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Digital Pathology quantitative image analysis and AI method detects the treatment effect of pegbelfermin in Cirrhosis patients with a performance that benchmarks manual histological assessment

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BACKGROUND and AIMS

Manual histological evaluation of liver biopsy is the gold standard for fibrosis staging in Non-Alcoholic Steatohepatitis (NASH), but it is limited by its inter- and intra-reader variability. Digital Pathology image analysis (FibroNest™) has the potential to overcome the current limitation of such standards. This exploratory post-hoc analysis compared FibroNest's continuous scores with NASH-CRN categorical stages in patients with NASH from the phase 2b FALCON 2 study (NTC03486912).

METHOD

STUDY DESIGN AND TREATMENT ARMS

Eligible adults were 18-75 years of age (N=145) with NASH diagnosed by histologic assessment of liver biopsy according to NASH CRN criteria and stage 4 fibrosis, defined as Cirrhosis. During the 48-week double-blind treatment period, patients received 10mg, 20mg, or 40mg pegbelfermin subcutaneous or placebo once weekly.

LIVER TISSUE HISTOLOGY

- Liver biopsies were obtained six months before or during screening and at week 48
- Formalin-fixed, paraffin embedded sections (~4 microns) of adequate liver biopsies were stained with Masson Trichrome for Collagen

WHOLE SLIDE IMAGING

- The same slides prepared for and reviewed by the pathologist were digitized at 40X (0.25 micron/pixel) on an Aperio AT2 WSI system. A total of 276 images were acquired.
- Each Digital Image was evaluated for adequacy, using 20 criteria specific to Biopsy tissue (quality, size), Histology Tissue processing (embedding, sectioning, mounting), Trichrome staining (uniformity, significant degrees of intensity) and scanning (white balance correction, stripes, dust), all resulting into a Digital Biopsy adequacy Score (DBA) ranging from 0 to 10. Digital Images that are not acceptable has DBA<5, and minimally acceptable is 5=<DBA<7.5.

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

NASH-CRN and ISHAK STAGING FOR FIBROSIS SEVERITY

- Biopsy slides are read by an independent pathologist trained on the NASH-CRN and ISHAK staging system
- NASH CRN F3 (severe fibrosis) and F4 (cirrhosis) and ISHAK (Stages 4, 5, or 6) are used to stage patients at baseline and end of treatment
- Responders are identified with at least a full categorical stage change

DIGITAL PATHOLOGY AND ARTIFICIAL INTELLIGENCE

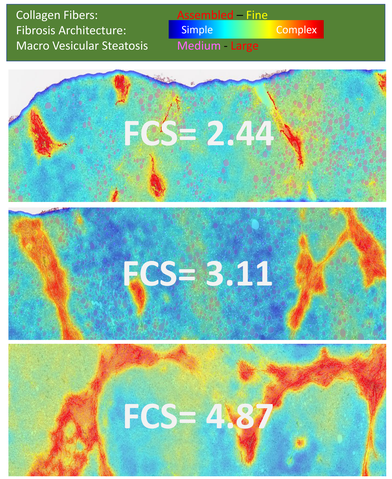
- The digital images were read using FibroNest™, a single-fiber, high-content, quantitative Digital Pathology image analysis and AI method. This provides a continuous phenotypic Fibrosis Composite Severity Score (Ph-FCS, FibroNest Digital Pathology biomarker for fibrosis) that ranges from 1 to 10 for 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. An exploratory biomarker PT-Ph-FCS was also normalized and calculated based on the Parenchymal (e.g. non-steatotic) Tissue topology.
- A 0.3 absolute reduction in Ph-FCS (4-fold higher than the analytical variability) identified any reduction in fibrosis [5].
- A 25% relative reduction of Ph-FCS (corresponding to an absolute change of 0.75 to 2 for 3<Ph-FCS<8) is used to identify significant reduction in responders [4,5].

DIGITAL PATHOLOGY METHOD

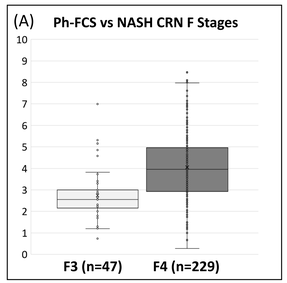
SINGLE-FIBER, HIGH CONTENT, QUANTITATIVE IMAGE ANALYSIS

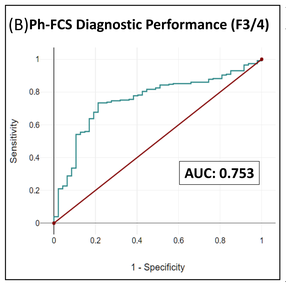
- Using Quantitative Image Analysis (FibroNest™) the fibrosis phenotype is described for its collagen content and structure (12 traits), the morphometric traits of the collagen fibers (13 traits), and fibrosis architecture traits (7). In each image, each morphometric and texture trait is represented by a histogram distribution.
- The histogram for each trait is described by seven quantitative fibrosis parameters (qFPs, 315 in total) to account for mean, variance, distortion and progression.
- Principal qFTs[1,2,3] 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 combined into a normalized Phenotypic Composite Fibrosis Severity score (Ph-FCS), a continuous quantifier of the fibrosis severity phenotype.

