

# REDUCING ATTRITION THROUGH EARLY ASSESSMENT OF DRUG SAFETY

## HIGHLIGHTS FROM THE SOCIETY OF MEDICINES RESEARCH SYMPOSIUM, HELD ON MARCH 13<sup>TH</sup>, 2014 – NATIONAL HEART & LUNG INSTITUTE, KENSINGTON, LONDON, U.K.

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### SUMMARY

*The Society for Medicines Research Symposium, sponsored by Amsbio, Pfizer Neusentis and Takeda, was held at the National Heart and Lung Institute, Kensington, London, U.K. The meeting, organized by Wendy Alderton, Julie Holder, Ruth Lock and David Pryde, focused on reducing attrition through the early assessment of drug safety and brought*

*together a range of international experts from both academia and industry to present the new technologies and their applications in early drug safety assessment.*

**Key words:** Drug toxicity – Risk management – Bioinformatics – Engineering – Metabolomics – Toxicology

### DRUG TOXICITY

Drug toxicity remains one of the main causes of compound attrition in the drug development process. Compounds displaying organ, mechanism-based or off-target toxicity are just some of the safety issues that have contributed to drug development failures, and the need for robust, early drug safety screening in an integrative manner is clear. In the last decade, an increased understanding of the molecular basis for some of these toxicities and the advancement of technologies designed to screen them has improved options for screening out potential compound liabilities earlier in the research phase. Advances include the use of human induced pluripotent stem (iPS) cells and miniaturized mimics of human organs. New markers of toxicity are being investigated at the RNA level using metabolomics and through application of bioinformatic approaches. In addition, technologies have been developed to gather data on broad compound promiscuity at the protein level, using panels of off-target activity as markers of general promiscuity.

### RISK MANAGEMENT OF MEDICINES: THE NEED FOR EARLY TOXICITY TESTING

Professor Frank Bonner, Chief Executive of SC4SM, delivered the opening lecture on “Risk management of medicines: the need for early toxicity testing”, where he gave a background perspective into

why the pharmaceutical industry is focusing on reducing drug attrition and what the industry is using to address this, as successes in drug development are low and are currently declining against a backdrop of decreased revenues, profitability and return on investment. He shared some slides on overall drug attrition and showed that 20% of drug failures between 1991 and 2000 were due to toxicology (1) (Fig. 1). The conclusion from his introductory section was that even modest improvement in drug attrition will result in cost savings for the pharmaceutical industry.

He then started to focus in on the challenges faced by industry on the ability to translate across experimental models and species, and the need to model a wide spectrum of genetic and epigenetic factors, which may not be supplied by a “one model fits all” approach. There was also a need to continue to identify opportunities to exploit novel technologies, highlighting the talks in the program, which could address these deficits at the drug discovery/development interface where there is the greatest area of opportunity for the impact of these novel strategies.

Professor Bonner then focused on the liver as an example, and described the complex organ pathology and the variety of cell types (> 15 cell types) and the specific regions that are responsible for the toxicological endpoints. To recapitulate this complex tissue functionality in any one model would be difficult, but he stressed that any model developed should have fit-for-purpose functionality. The model would need to recapitulate multiple metabolic processes and have the ability to respond to a wide variety of compounds and to model both acute and chronic effects of compounds. A one-size

