Novel Digital Pathology Quantitative Image Analysis and AI Method Detects Traits of Fibrosis Treatment Response

Li Chen[1], Louis Petitjean[1], Anne Minnich[2], Vipul Baxi[2], Edgar D. Charles[2], Zachary Goodman[3], Mathieu Petitjean[1], Arun J. Sanyal[4]

[1] Pharmanest Inc, Princeton, NJ, USA, [2] Bristol Myers Squibb, Princeton, NJ, USA, [3] Inova Health Systems, Falls Church, VA, USA, [4] Virginia Commonwealth University, Richmond, VA, USA

BACKGROUND and AIMS

Manual histological evaluation of liver biopsy is the gold standard method for fibrosis staging in Non-Alcoholic Steatohepatitis (NASH), but it is limited by its inter- and intra-reader variability. The use of single-fiber, quantitative and high-resolution Digital Pathology image analysis offers a way to describe specific traits that account for disease progression and/or regression or treatment response. In this exploratory post-hoc analysis, we used FibroNest™ digital pathology to identify fibrosis traits of treatment and dose response from the phase 2b FALCON1 study of pegbelfermin (PGBF) in NASH (NCT0348699).

METHOD

STUDY DESIGN AND TREATMENT ARMS

Eligible adults were 18-75 years of age (N=197) with NASH and stage 3 fibrosis diagnosed by histologic assessment of liver biopsy according to NASH CRN criteria. During the 48-week double-blind treatment period, patients received subcutaneous 10mg, 20mg, or 40mg PGBF or placebo once weekly. Liver biopsies were obtained up to six months prior to or during screening and at week 24.

LIVER TISSUE HISTOLOGY

- Liver biopsies were obtained six months before or during screening and at week 24
- 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.
- Each Digital Image and tissue was evaluated for adequacy, using 20 criteria specific to biopsy quality (length, area), tissue preparation (embedding, sectioning, mounting), staining (uniformity, rinse residual, 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 has 5=<DBA<7.5.
- Groups sizes ranged from 34 to 39 per group for patients with paired data following removal of those samples considered nonacceptable for Pharmanest algorithms (i.e., DBA < 5).

DIGITAL PATHOLOGY AND ARTIFICIAL INTELLIGENCE

- The digital images were read using FibroNest™, a single-fiber, high-content quantitative Digital Pathology image analysis and AI automated, full tissue method to provide 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 [1] as well as statistical quantification of change from baseline.

PREVIOUSLY REPORTED DIGITALPATHOLOGY RESULTS AND

- We previously reported that the Ph-FCS Digital Fibrosis Pathology Biomarker method detects the treatment effect of pegbelfermin with a performance (responder identification and mean % change from baseline) that benchmarks imaging based measurements (MRE) and Histological Staging Methods (NASH-CRN and ISHAK) [2], to confirm that the FALCON 1 did not meet histological endpoints with a statistically significant effect.
- Here, we investigated the effects of pegbelphermin on specific histological features / traits of the fibrosis phenotype and the changes during the 24-week study period.
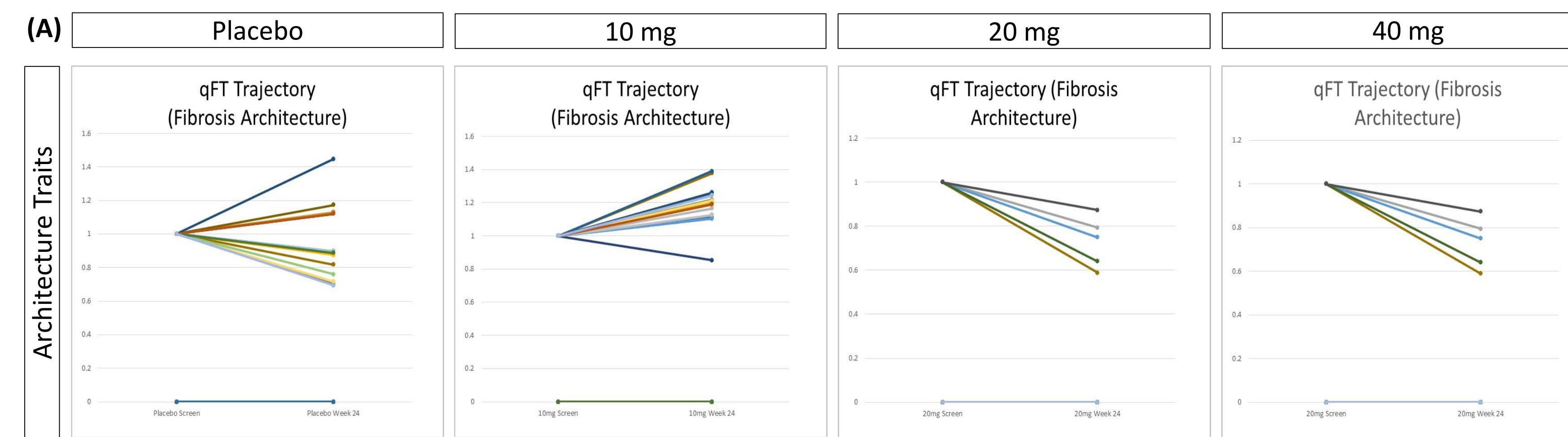
DIGITAL PATHOLOGY METHOD

