Digital pathology with artificial intelligence analysis provides insight to the efficacy of anti-fibrotic compounds in human 3D MASH model

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Rationale and Aim of the study

Metabolic dysfunction-associated steatohepatitis (MASH) is a severe liver disease characterized by lipid accumulation, inflammation, and fibrosis. The development of MASH therapies has been hindered by the lack of human translational models and limitations of analysis techniques for fibrosis. In this study, we have combined the use of the MASH HLM1 model and the FibroNest™ digital pathology platform to analyse the anti-fibrotic drug effects of two reference control compounds, activin receptor-like kinase 5 inhibitor (ALKS) and anti-TGF-β antibody (Ab), and the clinically tested drugs, Firsocostat (an acetyl-CoA carboxylase inhibitor) and Selonsertib (an apoptosis signal-regulating kinase 1 Inhibitor).

Aims:

We aimed to establish an algorithm for automated phenotypic quantification of fibrosis of Sirius Red stained histology sections of MASH HLM1Ts model using a digital pathology quantitative single-fiber artificial intelligence (AI) FibroNest™ image analysis platform. We aimed to quantify the severity of fibrosis and assess the reference and clinical compound effects in MASH HLM1Ts. The efficacy of the tested clinical drug candidates in the 3D MASH model is then related to their impact on fibrosis in clinical trials.

Methods

The MASH HLM1 model consists of primary human hepatocytes, Kupffer cells, liver endothelial cells and hepatic stellate cells. Upon exposure to defined lipotoxic and inflammatory stimuli such as free fatty acids and LPS in media containing high levels of sugar and insulin the 3D MASH model displayed key disease pathophysiological features within 10 days of treatment. Quantifiable markers were established for drug efficacy testing such as secretion of pro-collagens type III (HFPYR+EUSA) and tissue inhibitor of metalloproteinases (TIMPs)/matrix metalloproteinases (MMPs) (Luminex assay) as well as quantification of fibrosis using digital pathology quantitative single-fiber AI FibroNestTM image analysis platform (PharmaNest) based on the Sirius Red staining of histology slides (Figures 1A and 1B). Next generation sequencing was used for the assessment of the gene expression changes (Figure 1B). All the results were presented as a mean ± SD, (n=6 microslides, *p ≤ 0.05 p** ≤ 0.01, p*** ≤ 0.001, p**** ≤ 0.0001 ANNIOVA, Welch's cor.)

• The FibroNest™ algorithm for MASH HLM1Ts was validated using anti-fibrotic reference compounds with different therapeutic modalities - ALKS and anti-TGF-β Ab.

• The phenotypic quantification of fibrosis demonstrated that both reference compounds decreased the deposition of fibrotic collagens in alignment with effects on the secretion of pro-collagen type III, TIMP-1, MMP-3 and pro-fibrogenic gene expression.

• In contrast, clinical compounds, Firsocostat and Selonsertib, alone and in combination showed strong anti-fibrotic effects on the deposition of collagen fibbers, however less pronounced on the secretion of pro-fibrotic biomarkers.

• In summary, the phenotypic quantification of fibrosis of MASH HLM1Ts using FibroNest™ AI imaging platform based on histology Sirius red stained slides should be combined with secretion of pro-fibrotic biomarkers and transcriptomics for comprehensive assessment of efficacy of the anti-fibrotic compounds.