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Liver Forum 12

Disease Assessment Strategies to Accelerate Drug Development

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Assessment of Fibrosis: Experiences from Longitudinal Studies and Novel Approaches

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Presenter Disclosure Information

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Potential conflict of interest: N. Naoumov is an independent expert. He advises Histoindex, Hepion Pharmaceuticals and InSphero.

Disclaimer:

- *This presentation is based on publicly available information*
- *The views presented are the views of the presenter*
- *These slides are intended for educational purposes only and for personal use of the audience*

Unlabeled/Unapproved Disclosure:

This presentation includes investigational compounds which are in development and none of these are approved for treatment of NAFLD.

Why Using Digital Pathology with Artificial Intelligence (AI-DP) in Assessment of Fibrosis?

- AI-DP provides precise, standardized and reproducible **quantification** of liver fibrosis on a continuous scale
- Identifies more granularity of collagen fibers and changes in response to treatment;
- Avoids the inter- and intra-observer variations in the current subjective assessment and the limitations of categorical assessment in conventional scorings;
- Allows asking specific questions and obtaining details than can not be seen by the human eye and conventional microscopy.
- Allows assessment of fibrosis dynamics and treatment response by combining the AI-DP outputs with imaging, non-invasive tests and clinical parameters.

NB! AI digital pathology is not a replacement of the diagnostic assessment of liver histology

Digital pathology approaches for quantitative assessment of liver fibrosis

- **Unstained liver sections:**
  - Histoindex
- **Stained liver sections:**
  - PathAI
  - Biocellvia
  - PharmaNest
  - Reveal Bioscience
  - “In house” AI-based software/platform[1,2]
- **Virtually stained sections:**
  - Verily
  - Virtual Trichrome staining technology[3]

[1] R. Forlano et al. Clin Gastroenterol Hepatol. 2020;18:2081-2090.
[2] S. Gawrieh et al. Ann Diagn Pathol. 2020;47:151518.
[3] J. Levy et al. Modern Pathology 2021;34:808–822.

