

ADME Solutions from Drug Discovery to Lead Optimisation, Candidate Selection and Beyond

a report by

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Tailor-made Studies

One of the bottlenecks of modern drug discovery and development is the characterisation of absorption, distribution, metabolism and excretion (ADME) properties of new chemical entities (NCE) in the pipeline. As soon as hit molecules are found after screening thousands of molecules from combinatorial and natural product libraries or from rational synthesis, the ADME properties of these hit molecules have to be characterised. However, current *in vitro* (and *in vivo*) practices have concentrated on characterisation of ADME properties of late stage molecules. The protocols are not always suitable for early-stage molecules. The methods used involve high sample need and high level of validation and quantification ending up in high cost per compound and test. Also, *in vivo* activity screening, pharmacokinetic and toxicological studies are not integrated to support ADME characterisation to save time, resources and laboratory animals. It is clear that modified and tailored approaches are needed at different stages of drug discovery and development. The need is especially obvious for small- to medium-sized drug discovery and development companies outsourcing almost everything related to ADME. These companies usually do not have pharmacological and biopharmaceutical expertise of their own to judge what kinds of studies are needed, at what stages they should be performed and how the studies correlate with each other.

Tailored Studies Needed at Different Stages of Drug Discovery and Development

Hit/lead identification/selection, early ADME:

- quick and dirty, reveal-type studies;
- low-cost/compound/test; and
- low sample consumption.

Lead characterisation/optimisation:

- more in-depth studies; and
- medium cost/compound/test.

Candidate drug selection/characterisation:

- full validation and quantification;
- higher cost/compound/test; and
- full permeation, metabolism and interaction studies.

It is important during the hit/lead identification and selection phases to cost-efficiently rank different kinds of compounds by simple *in vitro* laboratory experiments. Quantification can be sacrificed at this point to save time and costs. Another important aspect is that as little as possible of the hit or lead compound is consumed, because only a few milligrams might be available. It is obvious that accuracy is being compromised as minute amounts of compounds are handled and quantification is omitted, but it does not matter at this point. As the accuracy is not crucial, quick and dirty reveal-type studies are utilised in these early stages. For practical purposes, it is enough to be able to state that a certain property has a value of, for example, about two, even though the right and accurate value might be 0.92 or 6.1. For a drug developer, it is important that the value obtained is not around 0.1 or 40; the right order of magnitude matters.

When drug development is advanced to lead optimisation or lead molecule characterisation, more thorough and in-depth studies are needed. The further the compound proceeds, the more accurate values for the properties are needed. The exact need is met by increasing the number of time-points, concentrations and replicates, and implementing quantification. Naturally, the study costs are increased as well as the need to consume more compound. The costly and sample-consuming studies are not done to every compound, but only to compounds having passed the reveal-type studies. Likewise, for the re-designed and re-synthesised compounds during the lead optimisation, ADME properties should first be studied with revealing type methods before thorough studies to avoid unnecessary experiments with failing compounds.

For drug candidate selection, and especially for candidate characterisation, validated methods with



quantification are needed. To be able to accurately predict *in vivo* properties of drug candidate and to plan pre-clinical and clinical studies, comprehensive *in vitro* permeation, metabolism in liver preparations of several species and human cytochrome P450 (CYP) related *in vitro* drug-drug interaction studies should be performed. Earlier studies should certainly be taken into account in planning and to avoid overlapping.

Priority of Studies

An emerging drug discovery and development company is often based on biochemical expertise on target identification, target validation and screening of potential hit molecules. Many times they experience difficulties in getting to the right track after that. They may have good hits or even lead molecules, but they are not certain how to proceed in investigating ADME properties. They are at the mercy of contract research organisations (CROs) and consultants. It is hard to give exact procedures regarding the characterisation of ADME properties, but general guidelines can be given. In reality, the studies are not conducted linearly but in parallel and, depending on the intended indication, certain studies are more valuable than others or prioritised over others. The following guidelines apply mainly to candidates intended for oral delivery.

An Example of Prioritising ADME Studies Needed

Basic Properties

- Solubility, pKa, lipophilicity;
- plasma protein binding;
- non-specific binding; and
- chemical stability and degradation.

Metabolism In Vitro

- Human liver preparations with all co-factors;
- several animal liver preparations; and
- metabolic stability and metabolite identification.

Permeation Studies

- Caco-2 and MDCK permeation;
- P-gp substrate and transporter substrate screen; and
- other permeation models (blood-brain barrier (BBB), epithelial, corneal, tissues).

Drug-drug Interaction Studies

- Metabolising CYP enzyme identification;
- CYP inhibition;
- CYP induction, nuclear receptor activation;

- Regulation of lipid and cholesterol metabolism; and
- aromatase inhibition.

