

Special Interest Group Notes: ADME/Tox Session, Wed., 7:45 –9:00 am, September 15, 2004, Miami 2, Gaylord Palms Convention Center.

Interest in this session was very high as evidenced by a standing room-only session that remained 5 minutes over the 9:00 am end time. Pauline Gee, special interest group chair for ADME/Tox invited Dr. Jean-Pierre Valentin, Sr. Director of Safety Assessment, AstraZeneca, UK and Dr. JoAnn Scatina, Asst. VP of Safety Pharmacology as keynote speakers. Dr. Claudia Schmidt had to cancel at the last minute due to weather concerns.

Dr. Valentin, reviewed pharmacologic and secondary ADMET profiling: its definition, why it is important; and what the strategies were used for secondary screening. He pointed out that Frost and Sullivan estimated that failures of drugs for ADME/Tox problems cost the industry \$2BN in 2003. His presentation reviewed strategies used by his group and the application of databases. Safety pharmacology groups at AstraZeneca are de-centralized somewhat and various groups use different strategies.

According to the ICH guidelines there are three types of drug safety liabilities: (1) pharmacology related resulting from mechanism of action, (2) mechanisms not related to target, and (3) those which are unanticipated and idiosyncratic. Adverse drug reactions (ADR's) in humans falls into 5 types that can be described as: A) dose-dependent, predictable that account for about 75% of the cases and usually not lethal; B) idiosyncratic responses not related to dose account for about 25% of the cases but often lethal; C) long term adaptive changes; D) delayed effects such as carcinogenicity or teratogenicity; E) rebound effects after discontinuation of the drug. He summarized a simplified model for drug exposure with cells interacting with primary target to yield a desired downstream effect. However, many drugs interact with other targets yielding secondary effects that may be desired, neutral or undesired.

The main reason for pharmacologic profiling is to anticipate potential drug liabilities earlier in preclinical development. Drug fail in development 23% for toxicology reasons, 12% for high clearance, 22% for poor efficacy, 23% for protein binding, and 20% for off-target PK. (ref. *Drug Discovery Today* (1997) 2:436-444; *British Medical Journal* (2004) 329:15-19). More than 75% of adverse drug responses can be predicted by primary and secondary safety pharmacology, while the remaining are idiosyncratic. Adverse drug responses are responsible for 12% of all drug failures in development and are the fourth leading cause of death in 1994.

Safety pharmacology profiling aides in decision making for synthesis of new chemical series and selection of drug candidates, evaluation of novel drugs against known undesired targets, proactive studies to precede advancement into official Safety Pharmacology groups. Safety pharmacology studies can also be applied retrospectively to interpret past data and uncover undesirable off-target effects to avoid.

Methods included primary screening against panels of target subtypes GPCR, ion channels *etc.*, dose response profiles (IC_{50} , slope, mechanism of action), *in vitro* cell and *in vivo* animal assays (e.g. radioligand binding and *in vivo* imaging) for drug distribution. These results have to consider the differences between human vs non-human, normal vs diseased, functional vs binding assays. *In vitro* cell functional assays can be performed rapidly in the laboratory but equilibrium is not achieved. For *in vitro* tissue assays, non-human tissues are used, so again an awareness of species differences and multi-target effects is required. *In vivo* animals

represent integrated physiology, as it samples multiple targets but requires relatively large amounts of compounds.

hERG channel screening is an example of a new pharmacological profiling that is used currently to avoid drug mediated QT-wave prolongation associated with the arrhythmias.

By front-loading *in vitro* screening assays, early input is given to chemist to allow them to prioritize between chemical series. This allows chemist to modify a series to remove off-target effects. His group has done pharmacology profiling against 130 targets to generate a “receptogram” showing the strength of interaction of compounds against each target. Key target assessments are made for H1 histamine receptors, COX1, hERG, and beta1-adrenergic receptors at 1 μ M of compounds. Assessments of therapeutic margin versus target IC₅₀ are thus provided. The therapeutic margins required differ for different therapeutic indications: GI, oncology, CNS could be low, for inflammation a >100X selectivity was required to avoid toxicity.

Not all off-target activities are negative. For example, a β 2-adrenergic receptor antagonist was also active on dopamine D2, which also helped in blood pressure reduction. Databases needed to correlate *in vitro* and *in vivo* pharmacology to clinical effects. Comparison of new drugs to the profiles of marketed drug aids in the search for desirable features. *In silico*, predictions are used for gene expression data and computation for molecular targets e.g. hERG and P450 without experimentation.

AstraZeneca outsources most of its safety assessment and radioligand synthesis needs. AstraZeneca has strict criteria for go/no-go decisions. HERG is done early in discovery and a100X selectivity against the desired target receptor is required.

Questions and Comments: This strategy was very rigorous and more so than other companies.

JoAnn Scatina: Discovery ADME support at Wyeth has 30 liquid chromatography mass spectrometers (LC-MS) in three supporting groups that perform all non-GLP drug metabolism and metabolite identification work for Wyeth.