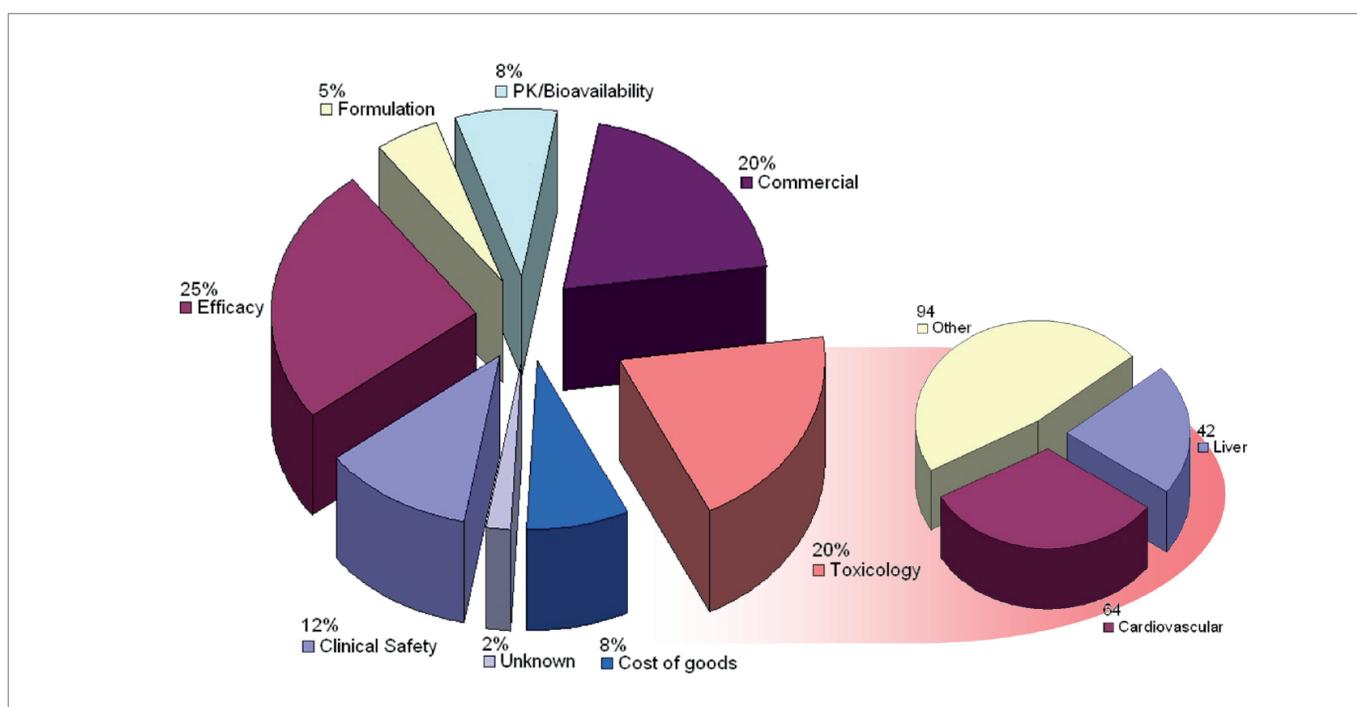
model may not fit all, and there is increasing awareness that there will be a need to capitalize on a number of models to build an integrated paradigm. The talk also touched on emerging 3D culture models as conferring a significant incremental improvement, which could transform the predictability of pharma to identify successful molecules.

### EARLY USE OF PHARMACOLOGICAL PROMISCUITY INDICES TO SELECT THE BEST COMPOUNDS

Dr. Gareth Waldron (Pfizer Neusentis) gave a talk entitled “Early use of pharmacological promiscuity indices to select the best compounds”, in which he explored the use of off-target promiscuity panels to inform potentially broader compound toxicity.

He started by describing adverse events (AEs) as a continuum of effects from the most common predictable dose-dependent drug-induced side effects, through non-predictable idiosyncratic findings, to long-term adaptive changes and delayed effects, including carcinogenicity.

