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EXPERT OPINION

Human induced pluripotent stem cell-derived hepatocytes for toxicology testing

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The need for more predictive *in vitro* toxicity models is a critical deficit in current preclinical pipeline safety evaluations. Current models employing tumor-derived cancer cell lines and isolated primary human hepatocytes (PHHs) afford an approximation of overt cytotoxicity but do not provide hepatotoxicity prediction owing to liabilities in metabolic activity along with phenotypic variability and instability in culture. Induced pluripotent stem cell-derived hepatocytes (iPSC-HCs) offer a long-term solution to accessing liver tissue from representative diverse as well as idiosyncratic patient populations and can be sourced indefinitely. iPSC-HCs are currently being evaluated as potential replacements for the existing cell models, but they have yet to prove superiority. It is acknowledged that iPSC-HCs are not functionally equivalent to PHHs and are somewhat mixed in terms of their gene expression profile, simultaneously displaying mature and immature markers *in vitro.* Combining iPSC-HCs with organotypic culture systems affords an opportunity to maximize the potential of both technologies where the cells benefit from more complex culture conditions while unlocking the potential of the culture systems by affording stability and reproducibility to provide the future of predictive *in vitro* toxicity models.

Keywords: CYP450, drug-induced liver injury, hepatocyte, induced pluripotent stem cell, organotypic, stem cell, toxicity

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1. Introduction

A recent examination of drug failures in clinical trials, particularly during the recent push to advance novel therapies at an accelerated rate as directed by the FDA via the Prescription Drug User Fee Act, reveals that the number of post-market withdrawals and black box labeling of drugs has concomitantly increased [1]. This resulting increase of unforeseen toxicity in late-stage clinical development and post-market release, particularly in the area of liver toxicity, highlights the need for highly predictive models of adverse clinical outcomes attributable to targeted and off-target drug toxicity. The pursuit of better models suggests the development of advanced *in vitro* human toxicity models for two primary reasons: i) the lack of a comprehensive predictive correlation between current *in vitro* and *in vivo* toxicity models and population-wide human outcomes; and ii) animal models are under increased pressure from regulatory agencies to be obviated from the drug development process from an ethical viewpoint. Thus, the pressure to work with predictive human patient population representative *in vitro* models has mounted over time. To address these concerns and enable a better clinical development path for drug safety as it relates to toxicity prediction and mechanistic investigations, the use of stem cell-derived tissues (i.e., cardiomyocytes, neurons and hepatocytes) has been an intense area of research interest. Whereas the application of stem cell-derived cardiomyocytes in drug safety assessments has already demonstrated their value to the

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point of garnering the attention of regulatory agencies [2,3], the use of stem cell-derived hepatocytes in ADME-T applications is still in an exploratory phase at present. This editorial will focus on the current state-of-the-art technology for the promise of human stem cell-derived hepatocytes in drug toxicity assessment.

