

Intelligent ADME Outsourcing

By John Nash, Alan Beresford and Dawn Yates at Biofocus

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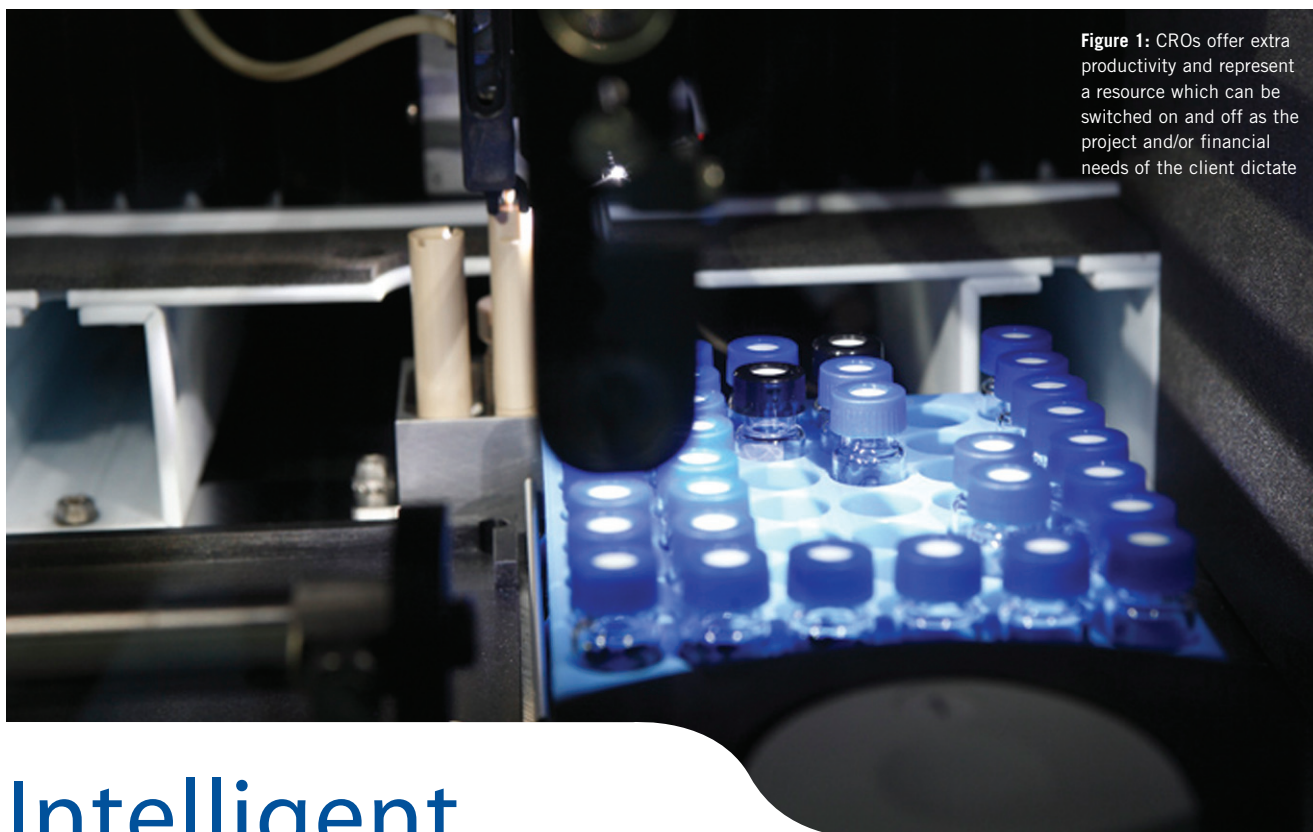


Figure 1: CROs offer extra productivity and represent a resource which can be switched on and off as the project and/or financial needs of the client dictate

Intelligent ADME Outsourcing

John Nash, Alan Beresford and Dawn Yates at BioFocus examine the benefits of *in vitro* ADME outsourcing for both large pharmaceutical and small biotech companies

In vitro absorption, distribution, metabolism and excretion (ADME) tests are used to characterise a compound's properties at an early stage in drug discovery. The aim is to reduce the risk of candidate compound failure due to poor pharmacokinetic behaviour before reaching clinical trials. Over the last 10 to 15 years, the industry has been performing ADME screening ever earlier in the stages of drug discovery. The drive for this testing remains as strong as ever. It is estimated that an improvement in predicting the failures by 10 per cent prior to clinical trials could save a company \$100 million in development costs per drug (1). ADME is now an established and essential part of integrated drug discovery and development programmes, providing indispensable information to enable iterative chemistry, target discovery and biology processes on which pipelines depend.

