Drug News Perspect 19(10), December 2006

Available on the web at: www.prous.com/journals

MEETING REPORTS

Highlights of the Society for Medicines Research Symposium held June 15, 2006, in Harlow, United Kingdom.

Translational Sciences—Turning Drug-like Molecules into Medicines

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Summary

On June 15, 2006, the Society for Medicines Research held a one-day meeting in Harlow, United Kingdom, entitled *Translational Sciences—Turning Drug-like Molecules into Medicines*. The meeting brought together speakers from Europe representing the pharmaceutical industry and provided an overview on some of the latest approaches in a range of areas such as predictive toxicology, translational biology, *in vitro–in vivo* extrapolation, pharmacokinetic/pharmacodynamic modeling, and the use of biomarkers and surrogate endpoints.

Dr. Colin Fish (GlaxoSmithKline, Welwyn, UK) gave the opening presentation which was entitled *Current Approaches to Predictive Toxicology*. He began by reminding the audience that the preclinical studies conducted on any new pharmaceutical agent are all intended to be "predictive." The aim of dosing animals with high doses of compounds is to identify potential hazards and characterize them in terms of dose–response, exposure–response, species selectivity, reversibility and the availability of early markers for use in man. This allows an informed assessment of any risks before administration of the new agent to humans, primarily through a thorough understanding of the dose (or exposure) margin between the anticipated therapeutic dose and the toxic dose. However, it is still not uncommon for "unexpected" side effects to occur in clinical studies, or for new hazards to be identified in longer-term animal studies being conducted while early human trials are underway. In extreme cases, these unexpected side effects may not show themselves until the drug is already on the market, and can lead the drug being withdrawn. Avoiding this late-stage attrition would not only bring financial benefits to pharmaceutical companies, but would also minimize the risks to human volunteers and patients. Dr. Fish then went on to describe potential advances in methodology that could help to give earlier predictions of what might happen in man or in longer term animal studies. The commonest organ systems which give rise to safety-related drug attrition are the liver and the cardiovascular system. Importantly, it has also been shown[1] that the highest incidence of overall concordance between animal studies and human toxicities is seen for the cardiovascular, gastrointestinal and hematological effects of drugs. The presentation therefore focused on some advances in the methods of evaluating cardiac and hepatic liabilities, utilizing a tiered approach from *in silico* to *in vitro* and *in vivo* studies.

Structural alerts form an important first step in the assessment of a compound's liabilities. Typically, this *in silico* approach can be used to identify potential DNA-reactive functional groups within a drug, allowing an informed prediction of the potential for causing genotoxicities. Other *in silico* approaches that can be used

early in the drug design phase, such as pharmacophore comparison, may help to predict the potential for a drug to interact with hERG and thus produce adverse cardiovascular effects. However, interaction with hERG represents only part of the story in terms of predicting the potential to cause prolongation of the QTc interval in the electrocardiogram and arrhythmias in humans. Both *in silico* and simple *in vitro* studies (e.g., dofetilide binding or single cell electrophysiology) on their own are insufficient predictors for this liability. The isolated, paced Langendorff-perfused female rabbit heart model (Screenit system) offers an improved *in vitro* assessment of the potential to cause ventricular arrhythmia.[2] This *in vitro* model provides detailed information on the overall profile of drug-induced electrophysiological effects and allows drugs to be classified based on their action potential morphology and conduction properties. Dog telemetry is still the standard *in vivo* study used to assess cardiovascular liability.

The liver is the first point of concentration of a drug following oral absorption, and drug-related idiosyncratic toxicity is still one of the major causes of acute liver failure cases in man. Dr. Fish described the recent advances in cell health assays and the increased utilization of "-omics" technologies towards identifying drugs with potential to cause hepatic toxicity. A recent study[3] has demonstrated high concordance of drug-induced human hepatotoxicity with *in vitro* cytotoxicity measured in a cell-based model using the new technology of high content screening. This technology is based on automated epifluorescence microscopy and image analysis of cells in a microtiter plate format. When applied to HepG2 human hepatocytes cultured in 96-well plates and loaded with four different fluorescent dyes, the authors were able to measure calcium concentration, mitochondrial membrane potential, DNA content, and could determine nuclear area and cell number and plasma membrane permeability. The study has shown very good concordance for more than 200 drugs known to cause hepatotoxicity in man. Within GSK, a large *in vivo* study has been conducted in the rat with the aim of identifying changes in gene and protein expression in the liver, which can then be used as potential targets or biomarkers for the prediction of human toxicity. So far, data has been generated on more than 100 drugs and known hepatotoxins. This approach, with data from liver microarrays, histopathology, urine NMR and serum SELDI, has provided an enormous challenge for the bioinformatics group. At present, the team has identified a panel of genes whose expression is modified by hepatotoxins, and these data are now used to aid the selection of candidate compounds prior to entry into development.

