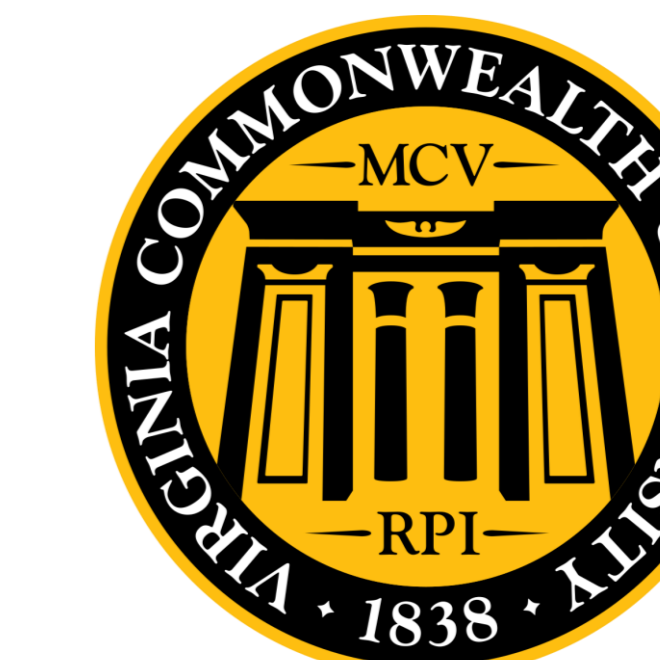


Novel Digital Pathology quantitative image analysis and AI method detects the treatment effect of NASH drug candidates with a performance that benchmarks Imaging based measurements.



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1 Introduction

Manual histological evaluation of liver biopsy is the gold standard for fibrosis and steatosis staging in Non-Alcoholic Steatohepatitis (NASH), but it is limited by its inter and intra-reader variability. Quantitative Digital Pathology image analysis and AI (FibroNest™) as well as quantitative MRI signal analysis methods have the potential to overcome the limitation of these standards

2 Aim

This exploratory post-hoc analysis compared FibroNest digital pathology scoring methods (Fibrosis and Steatosis) with NASH-CRN categorical stages and imaging-based scores (MRI Mean Liver Stiffness and MRI Mean Proton Density Fat Fraction (MRI-PDFF)) in patients with NASH from the Phase 2b FALCON1 study (NCT348699).

3 Method

- N=197 adults were 18-75 years of age with NASH and stage 3 fibrosis (NASH-CRN)
- 48-week double-blind treatment period, 10mg, 20mg, or 40mg PGBF subcutaneous or placebo once weekly.
- Liver biopsies (N=394) six months before or during screening and at week 24
- NASH-CRN categorical stages** (F0 to F4) are adjudicated for each biopsy
- MRI imaging resulting in **Mean Liver Stiffness (MRE)** and **MRI-PDFF** for most patients

Pathology, Digital Pathology and AI:

- 40X digital images of Masson Trichrome** stained of FFPE sections of liver biopsies at Baseline and week 24 (same as adjudicated by Pathologists)
- Digital Biopsy Adequacy Score (DBA):** each digital image was evaluated for quality along 20 dimensions (tissue processing, staining, and scanning) (FibroNest-Check™)
- Fibrosis severity continuous score (Ph-FCS, 1 to 10).** Quantitative image analysis extracts single-fiber quantitative traits (qFTs, N=315) from the fibrosis histological phenotype. A previously validated selection of principal qFTs [1] is normalized and combined to form the severity continuous score (FibroNest™ method).
- Additional **sub-Phenotypic scores** (fine and assemble fiber sub-classes, morphometry, architecture, fibrosis scar) are used to further describe the fibrosis phenotypes and its remodeling as fibrosis progress or regresses
- Steatosis Severity Continuous Score:** the non-fibrotic parenchymal tissue area fat ratio (A%) is measured. Its square root **SQRT(A%)** is used as an exploratory marker

