

Advancing the drug discovery pipeline with early stage *in vitro* ADME and toxicity testing

With increasing pressures on pharmaceutical companies to develop new, effective therapeutics across the board, and keep the costs of development down, the drug discovery industry needs to ensure that its standard processes and procedures are as streamlined and effective as possible.

**By Dr Maureen
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Bringing new drugs to the market in a timely and affordable manner is paramount to combatting numerous diseases. Late-stage clinical failures and post-market withdrawals can be accompanied by high profile patient injuries and even deaths, meaning there is a real need to understand the physiological consequences of new drugs before they come to the clinic. The stakes are a lot higher than just the loss of revenue from a failed development project.

Understanding the ADME (absorption, distribution, metabolism and excretion) and toxicity of new drugs are key parts of the compound testing process, during preclinical and clinical trials, providing insights into the disposition of a pharmaceutical compound, including its safety and efficacy. Advances in capacity to isolate and culture human primary cells and engineer complex human primary tissues at the bench-top are enabling drug developers to better understand human biological responses to drugs long before the drug even advances to the clinic.

One of the key challenges in ADME and toxicology is finding a meaningful model that can provide clinically relevant measures. Although animal models provide a good level of data, they are not

always the most appropriate model to use, since they do not always provide an accurate indicator of the human *in vivo* environment, and are not necessarily representative of a human response to the drug's impact, safety or efficacy. Culturing primary human cells enables researchers to benefit from a physiologically relevant model that expresses the correct gene profile and enzyme kinetics as the *in vivo* human liver. This allows the capture of relevant pharmacokinetics during the preclinical stages of testing, saving both time and resources by enabling the assessment of physiologically relevant human data early on.

Here we discuss how new *in vitro* approaches in both ADME and toxicity testing can be leveraged to increase efficiencies within the drug discovery pipeline to help companies take new drugs to the market in a faster manner, and potentially reducing costs.

The front line for *in vitro* ADME and toxicity innovation

The front line for understanding the potential for efficacy while avoiding off-target effects and toxicities in any small molecule drug development programme is the liver. As a complex structure, the



liver is composed of 80% parenchymal cells (hepatocytes) and 20% non-parenchymal cells (stellate cells of the connective tissue, endothelial cells of the sinusoids, Kupffer cells functioning as immune cells and macrophages). The hepatocytes are responsible for approximately 80% of all drug metabolism and biotransformation of small molecules in the body. In recent years, advances in the understanding of hepatocyte function, cell signalling, liver toxicity mechanisms and cell culture techniques have led to numerous advances in compound testing for both preclinical and clinical trial purposes. In this regard, the use of primary human hepatocytes in cell culture has become an integral part of preclinical drug development.

In general, the use of primary hepatocytes in cell culture is an established technology, but it still has limitations. When the liver tissue is disrupted as part of the hepatocyte isolation and purification procedure, the hepatocytes begin to rapidly lose their identity. The metabolic activity and hepatocyte-specific markers and functions of the cells immediately begin to decline. As a result, hepatocytes tend to under-predict clinical pharmacokinetics. Furthermore, because primary hepatocytes are removed from their native environment of support cells, they have been of limited use for the prediction of drug-induced liver injury (DILI). DILI remains a leading cause of clinical failure and market withdrawal and is poorly predicted in animal models. The mechanisms of DILI do not often point to the hepatocytes alone as the locus of disease, but include paracrine and endocrine relationships with other cell types throughout the system.

Current common hepatocyte formats

Hepatocytes in suspension taken directly after isolation have the highest metabolic activity and are used in this format to determine close to *in vivo* measurements of intrinsic clearance of a drug. However, these hepatocytes are limited to 2-4 hours of metabolic activity, so as drugs in development become more and more stable, assays in suspension hepatocytes can sometimes not provide any meaningful metabolic data.

Hepatocytes are used in plated formats when drugs are poorly metabolised in short-term suspension assays, or when mechanistic data regarding drug-drug interactions and mechanistic toxicity is needed. Hepatocytes plated in a monolayer on a collagen matrix have an extended life-span compared to cells in suspension and will maintain metabolic capacity longer.

In vivo, hepatocytes exhibit a polar morphology whereby cell surface proteins are different on the different 3D surfaces. This polar morphology can only be replicated *in vitro* by also providing an overlay of collagen or other basement membrane extracts such as Matrigel® (Corning) to the top of the hepatocyte monolayer. By 'sandwiching' hepatocytes between these basement membrane protein mixtures, hepatocytes form the correct basal surface structures and a small apical membrane pocket between cells that is known as bile-canaliculi which enables excretion of bile acids from the hepatocytes similarly to the *in vivo* processes. It is generally accepted that the sandwich culture most reflects 3D *in vivo* cell shape, and functionality in a 2D format, but nonetheless sandwich culture does not solve the issue of metabolic decline.