In setting out to improve the industry’s ability to predict for off-target-driven toxicity, four major pharmaceutical companies — AstraZeneca, GlaxoSmithKline, Novartis and Pfizer— shared their own individual approaches to screening for off-target or secondary pharmacology, as compounds were progressed through the safety screening funnel. They found that 19 targets were commonly used across all companies in their screening panels, with a further 25 targets used in 3 of the 4 companies. This composite list of 44 targets became the minimal screening panel comprising: G-protein coupled



**Figure 1.** Drug failure to toxicology. Adapted from Kola, I., Landis, J. *Can the pharmaceutical industry reduce attrition rates?* Nat Rev Drug Discov 2004, 3(8): 711-

receptors (GPCRs), kinases, enzymes, nuclear hormone receptors, ion channels and transporters (2). It was noted that the reliable translation of some of the targets of this panel, from binding through to functional and even clinical effects, was quite variable; whereas hERG activity in vivo could be well predicted from a robust binding assay built on years of experience, and thousands of data points, reliably predicting suicidality driven by cannabinoid receptor CB<sub>1</sub> binding activity or valvulopathy driven by 5-HT<sub>2B</sub> receptor affinity are very different scenarios. Caution was urged in relation to the translation of binding to functional data and in comparing assays of different design, and even endpoints.

Some easily measurable indicators of broad compound promiscuity were then discussed, including the Pfizer “promiscuity panel”, a low-cost and rapidly accessible panel of 15 targets which were a good indicator of potential compound toxicity, with activity at two or more members of this panel bringing a five-fold increase in the likelihood of seeing in vivo toxicity. Activity within the panel was also seen to correlate extremely well with a much broader off-target panel screen. Other markers of in vitro off-target promiscuity also included high lipophilicity (logP) and low polar surface area (PSA), now enshrined in the Pfizer 3/75 rule of targeting ClogP < 3 and PSA > 75 as a means of increasing the probability of seeing a non-toxic compound in chronic rodent in vivo toxicology studies.

The importance of dose and the primary pharmacological potency of the compound cannot be overstated, and indeed, it is often the mitigating factor for compounds that lack toxicity but fall outside of “ideal” compound parameters.

Pfizer is now using a composite approach of taking a compound's structure, its physicochemistry, its “promiscuity panel” profile and its hERG affinity to derive a Compound Safety Evaluator (CSE) score as an indicator of potential toxicity. This is a simple means to focus the design team on potential problems at a stage of the project when alternative compound structures can be brought forward to mitigate potential future problems when compounds are eventually taken in vivo.

Refinements are now being made in which the promiscuity panel has been adjusted for the acid chemotype to improve its performance against this chemical class. Analyses are also being performed for kinases that could inform for in vivo hemodynamic effects, and a safety/pharmacology alerter is being used to guide project teams on the most effective way to use their promiscuity data at an earlier stage.

The lecture closed with a reminder of the power of using promiscuity panel data and its analysis to predict for broader compound toxicity, and the future value of such data in beginning to inform the in vivo translation of such in vitro data.

## REDUCING ATTRITION VIA BIOINFORMATICS APPROACHES IN SAFETY AND TOXICOLOGY

Dr. Samiul Hasan of GlaxoSmithKline gave a talk entitled “Reducing attrition via bioinformatics approaches in safety and toxicology”, based on the use of evolutionary biology to make informed decisions on animal model choices for safety and toxicology studies early in the drug discovery/drug development continuum. This has a positive impact on the 3R's principle to reduce the number of animals used

in pharmaceutical research and development when the genomic variation, gene expression and pharmacokinetics are better understood in the relevant animal model and can lead to a better understanding of the translatability of the data obtained to man.

Dr. Hasan described the work that his group has done to assist in the publication of beagle dog and minipig genomic sequences (3), and these have proved to be of scientific utility, as the only available complete sequences deposited in the databases were from a boxer dog or a duroc pig (not commonly used toxicological animal models), increasing the genomic coverage in the case of the dog by 4- to 6-fold and in the pig by 79-fold. (The minipig and the beagle genome sequences are deposited in GenBank/DBJ/EMBL).

The availability of these data has allowed scientists to question: “Is my gene present in minipigs? What is the sequence of my gene in minipigs? Is the sequence well conserved? How many copies of my gene are in a minipig -1:1 or duplicated? Is my human gene a pseudo-gene in minipigs? Has my gene gone through positive selection/functional divergence in the minipig?”.

