Novel phenotypic image analysis of 3D NASH model generate quantitative and continuous scores for the evaluation of the human in-vitro InSphero 3D NASH Model

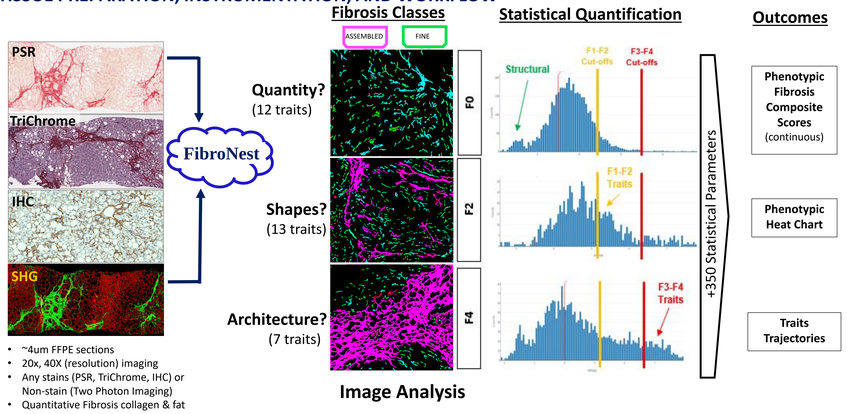
Mathieu M. Petitjean[1], Radina Kostadinova[2], Li Chen[1], Simon Ströbel[2], Eva Thoma[2]
[1]PharmaNest, Princeton, NJ, USA [2] InSphero AG, Schlieren, Switzerland

BACKGROUND

Human in-vitro InSphero 3D NASH tissue models have the potential to accelerate the discovery of new anti-fibrotic compounds. Up to now in vivo evaluation of the NASH progression and regression is mainly based on histological investigation of the deposited fibril collagens within the liver tissues. However, histological evaluation of the 3D NASH stained tissues of fibrosis is complex and still an emerging field. Therefore, automatic and "learning-free" computerized methods for the quantification of the severity and progression of fibrosis are of high interest. Here, a novel image analysis platform was used to quantify the fibrosis phenotypes which generates quantifiable fibrosis parameters (qFPs) and continuous phenotypic Fibrosis Composite Scores (Ph-FCS) that have the potential to become direct fibrosis endpoints.

METHOD

TISSUE PREPARATION, INSTRUMENTATION, AND WORKFLOW



- ~4um FFPE sections
- 20x, 40X (resolution) imaging
- Any stains (PSR, TriChrome, IHC) or Non-stain (Two Photon Imaging)
- Quantitative Fibrosis collagen & fat

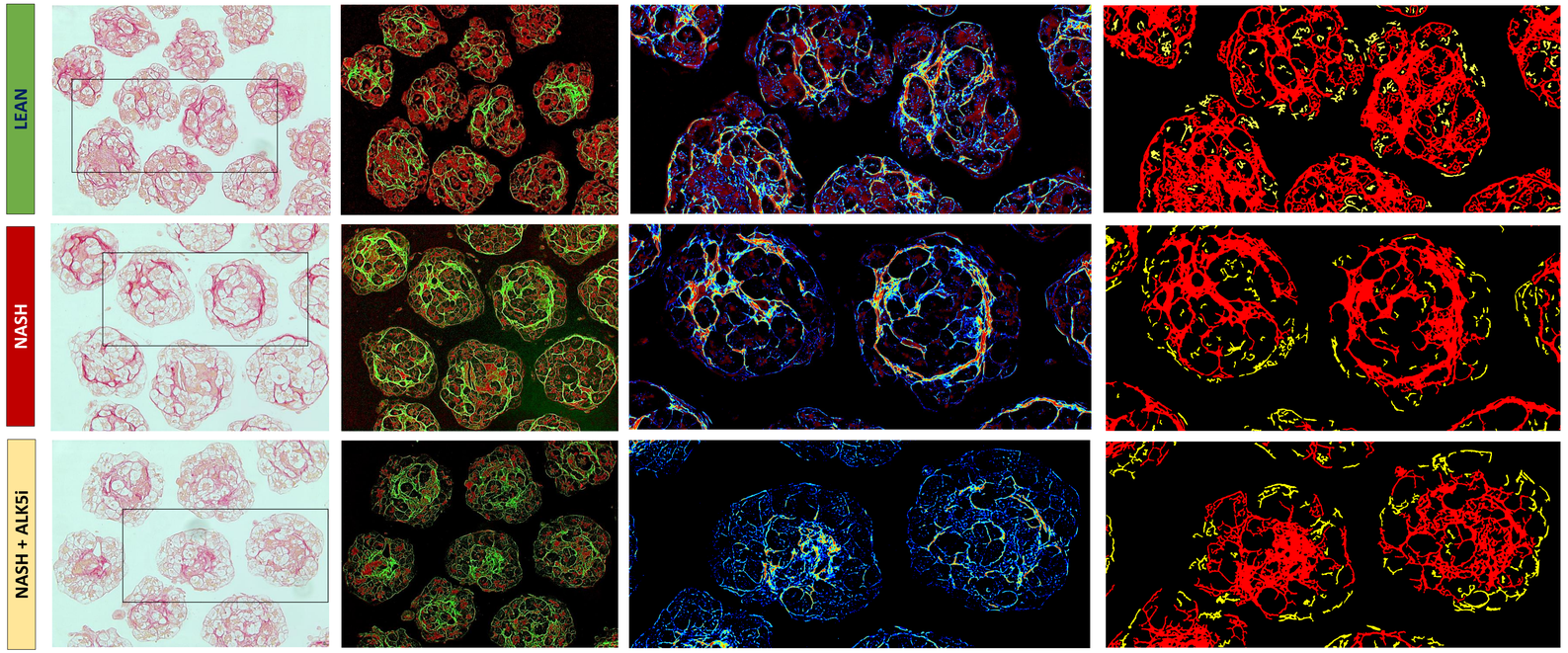
- Human in vitro 3D InSight™ liver microtissues containing primary hepatocytes, Kupffer cells, endothelial cells and hepatic stellate cells in 96-well plates and treated as described below.