Improvements to hepatocyte cultures

As pharmaceutical companies make significant strides towards developing compounds that are increasingly stable, there is a need for the hepatocyte metabolic capacity to be stable enough to detect relevant metabolism over longer terms. The sandwich culture model has become an invaluable tool and a common *in vitro* model to investigate drug-drug interactions due to their ability to maintain many of the structural features of the *in vivo* hepatocytes. While enabling query of the more relevant hepatocyte features such as polarity and appropriate drug transport kinetics, the sandwich culture format does not completely solve the technical issue of metabolic decline.

There have, however, been advances in other 3D culture methods that are gaining popularity in use. Spheroids are spherical cultures that can be produced by either embedding hepatocytes in non-adhesive hydrogels or allowing hepatocytes to self-assemble in low attachment cell culture plates or hanging drops. Studies have shown that the formation of multicellular hepatocyte spheroids in the 3D culture is a promising approach for enhancing liver specific functions. Hepatocytes cultures in spheroids resemble polarised cell structures and direct cell-cell contact and importantly can maintain steady metabolic function for more than 14 days. This approach also enables the self-assembly of multiple cell types in the liver creating a pseudo microtissue much more similar to the *in vivo* hepatocyte environment.

Another method shown to improve hepatocyte health and metabolic stability are fluidic flow system whereby cells in a monolayer are placed in chambers with media flowing and recirculating. While they may not be as amenable to higher throughput applications as spheroids, flow-based cell culture systems also stabilise metabolic activity for more than 14 days with the added benefit of allowing longer-term hepatocyte cultures without frequent media changes. Without frequent media changes, metabolites generated from slowly metabolised drugs are allowed to build up in the culture improving chances of detecting the metabolite during bio-analysis.

Importance of advanced culture formats in hepatotoxicity

With respect to hepatotoxicity, damage to hepatocytes is often secondary to immune responses or other endocrine and paracrine signalling. The complex signalling between cells and tissues during a toxic response to drugs and chemicals has implications in how well an *in vitro* liver model needs to

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Primary human hepatocytes in sandwich culture for five days. © Lonza, Basel, Switzerland

replicate the full liver function. Regulatory agencies do not require detailed mechanistic testing in a human model system for toxicity testing, so it is most often not performed. Human cellular toxicity testing that is required is limited to very simple models and tumour cell lines.

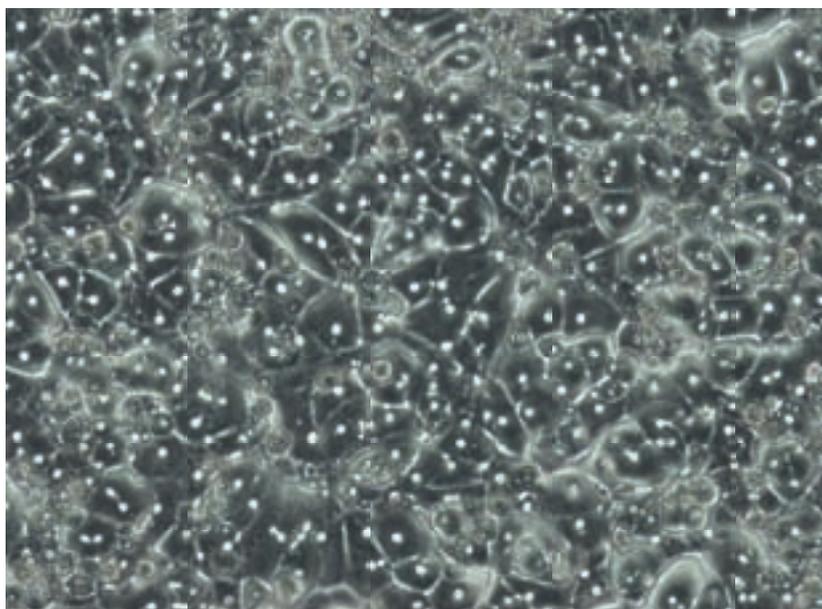
Toxic drugs that advance through the clinical pipeline often do so because of three main factors:

1. The length of time to generate the toxic response is long and therefore not modelled by the shorter term preclinical and early clinical testing.

2. The toxic response is specific for humans and therefore not captured in preclinical animal testing.

3. The toxic response is rare and population sizes used in preclinical and early clinical studies are too

Primary animal hepatocytes in sandwich culture for two days. © Lonza, Basel, Switzerland



small, or not diverse enough to capture the effect.