Dr. Hasan then described a tool available on EMBL, called ADME Sarfari (<https://www.ebi.ac.uk/chembl/admesarfari/>), which is an ADME predictor. Instructions on how to use the database were given in the lecture and how to generate clearance, C<sub>max</sub>, bioavailability, half-life, T<sub>max</sub> and volume of distribution for chemical structures based on data generated in multiple species for comparable compounds.

In conclusion, Dr. Hasan stated that ortholog calling remains a difficult area to work in, as there are complex gene families and missing data, as well as large gaps in using phylogenetic data analysis to drive safety projects, and that this is an area which could be addressed within the scientific community. The ADME Sarfari database described is a novel interface that reuses data from existing public resources to enhance animal model studies. It would be of interest to add tissue-specific protein expression from other model organisms when such data become available.

## ORGANS-ON-CHIPS

Dr. Tony Bahinski described the research of the Advanced Technology Group at the Wyss Institute for Biologically Inspired Engineering at Harvard University, who are developing engineered microchips containing living cells combined with mechanical activation and dynamic flow to reconstitute organ-level functions for application to the evaluation of preclinical safety and efficacy (4). These “organs-on-chips” containing functional human cells organized within physiological microenvironments offer potential capabilities to generate relevant, predictive human data during critical stages of the drug discovery process, which will help drive key decisions to prioritize drug candidates for clinical development. There are currently projects to apply this biomimetic microsystem approach to the lung, small airways, heart, bone marrow, liver, kidney, skin, gut, blood-brain barrier and eye. This presentation focused on the lung-, heart- and gut-on-a-chip models in detail.

The lung-on-a-chip model is engineered within a 100 micron long silicon rubber chamber, which is flexible, clear and biocompatible, and allows mechanical activation in three dimensions to reproduce rhythmic breathing movements (5). The chip contains parallel

microfluidic channels separated by a porous membrane, with human lung alveolar cells on one side and human lung capillary cells as the vascular channel on the other. Air flows above the alveolar cells, while the lower side is exposed to flowing nutrient medium containing human white blood cells. Mechanical stretching forces are applied to both cell layers to mimic physiological breathing motions. Lung inflammation can be recreated in this system by introducing TNF- $\alpha$  (50 ng/mL) or bacteria into the vascular channel. In the inflamed state, fluorescently labeled neutrophils can be observed as sticking to the vasculature and engulfing the bacteria. This can be suppressed by the addition of 20  $\mu$ M dexamethasone. The lung-on-a-chip model also has been modified to model complex human diseases, such as chemotherapy-induced pulmonary edema, by demonstrating vascular leakage and fluid infiltration.

Spontaneously beating myocytes are used in the heart-on-a-chip model, which exploits muscular thin film technology—biohybrid constructs of an engineered, anisotropic ventricular myocardium on an elastomeric thin film to measure contractility and quantification of action potential propagation (6). An alginate hydrogel-based membrane allows for long-term treatment for chronic toxicity applications.

The gut-on-a-chip model is constructed in a 300 micron channel that can mimic slow peristaltic motions, and Caco-2 intestinal cells form crypt and villi-like structures. The flow and stretch motion leads to increased cytochrome P450 3A4 (CYP3A4) expression and the model can also be co-cultured with intestinal bacteria, thereby mimicking the environment of the intestine. Interestingly, this co-culturing leads to improved barrier function and bacterial colonies form in the crypts. This chip has potential for use in drug transport studies.

Microdevices, such as the organ-on-a-chip model described here that reconstitutes tissue–tissue interfaces critical to organ function, may in the future expand the capabilities of cell culture models and provide low-cost alternatives to animal and clinical studies for drug screening and toxicology applications.