5 Conclusions

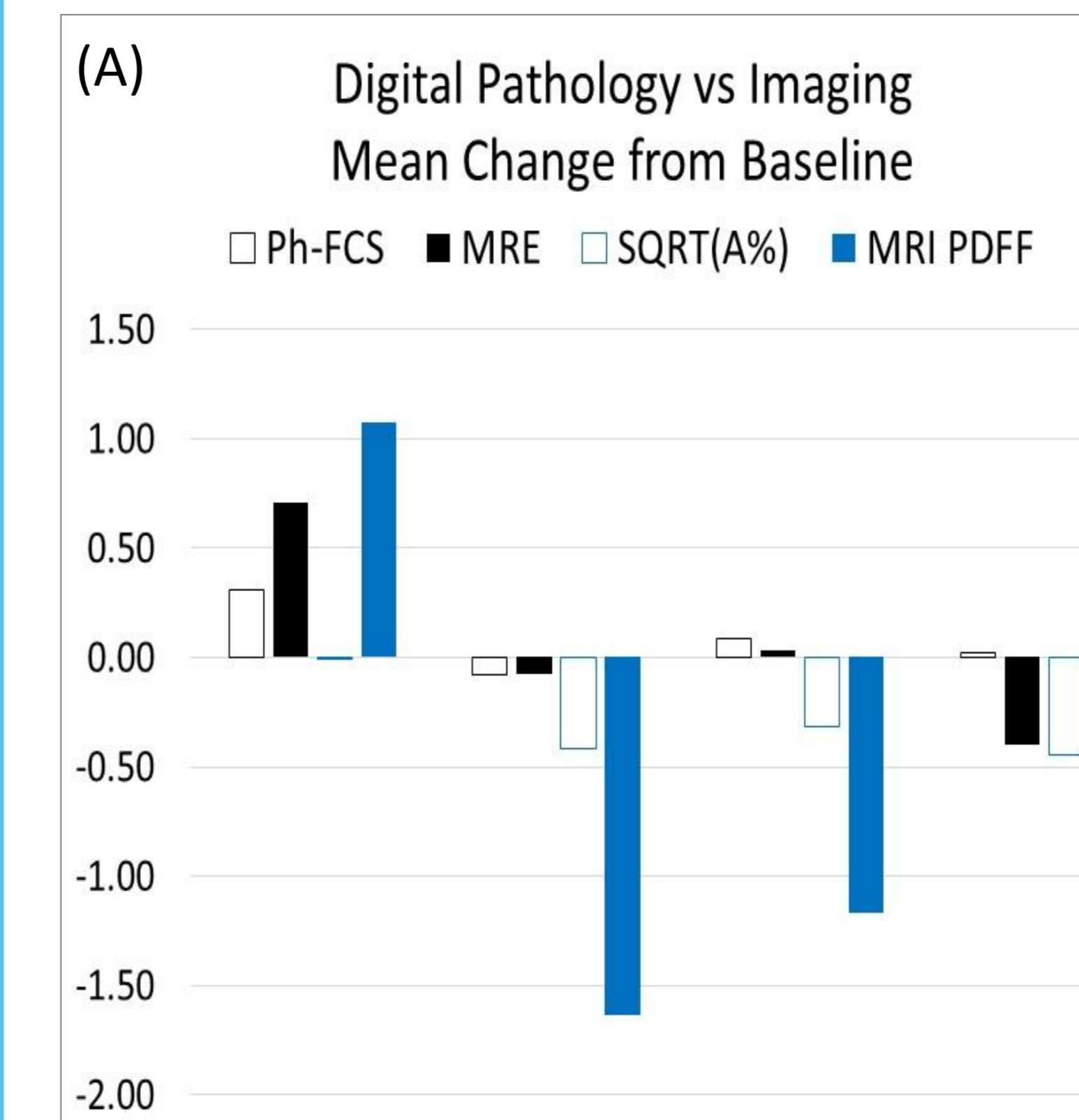
Combined to AI algorithms, quantitative digital pathology image analysis provides continuous read-outs of the histological parameters for severity and steatosis. This read outs are sensitive to subtle changes providing a more granular and accurate way to assess drug effects in clinical trials.