These late-stage failures are very costly, both financially and in terms of patient health.

With advances in primary cell culture technologies and bioengineering that enables the recreation of human physiology on the benchtop, there are opportunities to fill the gaps in preclinical testing. For the first type of toxicity failure, duration of response, hepatocytes and hepatic micro-tissues can be maintained for >40 days in a spheroid format and fluid flow cultures. For the lack of correlation between human response and animal models, there are examples where micropatterned hepatocyte with fibroblast co-cultures and spheroids out-predict animal models for clinical liver toxicity. For larger population-based idiopathic responses, cell culture systems such as iPSC-derived cells can be developed from biobanked tissue sources that number in the 10s of thousands.

There are of course other cell types that are important for ADME and toxicology testing, such as stellate, Kupffer and sinusoidal endothelial cells, which can be coupled in co-cultures to allow the analysis of complex communication networks *in vitro*. Such an approach could have enormous benefits, especially when expanded to couple entire microphysiological systems such as the liver, heart, lung muscle, adipose and kidney. Although years away from reality, the advantages of being able to analyse interactions and the impact of a drug compound more holistically across the body could be significant in terms of the future of drug development methods.

Impacting big pharma

It is thought that the regulatory agencies are beginning to explore and even embrace more *in vitro* testing technologies, as evidence continues to mount for their value. This has required a change of mind-set to include seeing each of these new *in vitro* technology as a functional unit, rather than something that will solve current preclinical testing failures by providing a one-to-one substitution for animal models.

All pharmaceutical companies have internal or external groups that address the needs for ADME and toxicology in their drug pipelines. In larger pharma companies, these are often within their own metabolism and toxicology groups of specialists where other discovery project groups within the company send their compounds for testing. Such groups will see every compound in development, enabling them to have a broad overview of the development pipeline, and be

highly specialised within their testing arena. Having specialists available internally enables pharmaceutical companies to have more integrated interactions between the ADME and toxicology testing teams, and the discovery scientists. By allowing all of these groups to discuss findings, goals and outcomes, the discovery process in its entirety can flow smoothly and be more efficient and quickly adapt to changing regulatory guidance and trends.

In contrast, smaller biotech and drug development companies often do not have the funds or resource to warrant an internal team of specialists specifically for ADME and toxicology testing and as such, these companies rely on outsourcing. Therefore the ability to adapt to newer technologies that can help streamline the process and better ensure clinical success is dependent on the various offerings of CROs.

Nonetheless, as with almost any company in the current financial climate, the pharmaceutical industry, small and large, is under increasing pressure to lower costs while maintaining high outputs of quality drugs. In order to achieve this, there is a need to lower the cost of research and development. Better prediction of clinical failures through innovative new processes in ADME and Toxicology settings, that can be implemented earlier in the development pipeline, could significantly improve overall costs for clinical trials and ultimately decrease costs to patients.

Innovation for the future

The FDA has issued guidelines for the use of ADME and toxicology within the drug discovery pipeline, and although it is always recommended to follow such guidelines, it is the ability to innovate that enables drugs to succeed in development. Companies whether small or large need to be able to look beyond the supplied guidelines and visualise the need for testing protocols at various stages of the pipeline, especially earlier on, to really benefit from the data that such techniques supply. Regardless of what the regulatory agencies require, if a technology proves to be more predictive than the currently required regulatory test, it makes sense for its use to be adopted. Ultimately eliminating compound destined to fail earlier in development saves money across the board from investors, to large pharma companies, all the way to the amount each patient has to pay for drugs that are determined safe and effective. The recent advancements in new *in vitro* liver microtissues and improved isolations of the core cellular components of the liver is one area that all drug companies can begin to benefit from.

Conclusion

In vitro, human-based ADME and toxicology methods have advanced over recent years, providing researchers with new and effective testing tools to use throughout the drug discovery pipeline, as an indicator of whether the drug candidate will succeed through clinical trials and be brought to the market. The implementation of more tailored, in-depth testing could save pharmaceutical companies both time and resource in the long run. By identifying unsuccessful candidates and ruling them out earlier on, only those compounds most likely to succeed are moved through to the later stages of the pipeline. This more streamlined approach enables the R&D process to be more effective, thus reducing costs and time spent testing unviable candidates, ultimately leading to reduced drug costs for patients in the future.

By ensuring that only the most promising candidates are progressed through to clinical trials, the whole drug discovery pipeline can become more streamlined and efficient. Optimising drug leads early on enables the cost and time implications of progressing unsuccessful compounds through to the later stages of development to be significantly reduced.

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