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Human Liver Organoid Platforms for MASH Drug Discovery: Evaluating Immunometabolic Fidelity, Pharmacological Predictivity, and Translational Readiness

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Abstract. Metabolic dysfunction-associated steatohepatitis (MASH) is a progressive form of metabolic dysfunction-associated steatotic liver disease characterised by steatosis, inflammation, hepatocellular injury and fibrosis. Human liver organoid platforms offer a controllable system for modelling selected MASH-related processes and evaluating candidate therapeutics, but their performance varies across biological fidelity, pharmacological predictivity and translational readiness. This review evaluates three major platform types: pluripotent stem cell (PSC) - derived multicellular organoids, expandable hepatocyte organoids and assembled multicellular systems. Current evidence indicates that PSC-derived models provide the broadest representation of multicellular MASH phenotypes, hepatocyte organoids are particularly suitable for genetic and pharmacological screening, and assembled systems offer greater control over cell composition and experimental conditions. However, no single platform currently combines adult-like hepatic function, robust multicellular disease modelling, scalable screening and demonstrated clinical predictivity. Major limitations include immature cell states, donor and batch variability, and limited representation of perfusion, zonation and extrahepatic metabolism. Overall, liver organoids are best viewed as fit-for-purpose tools for mechanistic studies, target validation and early candidate evaluation, with future progress requiring improved standardisation, patient-tissue benchmarking and clinically relevant validation.

Keywords: Liver Organoids, Metabolic Liver Disease (MASH / MASLD), Preclinical Drug Discovery, Immunometabolism

1. Introduction

Metabolic dysfunction-associated steatohepatitis (MASH) is an inflammatory form of metabolic dysfunction-associated steatotic liver disease (MASLD) characterized by hepatic steatosis, hepatocellular injury, and lobular inflammation, with variable degrees of fibrosis [1]. Beyond fat accumulation, the underlying mechanism is a vicious cycle of hepatocyte lipotoxicity, innate immune cell activation, hepatic stellate cell activation, and extracellular matrix accumulation [2, 3]. Fibrosis progression leads to cirrhosis and negative liver outcomes [2]. Therefore, effective treatments need to improve hepatic metabolic stress, inflammation, and fibrosis. Resmetirom acts on

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hepatic lipid metabolism by activating thyroid hormone receptor beta (THR-β). Semaglutide is a GLP-1 receptor agonist whose therapeutic effects in MASH are strongly linked to weight loss and improvements in systemic metabolism [4, 5]. These distinct mechanisms highlight the importance of selecting an in vitro model that can measure the intrahepatic response to a drug, and acknowledge that there are systemic contributions that may not be present in an in vitro model. Two-dimensional hepatocyte cultures can show lipid accumulation, but they do not capture hepatocyte, immune cell, and stromal cell interactions. Animal models recapitulate systemic complexity, but species-specific metabolic and immune differences may limit their applicability to humans [6]. Human liver organoids hold promise for bridging this gap.

Pluripotent stem cell (PSC)-derived organoids have been shown to exhibit sequential steatosis, inflammation, and fibrosis after free fatty acid treatment [6]. Kumar et al. used the fully defined HepMat matrix to maintain hepatocyte-like, macrophage-like, hepatic stellate, and endothelial cells which increases the inflammatory and fibrotic responses [7]. High-throughput genotyping of donors has shed light on gene and metabolic interactions [8]. Improvements in vascularization [9] and the development of a microwell system for organoid imaging [10] have increased structural complexity and continuous measurements. Both of these technologies make large scale organoid culture more feasible for drug screening. These advances have established that human liver organoids have utility in the laboratory setting. However, none of these technologies encompass the disease mimicry and high throughput screening potential.

The addition of multiple cell types alone does not result in an adult MASH model. Immature cellular phenotypes, batch to batch variation, acute exposure to high concentrations of fatty acids, and lack of perfusion, zonation, and immune cell infiltration are limitations present in current organoid systems [6, 10, 11]. Most models in the literature are capable of producing a disease-like phenotype or responding to a drug, but only a few have tested predictive validity in a compound panel of both approved and unsuccessful drugs. Hepatocyte organoids have been used to identify CRISPR target genes for steatosis [12], and liver organoids have been shown to have good predictive validity in a drug-induced liver injury model [13].

This review evaluates the immunometabolic mimicry, pharmacologic predictivity, and suitability of multicellular human liver organoids for MASH drug discovery. The paper compare the cell populations used, how disease is induced, pharmacologic end points, reproducibility, and scalability across different models. Based on the strengths and weaknesses identified, the review describes how these organoids should be used in basic research, target identification, and early drug candidate screening. Finally, the criteria required for clinical benchmarking and the experimental evidence needed to establish validation standards are discussed.