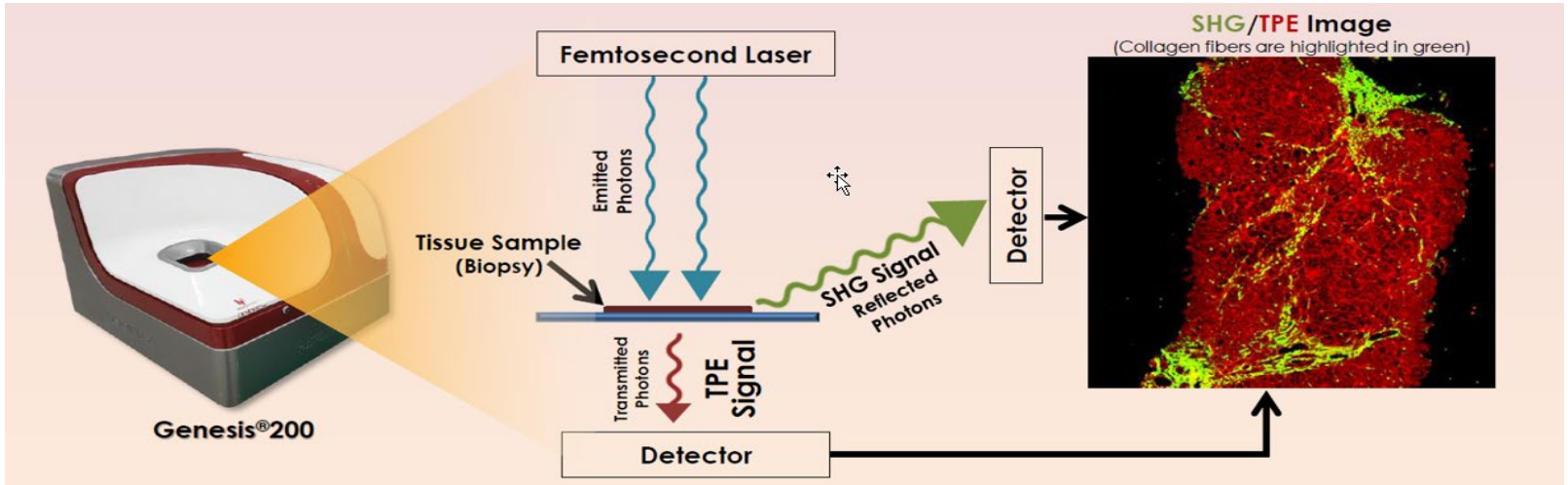
Conventional microscopy

Semiquantitative scoring of fibrosis in stained slides

Fibrosis stages

F0 – F1 – F2 – F3 – F4

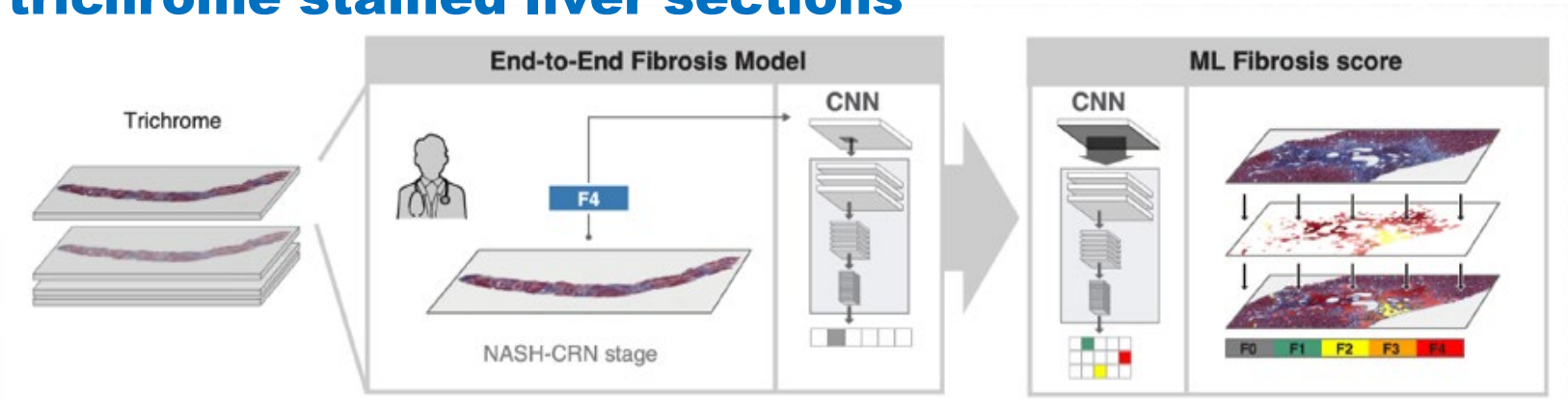
Second Harmonic Generation and Two Photon microscopy for quantitative fibrosis assessment of unstained tissue samples



128 collagen features are interrogated for scoring qFibrosis readout on a continuous scale; quantitation of zonal changes in collagen plus septa analyses with 12 specific parameters

W. Sun et al. J Biomed Optics 2008;13(6):064010. F. Liu et al. Hepatology 2020;71:1953-1966.

Machine Learning model for assessment of fibrosis in trichrome stained liver sections



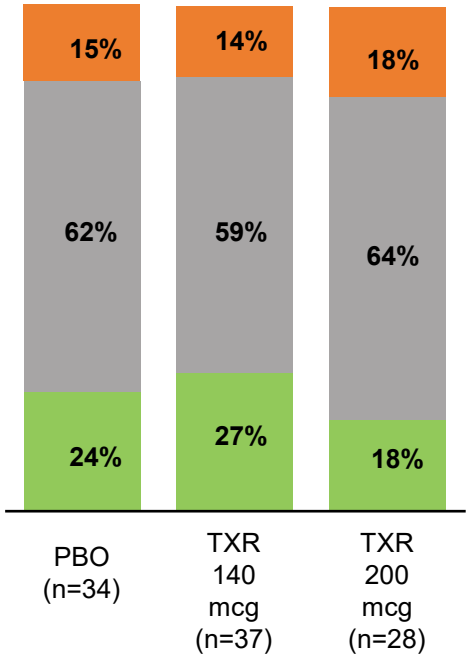
E2E model: fibrosis stage (NASH CRN or Ishak) directly from trichrome-stained slides; measures the proportionate area of fibrosis stage; computes DELTA Liver Fibrosis score measuring fibrosis changes