Dr. Fish concluded by saying that the overarching problems will continue to be the early prediction of human therapeutic dose and the translation of preclinical dose–response information into the clinical context. Prediction of human risk will remain a complex and uncertain business.

Dr. Andreas Reichel (Schering AG, Berlin) then discussed the *Current challenges towards assessing CNS penetration in man*. The discovery of new medicines to treat diseases of the central nervous system is one of the most challenging objectives for the pharmaceutical industry, and the rate of drug attrition in development is much higher than any other therapeutic area. Dr. Reichel gave a number of reasons for the high level of attrition in CNS drug discovery and development. Compounds may:

- Be given to the wrong subjects, or at the wrong dose or schedule
- Have significant effects only in the laboratory
- Show short-term favorable trends that may reverse over longer-term application
- Possess a very narrow therapeutic window.

All of these issues are further complicated by the many hurdles a drug has to overcome between oral administration and reaching the site of action in the CNS. In addition to the barriers of, and various mechanisms for, absorption, metabolism, excretion and distribution, CNS drugs have to be able to penetrate the blood–brain barrier. This is a selective barrier formed by the endothelial cells that line cerebral microvessels, and it acts as a "physical barrier" because complex tight junctions between adjacent endothelial cells force most molecular traffic to take a transcellular route into the CNS. Penetration into the CNS and the level of drug available to bind to its target is dependent upon the rate of entry through the blood–brain barrier, distribution within the CNS and the rate of removal from the CNS. Each of these functions may be passive, or driven by active uptake, protein binding, metabolism, degradation or efflux transporters. Discovery organizations therefore need to utilize data from many different *in vitro* and *in vivo* assays in order to be able to adequately evaluate and rank candidate compounds and provide confidence that these compounds will be CNS-penetrant in man. Although as yet there is no generally accepted *in vitro* model of the blood–brain barrier, it has been demonstrated[4] that an MDCK cell line provides a better correlation with *in vivo* data compared to a BMEC cell line. Using MDCK cells, it has also been possible to demonstrate that many known CNS drugs are, to a greater or lesser extent, substrates for the P-glycoprotein transporter.[5] Successful drug discovery depends upon optimizing both the rate and the extent of CNS penetration *in vivo*. Dr. Reichel reviewed the methods for *in vivo* determination of these parameters. A high rate of penetration depends upon the compound having high tissue permeability and low binding to brain tissue. Measurement of whole-brain drug concentration can be misleading and the extent of brain penetration is probably best determined by measuring the drug concentration in extracellular fluid and comparing this to the plasma concentration. It is important to take into account all of these factors when assessing the *in vivo* activity of candidate drugs in ani-

mal models. Dr. Reichel described how pharmacokinetic (PK) and pharmacodynamic (PD) data could be integrated using PK-PD modeling. This approach enables the prediction of dose to humans and facilitates, with the aid of safety assessment data, finding a safe starting dose for the first trials in man. It is important to take into consideration species differences in drug PK and metabolism and CNS penetration and disposition, especially when interpreting toxicology findings. The introduction of PET imaging has been of great benefit for CNS drug development, as it provides a non-invasive method for measuring target occupancy and brain penetration in man and can therefore guide dose selection. A fully integrative approach between research, preclinical and clinical departments is anticipated to produce improved success in the translation from animals to man, leading to reduced late-stage attrition in development for drugs targeted at the CNS.

