Evaluation of anti-fibrotic compounds effect in 3D human NASH model using quantitative digital pathology

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Introduction and Aim

Non-alcoholic steatohepatitis (NASH) is a progressive severe disease characterized by lipid accumulation (steatosis), inflammation (steatohepatitis) and fibrosis in the liver. The development of novel anti-fibrotic therapies has been hindered in part, by limitations of existing fibrosis analysis techniques of histology samples from in vivo and in vitro preclinical models. InSphero, a novel Digital Pathology Quantitative AI platform generates automatic, continuous and direct fibrosis endpoints to quantify fibrosis severity and compound treatment response in clinical NASH samples.

The aim of this study was to establish an algorithm for quantification of fibrosis in an in vitro NASH model using readouts from the Digital Pathology Quantitative AI platform, and to validate the algorithm using ultrastructural analysis and biochemical readouts from human NASH samples.

Method

Using proprietary Akusz™ 3D cell culture for 3D cell culture, we produced 3D human liver microtissues (3DMTI) using human primary cell lines relevant for NASH disease induction and progression, co-cultured with human primary stellate cells (HSC). To recapitulate NASH induction in vitro, microtissues were exposed for 10 days to defined lipotoxic and inflammatory stimuli, including free fatty acids, high sugar, insulin, and LPS. PIO-278 subtype 1 and 3 secretion were measured using PIO-278 type I pro-collagen III antibody and PIO-278 type II procollagen III antibody (INTRA). The digital collagen deposition and fibrosis architecture was evaluated using the 3D Quantitative AI, combined with confocal microscopy, and compared against standard histology slides and biochemical readouts.

Results

Alk5i treatment leads to decreased fibrosis evaluated by biochemical and digital pathology endpoints

Figure 1. InSphero technology and disease modeling: A. Akusz™ 3D cell culture technology. B. NASH disease induction and treatment schedule. C. Compositional vehicle treatment with NASH induction.

Figure 2. Assessment of anti-fibrotic effects of Alk5i. A. Increased pro-collagen type I & III deposition as well as TIMP-1 secretion (day 7-10). B. Days 1-10 collagen deposition normalized to control. C. Compositional vehicle treatment with NASH induction.

Figure 3. Assessment of anti-fibrotic effects of Alk5i. A. Anti-TGF-β inhibition with low (0.001 µM) and high (0.5) µM concentrations led to concentration-dependent decreases of pro-collagen type I & II and TIMP-1 secretion, mean ± SD, ***p < 0.001 vs test. B. Sirius red staining and phenotypic quantification of fibrosis (FibroNest, Pharmanan) indicate an increase collagen fibers deposition (PhibroNest) and fibrosis architecture (FibroNest) in disease conditions vs control. Alk5i decreases the fibrosis deposition in both disease conditions vs control. Mean ± SD, ***p < 0.001, **p < 0.01 vs test.

Figure 4. FibroNest fibrosis quantification of anti-fibrotic compounds. A. The FCS assay shows a significant detection threshold and dynamic range to evaluate the anti-fibrotic response of seven clinical compound treatment arms. p-values are calculated using the Student’s T-Test Method.

Summary and Conclusions

- Phenotypic quantification of collagen fibers using quantitative fibrosis trials (QFTS) complements the efficacy assessment of anti-fibrotic NASH compounds using biochemical quantitative assays.
- Proof-of-concept studies using anti-TGF-β AB and Alk5i demonstrate the power of 3D NASH model for efficacy assessment of compounds for inhibition of fibrosis such as collagen fibers deposition and pro-collagen type I secretion.
- The anti-fibrotic effects of Firsocostat (10 µM) and MGL-3196 (0.05 µM) on the deposition of fibrotic collagen are significant and comparable.
- The combined treatment of Selonsertib (10 µM) with the low dose of Firsocostat (0.05 µM) does not demonstrate any synergistic effect.
- The dose-response analysis are poorly detected except for the MGL-3196 arms (p<0.07), which demonstrate that the results is driven by the compounds, not the PI3K pathway.
- FibroNest algorithm can be used to quantify differences in the fibrotic phenotype in each group and quantify specific effects of each drug (and dose) on the collagen deposition, collagen fibers morphometry and fibrosis architecture.