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Histologic assessments by Pathologists and Digital Pathology describe the antifibrotic effect of LPCN 1144

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BACKGROUND and AIMS

LPCN 1144 is an oral prodrug of testosterone developed as a treatment for pre-cirrhotic non-alcoholic steatohepatitis (NASH) and recently completed the 36 weeks, blinded, placebo controlled, paired-biopsy LiFT Phase 2 clinical study (NCT04134091). Some subjects continued treatment (Open Label Extension, OLE, NCT04685993) for additional 36 weeks. This analysis evaluates the anti-fibrotic effect of the treatments using histological (pathologist) and Digital Pathology and artificial intelligence (AI) methods to optimize the design of a registration study.

METHOD

STUDY DESIGN AND TREATMENT AREMS

Group	Enrollment	36 w Initial Intervention	N	36 w Open Label Extension (OLE)	N
Placebo	NASH diagnosed by histologic assessment of liver biopsy and NASH-CRN stage 1-3 fibrosis.	Placebo Twice Daily for 36 weeks	15		15
Treatment A		142 mg eq. T (testosterone) Twice Daily for 36 weeks	15	9 patients from placebo group initiated on treatment , 3 elected for a final additional biopsy	18
Treatment B		142 mg eq. T + 238 mg d-alpha tocopherol Twice Daily for 36 weeks	14		14

LIVER TISSUE HISTOLOGY

FFPE sections (~4 microns) of Adequate liver biopsies were stained with Masson Trichrome for Collagen

THREE ASSESMENTS OF THE ANTIFIBROTIC EFFECT WERE STUDIED ON THE SAME SLIDES:

NASH-CRN STAGING

- Biopsy slides are read by an independent pathologist trained on the NASH-CRN staging system
- F1 stage sub-stages F1a (coded 1), F1b (coded 1.33), F1c (coded 1.66) are used in addition to F2 and F3
- Responders are identified with at least a full categorical stage change

RANKED ASSESSMENT

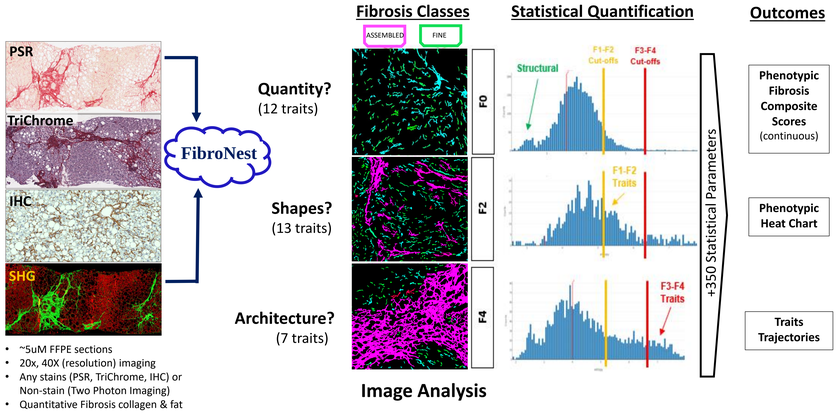
- Paired (baseline and 36W) biopsies, blinded to sequence and treatment are presented to the independent pathologist
- A qualitative ranked assessment (improvement | worsening | stable) is established
- Responders are identified if “improvement” is established

DIGITAL PATHOLOGY AND ARTIFICIAL INTELLIGENCE

- The same slides prepared for and reviewed by the pathologist were digitized at 20X (0.50 micron/pixel) on an Aperio AT WSI system.
- The digital Images were read using FibroNest™, a single-fiber, high-content quantitative Digital Pathology image analysis and AI automated, full tissue method providing a continuous phenotypic Fibrosis Composite Severity (Ph-FCS) score that ranges from 1 to 10 for the full spectrum of fibrosis severity observed in the liver. This allows identifying fibrosis improvements that may be missed by staging methods [5] as well as statistical quantification of change from baseline.
- A 0.3 absolute reduction in Ph-FCS (4-fold higher than the analytical variability) identified any reduction in fibrosis.

DIGITAL PATHOLOGY 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 & fat

WHOLE SLIDE IMAGING

- The same slides prepared for and reviewed by the pathologist were digitized at 20X (0.50 micron/pixel) on an Aperio AT WSI system.