A. Taylor-Weiner et al. Hepatology 2021;74:133-147.

Liver fibrosis dynamics as assessed by NASH CRN scoring and by digital quantification after 48 weeks treatment with Tropifexor (TXR): Results from Phase 2b (FLIGHT-FXR) trial

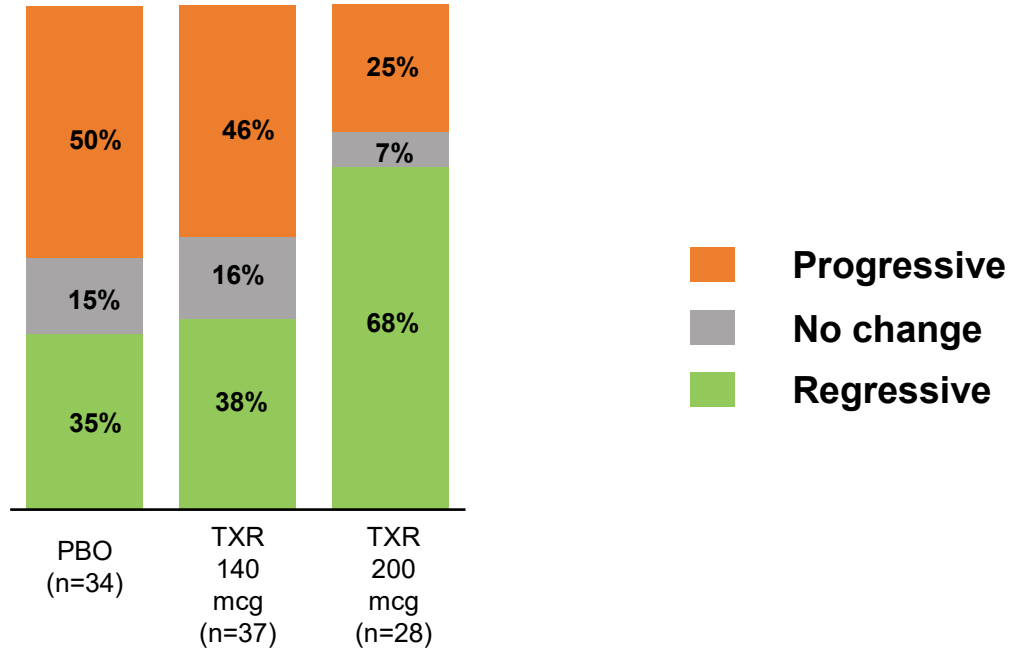
Proportion of patients in each group, categorized according to liver fibrosis changes from BL to EOT

a) **P/N/R based on NASH CRN scores from blinded paired reading**



P (progressive): increased by ≥1 stage fibrosis
N (No change):
R (regressive): decrease by ≥1 stage fibrosis

