

PRESENTATION TYPE: Symposium  
Secondary Pres Type - Proposal: Workshop  
IAT/ITS Designation: Innovations in Toxicological Sciences (ITS)  
CURRENT ENDORSERS

**TITLE: HIGH THROUGHPUT TRANSCRIPT PROFILING AND FUNCTIONAL ASSESSMENT: FROM SCREENING TO SYSTEMS BIOLOGY STRATEGIES FOR PERSONAL CHEMICAL SAFETY PREDICTIONS**

**ABSTRACT BODY:** High-throughput screening (HTS) assays are an important component of chemical safety evaluation programs carried out by a number of organizations. However, it is recognized that the assays do not sufficiently cover all potentially important pathways. In the last few years adaptation of gene expression profiling to high throughput formats is increasingly considered an attractive alternative to individual assays due to lower costs and the ability to measure essentially all pathways simultaneously. While microarrays have been used extensively in more focused lower throughput studies and comprise the bulk of large publically-available databases, technologies that can measure the targeted expression of the entire genome are emerging as attractive alternatives. New computational approaches are increasingly being used to move the field from using transcript profiles as hypothesis generation tools to accurately predicting effects. Functional genomics strategies that identify gene-chemical interactions in gene knockdown screens have proven valuable to validate predictions from transcript profiling and determine species-specific effects. These integrated high throughput genomics approaches will allow identification of relevant key events that are quantifiable in HT transcriptomic settings and predict cell and biological changes as well as human translational implications. This symposium highlights major advances in the field of using transcript profiling and functional genomics in a number of areas important in risk assessment. The first speaker will present recent results of a large-scale HT screen of 1000 chemicals in a human cell line allowing dose-response modeling on a massive scale for biological pathways. The second and third speakers will describe novel computational approaches utilizing both private and publically available databases to make predictions of molecular targets of chemicals and perturbations in gene networks that lead to toxicity. The last two talks will describe exciting work which identifies genetic modifiers of responses to chemicals allowing assessment of individual susceptibility to chemical injury. This symposium will be of wide interest to SOT members including scientists interested in the application of expression profiling and in vitro assays to regulatory decision making.

165 min total for the symposium

Chair Name: Chris Corton  
Chair Affiliation: EPA  
Chair Email: corton.chris@epa.gov

Co-Chair Name: Bob van de Water  
Co-Chair Affiliation: Leiden University  
Co-Chair Email: Bob Van de Water (b.water@CHEM.leidenUniv.nl)

**Introduction: Chris Corton**

**Presentation Title: Introduction to session**

Presentation Description: This short introduction will briefly outline the goals of the session. (5 min)

**Speaker 1: Imran Shah**

US-EPA

**Presentation Title: High Throughput Transcriptomics: From screening to pathways**

Presentation Description: The EPA ToxCast effort has screened thousands of chemicals across hundreds of high-throughput in vitro screening assays. The project is now leveraging high-throughput transcriptomic (HTTr) technologies to substantially expand its coverage of biological pathways. The first HTTr screen has measured the expression of 19,290 genes in MCF7 cells for more than 1,000 chemicals in concentration response format. We have developed a computational strategy to interpret the HTTr data in terms of pathway perturbations, the specificity of pathway perturbations and associated points of departure. A strategy for integrating these new transcriptomic technologies into high throughput toxicity testing will be presented and the challenges discussed. We believe HTTr technologies can be deployed in a tiered fashion to complement the existing suite of high-throughput in vitro screening assays to realize the vision of Toxicity Testing in the 21<sup>st</sup> Century. (30 min)

**Speaker 2: Chris Corton**

US-EPA

**Presentation Title: Identification of potential endocrine disruptors in compendia of gene expression profiles.**

Presentation Description: This talk will describe unique computational strategies to identify chemicals that act as potential endocrine disruptors in large compendia of gene expression profiles. Efforts are focused on building and testing gene expression biomarkers that can accurately predict molecular initiating events (MIE) or downstream key events (KE) in adverse outcome pathways (AOPs) with a focus on endocrine disruption. Gene expression profiles from chemically exposed cells are compared to the biomarkers using a correlation-based method which determines the significance of the overlapping gene expression and allows for prediction of modulation of that MIE/KE. We used predictive biomarkers that can accurately identify chemicals that modulate the activity of estrogen receptor or androgen receptor as well as the downstream key events of cell proliferation and oxidative stress (assessed indirectly through Nrf2 activation). Our screen identified many unique chemicals previously unknown to affect these pathways. Using the same methods, we can examine the effects of over 1500 genes either knocked down or overexpressed on the same endpoints to identify putative genetic modifiers. Our approach highlights the value of using transcript profiling to identify chemicals that could cause endocrine disruption. (30 min)

