Top five global trends direct strategic thinking

Drug discovery moves faster with molecular modelling
Identification of potential contaminants in parenterals
A xenobiotic (drug) entering the human body will undergo a series of biotransformations via phase I and phase II metabolic pathways. During phase I, enzymatic processes in the liver – such as cytochrome P450 – and other tissues will modify the chemical structure of the drug by introducing reactive or polar groups (hydroxyl, thiol, amino and carboxylic acid, for example). These biomodifications render the parent drug more water-soluble to allow the body to eliminate it more easily. The metabolites produced will have a similar chemical structure to the parent drug and may be more pharmacologically active at the therapeutic receptor sites.

In phase II, the drug is conjugated to produce a more water soluble and pharmacologically inactive metabolite. Phase II reactions can be catalysed by transferase enzymes, such as glutathione-S-transferases, with the conjugated metabolite usually exiting the body via a detoxification mechanism. Moreover, the majority of phase II reactions are catalysed by UDP-glucuronosyltransferases.

Other phase I/II biotransformations during the metabolism of the drug can take place and may involve a number of enzymes such as alcohol dehydrogenase, aldehyde dehydrogenase, ester and amide hydrolases, epoxide hydrolase and flavine mono-oxygenases. Others include the sulfotransferases, catechol-O-methyltransferase and N-acetyltransferase.

The safety assessment of a drug candidate relies strongly on the metabolism data generated from animal studies. These preclinical safety studies will produce a different metabolic profile in comparison with human studies. This is illustrated by the analysis of in vitro metabolite profiles from a single drug candidate in the presence of rat, dog and human hepatocytes (Figure 1).

This study showed that some of the in vitro metabolites formed in human hepatocytes were also present in both the rat and dog metabolic profiles. The main major difference was observed between rat and human hepatocytes, which generated slightly different metabolite profiles. One disadvantage of using rodents is that there is a broad range of metabolites produced. It could be said that the rodent is a metabolite factory. Some of these metabolites are produced in small quantities in animals but may be produced at higher levels in human hepatocytes.

Consequently, the true metabolic fate of the drug is uncertain when administered to humans. Therefore, additional testing may be required to investigate the potential effectiveness of a particular metabolite with its safety.
considerations against the drug's efficacy. It is therefore important to have confidence in the right animal study so that it becomes possible to generate metabolites in significant amounts. These can be assessed against the metabolic profile in humans in developing a first-in-man (FIM) study.

**MIST: Metabolite in Safety Testing**

The discovery of new medicines gives rise to issues regarding the toxicity of drug metabolites that are associated with the parent drug. The pharmaceutical industry and the regulatory agencies – the MHRA in the UK, the US Food and Drug Administration (US FDA), and the European Medicines Agency (EMA) – have all developed an interest in this area since the publication of the first MIST paper in 2002.

The next important regulatory guidance came from US FDA Center for Drug Evaluation and Research (CDER) in 2008. This was entitled "Safety Testing of Drug Metabolites" and aimed to clarify the FDA position on when metabolites should be identified and characterised. These guidelines apply only to small molecule, non-biological drug candidates. Anticancer agents, drug conjugates (other than acylglucuronides) and reactive intermediates are MIST exempt.

MIST focuses on stable metabolites circulating in human plasma and recommends that in vivo metabolite evaluation in humans be performed as early as possible. The FDA guidance places an important emphasis on these circulating metabolites; in particular, all metabolites exceeding 10% of the parent drug will require toxicological evaluation (see Figure 2).

In reference to the ICH Topic M3 (R2) on non-clinical safety studies for the conduct of human clinical trials and marketing authorisation for pharmaceuticals, guidelines, the evaluation of total exposure is when the metabolite has achieved 10% of parent drug systemic exposure at a steady state in order to initiate a toxicological investigation.

**carbon-14 radiolabelling**

Complete data on human metabolites becomes available only after human mass balance studies (AME/MB); in a majority of cases carbon-14 radiolabelled material is used. This information is typically utilised in parallel with clinical Phase II studies. Overall, the requirement for safety drug metabolite studies can potentially delay the start of Phase III trials.

Regarding the circulation of metabolites in humans that are either absent in the animal species or are present in humans at much lower levels, these MIST guidelines recommend that the metabolites whose AUC (area under curve) at steady-state is less than 10% of that of the parent need no further study.

In this context, MIST requires efficient production systems to permit the production of human drug metabolites. As conventional chemical synthesis cannot always produce these metabolites, biotechnological approaches are being developed that typically employ the recombinant expression of human drug-metabolising enzymes and whole-cell biotransformation processes.

The gold standard approach to quantify a metabolite is to synthesise a carbon-14 labelled version of the drug. The rationale for selection of carbon-14 in radiolabelling of drug substances lies in the ability successfully to substitute a particular carbon-12 atom in a molecule for carbon-14 to produce a chemically identical analogue. The introduction of this radiolabel in the carbon framework allows the pathway of the drug to be traced in a biological system.