Purpose and function at Wyeth:

At Wyeth, a DMPK member participates in each of 34 drug discovery teams. DMPK supports medicinal chemistry and guides design of more drug-like molecules improve bioavailability and lower clearance. *In vivo* tests aid proof of concept studies, estimate therapeutic window (AUC tot/AUC effective dose). Estimating PK and assessing the metabolic liabilities aid in go/no-go decisions (bioavailability, drug-drug interactions, reactive metabolites). Predicting human PK properties and estimating Allometric scaling from animal into humans allows recommendation of dose levels for first-in-man (FIM) studies. In addition, the purpose of profiling is to identify the “soft-spots” in a potential drug development program and provides rankings on compound primarily in the lead optimization phase.

Dr. Scatina discussed some *in vitro* experimental techniques and their limitations. For instance, in pharmacologic profiling for metabolic stability by incubation of compounds with various fractions with liver-like activity, where only loss of parent compounds are monitored

by LC-MS, no metabolite identification is performed. During this evaluation, the researcher needs to be aware of species differences and influence of the preparation used (crude microsomes have no cofactors, S9 fractions, or liver slices) on the final analysis. For instance there are metabolic differences in between the Zucker rat and Sprague-Dawley mouse as well as with male vs. females of a species, or if a female is pregnant. There are cases in rats where a compound was very unstable in rats, but was later proven that to have good PK and stability in humans.

Fluorometric assays using recombinant CYP450s commonly used to estimate IC₅₀ and K_i for CYP interaction. However, she emphasized that these assays do not measure Phase II transformations, and do not directly prove that compounds are substrates for the CYP450s but merely competitors of the indicator biotransformation.

Preliminary PK studies are often performed in rats, but must also be done in the appropriate model for the clinical indication. PK in non-rodent species is used to select doses. Species differences can affect apparent metabolic reactions.

In silico ADME properties, though computer programs are used for initial hypothesis generation. DEREK is a program to predict toxicity and potential genotoxicity. METEOR used in evaluating potential routes of metabolism.

In vivo effects are also monitored such as liver weight increase, thyroid growth, then organs are studied *ex vivo* to explain effects as they relate to metabolism complications.

In a retrospective study, GSK looked at 1200 compounds that had been taken to advanced lead optimization in DMPK that were found to have metabolic soft spots. Microsomal incubation detected and predicted 60% of these compounds. 10% were predicted to have poor stability and high clearance, from high protein binding and CYP inhibition, but actually did not have these deficits when tested in humans. Also, some compounds were very unstable in rat *in vivo* assays but were stable in humans.

In vivo pharmacological screens are performed at few time points. Full PK studies are used to determine bioavailability, clearance, volume of distribution, half-lives, *etc.* In drug discovery, these data are used to estimate drug exposure and fraction absorbed in tissue and organs. Portal vein measurements are used to determine gastrointestinal absorption. PK/PD combinations to determine plasma exposure appropriate for therapeutic regimen. Other measurements such as urinary/biliary excretion help with extrapolation used in allometric scaling. These exposure screens estimate the PK for multiple dosing and dose escalation support.

Dr. Scatina shared an example flow chart of an *in vivo* screen for an oncology program:

This combined an *in vivo* pharmacological profiling screen with *in vitro* enzyme and cell-based assays that feed into estimating drug exposure levels in efficacy and metabolic stability models in nude mice. Low PK and poor metabolic stability would “kill” development of a compound. Drugs that passed this filter advanced into a tumor efficacy model in a xenograft animal, with full PK and metabolism work up in rodents and non-rodents. Then to allometric scaling was used to select the maximum tolerated dose,

At Wyeth, they felt it was critical to have all ADMET and DM/PK organizationally under one department with participation in both discovery and non-GLP preclinical development teams. This department would utilize all necessary laboratory robotics and automation, state-of-art 96-well screens for in vitro metabolism indicator assays such as enzyme CYP assays as a core center of excellence for the entire organization.

They have judiciously applied technology to give relevant experimental input to evaluation of compounds. CaCo2 data are not used, since they feel these do not correlate well with their experience during development. They prefer immobilized membranes and PAMPA assays. They have also used *in silico* methods to predict metabolite trapping and potential liabilities associated with unintended target activities. Dr. Scatina provided the example of a 3-methyl-indole compound in which a series of biotransformations through a benzoic acid intermediate with a final acylglucuronidase mediated step, lead to metabolites that produced an unintended PK/PD effect.

Some of the future directions of the Wyeth group will be to look more closely including other uptake and efflux assays, such as PGP, and other efflux or uptake receptors that are being discovered (*e.g.*, OATP's) especially in the brain and liver, which lead to much of the drug-drug interactions. Finally, there is a push at Wyeth to look at "all the data" including the PK/PD, metabolic trapping, and metabolic profile, and gene expression on toxicity effects of "failed" compound to see if some of these will be amenable to *in silico* predictive tools to hopefully yield predictive models based on all the data, rather than just the successes.

Questions and Comments: Unfortunately, there was not enough time for a formal discussion at the end of the presentations however many participants stayed to follow up with the speakers individually.

Notes transcribed by Dr. Thomas Chung, Past President, SBS