### CIRCULATING miRNAs AS MARKERS OF TOXICOLOGY INJURY

Dr. Chris Goldring described ongoing studies into identifying serum microRNA (miRNA) markers of drug-induced liver injury. The development of stable, rapidly quantifiable, tissue-specific and minimally invasive biomarkers for drug-induced liver injury remains a primary aim in clinical settings to detect and monitor the level of a pathogenic insult to the liver. Furthermore, early identification of drug-induced liver injury could facilitate patient-individualized treatment strategies. Paracetamol overdose is the leading cause of drug-induced liver injury, causing more than 500 deaths and 56,000 visits to the emergency room in the U.K. a year, and it is therefore the focus of the studies described. MiRNAs are an abundant class of endogenous, small, 18-25 nucleotide non-coding RNAs which regulate gene transcription at the transcriptional or post-transcriptional level. Each species of miRNA is believed to target hundreds of different mRNAs, and an mRNA may be targeted by different miRNAs. Although some miRNAs are widely expressed in a number of tissues, certain miRNAs appear to be highly organ specific. Liver tissue expresses a number of distinct miRNAs, including miR-122, the most abundant hepatic miRNA, which is estimated to make up 70% of the total adult hepatic miRNA. MiR-122 has been shown to be under the

transcriptional control of several hepatic nuclear factors. The potential of miR-122 and miR-192 as blood-based biomarkers was demonstrated recently in a mouse model of paracetamol-induced hepatotoxicity, which showed that miR-122 was detectable earlier than the standard liver marker alanine aminotransferase (ALT) and with greater sensitivity and less variability (7).

In a clinical study in a total of 53 adult patients admitted to the Royal Infirmary of Edinburgh with acute liver injury secondary to paracetamol ingestion, it was demonstrated that miR-122 and miR-192 inform on human drug-induced liver injury and were significantly higher in the serum of paracetamol-poisoned patients than in controls or those with kidney disease (8). Circulating miR-122 levels correlated with ALT levels; however, the circulating half-life of miR-122 was found to be in the region of 24 hours compared to that for ALT, which was 48 hours. In a second clinical trial in 129 patients, it was shown that early measurement of serum miR-122 (< 24 hours after overdose) could distinguish patients who subsequently develop liver injury (9). The study found that plasma miR-122 levels were raised at first hospital presentation in some paracetamol-poisoned patients, while ALT was at “normal” levels and the early measurement of serum miR-122 correlated significantly with peak serum ALT activity. A study to define the normal range of miR-122 in the serum of a healthy human population in 130 healthy volunteers followed for 3 days demonstrated stable miR-122 levels, with no significant variation (coefficient of variation < 6%) across time and no difference across different age and male/female groups. Finally, a case study was described in a 25-year-old male who presented 4.5 hours after he had taken a single overdose of 35 g of paracetamol. Although miR-122 was significantly raised at presentation, the standard liver markers were normal and he was therefore discharged. However, he then presented 43 hours with vomiting and lethargy and very high ALT levels. In this case, miR-122 had correctly identified a life-threatening hepatotoxicity missed by current tests. Several emerging technologies have recently been described which may provide fast, sensitive and clinically robust methods for miRNA measurement, and therefore miR-122 represents a potentially sensitive and early biomarker of clinical paracetamol hepatotoxicity.

### USE OF METABOLOMICS TO ASSESS DRUG TOXICITY DURING PRECLINICAL RESEARCH

Dr. Gina Montoya of BASF SE and metanomics GmbH presented data on the development of a metabolomics database — MetaMap<sup>®</sup>Tox—, which is the largest metabolomics in vivo reference database worldwide. It houses metabolite profiles from rat plasma and comprehensive toxicological and pharmacological data. More than 500 chemical entities (chemicals, agrochemicals and pharmaceuticals) were thoroughly tested in vivo (28-day repeat-dose toxicity studies in Wistar rats) to generate and validate more than 100 metabolomic fingerprints of different toxicological modes of action (MoAs) (10). These MoAs cover several target organs, including the liver, kidney, thyroid, ovaries, adrenals, testis, bone marrow and the endocrine system. MoA profiles include microsomal enzyme induction, peroxisome proliferation, liver cell necrosis, steroid synthesis inhibition, primary and secondary hypothyroidism and many others. In-house evaluation of MetaMap<sup>®</sup>Tox predictivity against actual histopathological outcome showed an overall 83% success rate, with evaluation still ongoing.