b) **P/N/R based on digital quantification: qFibrosis as a continuous value**

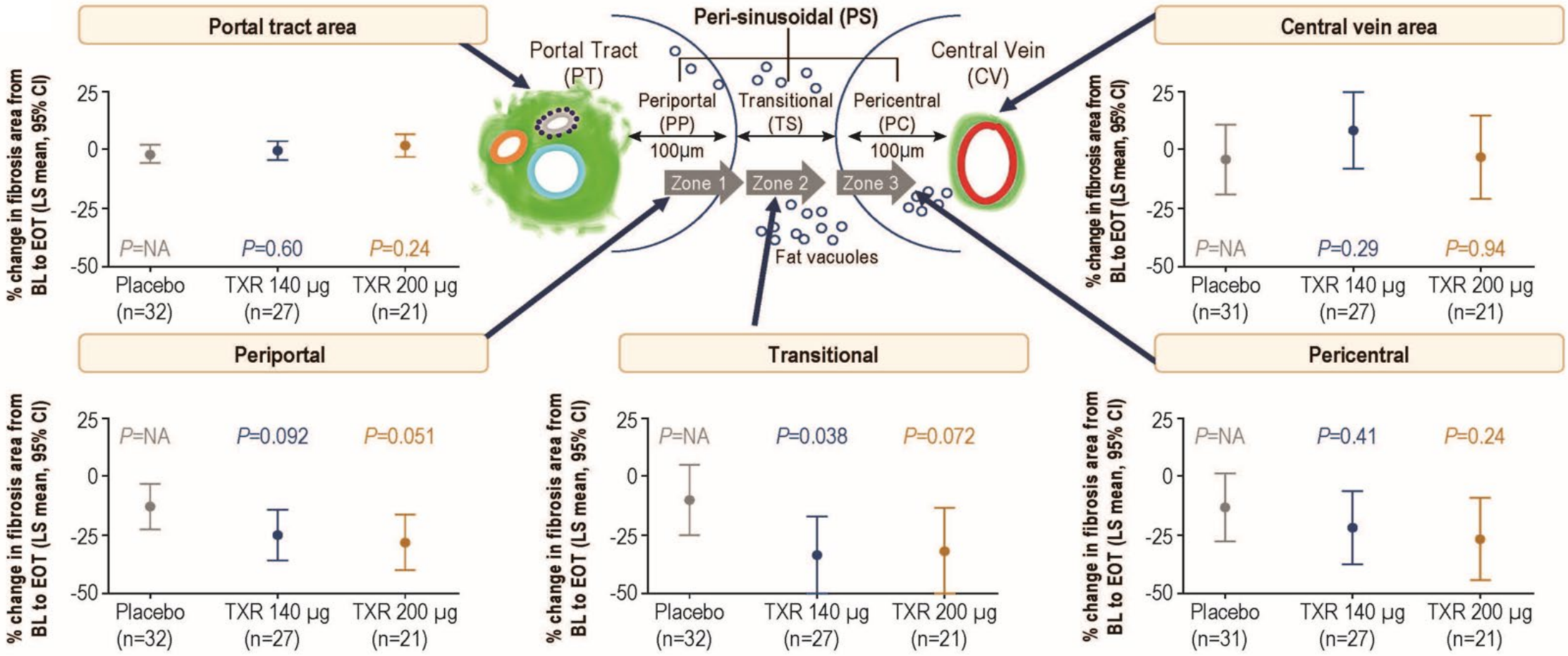


P (progressive): increased ≥1 Standard Error of Measurement (SEm)
N (No change):
R (regressive): decrease ≥1 Standard Error of Measurement (SEm)

BL, baseline; EOT, end of t treatment; FXR, farnesoid X receptor; n, number of patients; PBO, placebo; TXR, tropifexor

N. Naoumov et al. EASL/ILC 2021; Poster #889

Digital quantification of fibrosis changes in different zones of the liver lobule



P values for difference to placebo are from ANCOVA adjusted by baseline value, no multiplicity correction
ANCOVA, analysis of covariance; BL, baseline; CI, confidence interval; EOT, end of treatment; LS; least squares; TXR, tropifexor