Group	Description and treatment	Total N 17
LEAN-1	Human in-vitro 3D InSigh™ liver MT (microtissues) Lean - Control	N= 11
LEAN-2	LEAN Duplicate group	N=10
NASH-1	Human in vitro 3D InSight™ liver microtissues treated with a defined cocktail of free fatty acids, LPS and high levels of sugars used to model NASH progression	N=10
NASH-2	NASH Duplicate Group	N=9
NASH + ALK5i	Human in vitro 3D InSight ™ liver microtissues NASH model as described above, treated with anti-fibrotic inhibitor (SB525334, a Transforming Growth Factor β1 receptor (ALK5) inhibitor).	N=8

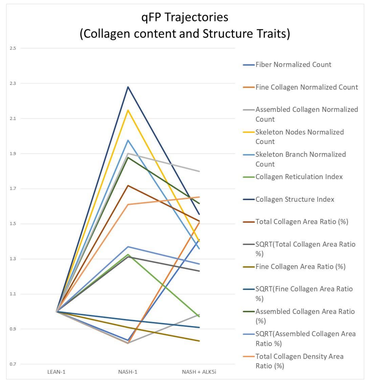
- FFPE sections (~4 microns) of spheroid aggregates were deparaffinized, stained for fibril collagens using an optimized Picro Sirus Red (no Hematoxylin bath) protocol and digitized at 20X (Zeiss, no image compression losses)
- Duplicate LEAN and NASH groups are studied to prove that the analysis and quantification are not sensitive to the experimental variability introduced by tissue processing, sectioning and staining procedures
- Using FibroNest™ the fibrosis phenotype is described for its collagen content and structure (12 traits), the morphometric traits of the collagen fibers (13 traits), and fibrosis architecture / texture traits (7). In each image, each morphometric and texture trait is represented by a histogram distribution (e.g. Fiber Skeleton Length histogram).
- The histogram for each trait is described by up to seven quantitative fibrosis parameters (qFPs, 315 in total) to account for mean, variance, distortion and progression.
- Principal qFPs are automatically detected to account for progression (LEAN vs NASH). They are combined into a normalized Phenotypic Composite Fibrosis Score (Ph-CFS), a continuous quantifier of the progression / regression of the fibrosis.
- Principal qFPS are also used individually to describe the progression / regression in phenotypes between the LEAN, NASH and Treatment groups.

RESULTS

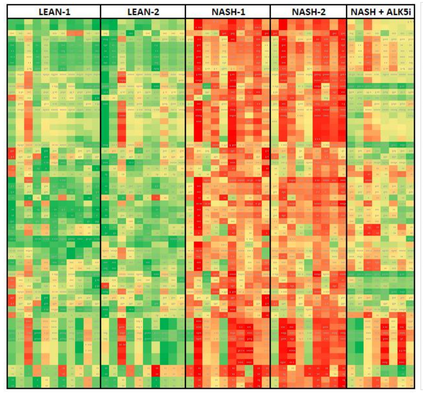
REPRESENTATIVE IMAGES AND FIBRONEST ANALYSES



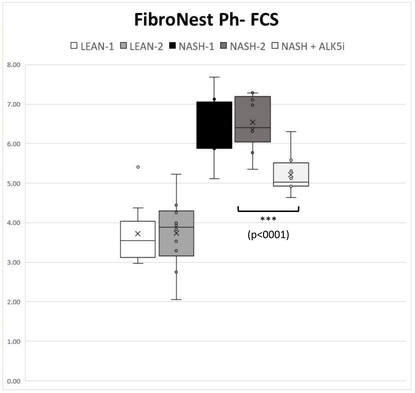
PHENOTYPIC QUANTIFICATION



qFP trajectories (Content and Structure traits, normalized to the LEAN phenotype) describe the differences between LEAN, NASH and TREATED Phenotypes



Phenotypic Heat charts (showing Principal qFPS only) describe each traits for each Spheroid and are used to support mechanisms of Action and identify experimental variability



The Phenotypic Composite score is assembled from Principal qFPs and quantify the severity of Fibrosis, its progression and regression under the effect of a treatment

Conclusion

FibroNest analysis was able to quantify the fibrosis phenotype in the Human in-vitro InSphero 3D NASH tissue, and anti-fibrotic changes induced by ALK5i.

Reproducibility between consecutive batches was demonstrated for the LEAN and NASH models. Experimental issues compromised the duplicate NASH + ALK5i dataset. A full assay validation study will be reported soon with superior results to confirm relevance, and reproducibility.

These data demonstrate the suitability of the method for quantification of a histology-based fibrosis endpoint in the Human in-vitro InSphero 3D NASH model and its possible translational application from in vitro to in vivo clinical situation. Studies are conducted to correlate these qFPs with other biomarker endpoints and with FibroNest scores validated in clinical NASH.