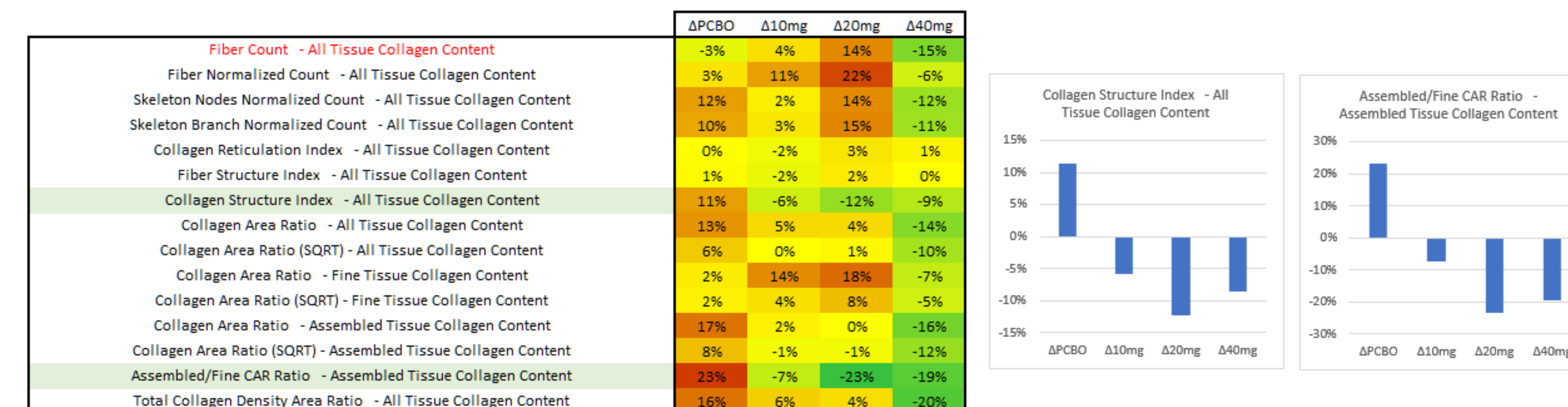### SINGLE-FIBER, HIGH CONTENT, QUANTITATIVE IMAGE ANALYSIS

- 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.

Histological Traits of Treatment Response

- Each of the 315 qFT (group mean) exhibits a relative % change from baseline (Fig. A, example of Architecture traits).
- Histological Traits of Treatment response are then identified when they exhibit a meaningful (>20%) and statistically significant change (p<0.01) from the placebo arm (Fig. B, example for histological traits of collagen deposition and reticulation)
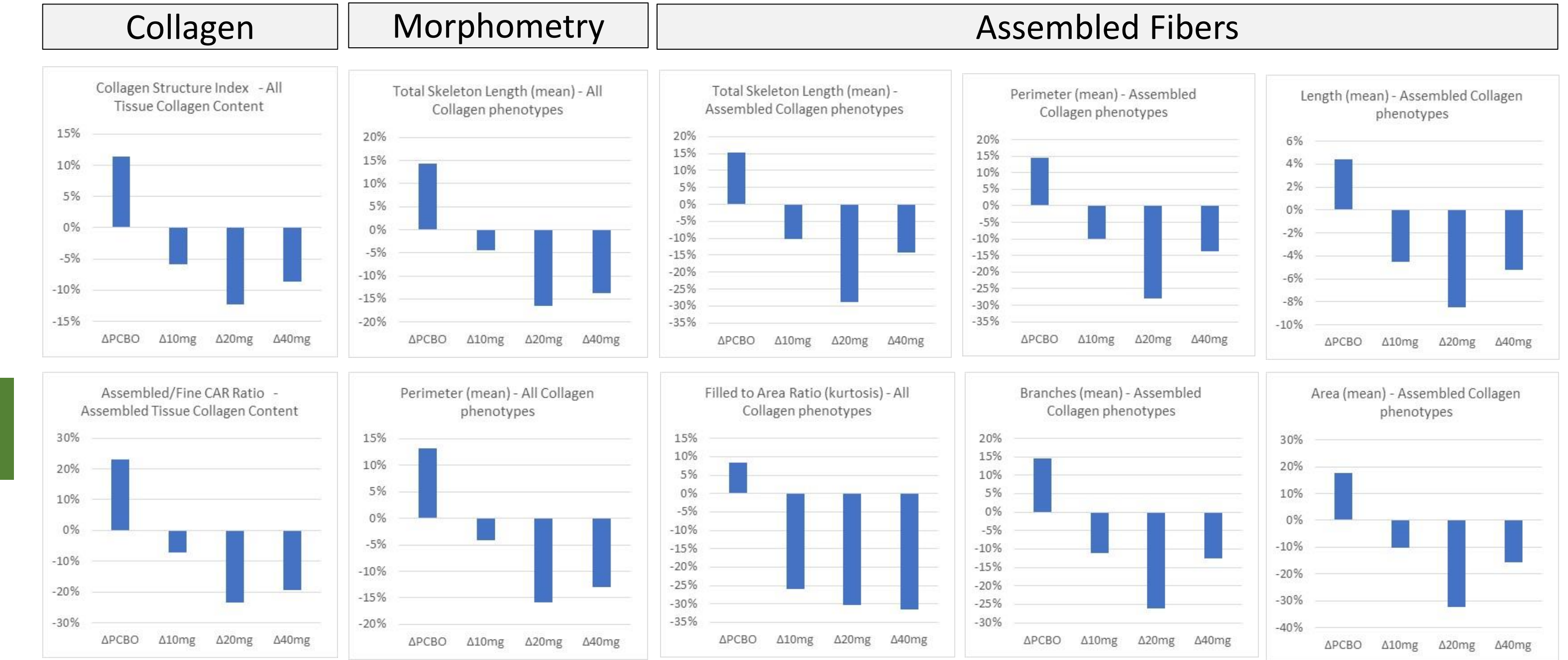