2. Human liver organoids for modelling MASH

2.1. Rationale and validation criteria for multicellular MASH models

MASH is driven by a continuous multicellular feedback loop. Lipid overload causes hepatocyte stress and injury leading damaged hepatocytes to release chemokines and damage-associated molecular signals. Macrophages amplify or regulate inflammation. Hepatic stellate cells shift into collagen-producing, myofibroblast-like states. Liver sinusoidal endothelial cells contribute to metabolic exchange, immune recruitment, and formation of the fibrotic microenvironment. Therefore, lipid droplet accumulation indicates steatosis but does not by itself demonstrate that a model has reproduced MASH.

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Evidence from patient tissue provides a benchmark for model evaluation. Single-cell studies show that NASH livers contain TREM2-high disease-associated macrophages and that hepatic stellate cells act as important signalling hubs [3]. Spatial multi-omics has further revealed heterogeneous macrophage states and an association between endothelial-stellate cell connections and advanced fibrosis [2].

Two-dimensional cultures are useful for studying hepatocyte-intrinsic mechanisms, while animal models are useful for systemic effect. The distinct value of multicellular human liver organoids is that they allow controlled study of human intrahepatic crosstalk. A model should include at least the cell combination required by the research question.It should also demonstrate interactions through co-culture dependence, cell-specific readouts, or perturbation experiments.

2.2. Current organoid platforms: classification and research progress

Current liver organoids platforms can be divided into three main groups: self-organizing multicellular models derived from PSCs, three-dimensional models assembled from primary cell lines, and expandable organoids composed mainly of hepattic epithelial cells or hepatocytes. These platforms emphasise cell diversity, experimental control, and screening efficiency, respectively. They should not be ranked only by the number of cell types. Instead, they should be compared according to their intended use.

PSC platforms provide the most direct support for multicellular MASH modelling. After fatty acid stimulation, the Ouchi et al. model showed lipid accumulation, inflammation, and fibrosis [6]. Kumar et al. used the fully defined HepMat matrix to maintain hepatocyte-like, macrophage-like, hepatic stellate, and endothelial cells. The co-culture response the response was stronger than observed in matched monoculture [7]. Kimura et al. expanded this apporach to 24 donors and showed that the effects of glucokinase regulator (GCKR) variants depended on metabolic state [8]. Studies of patatin-like phospholipase domain-containing protein 3 (PNPLA3)-associated iPSCs also show that geneic background can be included in these models [14]. The presence of multiple cell types does not guarantee adult-like functional maturity, which must be validated separately for each relevant hepatic function.

Expandable hepatocyte organoids are more suitable for genetic manipulation. Hendriks et al. combined a steatosis phenotype with CRISPR and drug screening to identify fatty acid desaturase 2 (FADS2) [12]. Adult stem cell-derived models can also produce stable lipid accumulation [15]. These platforms are useful for target discovery.However, because they lack immune and stromal cells, they mainly address mechanisms within hepatocytes and cannot represent complete immunometabolic crosstalk.

Assembled models provide greater experimental control. Bronsard combined HepaRG cells, primary macrophages,and LX-2 cells in a three-cell-type organoid to compare drug toxicity in a fatty liver background [11]. Vascularized PSC organoids have also advanced scalable production and tissue organization [9]. Guan et al. multi-lineage model can reproduce fibrosis [16], and a later microwell system allows continuous measurement of collagen and inhibitor effects [10]. These methods expand the options for safety testing, imaging, and antifibrotic screening. However, dependence on cell lines or the use of induction methods that are not specific to MASH limits the conclusions that can be drawn.

Overall, steatosis, multicellular inflammation, fibrosis,genetic susceptibility, and scalability are distributed across different platforms. No current model current performs optimally in disease fidelity, throughput, and clinical predictivity at the same time. The appropriate approach is to define

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the intended use first and then select the cell composition, induction method, and readouts. Liver organoids should not all be treated as the same category of evidence.