N Naoumov et al. EASL/ILC 2021; Poster #889

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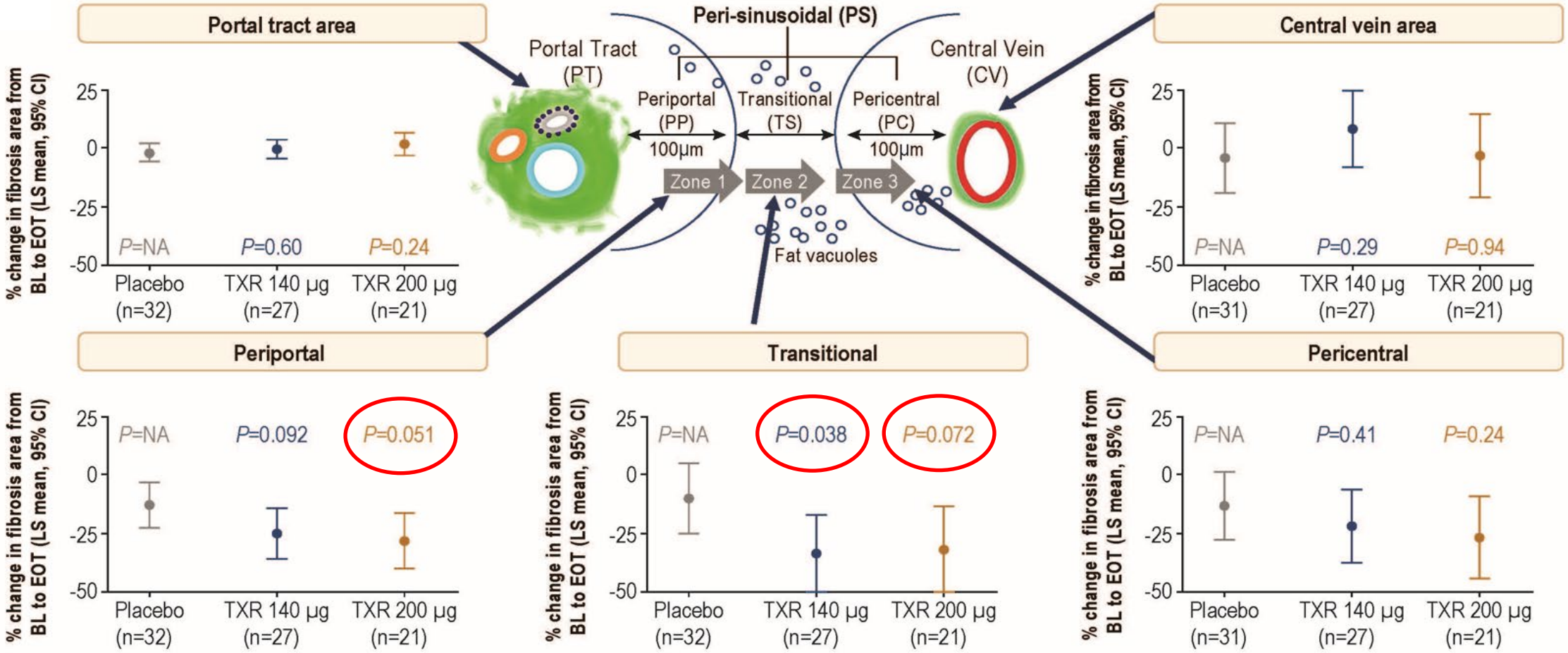