6 Contact information

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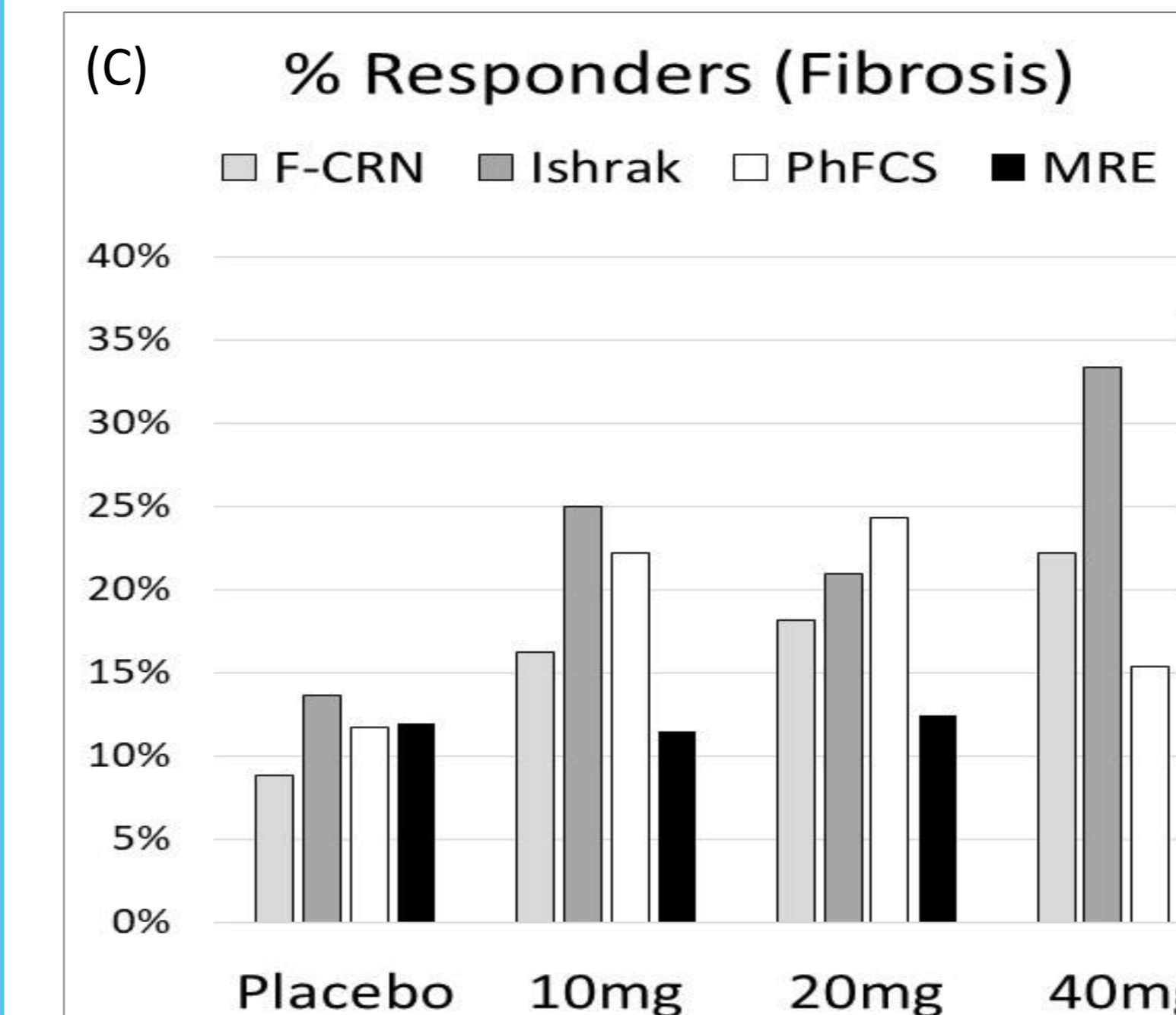
4 Results

- Patients with biopsies with a DBA lower than 5 (non-adequate, ~10% of the cohort) were not included.
- Groups sizes ranged from 27 to 40 patients per group.

