bayesian networks.

Table 4. Main risks and associated contingency plans Data Infrastructure Component

Risk	Likelihood	Impact	Risk score	Contingency
WP9: 'Omics raw data comes in many different formats preventing an integrated approach	Mid	Low	Low	- Process data from many different 'omics platform which are managed by the available platforms (partners 8 and 9) already today; - Resources havebeen planned for the development of parsers for data types which are not yet covered; - Dedicated Tasks for provision of integrative platforms; Reports on data standards
WP9: Phenotypic data comes in non- standardized format	Mid	Mid	Mid	- Organize workshops to train all partners submitting data, in the use of the ISA-Tab tools, through which meta-data will be captured and stored in a standardized format and with a common nomenclature; - Dedicated tool box for data submission (ISA-Tab); - Reports on use cases and ontologies
WP10: Analysis pipelines not ready in time for all data types	Low	Mid	Low	- Pipelines established in the diXa project are continuously enhanced to cover additional data types; - Leverage on previous projects

WP10: Data quality not sufficient for downstream analyses	Low	High	Mid	- Centralized QC procedures ensure common quality standards and allow early feedback to experimenters; - Early feedback to data providers
WP11: Experimental design not ideal for integrated statistics	Low	High	Mid	Close interaction between WP11 and WP8 (partner 1 in both WPs)
WP11: 'omics' - based AOPs derived from *in vitro* analysis are hard to validate *in vivo*	Mid	High	Mid	- Close interaction between WP11 and WPs 5/6; joined task forces on compound selection and data analysis; - Analyse the same compounds *in vitro* and *in vivo*; focus on compounds with clinical relevance

Remaining Workpackages

- **Workpackage 12: Predictive comparisons** will assess model performance in toxicity prediction, by comparing *in silico* predictions from Sub-project 1 with experimental results from Sub-projects 2-4, by performing simulations on parameter optimization.

- **Workpackage 13:** Training and dissemination activities.

Table 5. Main risks and associated contingency plans Data Infrastructure Component

Risk	Likelihood	Impact	Risk score	Contingency
WP12: No sufficient or inadequate data for benchmarkin	Low	High	Low	- Close interaction among all project partners; - Focus on high quality data; - Generate missing data
WP12: Combined molecular model lacks prediction power	Low	Mid	Low	- Detailed analysis of information content of different model layers; - Focus on sub-models; - Focus on specific layers of information
WP12: Model parameter space is too large	Mid	Mid	Mid	- Parts of the technology is already based on Monte Carlo simulations; - Use simulation techniques and massive parallel computation
WP12: Specification and conduction of use cases is difficult	Mid	High	Low	- Close interaction among all project partners; - Start with single-compound use cases; use established AOPs

WP13: Lack of synergy between different aspects of multi-scale models	Mid	Mid	Mid	Will organize dedicate workshop to coordinate interactive discussion on methodology for multi-scale models

MANAGEMENT STRUCTURE AND PROCEDURES

Workpackage 14 Management covers the management and coordinated execution of the project, emphasizing the setting up and strict operation of procedures ensuring efficient communications, monitoring the smooth flow of critical processes, foreseeing difficulties and intervening in a timely and efficient manner.

The management structure and procedures of this project has been described (in further detail) in the HeCaToS Consortium Agreement (CA). In the CA (according to DESCA) is specified with respect to the HeCaToS project the relationship among the Parties, in particular concerning the organization of the work between the Parties, the management of the Project and the rights and obligations of the Parties concerning inter alia liability, Access Rights and dispute resolution, thereby obtaining full transparency and a good overview. The Consortium Agreement will be signed before the end of project negotiations.

The HeCaToS project is a collaboration between leading institutions in Europe. To foster the truly collaborative nature of the HeCaToS project, the management structure has been designed to effectively balance the competing priorities for flexibility for consortium Participants, with the needs for robust reporting and project delivery. To this end the project will be delivered according to the management structure set out in Figure 2.

