DIGITAL PATHOLOGY IMAGE ANALYSIS ACCURATELY QUANTIFIES ANTI-FIBROTIC AND ANTI-STEATOTIC EFFECTS OF FXR AGONISTS USING MULTIPLE HISTOLOGICAL METHODS

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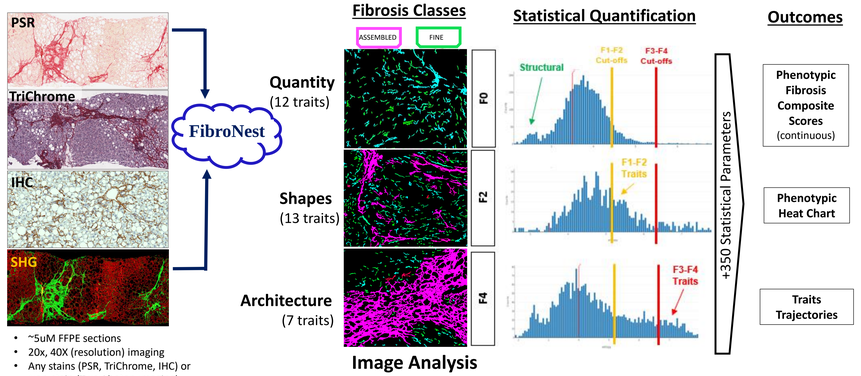
BACKGROUND and AIMS

Automated image analysis methods for the quantification of histological features in pre-clinical models of Non-Alcoholic Steatosis Hepatitis (NASH) are gaining significant interest as they provide sensitive and reproducible endpoints for the quantification of fibrosis and steatosis. There is still significant debate on the incremental value of Second Harmonic Generation (SHG) compared to conventional histology tissue staining in terms of cost, ease of use, and sensitivity in detection of fibrosis. This study characterizes the anti-fibrotic efficacy of investigative FXR agonists INT-2228 and INT-767 in a rodent NASH model with a focus on quantification of fibrosis phenotypes and steatosis detected by SHG (unstained) and conventional histology staining (IHC).

METHOD

TISSUE PREPARATION, INSTRUMENTATION, AND WORKFLOW



- ~5uM FFPE sections
- 20x, 40X (resolution) imaging
- Any stains (PSR, TriChrome, IHC) or Non-stain (Two Photon Imaging)
- Quantitative Fibrosis collagen &/or fat

AMYLIN LIVER NASH FIBROSIS MODEL (AMLN)

Leptin-deficient (lep 0b/0b) mice were allowed ad libitum access to normal chow (low-fat diet w no fructose nor cholesterol) or to modified ALIOS diet (high trans fat (40%), fructose (22%), cholesterol (2%) in food pellets) to induce NASH. Mice were biopsied and confirmed to have steatosis (score 3) and fibrosis (stage 2-3) before treatment (PO, QD) with vehicle (n=12), INT-767 (10 mg/kg, n=11), or INT-2228 (1 mg/kg, n=7) for 8 wks. The anti-fibrotic and anti-steatotic efficacy of the investigative FXR agonists INT-2228 and INT-767 are measured.

TREATMENTS	STRAIN	N	DOSE
NASH (Vehicle)	Lep 0b/0b	12	--
INT-767	Lep 0b/0b	11	10 MG/KG
INT-2228	Lep 0b/0b	7	1 MG/KG

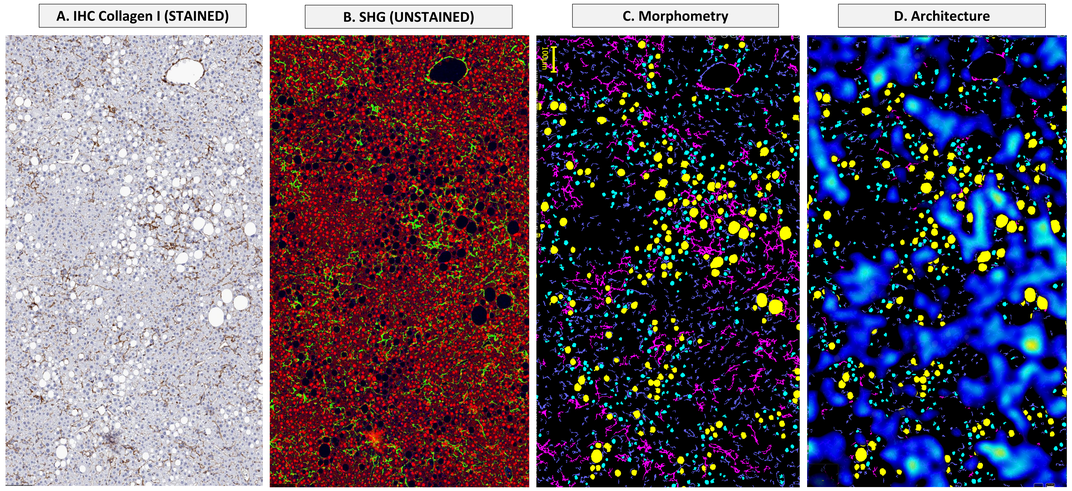
- Liver histological sections from the same animal were imaged with Collagen I (IHC-Coll1) stains or imaged with unstained using Genesis®200 Two Photon microscope (SHG) for collagens I and III.
- FibroNest®, a cloud-based image analysis platform, was used to quantify the fibrosis phenotype including 32 traits for collagen content, fiber morphometry, and architecture. Principal quantitative fibrosis traits (up to 315 qFTs in total) are automatically detected and combined into a normalized Phenotypic Composite Fibrosis Score (Ph-FCS).