(A) qFT Trajectory plots — Placebo, 10 mg, 20 mg, 40 mg (Fibrosis Architecture)

(B) Histological traits of Collagen deposition and reticulation

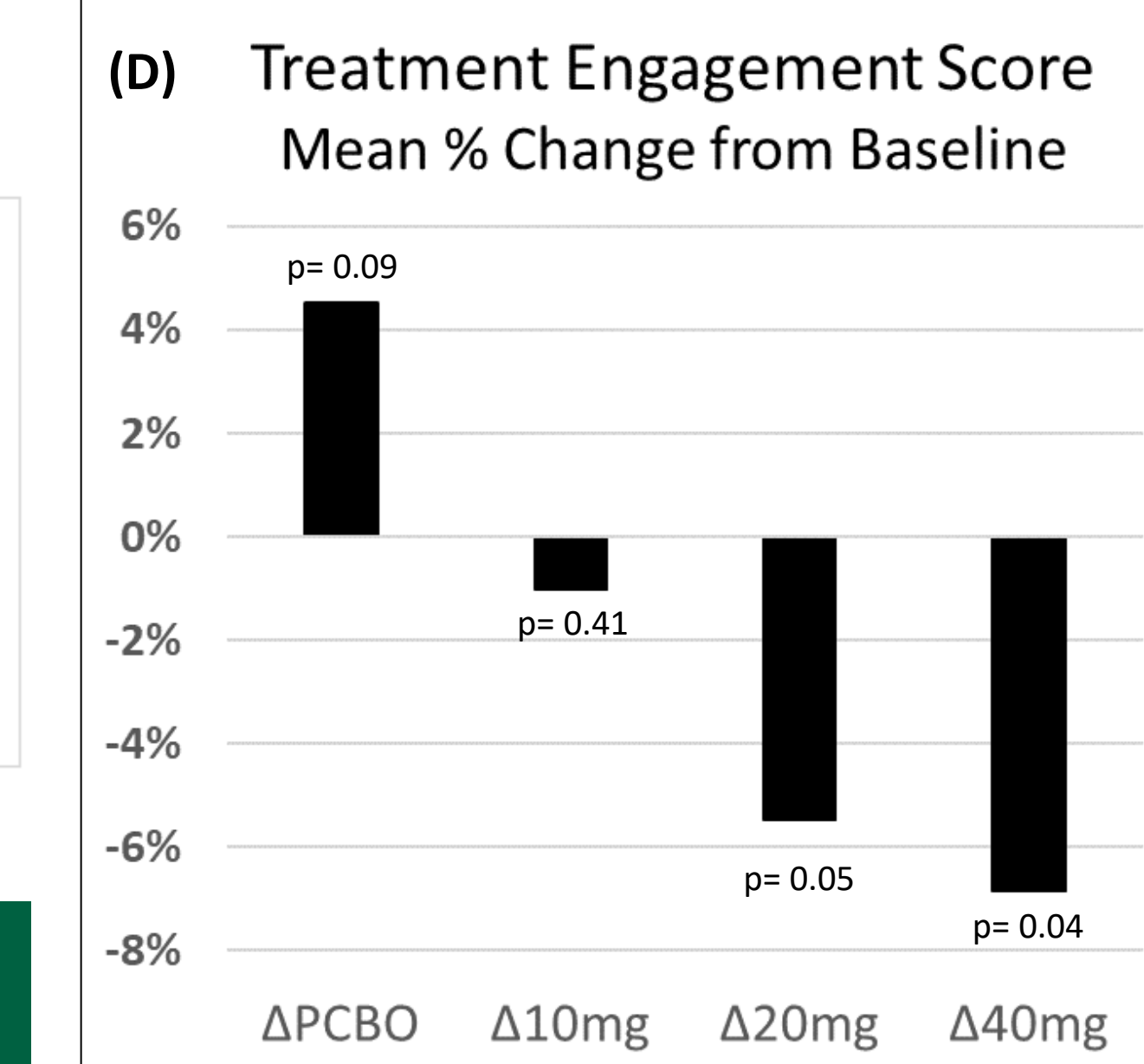|	ΔPCBO	Δ10mg	Δ20mg	Δ40mg
Fiber Count - All Tissue Collagen Content	-3%	4%	14%	-15%
Fiber Normalized Count - All Tissue Collagen Content	3%	11%	22%	-6%
Skeleton Nodes Normalized Count - All Tissue Collagen Content	12%	2%	14%	-12%
Skeleton Branch Normalized Count - All Tissue Collagen Content	10%	3%	15%	-11%
Collagen Reticulation Index - All Tissue Collagen Content	0%	-2%	3%	1%
Fiber Structure Index - All Tissue Collagen Content	1%	-2%	1%	2%
Collagen Structure Index - All Tissue Collagen Content	11%	-6%	-12%	-9%
Collagen Area Ratio - All Tissue Collagen Content	13%	5%	4%	-14%
Collagen Area Ratio (SQRT) - All Tissue Collagen Content	6%	0%	1%	-10%
Collagen Area Ratio - Fine Tissue Collagen Content	2%	14%	18%	-7%
Collagen Area Ratio (SQRT) - Fine Tissue Collagen Content	2%	4%	8%	-5%
Collagen Area Ratio - Assembled Tissue Collagen Content	17%	2%	0%	-16%
Collagen Area Ratio (SQRT) - Assembled Tissue Collagen Content	8%	-1%	-1%	-12%
Assembled/Fine CAR Ratio - Assembled Tissue Collagen Content	23%	-7%	-23%	-19%
Total Collagen Density Area Ratio - All Tissue Collagen Content	16%	6%	4%	-20%

