

# HeCaToS Newsletter

Hepatic and Cardiac Toxicity Systems modelling (HeCaToS)

European Commission Research

and Innovation FP7 programme

Project number: 602156

Issue Number 4

April 2018

## In This Issue

- Editorial Note
- Metabolomics: new insights in the assessment of drug-induced liver injury (DILI)
- BioStudies database for HeCaToS
- Who's who?
- Recent publications

## Editorial Note

Welcome to the fourth issue of the HeCaToS Newsletter!

For the 4th consecutive year the HeCaToS Annual Consortium Meeting was successfully held at the Instituto de Investigación Sanitaria La Fe, in Valencia, Spain, in November 15-17, 2017. On the first day partners had the opportunity to attend parallel work package meetings and the poster pitch session featuring outstanding work from partners. Highlights of the day were the presentations on Proof of principle on cardiotoxicity and liver toxicity, demonstrating the impact of separate partners' work towards the goal of the HeCaToS project. On the next day the focus was given on the progress of separate work packages divided into four sub-projects, specifically "multi-scale modelling", "samples", "analysis" and "data infrastructure". The last day of the meeting a productive workshop on predictive comparisons based on *in silico* predictions compared with experimental data results was held incorporating anthracycline-induced cardiotoxicity as a case study. The meeting closed with the poster prizes awarded to Eirini Kouloura from Imperial College London and Raoul Sánchez from Instituto de Investigación Sanitaria La Fe.

The excellent organization of the meeting and the social events was coordinated by Prof. Jose Castell.



HeCaToS, is a private-public partnership funded by the EU Commission, aims at developing multi-scale integrative *in silico* tools and models for predicting human liver and heart toxicity, combining computational chemistry and systems toxicology.

# Metabolomics: new insights in the assessment of drug-induced liver injury (DILI)

By Prof. Dr. José V. Castell, Unit of Experimental Hepatology, University Hospital La Fe, – HULAFE–, Valencia, Spain

## Current research

Drug induced Liver injury is a matter of health concern. It occurs, not only at early clinical stages of drug testing, but also under normal usage of already approved drugs. Predicting the potential hepatotoxicity of a new drug constitutes a challenging exercise for this research group. Using human hepatocyte cultures and different advanced analytical approaches it is possible to assess hepatotoxicity by monitoring changes in functionality of cells and metabolic performance. Assays to measure drug metabolism and CYP bioactivation, oxidative stress biomarkers, bile acids production, and other relevant metabolic changes are established as routine assays at HULAFE Analytical Unit. The study of the more complex idiosyncratic DILI, by generating hepatocytes through direct reprogramming of somatic cells of DILI patients, constitutes a major research topic of this Unit. The use of metabolomic analysis in various fields (i.e., toxicometabolomics *in vitro* and in clinical samples, follow up of DILI patients, and assessing the quality of a liver graft prior transplantation) has become a powerful tool in our hands.

Within HeCaTos, the research group at HULAFE is currently involved in exploiting the potential of metabolomics for detection of hepatotoxicity of specific drugs in human liver spheroids and comparison with the *in vivo* metabolome changes observed in sera of patients to identify early and precise new biomarkers of DILI.

## Why Metabolomics?

Metabolomics is a forcefully emerging tool capable of analysing hundreds of metabolites in biological samples. As multiparametric measurement, it enables a comprehensive examination of the alterations occurring in cells or tissues upon exposure to a given drug. In combination with bioinformatic tools, metabolomics represent a breakthrough strategy for identifying early toxicity metabolic fingerprint, occurring in cells or patients' biofluids, which could serve as specific indicators of liver damage, and best describing what is really happening within the cells or tissues. DILI shares many features of other liver diseases and there is no specific test available for its unequivocal diagnosis. DILI is diagnosed in patients by exclusion criteria, i.e., after excluding other possible causes of liver injury, and this is basically due to the fact that it does not display specific features regarding the currently used clinical biomarkers.