A typical preclinical drug safety evaluation for liver toxicity will employ cell-based and animal models as indicators of potential liabilities. Focusing specifically on *in vitro* models, a number of cancer-derived cell lines (e.g., HepG2 and Huh7) can be employed in hepatotoxicity assessments, but these cells are limited in terms of their drug-metabolizing functions [4,5]. This functional liability makes these lines questionable in terms of their predictive nature for normal, healthy hepatocyte drug sensitivity in relation to toxic metabolite formation. More recent advances in the creation of the hepatoma-derived HepaRG™ cell line represents an improvement over previous cell line models in terms of hepatocyte-like functions, particularly in CYP function and nuclear receptor pathways [6]. However, this cell line still finds its origin in a tumor background resulting in reduced sensitivity to toxic insult. In addition, it is also limited to a single genotype available for toxicity investigations, and thus is not representative of a broad patient population. Access to primary human hepatic tissue in the form of isolated primary human hepatocytes (PHHs) has significantly improved with the advent of methods to stably cryopreserve the primary isolates [7]. However, once removed from the *in vivo* setting, these cells do not adapt well over time to the tissue culture environment. PHH in static two-dimensional (2D) culture routinely require Matrigel™ or another extracellular matrix protein overlay in standard sandwich culture to survive and still rapidly decline in terms of their function. PHHs attain a dispersed fibroblast-like morphology over a week's time, with CYP function declining even more rapidly over 48 h in culture [8,9]. While the opportunity to obtain a broad panel of representative donor-derived hepatocytes exists, the degradation of these cells in culture clearly limits their predictive capability, particularly in terms of chronic compound dosing experiments designed to explore metabolite formation and repeated low-dose exposure-related toxicity. Additionally, there is tremendous donor-to-donor and preparation-to-preparation variability in the overall cell quality with some lots significantly outperforming others in their ability to form adherent cultures and exhibit hepatocyte functions *in vitro*. Thus, researchers are forced to screen lots to identify the most suitable PHH material available at the moment for a given application in toxicity and drug metabolism assessment. Once a 'good lot' is identified, the investigator is compelled to secure as much of the lot as possible to ensure a semidurable supply, which will inevitably have to be replaced with similarly performing material as empirically determined by functionality assays. In attempts to capture the drug-sensitivity picture for a broad patient population and extend the supply, PHH lots also are frequently pooled to provide an averaged predictive assessment.

With the advent of footprint-free episomal primary tissue reprogramming to induced pluripotent stem cells (iPSCs) [10], the opportunity now exists to secure an infinite supply of differentiated tissues, including hepatocytes by multiple reported strategies [11-15], from a panel of donors with varying drug sensitivities. Several recent publications illustrate the predictive sensitivity of iPSC-derived hepatocytes (iPSC-HCs) to distinguish known hepatotoxins from compounds that do not negatively impact the liver across multiple toxicity end points and readouts [16-18]. The iPSC-HCs are sensitive to a diverse range of chemical and drug target classes spanning a varying range of toxicity mechanisms and, most importantly, are capable of generating the relevant toxic metabolites from known hepatotoxic agents (e.g., aflatoxin B1) in sufficient concentrations to reveal their cytotoxic effects. The data suggest that the relevant drug-metabolizing machinery is in place, although potentially limited as compared with the most highly functional PHH lot expressing a particular CYP of interest as identified by lot screening. One distinct advantage of iPSC-HCs over PHHs is the ability to sustain long-term viability and function in culture, affording the opportunity to explore lower, more clinically relevant dosing of compounds *in vitro* to reveal the impact of long-term exposures. This type of chronic dosing more accurately reflects the human *in vivo* drug exposure profile and further enhances the predictive power of *in vitro* toxicity testing. In the work of Holmgren *et al.*, chronic drug exposure of iPSC-HCs over 14 days in culture led to increased sensitivity as observed in the reduction of resulting EC50s by more than an order of magnitude as compared to a 48 h exposure [19]. The panel of compounds examined included amiodarone, aflatoxin B1 and troglitazone, and suggested a more sensitive repeat dose hepatotoxic assessment is possible in iPSC-HCs as compared to HepG2 cells in this example. It should be noted that this type of sustained exposure would not be possible with PHHs over the extended culture time, hence the comparison of iPSC-HCs to a cancer cell line.

By generating iPSCs from clinically identified donors exhibiting a range of drug sensitivities and metabolizing backgrounds, one can now secure access to a population representative, diverse, toxicity predictive hepatocyte panel and access the identical hepatocytes from that panel in perpetuity for drug testing [20]. Thus, one can now obtain hepatocytes reflecting a range of drug sensitivities for preclinical *in vitro* screening. In addition, and of potentially greater significant value, researchers can now perform a retrospective clinical analysis to understand why an unforeseen clinically sensitive population responded negatively to a development candidate during clinical trials or after approval. By identifying the genetic factors and *in vivo* mechanisms that contribute to idiosyncratic drug-induced liver injury (DILI), the drug development process can be better informed to avert such negative clinical outcomes. Efforts are underway to generate such iPSC-derived cells, initially including hepatocytes and Kupffer macrophages, from donors identified in the DILI

Network via collaboration between Cellular Dynamics International and the Hamner Institutes for Health Sciences [21]. Additionally, the Innovative Medicines Initiative has funded the MIP-DILI effort to generate iPSC-derived hepatocytes from sensitive patient populations and explore 2D and 3D culture conditions to develop clinically predictive models of toxicity.

