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

Radina Kostadinova[1], Simon Ströbel[1], Louis Petitjean[2], Li Chen[2], Jesús Glaus[1], Philip Vonschallen[1], Francisco Verdeguer[1], Mathieu Petitjean[2]
[1]InSphero AG, Schlieren, Switzerland, [2]Pharmanest, Princeton, NJ, USA, Corresponding Author*

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. FibroNest, 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* InSight™ three-dimensional (3D) human NASH microtissue model using novel digital pathology quantitative single-fiber artificial intelligence (AI) platform FibroNest (PharmaNest). The algorithm was further validated using tool anti-fibrotic compounds such as Alk5 inhibitor and anti-TGF-β antibody (AB).

Method

Using proprietary Akura™ 96 plate technology for 3D cell culture, we produced 3D human liver microtissues (hLiMTs) using human primary cell types relevant for NASH disease induction and progression: hepatocytes (PHH), Kupffer cells (KC), liver endothelial cells (LSEC) and hepatic 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 levels, insulin and LPS. Procollagen type I and III secretion were measured using procollagen type I propeptide HTRF assay and procollagen type III propeptide (PIIINP) ELISAs (CisBio), respectively. TIMP-1 and MMP-2 were measured using ProcartaPlex™ kit and Luminex technology. For FibroNest fibrosis quantification, spheroid FFPE sections were stained with Picro Sirus Red and scanned at 40X. Around 200 principal quantitative fibrosis traits (qFTs) are automatically detected and combined to generate a normalized Phenotypic Composite Fibrosis Score (Ph-FCS). Additional sub-phenotypic fibrosis scores related to collagen fibers, morphometry and architecture are used to further describe the fibrosis phenotypes and its remodeling as fibrosis progress or regresses.

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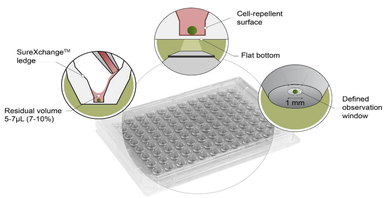

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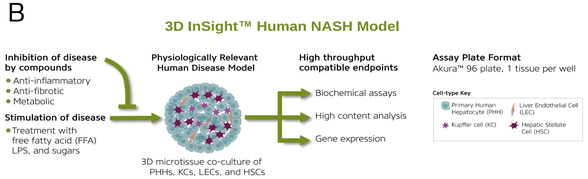

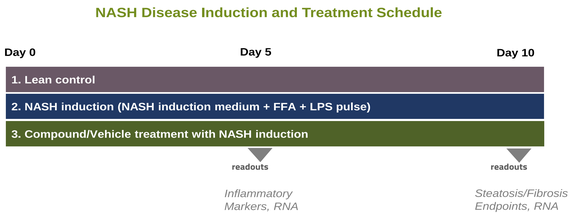

Figure 1. InSphero technology and disease modelling. A. Akura 96-well plate technology **B.** Description of three NASH model treatment conditions (Lean control, NASH induction, NASH induction with treatment) and schedule for endpoint measurement. Compound efficacy studies were performed using NASH induction medium and stimuli to investigate the preventive role of compounds in NASH disease progression

Results

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

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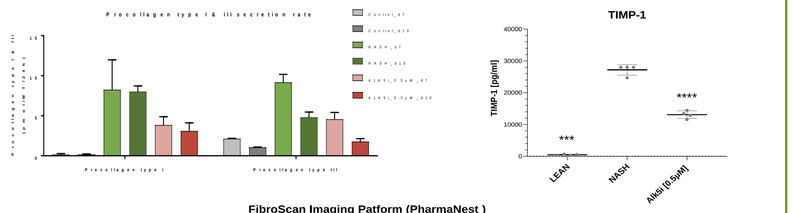

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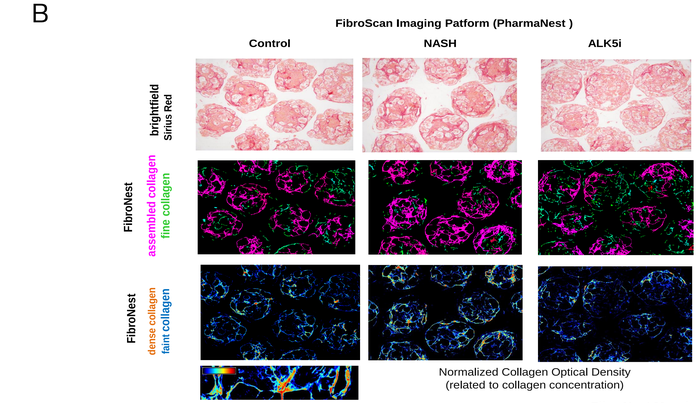


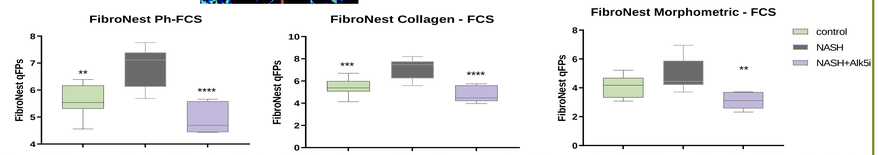
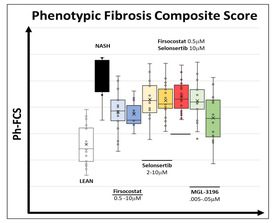