3. Evaluation of human liver organoids for MASH drug discovery

3.1. Evidence for immunometabolic fidelity

Among current multicellular systems, Ouchi et al. provide the clearest evidence for a sequence resembling MASH progression. The study used 11 healthy or diseased PSC line. The resulting organoids contained hepatocyte-like, stellate, and Kupffer-like cells. Free fatty acids caused sequential lipid droplet accumulation, inflammation, and fibrosis,while reggregated co-culture spheroids did not show the same response. Tissue stiffening measured by atomic force microscopy also matched the degree of fibrosis [6]. Patient-derived organoids with lysosomal acid lipase deficiency showed a more severe phenotype that was reduced through the fibroblast growth factor 19/farnesoid X receptor (FGF19/FXR) axis. These results suggest that three-dimensional self-organisation and genetic background change disease responses. However, rescue of a rare monogenic disorder does not establish a general mechanism for common metabolic MASH.

Kumar et al. directly tested cell interactions in a fully defined HepMat matrix. hepatocyte-like, stellate-like, macrophage-like, and endothelial-like cells were all kept together. Transforming growth factor beta (TGF-beta) elevated expression of fibrotic genes, such as collagen type I alpha 1 chain (COL1A1) and collagen type III alpha 1 chain (COL3A1), as well as expression of inflammatory genes such as tumour necrosis factor alpha (TNF-alpha), interleukin-1 alpha (IL-1alpha), and interleukin-6 (IL-6). Fatty acids also led to increases in both fibrotic and inflammatory genes [7]. This stronger response in co-culture compared with the matched monoculture indicates some level of cell-to-cell dependence. However, since exogenous TGF-beta directly induces fibrosis, it does not tell us whether the model adequately represents the progression from hepatocyte injury, to immune activation, to stellate-cell activation. In addition, Kimura et al. demonstrated in a panel of 24 donors that the GCKR rs1260326-T variant gave rise to a unique lipid, inflammatory, and mitochondrial phenotype when exposed to an insulin-resistant environment [8]. While the lipid phenotype was compared to a clinical cohort for external validation, the use of only 24 donors means that the panel cannot represent low-frequency variants. The panel is therefore more informative for studying genotype–phenotype interactions across donors than for making individual-level predictions.

Compared with patient tissue, the main gaps remain in the reproduction of disease-relevant cell state and spatial organisation. TREM2-high macrophage populations, macrophage heterogeneity, and spatial interactions between endothelial and stellate cells have been identified in patient livers [2, 3]. However, these features have not been systematically matched in models above by combined single-cell and spatial methods. Increases in alpha-smooth muscle actin (alpha-SMA), COL1A1, or total collagen also do not confirm that stellate cells have reached an adult MASH state. Isolated liver organoids also adipose tissue, the gut, endocrine inputs, and circulating immune-cell recruitment. Current evidence therefore provides relatively strong support for some functional links in the intrahepatic lipotoxicity-innate immunity-fibrosis axis. It provides weaker support for adult maturity, tissue zonation, and extrahepatic metabolic fidelity.

3.2. Evidence for pharmacological predictivity

Pharmacological predictivity should not be inferred solely from a reduction in a disease phenotype. This review evaluates the evidence at three tiers. A tier 1 finding would be pharmacological

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responsiveness; a reproducible or dose-related effect. Tier 2 findings would be consistent with the expected mechanism of action on the target. Tier 3 findings would indicate the potential for predictive validity, defined as the ability to discriminate between compounds with clinically demonstrated efficacy and compounds that failed to demonstrate efficacy, within a predefined context of use. Most MASH organoid studies currently reach the first or second level, but not the third [17].

For example, Ouchi et al. showed that intervention through the FGF19/FXR axis reduced oxidative stress and improved the steatohepatitis phenotype in organoids with lysosomal acid lipase deficiency [6]. Thus, while the data demonstrated the potential of the system to provide evidence of rescue of a pathophysiologic mechanism, it was a proof-of-concept study based on one genetic disease model. In contrast, Kimura et al. showed that metformin failed to reverse GCKR-induced mitochondrial dysfunction, whereas co-treatment with nicotinamide riboside and nitazoxanide restored oxidative adaptation [8]. The data indicate that the metabolic environment can influence pharmacologic responses, but this particular intervention has yet to become a clinical standard for MASH.

In a more comprehensive set of experiments, Hendriks et al. used primary human hepatocytes to generate organoids and tested 17 drug candidates at four concentrations. They found that lipid droplet content was reduced following treatment with inhibitors of acetyl-CoA carboxylase (ACC), fatty acid synthase (FAS), diacylglycerol acyltransferase 2 (DGAT2), and agonists of FXR and FGF19. Furthermore, the ranking of the efficacy of these drugs appeared to correlate between organoids generated using distinct triggers of steatosis [12]. While the study provided a more rigorous and systematic approach to pharmacological screening, the primary endpoint measured was the reduction of hepatocyte lipid content, without inclusion of inflammatory and fibrotic components. However, Guan et al. subsequently used micro-organoids to distinguish responses to TGF-beta- and platelet-derived growth factor B (PDGF-B)-driven fibrosis. Inhibition of the respective signaling pathways (TGF-beta and PDGF receptors) blocked the corresponding induction, whereas p38 MAPK and GSK3beta inhibition blocked both types of induction [10]. The data suggest the capacity to measure different degrees of pharmacological predictivity. However, this was still an in vitro screen using exogenous growth factors to drive the fibrotic process.