PHARMACEUTICAL INDUSTRY STRATEGY

The pharmaceutical industry has long been reviewing and reshuffling its available resources. With cost issues driving a lot of research overseas into China and India, those groups remaining in Europe need to ensure that business remains profitable. The current global economic downturn has prompted a renewed drive to maximise the efficiency of the drug discovery pipeline and reduce associated costs. Since the drug discovery process is lengthy as well as costly, the twin goals of researchers have, for some time, been to

identify potential drug candidates earlier and terminate less promising projects sooner in order to improve efficiency and productivity. With competitive pressures to maintain their efficiency, minimise costs and stay abreast of current technologies, contract research organisations (CROs) offer that extra productivity and represent a resource which can be switched on and off as the project or financial needs of the client dictate. Most outsourcing companies have been built to adapt and tailor their services to the requirements of different clients, whereas change within a large research organisation can be a slow and difficult process. Hence, outsourcing in research is increasingly being seen as a way to control costs and focus spending as well as tap into areas of additional expertise and experience, both technical and scientific, which can augment that available in-house.

The availability of relatively high-throughput ADME assays has led to vast amounts of data being generated in large pharma companies, but has not greatly improved their success rate. In some cases, tests will have been conducted on compounds which were never likely to make the grade, and those numbers have been inflated by the output of combinatorial chemistry approaches. Therefore, there is a need for rational and intelligent testing. For many projects, the number of compounds being submitted for ADME testing has fallen again in recent years. Smaller, focused libraries are being

made and, rather than a blanket screening approach, rational ADME screening cascades are designed to address those issues which are expected, based on calculated properties of the molecules and the location of the target in the body, or those problems revealed by preliminary *in vivo* pharmacokinetic studies.

EFFECTIVE COMMUNICATION

Lead generation incorporating early ADME screening generated \$540 million for CROs in 2007, and these revenues are predicted to increase at an annual rate of more than 20 per cent (1). However, the industry needs such investment to yield drug candidates and throwing money at the problem has not, to date, solved the issues. Intelligent testing is key to making the most of that investment. There is a huge amount of ADME experience in large pharma companies and this acts as a steady and constant supply of expertise to biotechnology startup and spin-out companies, as well as CROs. But ADME CROs have matured too and are also a source of expertise and experience; and whether the client is a small startup company with no internal ADME know-how or a large multi-national with extensive in-house ADME resources, communication is vital.

Many companies face the issues of intellectual property (IP) protection and confidentiality, which do not permit extensive sharing of information with an external company. As a result, a CRO may be employed solely for the data generation step, which can limit their input into screening design and appropriate assay implementation. Supplying structural information, where possible, in addition to the plans for the downstream use of a candidate, would aid the project significantly, facilitating the data interpretation process. With long-standing expertise, most CROs have a diverse client base across multiple projects. They have encountered numerous technical and scientific issues before, some outside the experience of more focused in-house teams, and are therefore well positioned to aid with the problem solving aspects of lead optimisation.

Figure 2: Caco-2 cell lines express the features characteristic of mature intestinal cells



ADMET ASSAYS

There are now a wide variety of *in vitro* ADME assays available, covering measurements from physico-chemical properties, such as solubility, lipophilicity and pKa, through to metabolic enzyme induction and cell toxicity, extending the acronym to ADMET. With this variety in mind, it is essential that any outsourced company is provided with enough information to enable them to perform the appropriate assays to assist the screening programme. This process should be optimised to avoid unnecessary ADMET tests. Some

data is 'nice to have', but generation of such information has to be viewed against the need to reduce costs, streamline the process and ensure that time and money are spent productively and that generating decision-making results is a priority.

The spectrum of ADMET assays available is continually expanding as understanding of the factors governing drug disposition increases. At present, there is much interest and effort directed towards the role of active drug transporters in this process, and assays to assess their impact on new drug molecules are steadily being added to the CRO repertoire. But, for many drug discovery programmes, the basic problems, such as poor compound solubility and high metabolic instability, remain a serious hurdle and some of the early tests appropriate to many projects are summarised below.

Solubility

Data on solubility should be obtained for every compound screened, or at least for representative examples within a chemical series. A kinetic assay, where a DMSO stock solution of test compound is mixed with an aqueous buffer, is usually sufficient at the early stages of drug discovery. Many *in vitro* screens for measuring target potency and selectivity, as well as ADME assays, will be based on dilution of DMSO stocks into an aqueous medium and, as noted above, knowing compound solubility under these conditions is important in generating meaningful data, as well as a guide to potential downstream issues for *in vivo* studies.

Absorption Assays

Various absorption assays can be performed to provide some confidence that a compound will successfully penetrate cell membranes and be absorbed into the blood stream for effective distribution to the targeted tissue. One example of this is the use of a colonic adenocarcinoma (Caco-2) cell monolayer. Caco-2 cell lines express the features characteristic of mature intestinal cells. Measuring the rate at which a compound passes through these cells can provide an indication of a compound's intrinsic intestinal permeability, as well as the involvement of some active uptake or efflux processes.

Plasma Protein Binding

Plasma protein binding (PPB) is largely governed by lipophilicity and frequently the physico-chemical properties required for good target activity will be a feature of the entire chemical series being tested. Hence, the level of PPB (very high, high, moderate or low) may be difficult to change while retaining good potency. However, only the unbound fraction of drug in circulation is able to pass into tissues, and knowing the extent of this issue is important for any project. Very high PPB will need to be offset by high target potency. However, as PPB may only change notably after significant alterations in chemistry, these assays only need to be performed periodically to monitor such changes as the project proceeds.