The potential for iPSC-HCs to engender donor diversity in unlimited long-term supply along with stability and consistency in the results generated during compound testing is already being realized. Stem cell culture techniques have advanced to maintain genetically stable cultures of differentiation ready expandable cell populations to ensure consistent starting material is entering the cell production process to ensure consistency in the resulting differentiated tissues. However, there is still room for improvement in this model system as it is applied to predictive hepatotoxicity beyond compound assessment in monocultured hepatocytes in a 2D sandwich format. For example, CYP induction pathways have been shown to be limited in iPSC-HC as compared to PHH suggesting that nuclear receptors pathways may not be fully functional. To that end, a variety of efforts are underway to identify mediators of enhanced maturity and more liver representative functions *in vitro* for drug-sensitivity testing. These efforts include: the identification of small molecules [22], heterologous cell co-culture systems [23], flow-based models [24], and enhanced matrices and 3D culture formats [25,26]. All such efforts are aimed at achieving a more completely functional liver-like tissue *in vitro* [27,28]. Many of the engineered systems being explored have been designed historically to improve and preserve the functions of PHHs *ex vivo* and now these efforts are significantly benefiting in return from the inclusion of iPSC-HCs as the source tissue. Bioengineers developing organotypic culture systems have previously been limited to employing suboptimal cancer-derived cell lines or PHHs in their models and these hinder the potential of these culture systems by adding noise to already complex processes. iPSC-HCs offer a solution to both donor and lot specific concerns by sourcing from a consistent genetic background combined with controlled production systems resulting in uniform cell performance. This combination of technologies can synergize to advance both *in vitro* liver bioengineering and iPSC-derived hepatocyte technologies. This advancement can be specifically envisioned in two ways: i) for liver bioengineering, employing uniform reagent cells in the form of iPSC-HCs along with accompanying relevant same donor-derived cells in co-culture removes the inter-lot/donor variability that currently hampers development, cross-system comparisons and routine implementation; and ii) for iPSC-HCs, organotypic culture systems, by increasing the complexity of the culture conditions, can enhance the functional maturity of iPSC-HCs by providing a more complete liver-like environment and molecular cues, thus increasing their predictive power.

2. Expert opinion

While the application of iPSC-HCs in toxicity testing represents a paradigm shift in *in vitro* drug development, it should be acknowledged that the systems are not perfect. By design, *in vitro* iPSC differentiation protocols are aimed at recapitulating ontological processes on an abbreviated timescale. While the resulting cells attain many of the functional characteristics of PHHs, current differentiation processes yield a cell population that is of a mixed phenotype: iPSC-HCs that express adult markers (e.g., high levels of albumin expression) while maintaining the simultaneous operation of aspects of the fetal gene program (i.e., α-fetoprotein and CYP 3A7 expression). While this mixed phenotype has critics concerned about the predictive power of iPSC-HCs, the advantages outlined throughout this editorial outweigh such skepticism and perceived limitations. It can be argued that, while the cells are not perfect, they represent a vast improvement over a tumor-derived cell line or PHH that is declining in function and in the process of de-differentiating into a different cell type altogether. The current state-of-the-art iPSC-HC models have already demonstrated value for predictive *in vitro* toxicity. Future advancements in culture systems will certainly serve to elevate iPSC-HCs further as a model not currently available for predictive toxicity evaluation that represents a broad patient population.

Declaration of interest

The author is an employee of Cellular Dynamics International, Inc. The author has no other relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript other than those disclosed.

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