## What are we working on?

Our group is currently working on the discovery of specific serum biomarkers of hepatotoxicity. The strategy combines the analysis of sera of DILI patients displaying the various phenotypes of the disease (hepatocellular damage, cholestatic or mixed-type), with the metabolic changes observed in 3D human hepatocyte cultures exposed to hepatotoxic drugs. Through a systematic search at the University Hospital La Fe (the largest reference hospital in a region of ca. 5 million inhabitants), patients unequivocally diagnosed with DILI are monitored along the time course of the disease and biological samples (serum and, occasionally, liver tissues) are systematically collected. More than 400 samples of 99 patients have been collected along the duration of the project.

The samples are analysed using LC-MS based untargeted metabolomics approaches to identify differences in metabolites between DILI patients and healthy controls, providing a dynamic snapshot of the metabolic profile. Then, bioinformatic analyses are used to group the patients according to the type of liver damage and to disclose the biomarkers specific of the injury type (hepatocellular, cholestatic or mixed).

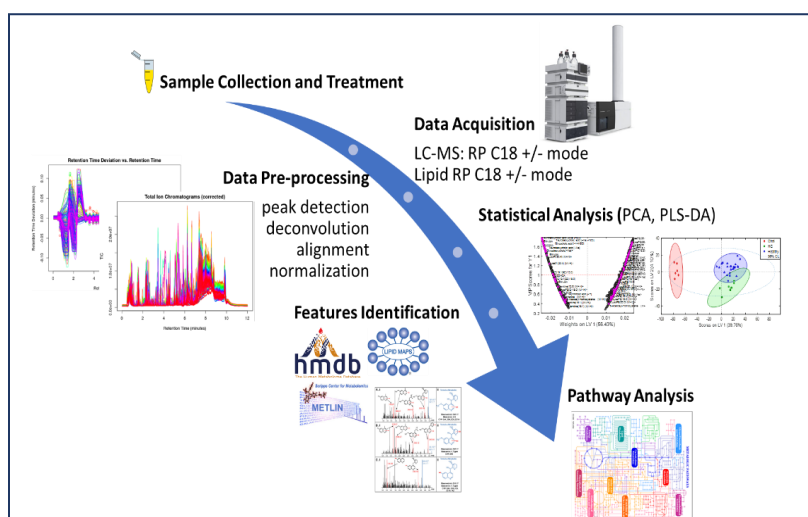


Figure 1: Metabolomic analysis workflow

Within HeCaToS, we optimized the handling and extraction of serum samples for the untargeted approach, where more than 1,500 features can be detected in a single analysis. Following the extraction, serum samples are fingerprinted by employing liquid chromatography coupled to high resolution mass spectrometry (LC-QTOF-MS), operating in full scan and MS/MS mode, in ESI(+) and ESI(-) ionization modes. Subsequently, the large data sets generated require extensive pre-processing steps (filtering, peak detection, alignment, scaling centring, normalization, transformation), which are performed by means of multiple software packages, prior to undergo any statistical analysis. Correlation and modelling studies employing Multivariate Data analysis tools are used to discover features of significant interest. The final, and perhaps most crucial step, is metabolite assignment, which typically is accomplished by comparison with spectral libraries of known metabolites (LipidMaps, METLIN, IIMDB, HMDB). Furthermore, to set the information into biological context, a pathway analysis of the annotated metabolites is performed using the Kyoto Encyclopedia of Genes and Genomes (KEGG)-ID to detect the pathways involved (Figure 1).

## Preliminary Results

We have envisaged and conducted an exploratory research based on untargeted mass spectrometry metabolomic analysis of serum samples from DILI patients, to determine whether a) the extent of necrosis and cholestasis can be accurately estimated and monitored, allowing patient stratification according to the type of injury; b) biomarkers can be identified (alone or in combination) to set up a predictive model for diagnosis and anticipation of the clinical evolution of DILI patients.