Bronsard et al. evaluated drug safety in a steatotic context using tricellular organoids composed of HepaRG cells, LX-2 cells, and primary human macrophages. They then ran ATP-based dose response assays with five compounds: 5-FU, paracetamol, valproic acid, voriconazole, and troglitazone. Steatotic organoids were found to be more sensitive to troglitazone [11]. These findings show that the steatotic background modifies drug toxicity; however, the study did not assess predictive efficacy for MASH therapeutics. Shinozawa et al. evaluated 238 marketed drugs at four concentrations using viability, cholestasis, and mitochondrial-toxicity endpoints. The study reports a sensitivity of 88.7% and specificity of 88.9% [13]. The main strength of this work is showing that you can validate a model using a defined set of compounds with multiple endpoints and defined cutoff values. These data however are not applicable to the prediction of efficacy for MASH. A true test of predictive power would use known effective drugs and late stage failures, run them at unbound concentrations similar to what is seen in humans, and blind the study and validate the results independently. No representative multicellular model has been tested in this way. Additionally, a valid drug model will depend on where the drug works. Resmetirom, a liver-specific THR-beta agonist [4], might be amenable to liver models, whereas the mechanism of action for semaglutide involves appetite, weight, and adipose [5]. In a non-organoid perfusion model,

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resmetirom reduced hepatic fat and partially normalized insulin response, but it also increased chemokine ligand 1 (CXCL1) and interleukin-8 (IL-8) [18].

3.3. Evidence for technical and translational readiness

Technical readiness can be assessed in terms of scalable production, automated readouts, and control of experimental variability. Kimura et al. pooled cryopreserved foregut progenitors and monitored 24 donors, Technical readiness can be assessed in terms of scalable production, automated readouts, and control of experimental variability [8]. Kumar's completely defined HepMat matrix decreases the uncertainty of matrix composition [7]. Guan et al embedded collagen reporting and single-cell imaging in a microwell format [10]. Harrison created organoids without extracellular matrix in 125 or 500 mL suspension flasks and quantified cytochrome P450 (CYP) 1A2 and 3A4 activity, albumin, and urea production [9]. These works focus on cell source, matrix composition, readouts, and production scale, respectively. None combine these elements into one MASH screening workflow. Minimum quality control for drug development must include more than the statement'organoids formed'.

Every batch should state the percentage and type of hepatocytes, macrophages, stellate cells, and endothelial cells. It should provide data on albumin, urea, and major CYP activities, along with baseline inflammation and fibrosis prior to induction. It should further report changes in lipid droplets, cytokines, and collagen following induction, as well as organoid diameter, presence of necrotic cores, donor-to-donor variation, batch-to-batch variation, and the concentration of unbound drug, solvent, and treatment time. High throughput assays should similarly provide information on intra- and inter-well variation, positive and negative controls, and the threshold for acceptable batches. The required number of replicate wells, data-exclusion criteria, and expected effect ranges should be prespecified. The current studies describe only some of these metrics. Thus, scaling up one platform does not necessarily imply that its MASH phenotype will be reproducible. To validate a specific MASH context of use requires more robust evidence. The intended context of use and primary endpoints should be defined before testing.

Then the test the drug rankings using the same panel of benchmark drugs in blinded trials, with multiple donors and laboratories, and compare the results with either patient tissue samples or clinical outcomes. Mechanistic studies may rely on cell-specific endpoints. Establishing ranking criteria and rules for repetition before assessing candidate drugs. Shinozawa et al. 384-well DILI assay and validation with 238 drugs [13] demonstrate that such an approach is possible, although the endpoint of interest differs.There is now sufficient technical capability to perform mechanistic studies and limited screening with multicellular organoids.