Figure 2. Assessment of anti-fibrotic effects of Alk5i. A Increased procollagen type I and III as well as TIMP-1 secretion (day 5-7 and 7-10 of treatment) indicates presence of fibrosis in NASH-treated models. ALK5i decreases the procollagen type I, III and TIMP-1secretion. Mean +/-SD, n=4-12 models, **** p ≤ 0.0001, *** p ≤ 0.001, ** p ≤ 0.01 NASH vs lean control, *** p≤0.001, NASH vs NASH+ALK5i; 1 of 3 exp. (t-test). **B**. Sirius Red staining and phenotypic quantification of fibrosis (FibroNest, PharmaNest) indicate an increase collagen fibrils deposition in NASH conditions vs control. ALK5i decreases the fibrils deposition vs NASH. Ph-FCS (phenotypic fibrotic composite score). Mean +/-SD, n=7-9, **p≤0.01 ***p≤0.001, ****p≤0.0001 (t-test), NASH vs control or NASH+ALK5i.

Anti-fibrotic clinical compound efficiency assessment by FibroNest



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									

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

Proof-of-Concept: Anti-TGF-ß antibody as an anti-fibrotic biotherapeutic

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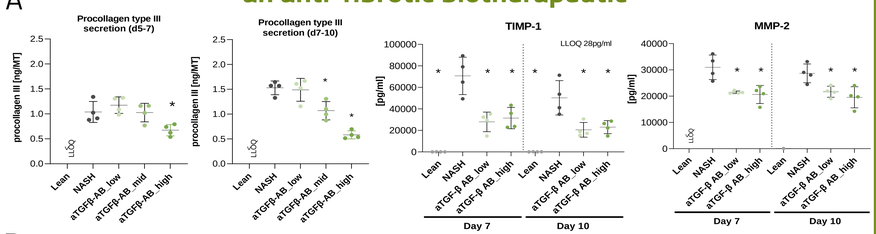

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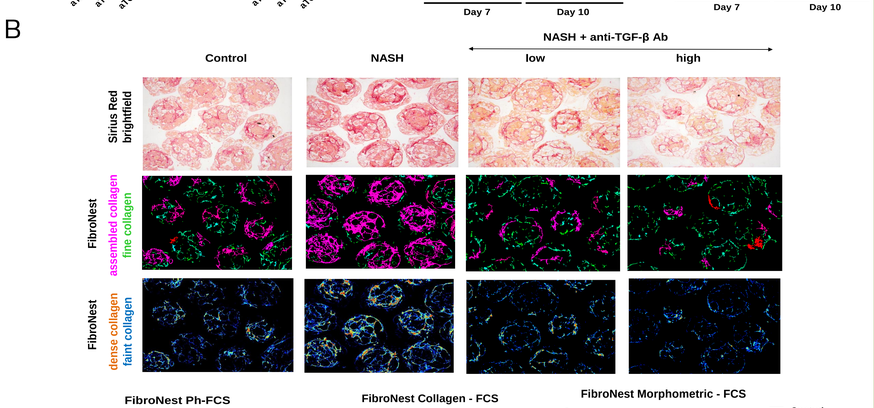

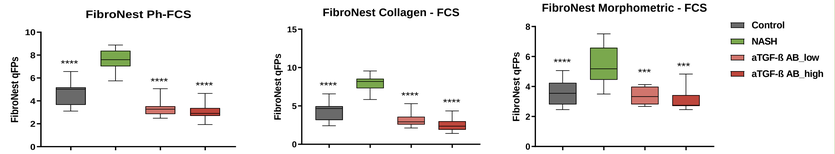

Figure 3. Assessment of anti-fibrotic effects of anti-TGF-β AB. A. Anti-TGF-β AB treatment with low (0.001 µM) and high (0.1 µM) concentrations led to concentration-dependent decrease of procollagen type III, TIMP-1 and MMP2 secretion. Mean +/-SD, n=4 models, * p ≤ 0.05 (t-test), NASH vs NASH+anti-TGF-β AB. **B.** Sirius Red staining brightfield (BF) and phenotypic quantification of fibrosis (FibroNest, PharmaNest) indicate increase of collagen fibrils deposition in NASH conditions vs control. Anti-TGF-β AB decreases the fibrosis vs NASH-treated samples. Mean +/-SD, n=7-10 models, **p ≤ 0.01, ***p ≤ 0.001, ***p ≤ 0.0001 (t-test), NASH vs control or NASH+anti-TGF-β AB.

Summary and Conclusions

- Phenotypic quantification of collagen fibers using quantitative fibrotic trials (qFTs) complements the efficacy assessment of anti-fibrotic NASH compounds using biochemical quantitative assays.
- Proof-of-concept studies using anti-TGFβ AB and Alk5i demonstrate the power of 3D NASH model for efficacy assessment of compounds for inhibition of fibrosis such as collagen fibrils deposition and pro-collagen type I/III secretion.
- The antifibrotic effects of Firsocostat (10 µM) and MGL-3196 (0.05 µM) on the deposition of fibrillated collagens are significant and comparable.
- The combinatorial treatment of Selonsertib (10 µM) with the low dose of Frsocostat (0.5 µM) 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.
- FibroNest algorithm can be used to quantify differences in the fibrosis phenotype in each group and quantify specific effects of each drug (and dose) on the collagen deposition, collagen fibers morphometry and fibrosis architecture.

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