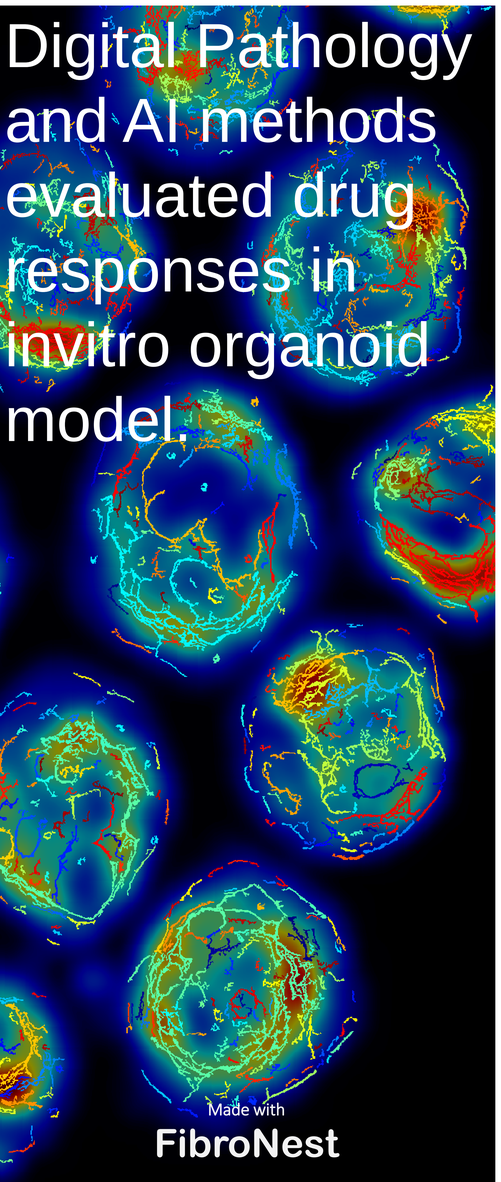

Quantitative digital pathology of 3D human NASH models establish continuous scores to evaluate the antifibrotic effects of Selonsertib, Fibrocystin and Resmetirom

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1 Introduction

Human in-vitro 3D NASH tissue model have the potential to accelerate the discovery of new anti-fibrotic compounds. Previously, we have reported the performance of novel Digital Pathology Quantitative AI to generate automatic, continuous and direct fibrosis endpoints to quantify fibrosis severity and compound treatment response.

2 Aim

Here, we expand this validation effort my measuring increasing concentrations of selonsertib, firsocostat, resmetirom (MGL-3196), and their combinations.

3 Method

- 9 groups (n=18 to 21 in each group).
- 3D liver tissues were either treated for 10 days with free fatty acids and LPS or not to generate **NASH** and **lean** conditions respectively
- 3D NASH tissues were simultaneously treated with:
  - Selonsertib (2µM and 10µM)
  - Firsocostat (0.5µM and 10µM)
  - A Selonsertib (10µM) and firsocostat (0.5µM) combination
  - Resmetirom (MGL-3196, 0.005µM and 0.05µM)

Spheroid Information

- Human in vitro 3D InSight™ liver microtissues
- Contain primary hepatocytes, Kupffer cells, endothelial cells and hepatic stellate cells
- Used a defined cocktail of free fatty acids, LPS and high levels of sugars

Pathology, Digital Pathology and AI:

- Spheroid FFPE sections were stained with **Picro Sirus Red** and scanned at **40X**
- **Fibrosis severity continuous score** (**Ph-FCS**, 1 to 10). Quantitative image analysis extracts single-fiber quantitative traits (qFTs, N=315) from the fibrosis histological phenotype. Principal qFTs are automatically detected and combined into a normalized Phenotypic Composite Fibrosis Score (Ph-FCS) (visualized in Figure D).
- **Spheroid Adequacy:** each spheroid was evaluated for quality by size.
- Additional **sub-Phenotypic scores** (fine and assemble fiber sub-classes, morphometry, architecture, fibrosis scar) are used to further describe the fibrosis phenotypes and its remodeling as fibrosis progress or regresses
- Each qFT is described individually for relative severity (green to red) in Phenotypic Heat charts (Figure A).

5 Conclusions

The combination of FibroNest™ imaging analysis for automated quantification of histological fibrosis severity phenotype within vitro 3D InSight™ human NASH model provide powerful platform for anti-fibrotic drug-candidates response evaluation.