A selected number of serum samples were used for the model construction applying unsupervised clustering analysis, which led to the recognition of three main groups. Through PLSDA, the three classes were used to build a classification model, which could accurately categorize the degree of cholestasis (blue) or hepatocellular (red) damage; the mixed samples (green) appeared in between the two major groups (Figure 2). Unclassified samples (gray) loaded into the model fell into the different group areas, which could better define their nature. The metabolomic study revealed the presence of several biomarkers (of which ca. 20 positively identified, bile acids (BA) and lysophosphocholines (LysoPC)) responsible for the discrimination of the main hepatotoxic patterns (Figure 2).

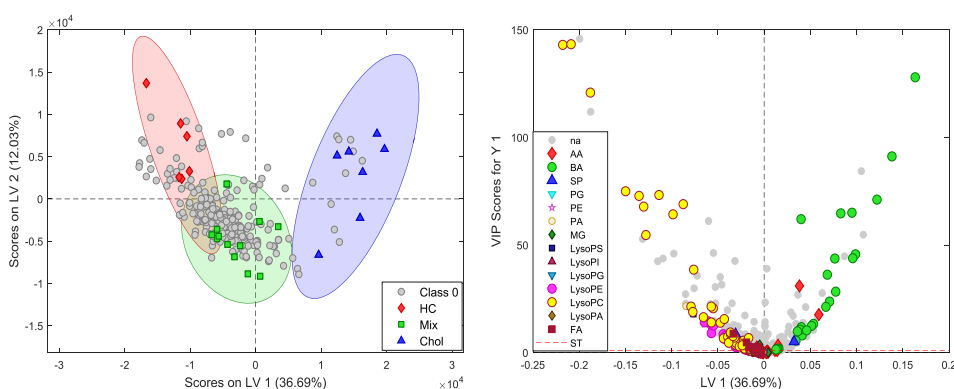


Figure 2: PLSDA scores and loadings plots of cholestatic (Chol), hepatocellular (HC), and mixed-type (Mix) serum samples. The loadings plot on the right shows mostly bile acids (green dots) and lysophosphocholines (yellow dots) to be responsible for the separation of cholestatic and hepatocellular pattern.

As shown in Figure 3, when the model is applied to unclassified samples the prediction probability of the disease class could be translated as the percentage of hepatocellular versus cholestatic pattern. Hence, the metabolomic analysis of sera from DILI patients has resulted in a much more powerful discriminating tool comparing to the currently used clinical biomarkers (typically ratio of ALP and ASP enzymes and bilirubin) and revealed a more comprehensive classification of DILI phenotypes.

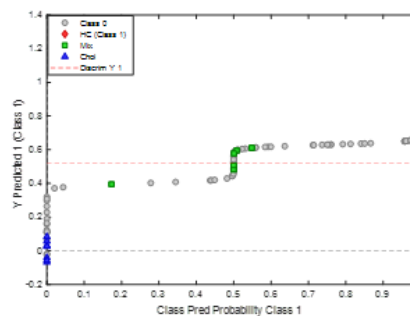
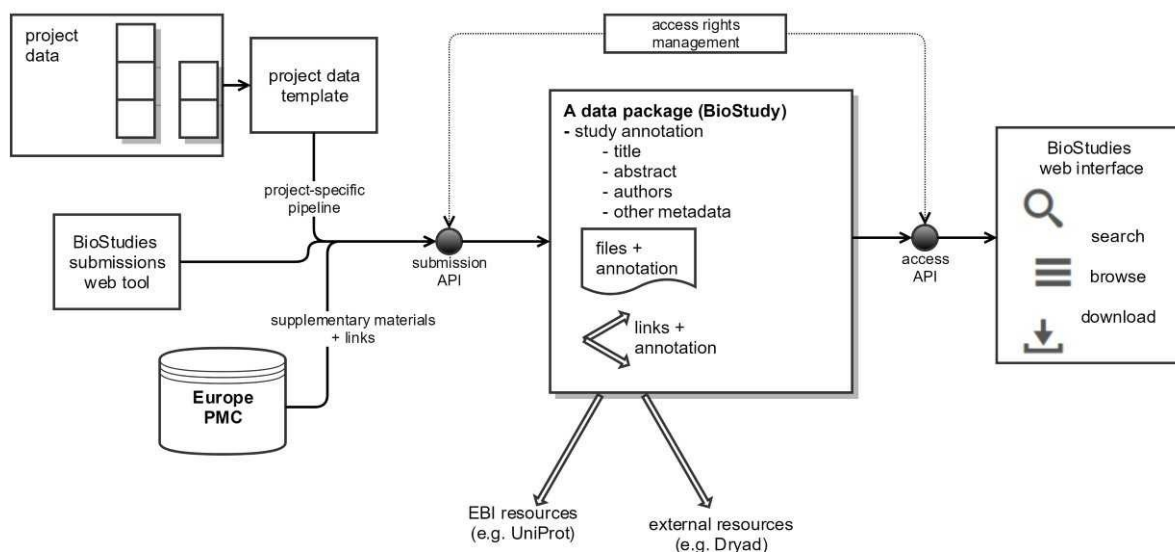