One of the primary reasons for choosing carbon-14 over other common radioisotopes, such as tritium, is that the exact position of the label can be selected based on the synthetic route employed for labelling. Carbon occurs in the skeleton of nearly all drug molecules, and therefore allows choice of the radiolabelling site in a position more likely to be metabolically stable.

Additionally, carbon-14 labelled compounds generally exhibit greater radiochemical stability than their tritium labelled counterparts, owing to the higher specific activity of tritium labelled material. This increases the risk of significant auto-radioysis (radiochemical decomposition), during storage or usage of the radiolabelled compound. Carbon-14 is also detectable at very low levels using scintillation counting, which makes it an ideal choice for animal/human studies where doses close to the pharmacological threshold are frequently used.

**carbon-14 radiolabelled metabolites**

The starting point for the carbon-14 radiolabelled drug is in vitro preclinical metabolism studies using hepatocytes from humans and animals. This study can be implemented using standard protocols to test for metabolites – which are radiolabelled at concentrations of 10µmol/L – when incubated with the hepatocyte's cells for a period of several hours.

The above approach is needed to evaluate the absolute concentrations of circulating metabolites. According to Smith and Obach, for human metabolites that are structurally distinct from the parent drug but are present in circulation at high concentrations (i.e. greater than 1µm), additional safety evaluation for an appropriate risk assessment may be required, especially if human exposure is greater than that observed in animals. The premise of these studies is to observe whether metabolites present in human in vitro systems are also found at comparable levels in animals. This enables the safety testing of metabolites (MIST) to be carried out correctly before entering human AME/MB studies.

**analytical methods**

The isolation of carbon-14 radiolabelled metabolites from preclinical plasma pooling methods can be analysed from an array of analytical techniques. These may include a combination of high performance liquid chromatography/ultraviolet/mass spectrometry (LC/UV/MS) using a radio-detection configuration to obtain reliable AUC values of metabolites present in the plasma of preclinical safety studies.

The application of HPLC used to collect radioactive fractions guided by real-time peak analysis is one approach. Quantification of major peaks was also conducted in the metabolites.
metabolites in human plasma from single and escalating repeat dose studies can also be carried out using nuclear magnetic resonance spectroscopy (NMR). The labelling of drugs with carbon-14 can facilitate the metabolite detection, ease purification and enable the radiochemical concentration of the metabolite. The data generated will help to provide metabolite profiles and give clues as to the chemical structure of the metabolite. This makes it more valuable for use in in vivo metabolism studies where drug doses close to the pharmacological threshold are normally used.

**low dose AMS studies**

Carbon-14 labelling has recently become an even more useful tool as a result of advances in Accelerated Mass Spectrometry (AMS). Although currently expensive, it is a very sensitive technique for the detection of carbon-14 and other radiolabels. The high sensitivity of AMS allows human micro-dosing to be carried out with sub-therapeutic doses and much lower levels of radioactivity (50nCi). The supply of small amounts of carbon-14 labelled API for first-in-man AMS studies typically use in the order of 10 kBq per study to analyse the ratio of carbon-14 to carbon-12, and provide information on drug metabolism and pharmacokinetics (DMPK). In comparison, human mass balance studies require doses in the order of 4 MBq of carbon-14 labelled investigational medicinal product (IMP) per individual.

The advantages of these low dose AMS studies are in their ability to be performed at the earliest stage in the clinical development of the drug candidate. These studies would provide information on the metabolism profile and rapidly highlight any difficulties in order to aid phase II studies by selecting the correct preclinical studies. These measures would benefit the sponsor regarding project time lines and clinical costs in later stage development.

**conclusions drawn**

The guidelines set out by the MIST and ICH Topic M3 (R2) documents for the safety testing of human metabolites have provided an approach for drug safety testing. The emphasis of MIST is to obtain essential preclinical human data as early as possible on the pharmacokinetics of an investigational drug. These guidelines can be met using in vitro studies, implementing limited animal studies and ending with human AME studies using carbon-14 labelled drugs.

The radiolabelling approach would help to exclude potential reactive metabolites and to find out which metabolizing metabolites may require MIST investigation to assess the early impact of metabolites on drug safety.

The presence of low levels of parent compound, as a consequence of extensive metabolism, highlights some challenges in assessing all metabolites that are present in greater than 10% of the parent drug in accordance with the recommendations set out in the 2008 FDA MIST guidelines. Moreover, the recent revision of the ICH M3 guidance can be applied to studies for the selection of circulating human metabolites that require separate safety assessment criteria.

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**Figure 2: MIST guidance decision flow diagram**

**References**


**contact**

Dr Sean Kitson
Almac Group
Almac House, 20 Seagoa Industrial Estate
Craignavon BT63 5QD, UK
Tel: +44 28 3833 2200
Email: science@almacgroup.com
Web: www.almacgroup.com