6 Contact information

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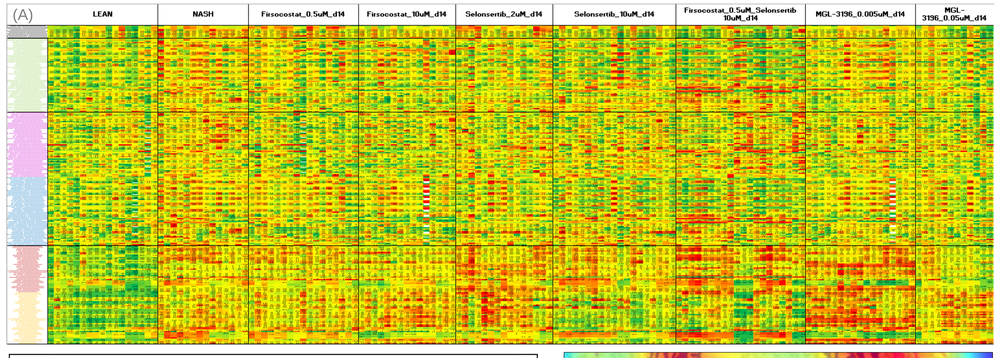
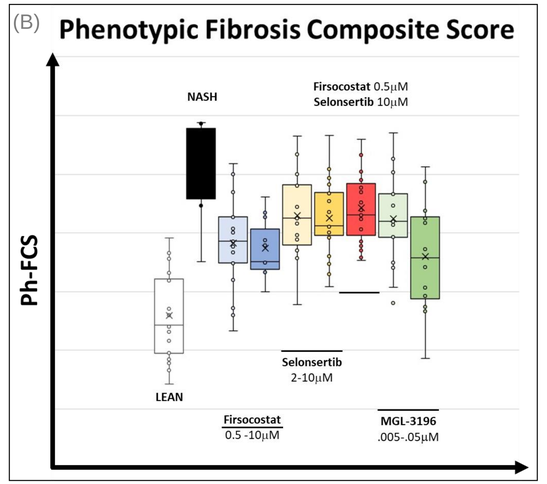
4 Results

- The Ph-FCS offers a significant detection threshold and dynamic range to evaluate the antifibrotic response the seven treatment arms (box plot chart (B) and p-value table below (C)).

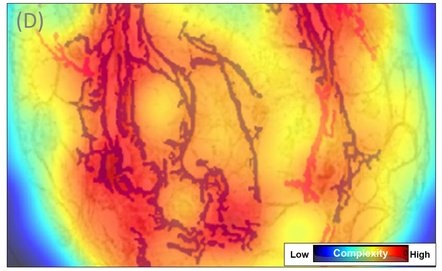




(C)

p Values	LEAN	NASH	Firsocostat 0.5µM	Firsocostat 10µM	Selonsertib 2µM	Selonsertib 10µM	Firsocostat-0.5µM Selonsertib 10µM	MGL-3196 0.005mM	MGL-3196 0.05mM
LEAN		0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.008
NASH			0.000	0.000	0.010	0.003	0.015	0.006	0.000
Firsocostat 0.5µM				0.692	0.095	0.086	0.017	0.133	0.386
Firsocostat 10µM					0.022	0.011	0.001	0.033	0.669
Selonsertib 2µM						0.869	0.582	0.843	0.057
Selonsertib 10µM							0.401	0.955	0.057
Firsocostat 0.5µM Selonsertib 10µM								0.433	0.018
MGL-3196 0.005µM									0.075
MGL-3196 0.05µM									

P-Values are calculated using the Student's T-Test Method



- Firsocostat (10µM) and MGL-3196 (0.05µM) antifibrotic effects are significant and comparable.
- The combination of selonsertib (10µM) with the low dose of firsocostat (0.5µM) does not demonstrate any synergistic effect.
- The dose response effects are poorly detected except for the MGL-3196 arms (p=0.075), which demonstrate that the result is driven by the compounds, not the Ph-CFS score and method.
- Each principal qFT is described individually for relative severity (green to red) in phenotypic heat charts (A)
- Can be used to quantified differences in the fibrosis phenotype in each group, and quantify specific effects of each drug (and dose) on the collagen distribution, collagen fibers morphometry and fibrosis architecture.