Conclusion

Histological features impacted by FXR agonists INT-2228 and INT-767 can be accurately quantified by both digital pathology methods. Using FibroNest® as an image analysis platform, SHG and conventional staining provide not identical but similar performance and data quality. However, SHG are quite costly and requires special technician and imaging machine that is not widely available. This study shows that the standard conventional staining method can still be a superior choice for histological therapeutic drug efficacy evaluation.

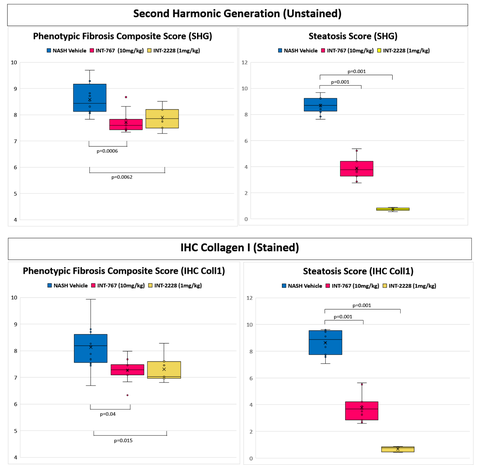
RESULTS

REPRESENTATIVE IMAGES AND FIBRONEST ANALYSIS



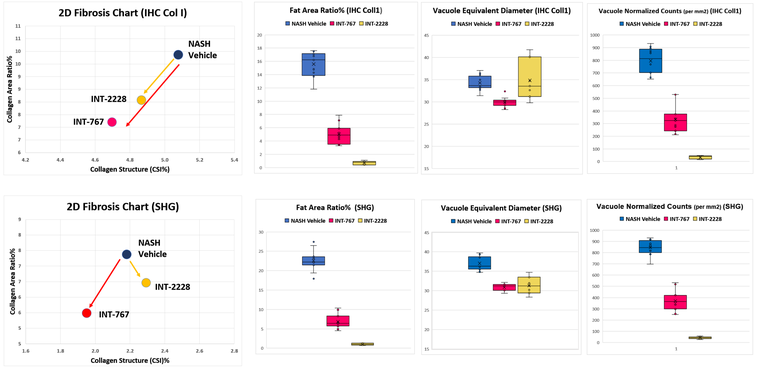
Mouse NASH liver histology. A) Liver is stained with immunochemistry for collagen I (brown). B) Second harmonic generation (SHG) for collagens I & III (green) and tissue (red). C) Morphometry. Image analysis for fibrosis and steatosis. Assembled Collagen (pink, highly reticulated fibers) and Fine Collagen (blue, smaller fibers). Large Fat Vacuoles (yellow, >14 uM diameter) and Small Fat Vacuoles (sky blue, <14uM diameter). D) Architecture measures the pattern and organization of the collagen fibers. Blue cloud overlay with image in (C) depict the area that have more complex compacted and organized fibers.

FIBROSIS AND STEATOSIS PHENOTYPIC QUANTIFICATION



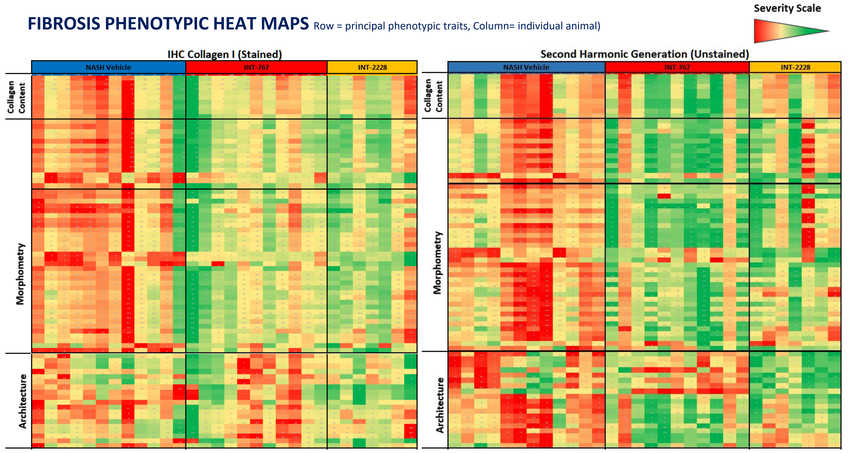
Phenotypic fibrosis score is an aggregate of collagen content, fiber morphometric, and architecture shown in the heat charts (below). INT-767 and INT-2228 both significantly improve fibrosis scores in NASH induced liver fibrosis from both IHC and unstained SHG images. INT-2228 exhibits a superior anti-steatotic effect than INT-767 as measured in the FibroNest® Steatosis Score from IHC and SHG images.

LIVER FIBROSIS AND STEATOSIS QUANTITATION



INT-767 and INT-2228 improves NASH induced liver fibrosis and steatosis. Fibrosis: INT-767 reduces both collagen content and structure, while INT-2228 reduces collagen content and not structure in SHG images. Steatosis: For INT-767, fat area ratio reduction is due to decrease in fat vacuole counts and size, while INT-2228 is due to the counts rather than the size in the IHC images.

FIBROSIS PHENOTYPIC HEAT MAPS Row = principal phenotypic traits, Column= individual animal)



Phenotypic fibrosis heat maps shows that overall NASH groups have most severe (red) fibrosis than treatment groups INT-767 and INT-2228.

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