Dr. Montoya went on to describe how by utilizing the MetaMap®Tox database alongside preclinical in vivo metabolite profiling for a new chemical entity (NCE), a “pattern ranking” and “profile comparison” can be generated, which allows you to compare the similarity of test compound-induced changes to known toxicity patterns, and also to get a statistical correlation ranking for an MoA based on biological similarity. Together, these data allow for a faster and better assessment of toxicological profiles of NCEs.

A series of case examples were presented whereby metabolomics was used to predict thyroid toxicology and how it was possible to distinguish between different MoAs resulting in hypothyroidism (11). Concluding, results confirmed that metabolome data can be used to predict toxicological effects in rats. The often deeper mechanistic insight into the observed effects gained within the same single study saves additional mechanistic follow-up studies in vivo, and hence enables a reduction in animal use. MetaMap®Tox is routinely used at BASF, contributing to faster decision making in development through its predictability, understanding of the toxicological mechanism and the ability to translate to clinical use.

#### HUMAN PLURIPOTENT STEM (IPS) CELLS EARLY IN DRUG DISCOVERY TO INFORM ON TOXICOLOGICAL HAZARD

Professor Marc Peschanski gave a talk on human pluripotent stem (iPS) cells based on the I-Stem mission of using iPS cells as an enabling technology in all therapeutic aspects of biomedical research.

The lecture focused on the use of iPS cells to both characterize and predict myotoxicity of compounds from the statin class of HMG-CoA reductase inhibitors, a side effect that led to withdrawal from the market of cerivastatin in 2001. iPS cells can be obtained in large quantity from human donor biopsy samples or from embryos. Given the observation that proliferative precursor cells are more sensitive to statins than differentiated muscle cells, it was proposed that the impaired muscle capacity induced by some statins may be due to a decrease in the regeneration capacity of muscle stem cells.

Initial results showed that the acute toxicity pattern of cell number reduction after single doses of statins was similar in both myoblasts and muscle progenitor cells. Repeat dosing demonstrated three patterns of effect, depending on dose; low doses of < 1 µM showed a delayed low toxicity cytostatic effect followed by adaptation, intermediate doses of ~1 µM showed both early and chronic cytostatic effects, and the highest dose tested of 2.5 µM showed early acute cell mortality which then persisted. The cytostatic effects for the low and intermediate doses were reversible upon cessation of drug treatment. These data suggested that different mechanisms of effect can be produced, depending on the dose and the duration of compound treatment.

Biomarkers of statin toxicity were then sought using several complementary approaches. Receptor binding domain technology was used to create a panel profile of nutrient transporters in response to statins at different concentrations and duration of treatment. Affymetrix gene expression arrays were then used to generate patterns of response to statin treatment, from which hypotheses of the pathways modulated by statin toxicity were obtained in collabora-

tion with the Karolinska Institute. For example, the biggest gene changes were seen at the higher acute doses tested, affecting pathways involved in cholesterol biosynthesis, oxidative phosphorylation and mitochondrial dysfunction, among others. Chronic dosing affected different pathways, which included the immune response, cell adhesion and skeletal and muscle pathways. From this analysis, several specific biochemical targets were then proposed as affecting the muscle cell condition in response to statin treatment, which included atrogen-1 as a marker of atrophy.

This approach of using in vitro methods to assess compound toxicity and then functional genomics to identify toxicity mechanisms could be applied broadly to the development of predictive biomarkers.

#### DISCLOSURES

W. Alderton, J.C. Holder, R. Lock and D. Pryde are paid employees of their respective companies. All authors are SMR Committee members for which no remuneration is paid.

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