In addressing the challenge of *In vitro–in vivo extrapolation in pharmacokinetics from known knowns to unknown unknowns*, Dr. Trevor Johnson (Simcyp Ltd., Sheffield, UK) outlined the used of predictive modeling techniques using the novel program Simcyp®. Simcyp® software provides the opportunity to make an informed link between *in vitro* and early human studies by simulating and predicting the population distribution of drug clearance and the effects on this distribution of enzyme inhibition and induction. The program incorporates extensive data on patient demographics, disease states, anatomical, physiological and biochemical variables, as well as input of information on *in vitro* drug metabolism. It has the potential to make the transfer of information between preclinical and the early phase of clinical drug development as seamless as possible. Dr. Johnson described the process by which the Simcyp® program was developed, validated and then revised based on the inclusion of literature data combined with new data generated by the company that was either missing from the literature or incorrect. Examples of predicting human clearance from *in vitro* data, pediatric pharmacokinetics and comparative differences in different ethnic populations were presented. In addition, the utility of using Simcyp® to understand and predict drug–drug interactions including mechanism-based inhibitors were also highlighted. Application of these modeling approaches has enabled the group to advise not just on key "go–no go" decisions in drug discovery and development but also on the optimum design of clinical studies to investigate drug–drug interactions in appropriate patient populations, thereby improving the chances of success while reducing overall development time and cost.

Dr. Sophie Dix (Eli Lilly, Windlesham, UK) gave an excellent review of the field of behavioral neuroscience as it applies to seeking new therapies for cognitive impairment (*Challenges of translating preclinical cognitive assays to the clinic*). The major cause of cognitive decline is Alzheimer's disease (AD), although cognitive function is also disturbed in other conditions such as schizophrenia and depression. The societal burden of AD is set to increase substantially with the increasing aged population: an estimated 13 million people in the United States will have AD by the year 2050 if current trends continue—the current figure is about 5 million. Current therapy for AD consists mainly of acetylcholinesterase inhibitors such as donepezil, rivastigmine and galantamine, although memantine, an N-methyl-D-aspartate antagonist, has recently been approved. These drugs offer only a very modest enhancement in cognitive enhancement, between 1.5–3.9 points on the ADAS-cog rating scale.

Thus, there is a significant unmet medical need for better therapies. Dr. Dix reviewed the therapies available to AD patients (four drugs targeting two mechanisms) with depression and anxiety (71 prescribed treatments for depression and anxiety representing 16 different therapeutic approaches) to highlight the magnitude of the difficulty facing the field. One of the difficulties has been the complexity of the *in vivo* testing paradigms available to detect cognitive enhancement preclinically. Cognitive functioning can only be assessed by observing and measuring a behavior—and behavior is subject to a range of confounding variables such as sensory perception, motor function, fear and anxiety, arousal and motivation.

Dr. Dix outlined the advantages and disadvantages of a range of different behavioral assays that are commonly used in the field, and outlined the major reasons for failure—Poor choice of behavioral test: not measuring the right thing or not accurately measuring the right thing, low signal-to-noise ratio, inappropriate baseline, interpretation confounded by competing responses, intra- and inter-lab reproducibility rarely tested. The field is also in a difficult phase because agents that provide robust cognitive enhancement are not available to validate existing or new models. Thus, it is difficult to interpret negative data—is a lack of effect due to the agent or an inappropriate choice of model? The other striking observation is that cognition is not a single domain but rather the summation of a range of cognitive abilities: attention.

Short-term/working memory; long-term learning and higher order functions such as decision making, rule learning, planning and execution.

Dr. Dix then outlined the types of tests that can be used to tap into these processes to enable a full pharmacological profiling of novel agents and mechanisms:

- **Attention**
  - –Five-choice serial reaction time task
  - –Two-choice sustained attention test
- **Working memory**
  - –Delayed (non)matching-to-position
- **Long-term learning and memory**
  - –Object Recognition
  - –Morris Water Maze
  - –Radial Arm Maze
  - –Reference memory tasks

- **Higher order functions**
  - –Reversal tests
  - –Stop-signal reaction time test

This presentation elicited considerable interest from the audience, including the question: how do you make decisions about the development of novel compounds into the clinic? This is the key question that faces many pharma companies as they attempt to prioritize their clinical portfolio. Unfortunately, there is no easy answer!