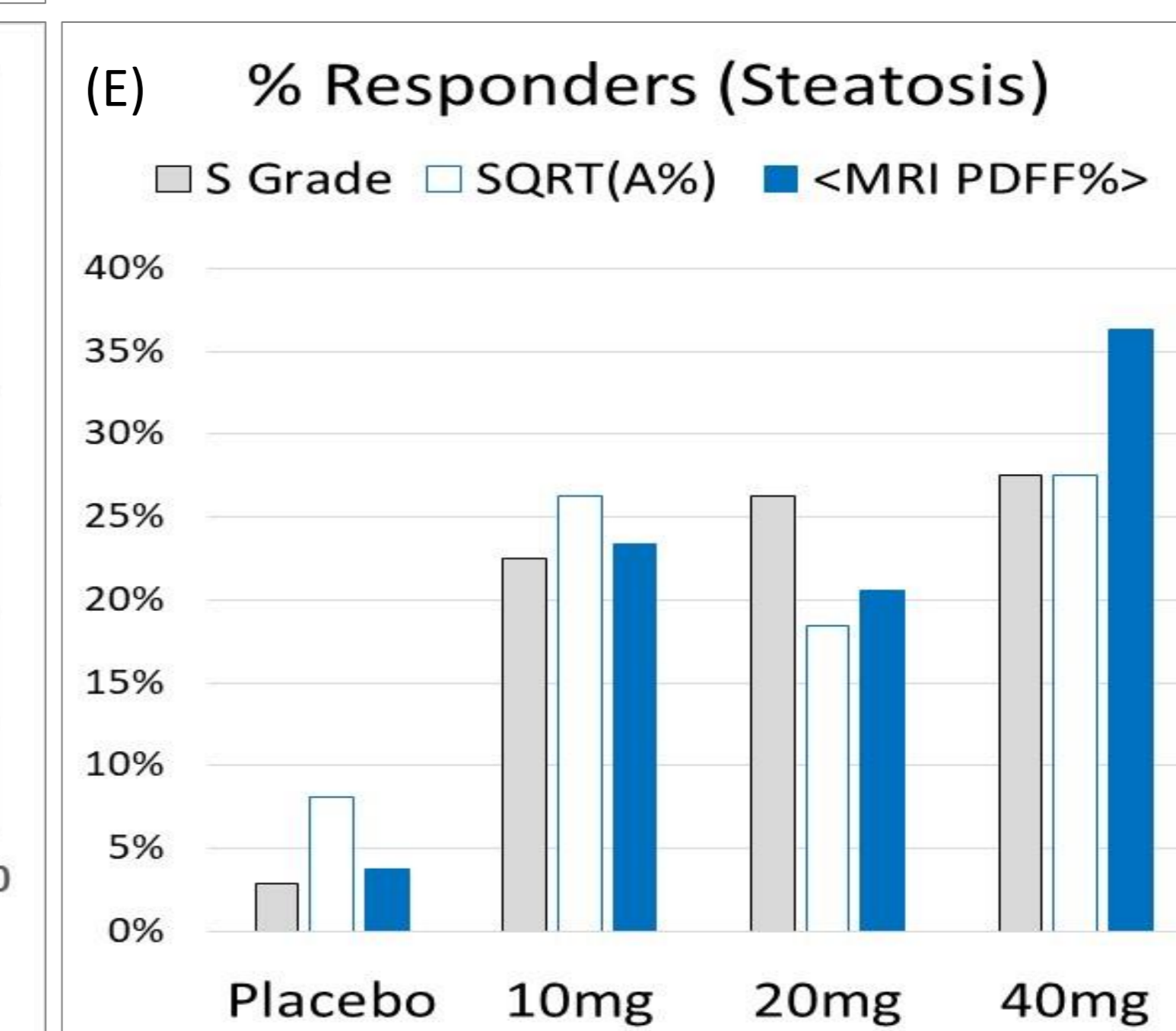
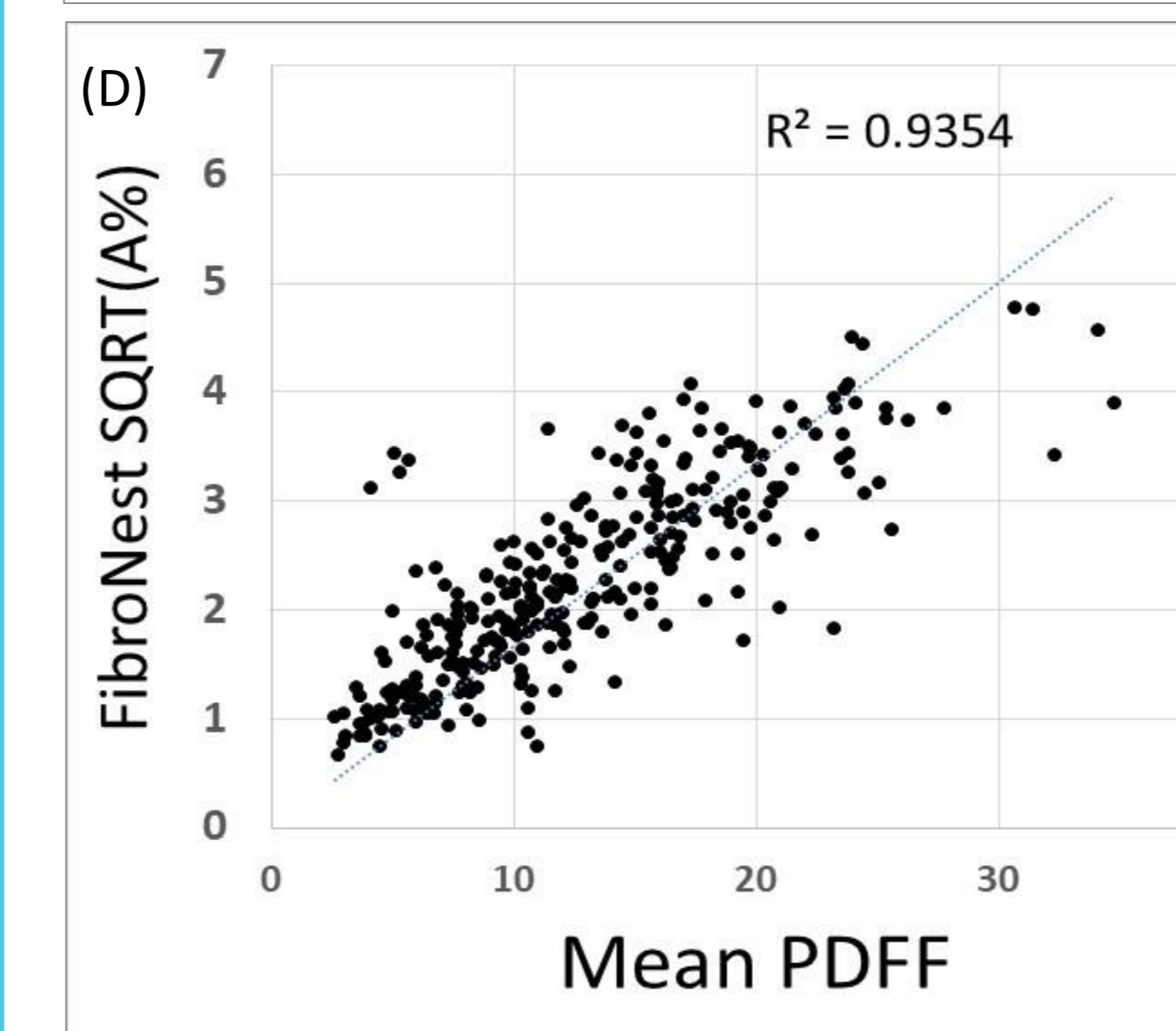