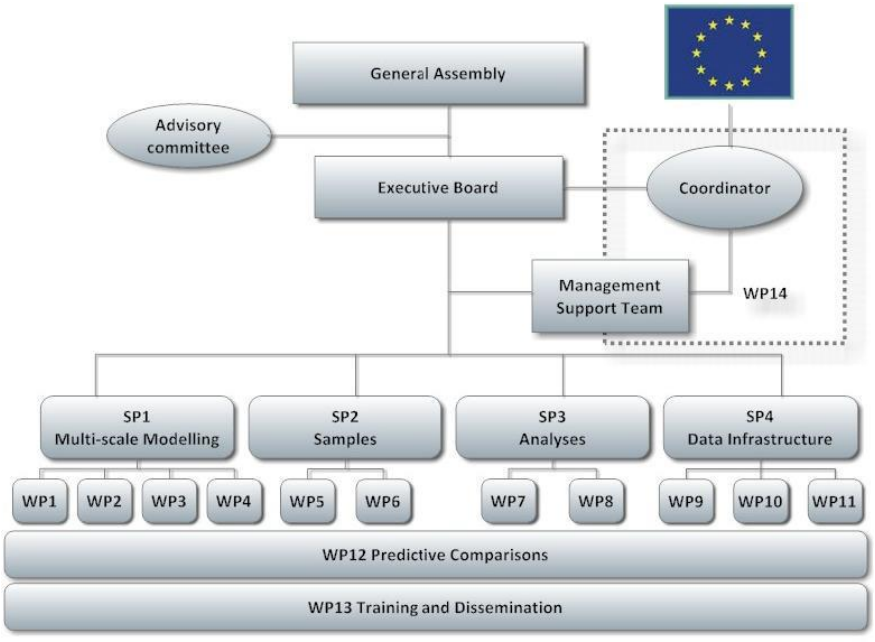

Figure 2: Organisational structure of the HeCaToS project

The management of the HeCaToS project has been organized as a separate Workpackage (WP14) which will bring together all organisational, administrative and communication activities necessary to ensure a smooth progress and completion of the project. The objectives of the Management structure and procedures will be to combine efficiency in planning and monitoring the project´s activities, flexibility in enabling effective intervention to avert problems and delays, partner accountability and transparency of decision making.

The HeCaToS Consortium has established formal procedures to stimulate co-operation through several types of meetings. We have at least every 3 month an Executive Board Meeting and once a year a full consortium meeting together with an external international Scientific Advisory Board.

The secure intranet-based communication system will be offered as a management tool for the HeCaToS project. It will be for internal use only, i.e. only partners of the HeCaToS project will have access to it. It will support the coordination of the partners' cooperation in order to ensure that results achieved by the different sub-projects and partners are comparable, and can be easily exchanged and consolidated.

We have installed and running the Xerox DocuShare® software. DocuShare© is one of the most flexible, easy-to-use content management platforms on the market today. It manages a wide range of paper and digital content and automates the daily project processes so partners can efficiently access, share and process project critical information 24 x 7. It contains all templates for Milestones, Deliverables, Meetings, papers, etc.

In DocuShare© Electronic Masters of Contracts and Management documents will be stored in the Management folder. The standard contractual documentation related to the project consists of the EC Contract plus Annexes (including Annex 1) and the Consortium Agreement. Additionally, there may be other contractual documents, such as, software licence agreements, other legal agreements between partners, or between the Consortium and external bodies (e.g. liaison agreements with collaborators). The original signed paper versions of all documents must be kept according to the institutional rules and regulations of the partner organizations. Electronic masters of contractual documents and version tracking and updating for amendments, are the responsibility of the Project Administrator. Maintaining repositories of Key Management Documentation (e.g., EPMB and PMB minutes, correspondence with the EC) is the responsibility of the Project Administrator.