Dr. Wendy Alderton (Daniolabs, Cambridge, UK) presented data on the applicability of the zebrafish to pharmaceutical research (*Translational biology using the zebrafish model organism*). Dr. Alderton started her presentation by reviewing some of the logistical advantages of using zebrafish: they are highly fecund; they develop rapidly and are transparent; they have most of the organs found in mammals (with the exception of lungs, breasts and ovaries) and their genome is close to being fully sequenced. It is also possible to keep them in 96 well plates in 100 μL of water and treat them with compounds which they absorb directly through the skin—of particular interest to chemists, they are tolerant to up to 2% volume DMSO!

Using morpholinos, it is possible to knock down genes of interest, and thus zebrafish can be used in pathway analysis experiments. Of note was that the similarity between the zebrafish and human genomes allows the translation of many common pharmacologies. For example, tachycardia can be induced by carbachol, sedation mediated by diazepam and apparently zebrafish can also be intoxicated with ethanol (presumably by 'drinking like a fish'). Daniolabs have set up some interesting assays to measure QT interval prolongation (the key domains in the Herg channel homologue are well conserved). Dr. Alderton demonstrated that QT prolongation leads to a characteristic change in the atrial to ventricular beat ratio to 2:1 and that the man-to-zebrafish pharmacology translation is very good. This enables rapid and inexpensive testing of compounds to assess their liability to cause torsades de pointes.

Dr. Alderton also showed data on the use of zebrafish in ophthalmology. The organization of the retina in zebrafish is very similar to man, and zebrafish have excellent color vision. Daniolabs have set up some very innovative assays to detect optokinetic and optomotor responses, as well as a facile assessment of visual acuity. These assay systems are allowing Daniolabs to seek agents for age-related macular degeneration and also, conversely, to ensure that agents do not cause ocular toxicity.

Another area being investigated is epilepsy: Dr. Alderton showed a video where zebrafish were induced to have epileptic fits with pentylenetetrazole. This model was shown to be responsive to common antiepileptic agents such as lamotrigine and gabapentin.

In summary, Dr. Alderton provided an enlightening description of the use of zebrafish as a relatively inexpensive model organism that is remarkable adaptable to many of the needs of pharmaceutical research.

The presentation by Dr. Piet van der Graaf (Pfizer, Sandwich, UK) highlighted the importance of *Preclinical PK-PD modeling as an enabler of translational pharmacology*. Dr. van der Graaf opened his talk with the view that PK-PD is the language of translational research; translation not only safety and efficacy but also, potentially, differentiation. Indeed, *in vivo* PK-PD studies provide the all-important link between the three disciplines of pharmacology, physiology and medicine. The keys to enabling PK-PD studies are experimental design, conduct and analysis, coupled to modeling and simulation programs. PK-PD can be classified as any quantitative *in vivo* pharmacology experiment which links drug concentration to a biological effect and the corresponding time course of these events. Translating these measurements from animals to man is the key to successfully reducing attrition in drug development. PK-PD experiments can be conducted either to predict for efficacy or for potential side effects. Dr. van der Graaf gave an example of applying these principles to the translation of *in vivo* cardiovascular side effects, using dofetilide and cisapride. He then went on to discuss the many complexities of PK-PD modeling, focusing on the issues associated with drugs where the pharmacodynamic time course does not match the pharmacokinetic time course. In many cases, the drug effect is not directly associated with plasma concentration, but is delayed—for example, when the drug needs to reach a target in the brain or when the mechanism itself takes time to elicit its effect. These types of relationships lead to hysteresis in the concentration–effect plots, which can range from minutes to hours, and therefore require more a complex mathematical resolution. Advocating an iterative approach to PK-PD modeling, Dr. van der Graaf described how this approach spans across the preclinical–clinical landscape beginning with a simple preclinical dose–response experiments that are refined into exposure–response studies incorporating a time course component. This is then further refined to include multiple dose levels and the development of a PK-PD model that predicts a clinical outcome. Before applying the model in anger, it should then be benchmarked with clinical data on competitor and/or comparator compounds. Such an approach increases the understanding of the behavior of the drug, thus reducing the risk of failure around choosing the wrong compound or the wrong dose, leaving only the thorny question of target selection to be answered.