In Vivo ADME

- Integrated to *in vivo* efficacy studies (bioavailability, distribution, metabolism, elimination);
- integrated to animal toxicity studies (bioavailability, distribution, metabolism, elimination); and
- integrated to clinical trials (metabolism, elimination).

First, determination of basic physicochemical properties is the prerequisite for every subsequent property determination. When developing adequate analytical methods for NCEs, it is important to know solubility, pKa and lipophilicity, and also chemical stability. A cost-effective approach is to integrate method development, simple degradation study and physicochemical property characterisation together with plasma protein-binding and non-specific binding. It is more feasible to start *in vitro* metabolism and permeation studies after the physicochemical, degradation and binding properties of an NCE are already known. It is easier to incubate, analyse and most importantly to predict the *in vivo* situation, if possible problems are dealt with before starting the experiments. Furthermore, it is clear that compounds with poor physicochemical, degradation and binding profiles are hardly suitable as drugs.

Second, metabolism data using human liver preparations are irreplaceable if the drug candidates are intended for human use. As rat is not human, it is not feasible to start metabolism studies with rat or any other animal liver preparations; their time is later when animal models for toxicological studies are selected. A good practice is to start metabolism studies with human and several most obvious animal models, but resources might be wasted if the compound is soon to be rejected. The first human *in vitro* metabolism studies should be conducted with a system involving all the possible drug metabolising enzymes and co-factors for the enzymes. High initial concentrations of parent compound should be used and all the possible (predicted and unpredicted, and major and minor) metabolites should be found out. Special attention needs to be addressed to those metabolites potentially associated with toxicity, such as acyl glucuronides, epoxides, glutathione pathway metabolites, hydroxyl amines etc., because their presence indicates further need for lead optimisation.

Third, permeation studies should be conducted after the initial metabolism studies. In most cases, if there is something wrong with permeation leading to

problems in absorption, it may be possible to correct the problem with drug formulation. But if there is something wrong with metabolism, it is usually fatal for the compound and can be corrected only via lead optimisation. Early permeation studies might be a waste of money even though permeation studies are generally more inexpensive than metabolism studies and very suitable for screening purposes. By starting permeation studies with the real lead molecules, comprehensive studies can be done readily and cost-effectively. In addition, the involvement of efflux pumps (P-gp) and transporters (multidrug resistance-associated protein (MRP), organic anion transporting polypeptides (OATP) and organic cation transporting polypeptides (OCTP)) in absorption should be investigated. Furthermore, depending on the intended indication or route of administration blood brain barrier, epithelial or corneal permeation are studied.

Fourth, the prediction of potential drug-drug or drug-food (supplement) interactions is a hot topic concentrating mainly on different kinds of CYP-related interactions. CYP inhibition and induction properties describe the ability of an NCE to inhibit or induce CYP enzyme function, respectively. In the case of inhibition or induction, the NCE creates problems for other co-administered drugs and their metabolism. CYP inhibition is easily measured in *in vitro* human liver microsome systems, but induction is more difficult as it requires hepatocyte incubations and access to human hepatocytes is limited. Another approach emerging from basic research utilises nuclear receptor (pregnane X receptor (PXR), constitutive androstane receptor (CAR) and peroxisome proliferator-activator receptor (PPAR)) activation and can become standard in induction studies. Later, the effect of NCE to lipid or cholesterol metabolism and to aromatase activity can be investigated with already established methods. Other drugs can also create problems for NCEs and their CYP-mediated metabolism if they inhibit or induce the enzymes producing the metabolites of the NCEs. CYP enzymes responsible for metabolising candidate molecules can be identified either by incubating with expressed CYP enzymes or by co-

incubating with known CYP-specific inhibitors in human liver microsome system. Potential major drug interactions should be verified with *in vivo* clinical trials and they may affect the labelling of the drug.

Last but certainly not least, integration of ADME studies to *in vivo* pre-clinical and clinical studies conducted anyway can considerably save resources and create a more thorough picture of the properties of the candidate. During drug discovery and development, drug efficacy is tested in laboratory animals, drug toxicity is examined in several different studies with multiple species and, later, human clinical trials are conducted. Sampling protocols for bioavailability, distribution, metabolism and elimination could be easily implemented to standard study protocols in animal efficacy and toxicity testing. Rational collection of serum, plasma, urine, fecal and/or tissue samples does not require much effort, but the information obtained by analysing the samples can be very valuable. The same applies for rational collection of metabolism samples (excluding tissues) from clinical trials. ■

All the studies and strategies presented in this article are included in the service portfolio of Novamass. The company has formed a comprehensive approach to characterise absorption, distribution, metabolism and excretion properties of new chemical entities to support drug discovery and development. Its special interest is to serve the drug development companies and offer them a full range of ADME services for the whole duration of their drug discovery and development.

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