Figure 3: Class Prediction Probability of the samples to belong to the Class 1 (Hepatocellular damage)

## BioStudies database for HeCaToS

By Dr. Ugis Sarkans, Jasmine Minguet, EMBL-EBI

BioStudies ([www.ebi.ac.uk/biostudies](http://www.ebi.ac.uk/biostudies)) is a new database built and maintained at EMBL-EBI that organizes data from biological studies. Often a study is associated with a publication, however, when BioStudies is used to support the data management needs in a large project like HeCaToS, a study will encapsulate data from a meaningful unit of work, e.g., all proteomics data from doxorubicin treated samples. BioStudies offers a simple way to describe the study structure, and provides flexible data deposition tools and data access interfaces.



*The main concepts of BioStudies, and data submission routes*

Research in the life sciences is supported by a variety of specialised resources that house an increasingly large volume of biological research data. Additionally, data supporting specific studies in biology live as supplemental data linked to articles, or in several general-purpose repositories such as Dryad and Figshare. When a number of data types is generated in a large project, data management becomes an issue: data sharing and access across resources is difficult, and different data outputs can become dispersed and disconnected. The BioStudies database aims to address this concern. It holds high-level metadata descriptions of biological studies, as well as annotated data files. Links to the data in specialised life science databases at EBI or elsewhere can be included. This approach also simplifies the citation of related data in a meaningful way.

BioStudies is used in several ongoing projects like HeCaToS as a data management platform. Datasets are currently held privately, accessible only to project members, and will be released publicly according to the data management plans of those projects. Capturing project data early in a resource like BioStudies solves the problem of sustainable data management. We are also learning from the HeCaToS project partners how to organize data capture and distribution, and what tool support is needed. We provide the data management service to the consortium, and are also further developing the generic BioStudies data submission tool so that it could be easily used by the project partners.

For data access, the BioStudies web interface allows data browsing and searching both within an individual project and across the entire database. The search is supplemented by auto-completion and ontological expansion. A data filtering mechanism gives HeCaToS users an overview of the types of data available in the system, and facilitate finding relevant datasets. Data files can be downloaded either individually or by selecting a subset. For large studies, users can explore the data files by filtering on a keyword and sorting on one of the associated file annotations. An authentication and authorization mechanism allows management of access rights; in HeCaToS, a single read-only user is available for access to all the data in the project, although more sophisticated authorization policies could be set up if necessary.

## References

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## Who's who?



Prof. Dr. José V. Castell (PhD, MD, BPharm) is leader of the Experimental Hepatology Unit, an integrated research unit of the University of Valencia and the Hospital La Fe. During his long research career he has devoted continued efforts to understand the mechanisms of drug induced liver toxicity. Pioneer in the development of human hepatocyte cultures and its applications to drug development, he has been involved in over 25 EU projects and authored more than 360 papers describing the use of these models in DILI studies. He promoted the creation of a Hepatotoxicity Clinical Unit at the hospital La Fe and the Hepatocyte Transplantation Unit to treat liver metabolic diseases in children and liver metabolic failures in adults. In the framework of HeCaToS project, Dr. Castell is committed to examine metabolome changes in 3D hepatocytes exposed to known hepatotoxicants, and to correlate findings with the clinically observed new DILI biomarkers in sera.