AUGMENTED DIGITAL PATHOLOGY IMAGES



F3-F4 Diagnostic Performance of the Ph-FCS



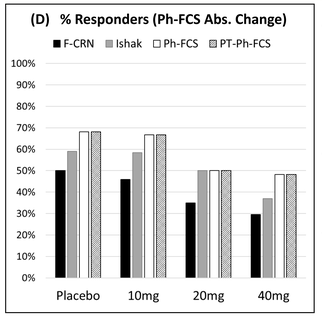


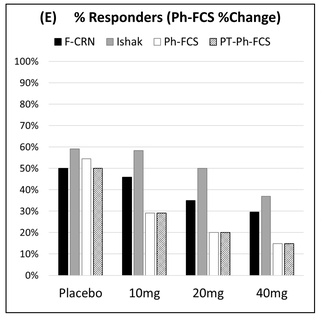
(C) *Ph-FCS*

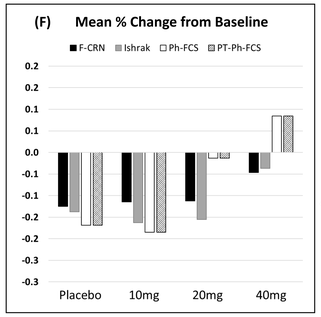
	Stage F3	Stage F4
Mean	2.75	4.05
Standard Error	0.17	0.11
Median	2.56	3.96
Standard Deviation	1.16	1.74
Sample Variance	1.36	3.02
Kurtosis	3.19	6.68
Skewness	1.45	1.30
Range	6.26	14.98
Minimum	0.73	0.27
Maximum	6.99	15.25
Count	47	229
CI (95.0%)	0.34	0.23

- Ph-FCS corresponds with NASH CRN F3 and F4 stages (Fig. A) and highlight the span of the Cirrhosis spectrum (Min/Max, Standard deviation values in Table C).
- The performance of the Ph-FCS as a diagnostic biomarker to classify F3 from F4 is moderate (AUROC=0.753) but is on-par with histological methods and Non-Invasive Tests.

RESULTS







- Groups sizes with paired biopsies were 22, 24, 20, 27 for the placebo,10mg, 20mg, 40mg groups following removal of images considered non-evaluable for FibroNest algorithms (i.e., DBA < 5).
- Responders were identified with a 1-unit reduction for the histological stage (Fig. D-E).
- Using an absolute reduction of 0.3 (4-fold higher than the analytical variability), the Ph-FCS resolved 15% to 20% (resp. 0% to 10%) more responders than NASH CRN (resp. Ishak) categorical stages which is consistent with an increased detection threshold (Fig. D).
- A 25% relative reduction of Ph-FCS (corresponding to an absolute change of 0.75 to 2 for 3<Ph-FCS<8) detected fewer responders than when using NASH-CRN or Ishrak (Fig. E).
- While the Ph-FCS absolute reduction cut off of 0.3 and relative reduction cut off at 25% are consistent with the one defined in the context of pre-cirrhotic patient studies [5], it might be appropriate to change them in the context of a study in cirrhotic patient to adjust the amount of change to the severity of the disease. The definition of a "meaningful Ph-FCS change" is still under development and will probably be settled with the study of retrospective cohorts with long term follow up and liver related events.
- There was no difference between the Ph-FCS and the PT-Ph-FCS which is attributed to the lack of antisteatotic effect of the treatment in this study, as reported elsewhere.

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] Ratziu V. et al.** Multimodality assessment of hepatic fibrosis: Ranked paired reading and artificial intelligence identifies fibrosis improvement with Aramchol missed by conventional staging. *International Liver Congress, EASL* (2022).

Conclusion

Quantitative digital pathology image analysis and AI generates continuous scores for fibrosis that enhance conventional histological staging and resolve the continuum of cirrhosis. The definition of meaningful change criteria using this continuous scoring remains to be improved.

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