4. Current limitations and future directions

4.1. Overall limitations and fit-for-purpose applications

The main problem is not the lack of a certain type of cell, but that disease fidelity, screening performance and system-level coverage have not been unified. Although PSC-derived multi-cell-type models are well suited for studying multicellular responses, their validity as adult tissues has yet to be confirmed [6-8], and batch-to-batch consistency is also a concern. On the other hand, hepatocyte-dominant organoids can be expanded, edited and screened at scale, but they do not allow direct study of immune and stromal responses [12, 15]. Although assembled/engineered models are more controllable in terms of cellular components and experimental conditions, the available

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evidence comes from different areas (drug safety, general fibrosis, tissue engineering), which are not directly applicable to predicting MASH efficacy [9, 10, 11, 16]. Therefore, the relationship between model complexity and usefulness for drug discovery is not a simple linear relationship: additional cell types may provide more biological information, but they may also lead to higher variability, lower throughput and more complex data interpretation.

Additionally, model utility depends heavily on the target application. Stable lipid droplet production enables studies of steatosis only. An increase in collagen or alpha-SMA only indicates a fibrotic-like response. Reversal of a single endpoint by a drug shows drug responsiveness, but does not necessarily mean the model is predictive of clinical efficacy. If each of these studies aggregates results across all of these evidence levels and concludes that a model has 'reproduced MASH' or 'is predictive of efficacy', they are claiming more than they have achieved. The real question is not whether an organoid is better than a 2D monolayer in every way, but whether the organoid can make a particular decision more reliably than a simpler model would.

On the basis of existing evidence, this review suggest the following four levels of applications for multicellular liver organoids. Strongest evidence supports using these organoids to study intrahepatic cell crosstalk, disease processes and target validation. Early-stage comparisons of antisteatotic and antifibrotic drugs, along with safety evaluation under disease conditions. Donor-derived organoids can also be used to study the interaction of genetic background and metabolic conditions, but they are currently more suitable for stratified populations rather than personalised dosing [8]. Predicting the clinical efficacy of systemic metabolic drugs, predicting long-term disease outcome, or even fully replacing animal experiments, remains unproven in isolated liver organoids. In short, the proper role of multicellular organoids should not be as a whole human MASH model, but as fit-for-purpose evidence modules in a multi-model drug discovery process.

4.2. Priorities for future development

Future development should prioritise a clearly defined context of use and a staged validation framework. Biological validation should compare cell states and spatial interactions with patient tissues and require both baseline hepatic function and MASH-relevant responses [2]. Analytical validation should quantify donor, batch, and interlaboratory variation using shared procedures, reference materials, and analysis methods. Pharmacological validation should use blinded panels containing clinically effective drugs, late-stage failures, and mechanism-specific negative controls at clinically relevant unbound exposures. Endpoints and ranking criteria should be specified before testing. The multi-compound, multi-dose DILI study by Shinozawa et al. demonstrates the feasibility of this design, although a MASH-specific benchmark panel remains to be established [13].

Optimization should address the limitations most relevant to each application. Greater cellular maturity, perfusion, zonation, and immune recruitment may strengthen mechanistic fidelity, whereas defined matrices, scalable cell production, automated imaging, and stable endpoints are more important for screening. A universal MASH organoid remains a long-term possibility, but current evidence more strongly supports a tiered set of complementary models. Hepatocyte-enriched organoids can support target discovery and high-throughput screening; multicellular organoids can test intrahepatic mechanisms and confirm candidates; and microphysiological, multi-tissue, or animal models can assess systemic effects. This approach expands organoid use without extending conclusions beyond validated boundaries.

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5. Conclusion

This review examined major human liver organoid platforms for MASH drug discovery by evaluating their immunometabolic fidelity, pharmacological predictivity, and technical and translational readiness. By comparing the distinct strengths and limitations of these platforms, the analysis clarifies their most appropriate roles within different stages of MASH research and drug development. Multicellular human liver organoids provide the strongest evidence for modelling selected intrahepatic processes in MASH, particularly the links among hepatocyte lipid stress, macrophage responses, stellate-cell activation, and fibrosis. They are therefore valuable for mechanistic studies, target validation, and early candidate comparison. However, most pharmacological studies demonstrate responsiveness or mechanistic concordance rather than the ability to distinguish clinically successful from unsuccessful drugs. Immature hepatocyte phenotypes, donor and batch variation, limited perfusion and zonation, and the absence of extrahepatic metabolism further restrict prediction of systemic or long-term outcomes. Multicellular liver organoids should therefore be treated as fit-for-purpose components of a broader drug-discovery workflow, not as complete human MASH models or independent replacements for animal studies. Their translational role will depend on patient-tissue benchmarking, interlaboratory reproducibility, and blinded validation with clinically informative compound panels.

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