**Speaker 3: Jim Stevens**

## **Eli Lilly & Company**

### **Presentation Title: Reducing noise and boosting biological signal detection in large transcriptomic datasets.**

Presentation Description: High throughput transcriptomic data presented significant challenges computationally due to the dimensionality of the data and in assigning biological interpretations necessary to construct mechanism-based risk assessments. Classification methods, e.g. signatures, have been developed to allow read across from training sets of compounds to characterize risk for test compounds. Other methods focus on characterization of the underlying biological response pathways that are activated or repressed by exposure to xenobiotics. Among the latter methods, unsupervised approaches that organize large datasets into a modular format based on coalescent properties of biological systems have advantages both computationally by reducing the dimensionality of, e.g. from  $10^4$  genes to  $10^2$  modules, and for data interpretation since they elucidate the underlying modular structure of the biological systems of interest. We have used one such method, weighted gene co-expression network analysis (WGCNA), to create a modular representation of gene expression for liver and kidney transcriptomic data using DrugMatrix and TG-GATES data. We identify modules of co-expressed genes that represent biological pathways and illustrate how perturbation of the modules reveal important mechanistic details for individual compounds as well as more general adaptive responses of tissues to xenobiotic-induced toxicity. (30 min)

#### **Speaker 4: Todd R. Golub**

**Broad Institute of Harvard and MIT, Cambridge, Massachusetts, USA.**

[golub@broadinstitute.org](mailto:golub@broadinstitute.org)

### **Presentation Title: High-throughput identification of genotype-specific vulnerabilities to drug treatment.**

Presentation description: For further personalized medicine and safety large numbers of patient-derived cell types are required to identify genotype-specific vulnerabilities to chemical treatment. As a first step the systematic assessment of the susceptibility of hundreds of genetically characterized cell lines to thousands of chemicals allows the discovery of genotype-specific vulnerabilities to chemicals. We have established high throughput methods, named PRISM (Nat Biotechnol.34:419, 2016), to perform pooled screening of mixtures of cell lines by labelling each cell line with 24-nucleotide barcodes. Such screens allow the interrogation of hundreds of cell lines in pooled survival screens against thousands of human relevant compounds (Nat Medicine 23:405, 2017). Correlation of the susceptibility of the various cell lines to the diverse chemicals, allows the identification of novel genetic vulnerabilities of cell lines and hence the mechanistic understanding of specific genetic traits to drug treatment outcome. (30 min)

#### **Speaker 5: Bob van de Water**

**Leiden University**

### **Presentation Title: Functional genomics of cellular stress pathways: towards a personalized chemical safety assessment.**

Presentation Description: The activation of adaptive stress response pathways is a key event in chemical-induced tissue injury. Toxicogenomics has established the various key stress pathways and their downstream constituents that define cell repair and defence of different target tissues, including liver, kidney and heart. These involve the oxidative stress response pathway, the unfolded protein response pathway, DNA damage response and immune signalling response pathways. While the critical core regulators and transcription factors that drive these pathways have been well described, the overall signalling networks that control these networks and, likewise, adaptation to chemical insults, are largely unclear. We have integrated imaging-based quantitative phenotyping of adaptive stress pathway activation with large scale RNAi-based functional genomics to identify individual signalling molecules, kinases, phosphatases, ubiquitinases and transcription factors, that define the amplitude of oxidative stress response, unfolded protein response and cytokine immune signalling response. We have further assessed the role of these individual signalling components in the onset of cytotoxicity. Human whole genome sequencing data allows the assessment of the variation in the copy number variation and/or genetic polymorphisms in these individual signalling components. This will drive our understanding of the individual susceptibility to chemical injury and a further personalized chemical safety assessment. (30 min)

**Discussion and audience comments/questions (10 min)**