References

[1] 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).

[2] 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).

[3] 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).

[4] 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).

[5] 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).

RESULTS

Collagen | Morphometry | Assembled Fibers | Architecture — results bar charts (ΔPCBO, Δ10mg, Δ20mg, Δ40mg)

(C) Traits of Fibrosis Regression & Treatment Response (FALCON 1)

Collagen Composition	Collagen Structure Index
	Assembled/Fine CAR% Ratio
Fibers Morphometry	Total Skeleton Length (mean) - All Fibers
	Width (std) - All Fibers
	Perimeter (mean) - All Fibers
	Filled to Area Ratio (kurtosis) - All Fibers
	Skeleton Length (Count of Long) - Fine Fibers
	Branches (mean) - Assembled Fibers
	Length (mean) - Assembled Fibers
	Skeleton Length (mean) - Assembled Fibers
	Perimeter (mean) - Assembled Fibers
	Area (mean) - Assembled Fibers
Fibrosis Architecture	Inertia (Contrasted Area Ratio)
	Inertia (std)
	Entropy (Rough Area Ratio)
	Entropy (std)

(D) Treatment Engagement Score Mean % Change from Baseline

p= 0.09 (ΔPCBO), p= 0.41 (Δ10mg), p= 0.05 (Δ20mg), p= 0.04 (Δ40mg)

- We identified 26 traits of response, 16 of which were readily interpretable (Table C).
- These traits are normalized and combined into in a composite Treatment Engagement Score (TrES) (Fig. D).
- While the study primary and categorical histological end-points for fibrosis resolution (1 stage reduction in NASH-CRN fibrosis stage) were not met, the TrES shows that the histological phenotype of fibrosis was remodeled in a dose-related way.
- These results might help close the existing gap between the response identified in circulating fibrosis biomarkers and histological end points.

Conclusion

Twenty-six histological traits of treatment response are identified with high-resolution digital pathology method using FibroNest™ and evaluated in the context of the PGBF intervention. The related Treatment Engagement composite continuous score detects the antifibrotic effects of PGBF treatment with moderate performance as seen for similar outcomes (Histology and fibrotic biomarkers) reported for this study.

Contact and presenting author: louis.petitjean@pharmanest.com