Microsomal Stability

The microsomal fractions from liver cell homogenate (hepatic microsomes) are a very useful tool for investigating the metabolism of drugs, their relative stability and their possible interactions at the level of metabolic enzymes. As such, the majority of active molecules in a programme may progress to testing in a microsomal system. The rate at which a compound is metabolised by microsomes is likely to influence the duration

and intensity of the pharmacological action *in vivo*; the more rapid the metabolism, the lower the systemic exposure. In addition, the metabolites produced need to be inactive and non-toxic. Identification of metabolites and the risk that these may be reactive chemical species, can also be assessed in microsomes.

In many projects, the attrition rate of compounds can be quite high in this 'first-tier' of assays and lower numbers will progress to more detailed, and often more expensive, testing. For example, those candidates that return favourable results in microsomes are then usually subjected to a second tier of testing in hepatocytes. Microsomal metabolism is primarily oxidative, but drugs can also be conjugated by the metabolic addition of polar substituents to form glucuronides and sulphates, for example, which assist their elimination from the body. Hepatocytes provide a comprehensive set of metabolic enzymes to more thoroughly explore how the test compound will be metabolised *in vivo*.

When moving into the pre-clinical development phase, hepatocyte studies can provide useful data for the selection of the animal species where the metabolic profile of the drug most closely mimics that in humans.

The principal family of enzymes in microsomes are the cytochromes P450. Inhibition or induction of cytochromes P450 by a new drug can be a serious concern with regard to potential interactions with other drug molecules known to be principally cleared by the same enzymes and can lead to drug-drug interactions (DDIs) in the clinic. Studies can be performed using specific substrates for individual human P450s to assess the likelihood of such interactions for an NCE.

Transporter Assays

Transporter systems are increasingly being implicated in drug resistance, DDIs and adverse drug reactions (ADRs) in the clinic, and are becoming an essential part of the ADME assay portfolio. There is no universally accepted system in which to study the diverse classes of transporters, but selective inhibitors and competitive substrates can be used with the Caco-2 system to investigate the influence of a number of transporters constitutively expressed in these cell lines. Alternatively, cell lines can be used in which individual transporter proteins are over expressed, such as MDR-1/P-glycoprotein in MDCK (Madin-Darby canine kidney) cells.

Pharmacokinetic Studies

In vivo pharmacokinetic studies should be initiated as soon as possible in a programme to determine the degree of *in vitro*/*in vivo* correlation. It is important to know that the ADME tests being performed *in vitro* are providing the appropriate information to select the right compounds for progression and ensure there are no underlying issues *in vivo* which are not being addressed. This combination of *in vitro* and *in vivo* testing also provides cross-species comparative data and helps predictions of likely drug behaviour in the clinic.

CONCLUSION

The current financial climate has increased caution and further encouraged pharmaceutical and biotechnology companies to consider the benefits of outsourcing across the board, through drug discovery and development, manufacture and packaging.

About the authors



John Nash, MPhil, PhD, joined BioFocus in October 2009 to head up the ADME Division. Until recently, John was at a major CRO in Canada, as Director of DMPK where he was responsible for the strategic direction of the commercial, scientific and technical aspects of the ADME, QWBA, IVDM (*in vitro*), metabolite ID and PK/TK groups within the department.



Alan Beresford, BSc, PhD is a Senior Research Fellow, ADME, at BioFocus. Alan is responsible for the design and implementation of client ADME screening. Acting as consultant and interpreter of ADME data, he advises on both internal and external projects concerning the appropriate use and application of *in silico* and *in vitro* tools for drug discovery.



Dawn Yates, BSc, is Director of Operations, ADME, at BioFocus. Dawn is responsible for the delivery of high quality ADME/PK data. During her time with the group, Dawn has developed the comprehensive suite of *in vitro* assays to support all discovery programmes and continues to develop and add to them in order to meet new requirements.
Email: info@glpg.com

In order to keep pace with technological developments and evaluate potential drug compounds more efficiently, companies are increasingly turning to outsourcing to streamline the process and reduce the overall costs of drug discovery.

Outsourcing ADME can be highly advantageous to both large pharmaceutical and small biotechnology companies. It enables them to operate in a more cost-effective manner, saving resources for other projects and reducing the requirement for highly trained in-house ADME expertise. Some companies outsource the routine aspects of ADME testing, freeing up in-house expertise to work on more challenging issues, while others prefer to run routine assays through their optimised in-house protocols and use the CRO's experience to develop custom or project-specific methods. By outsourcing to CROs, smaller organisations gain access to extensive ADME testing resources.

The goal is to implement high quality assays to assess physico-chemical and key ADME properties appropriate to the specific project, at a throughput which matches the project demand for decision-making data. As a consequence, potential development liabilities should be eliminated early on in the drug discovery process, removing the cost associated with downstream testing of unsuitable candidates. In developing a good relationship with CROs, both large pharmaceutical and small biotechnology companies will reap the benefit.

Reference

1. Anscomb A (ed), *Outsourcing in Drug Discovery*, A Kalorama Information Market Intelligence Report, 2008