- The quantification of the antifibrotic effect of the treatments is similar using the mean change from baseline of the Ph-FCS and MRE (Fig. A).
- SQRT(A%) highly correlates to PDFF (N=334) and quantifies the anti-steatotic effects for each group with the same performance as PDFF (Fig. A, Table B)

	Placebo	10MG	pValue	20MG	pValue	40MG	pValue
FibroNest Fibrosis Scores	N = 34	36		37		39	
Ph-FCS	0.312	-0.082	0.1534	0.089	0.4188	0.020	0.2735
Scar - FCS	0.117	-0.148	0.4268	0.086	0.9292	0.056	0.8721
Morpho FS	0.296	-0.034	0.2430	0.089	0.4493	0.014	0.2928
Morpho FS - FINE	0.348	-0.069	0.1911	0.190	0.6207	-0.011	0.2380
Morpho FS - ASBL	0.265	-0.008	0.2271	0.085	0.4331	0.050	0.3474
Architecture FCS	0.401	-0.264	0.0688	0.207	0.6248	0.024	0.2881
MRE - MRI Mean Liver Stiffness	N = 25	26		32		33	
MRE	0.707	-0.078	0.0749	0.035	0.1261	-0.399	0.0250
FibroNest Steatosis Scores	N = 37	38		38		40	
SQRT(A%)	-0.005	-0.421	0.0159	-0.316	0.0732	-0.449	0.0068
Steat-CS	-0.003	-0.306	0.0740	-0.255	0.1418	-0.268	0.0987
MRI - Mean PDFF	N = 27	30		34		33	
MRI PDFF	1.070	-1.637	0.0174	-1.171	0.0554	-1.512	0.0304



- Responders were identified with a relative reduction from baseline as summarized in Table F.
- The experimental error of the FibroNest method (related to staining and tissue processing variability) was estimated between 5% and 7% [1]. A 25% relative reduction from baseline is chosen for fibrosis, and 30% for steatosis to fully align to MRI-PDFF.



(F) Responder Criteria

Parameter	Relative Reduction from Baseline
MRE	>=15%
Ph-FCS	>=25%
MRI-PDFF	>=30%
SQRT(A%)	>=30%
NASH CRN F Stage	Reduction in 1 stage or more

[1] EASL2020: Evaluation of a novel histology-based fibrosis phenotypic composite score and its correlation with NASH-CRN Fibrosis scores in patients with NASH. Li Chen (1), Michael Lung (2), Cynthia Behling (2), Arun Sanyal (3), Mathieu Petitjean (1), 1 - PharmaNest, Princeton, NJ, USA; 2 - University of California, San Diego, NAFLD Research Center, Division of Gastroenterology; 3-Virginia Commonwealth University, Richmond, VA, USA.