Adrian Roth is Global Head of the Department of Drug Disposition and Safety at the Roche Innovation Centre in Basel, Switzerland and an Associate Professor at the University of Basel. His team is responsible for all *in vitro* activities addressing safety as well ADME questions supporting therapeutic modalities from small to large molecules and oligonucleotides across Roche's portfolio. The group continuously invests in establishing improved tools to experimentally predict and understand safety liabilities allowing for prioritization of drug candidates early on in development and de-risking of findings in later stage drug development programs up to clinical phases. This is done by use of human primary & stem cell based or complex models (3D, microfluidic, Organs on Chips) as well as technologies for analysis such as Mass-Spec, High content imaging, Genomics among others. Within HeCaToS, Adrian's team performs *in vitro* experiments using human 3D cell models.



Dr. Isabel Conde Amiel (MD) is medical specialist in Gastroenterology and Hepatology, trained at University Hospital La Fe and active member of the Spanish association for the study of liver. Within the HeCaToS project she is in care of the Hepatotoxicity Clinical Unit and is responsible for the diagnosis and recruitment of patients displaying drug-induced hepatic toxicity. She is responsible for the monitoring of DILI patients until recovery as well as for the collection of valuable human samples and clinical data from such patients along the course of the toxic event.

## Recent Project Publications

A number of publications since the previous issue of the HeCaToS Newsletter in November 2017 is listed below, demonstrating the progress of the scientific work and the high impact in the scientific community.

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Frantz, S. , Falcao-Pires, I. , Balligand, J. , Bauersachs, J. , Brutsaert, D. , Ciccarelli, M. , Dawson, D. , de Windt, L. J., Giacca, M. , Hamdani, N. , Hilfiker-Kleiner, D. , Hirsch, E. , Leite-Moreira, A. , Mayr, M. , Thum, T. , Tocchetti, C. G., van der Velden, J. , Varricchi, G. and Heymans, S. (2018). "The innate immune system in chronic cardiomyopathy: a European Society of Cardiology (ESC) scientific statement from the Working Group on Myocardial Function of the ESC" *Eur J Heart Fail* 20: 445-459.

Cordes, H., Thiel, C., Baier, V., Blank, L. M., & Kuepfer, L. (2018). "Integration of genome-scale metabolic networks into whole-body PBPK models shows phenotype-specific cases of drug-induced metabolic perturbation" *NPJ Systems Biology and Applications*, 4, 10.

Verdonschot, J. A. J., Hazebroek, M. R., Derks, K. W. J., Barandiarán Aizpurua, A., Merken, J. J., Wang, P., Bierau, J., van den Wijngaard, A., Schalla, S. M., Abdul Hamid, M. A., van Bilsen, M., van Empel, V. P. M., Knackstedt, C., Brunner-La Rocca, H.-P., Brunner, H. G., Krapels, I. P. C. and Heymans, S. R. B. (2018). "Titin cardiomyopathy leads to altered mitochondrial energetics, increased fibrosis and long-term life-threatening arrhythmias." *Eur Heart J* 39 (10): 864-873.

Thiel, C., Cordes, H., Fabbri, L., Aschmann, H. E., Baier, V., Smit, I., Atkinson, F., Blank, M. L., Kuepfer, L. (2017). "A Comparative Analysis of Drug-Induced Hepatotoxicity in Clinically Relevant Situations". *PLoS Computational Biology*, 13(2), e1005280.

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Fernandez-Chas, M., Curtis, M. J. and Niederer, S. A. (2018). "Mechanism of doxorubicin cardiotoxicity evaluated by integrating multiple molecular effects into a biophysical model." *Br J Pharmacol* 175(5): 763-781.

Thiel, C., Cordes, H., Baier, V., Blank, L. M. and Kuepfer, L. (2017). "Multiscale modeling reveals inhibitory and stimulatory effects of caffeine on acetaminophen-induced toxicity in humans." *CPT Pharmacometrics Syst Pharmacol* 6(2): 136-146.