The cost of getting new medicine to market continues to rise, yet output continues to decline. On top of this, approximately 50% of patients fail to respond to drugs they are prescribed. Approaches to increase the probability of success, reduce expenditure and increase the proportion of the patient population that respond to new drugs are therefore highly desirable. Stefan Schwoch (Eli Lilly, Windlesham, UK) reviewed the use, and the potential for

the use of biomarkers in medicines research. A biomarker can be defined as a laboratory or a physical sign used as a substitute for a clinically meaningful end point; changes induced by a therapy on a marker endpoint can be expected to reflect changes in a clinically meaningful endpoint. While marker endpoints may not be the true predictor of a genuine clinical efficacy, they may provide initial indication on whether the intervention is sufficiently promising to justify the conduct of larger-scale, longer-term and more expensive clinical trials. Clearly, only reliable biomarkers can be used to guide decisions to progress compounds to further development. Useful biomarkers should be straightforward to assess in a noninvasive (or moderately invasive) fashion, be detectable in living subjects and generate reproducible and reliable results. Overall, biomarkers should correlate with disease pathophysiology (a disease-based surrogate marker) or be linked to the mechanism of action of a potential new therapy and therefore be of use in determining central penetration and/or optimal dose (a mechanism-based marker, or biomarker). Regardless of the process, the relation between marker endpoint and intervention should have a biological relevance. Biomarkers can also help improve diagnosis accuracy, reduce the sample size, duration and cost of clinical trials, predict adverse events, identify genetic differences in rates of drug metabolism, and allow treatments to be assessed in situations where the use of primary outcomes would be excessively invasive, unethical, long or expensive.

There is currently a safety gap in terms of patient expectations and the drugs delivered to market. Thus, 2.2 million Americans suffer adverse reactions to prescription drugs. Of these, 100,000 die, making side effects a leading cause of death. What's more, treating adverse drug reactions in the United States totals USD 4 billion annually and 45 of the 548 drugs the U.S. FDA approved (1975 and 1999) acquired one or more new black box warnings, and 16 drugs were withdrawn from the market (half of them within 2 years of being launched). Safety is therefore a serious and expensive problem, with 20% of all new drugs eventually demonstrating serious side effects which are unknown or undisclosed at the time of their approval. This is an area that can benefit greatly from the effective use of biomarkers. A good example is Roche's platform technology to individualize drug dosing based on metabolic profiling with the AmpliChip® CYP450 test. It constitutes the first genetic test approved by FDA for analysis of CYP2D6 and CYP2C19, two genes in the cytochrome P450 system that can greatly influence drug metabolism. The AmpliChip® CYP450 test identifies a patient's genotype and, based on this analysis, provides their predicted phenotype—either poor, intermediate, extensive or ultrarapid metabolizer. The test can therefore help physicians adjust dosing and select drugs by predicting a phenotype based on a genotype so that patient treatment can be individualized to get the best therapeutic results possible.

The importance and potential benefit of good biomarkers is now widely acknowledged. Regulatory acceptance of biomarkers will be based on robust scientific data and an assessment of its utility in the clinic. Examples of the successful use of biomarkers include tailored clinical trials with Genentech's *Herceptin* (trastuzumab) for the treatment of breast cancer. The Her2/neu gene is amplified in 20–25% of invasive breast cancers and trastuzumab binds Her2/neu and blocks its function. Extensive preclinical work validated target/biomarker/antibody relationship, but initial diagnostic tests were not very good. Genentech therefore made a strategic decision to accept only Her2/neu-positive patients in phase 3 clinical trials. Results showed a 50% response rate to trastuzumab (vs. an estimated 10% response rate in the general population) in a trial with 470 patients in phase 3 (vs. an estimated 2200 without patient selection). In addition, patient follow-up took place over 1.6 years, as opposed to an estimated 10 years without patient selection.

In summary, biomarkers offer great potential on a number of fronts, including:

1. Target validation
2. Informing early proof-of-concept decisions
3. Enhancing understanding of the basis of why some individuals respond to a drug and some do not
4. Monitoring therapeutic activity and predicting response
5. Improving the benefit/risk ratio by supporting dose selection
6. Identifying the real target population to both reduce development time (and cost).

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The SMR Committee organizes conferences on behalf of the Society for Medicines Research four times a year, with a focus on different aspects of medicines research. Details about forthcoming meetings can be obtained from: the SMR Secretariat, 840 Melton Road, Thurmaston, Leicester, LE4 8BN, U.K. Tel: +44 (0)116 269 1048; Fax: +44 (0)116 264 0141; E-mail: secretariat@smr.org.uk; URL: http://www.smr.org.uk.