SINGLE-FIBER, HIGH CONTENT, QUANTITATIVE IMAGE ANALYSIS

- Using Quantitative Image Analysis (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 traits (7). In each image, each morphometric and texture trait is represented by a histogram distribution (e.g. Fiber Skeleton Length).
- 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.
- As reported previously [1,2,3], principal qFTs are automatically detected to account for disease severity if their group mean value difference is statistically (P<0.05, T-Test) greater than 20%.
- Principal qFTs are used individually and collectively to describe the differences in phenotypes between groups. They are combined into a normalized Phenotypic Composite Fibrosis Severity score (Ph-FCS), a continuous quantifier of the fibrosis severity phenotype.
- The correspondence between the Ph-FCS with NASH-CRN stages has been reported previously [4,5]

References

1. Petitjean, L. *et al.* Evaluation of the performance of a novel Digital Pathology method for the continuous quantification of Steatosis, Ballooning and Inflammation in liver biopsies and its correlation with NASH-CRN scores in patients with NASH. *International Liver Congress, EASL* (2022).

2. Chen, L. *et al.* Evaluation of a novel histology-based fibrosis phenotypic composite score and its correlation with NASH-CRN Fibrosis scores in patients with NASH. *International Liver Congress, EASL* (2020).

3. Chen, L. *et al.* Evaluation of the multivendor performance of a novel histology-based fibrosis phenotypic composite score and its correlation with NASH-CRN Fibrosis scores in patients with NASH. *Hepatology* **74**, 1S, 953A-954A (2022).

4. Chen, L. *et al.* Continuous staging of NASH Patients at low (F1) Fibrosis Severity: Evaluation of the performance of a novel histology-based fibrosis phenotypic composite score and predictive AI tools. *Hepatology* **74**, 1S, 945A-946A (2021).

5. Chen, L. *et al.* Novel Digital Pathology quantitative image analysis and AI method detects the treatment effect of NASH drug candidates with a performance that benchmarks Imaging based measurements. *International Liver Congress, EASL* (2022).

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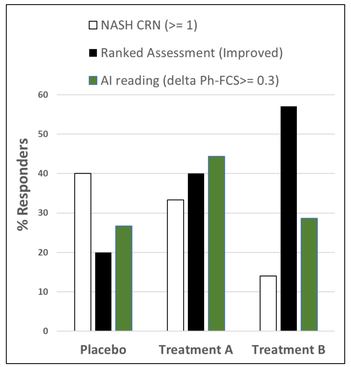
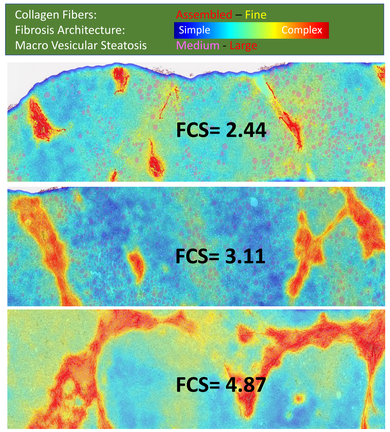
RESULTS

The assessment by a pathologist revealed higher rates of baseline fibrosis in the placebo group compared to Treatment A or B (Table). Ph-FCS values ranged from 1.05 to 5.27 (mean=3.16, STD=1.05) for the study. 36 weeks of monotherapy with Treatment A resulted in improved rates of fibrosis as measured by 2 of 3 histological techniques compared to placebo, although statistical significance was not met for any comparison. AI evaluation was consistent with paired reading.

Proportion of Patients with Fibrosis Improvement for Each Reading Methodology after 36 weeks of Treatment with LPCN 1144



Augmented Digital Pathology Images



Biopsy results using three histopathology reading methodologies

Responders Quantification	Placebo	Treatment A	Treatment B
	N=15	N= 18 (with OLE subjects)	N=14
Baseline fibrosis (NASH CRN)	2.1	1.6	1.4
Fibrosis Reduction at 36 Weeks	**% (N)**		
NASH CRN (>= 1)	40% (6)	33.3% (6)	14.3% (2)
Ranked Assessment (Improved)	20% (3)	40% (6)*	57.1% (8)
AI reading (delta Ph-FCS>= 0.3)	26.7% (4)	44.4% (8)	28.6% (4)

**N=15; ranked assessment not performed on OLE biopsies*

Conclusion

Results of this proof-of-concept study support potential antifibrotic effects of LPCN 1144, which require confirmation in a larger and longer study.

Both ranked assessments and AI evaluations identified more subjects with fibrosis improvement than the current FDA primary outcomes needed for accelerated approval.