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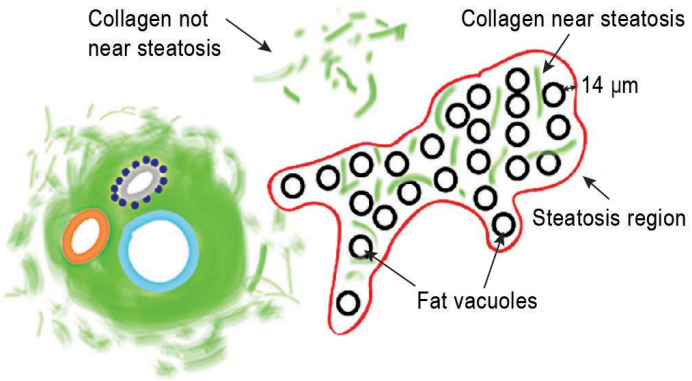
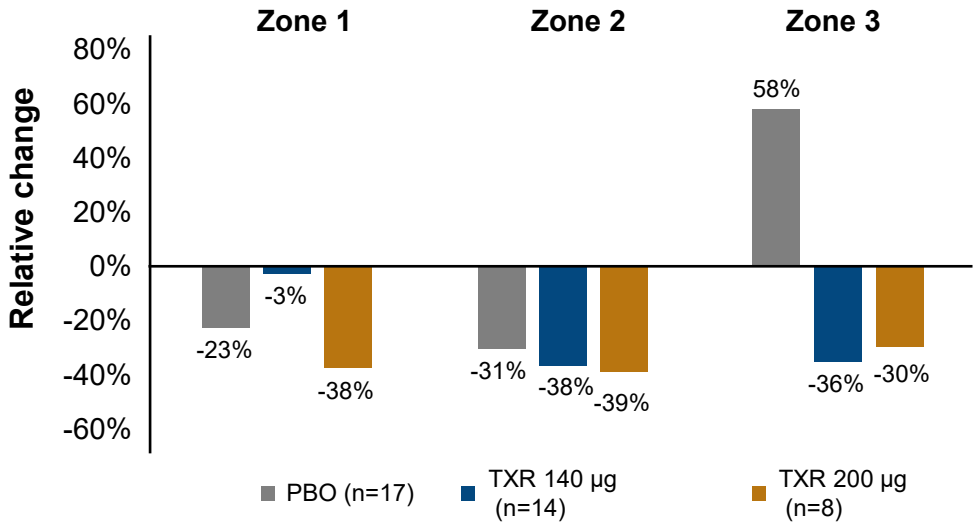
ANCOVA, analysis of covariance; BL, baseline; CI, confidence interval; EOT, end of treatment; LS; least squares; TXR, tropifexor

N Naoumov et al. EASL/ILC 2021; Poster #889

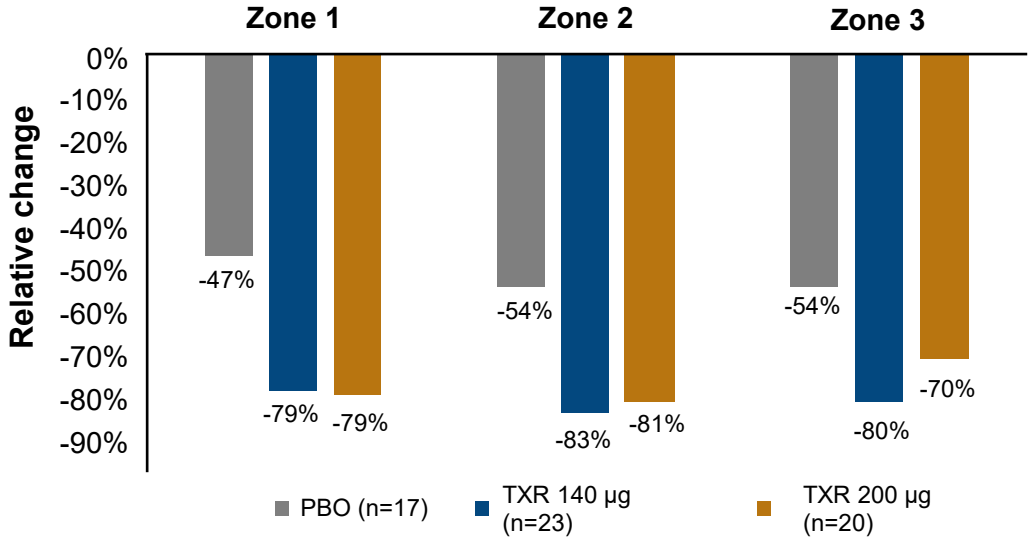
Colocalization of qSteatosis and qFibrosis changes in the liver lobule



Schematic illustration of the approach for concomitant quantitation of steatosis and fibrosis in the same area of the liver lobule. Collagen near steatosis is the collagen in the area within 14 µm around fat vacuoles.



Change of fibrosis near steatosis in patients with unchanged or increased qSteatosis score (n=39)

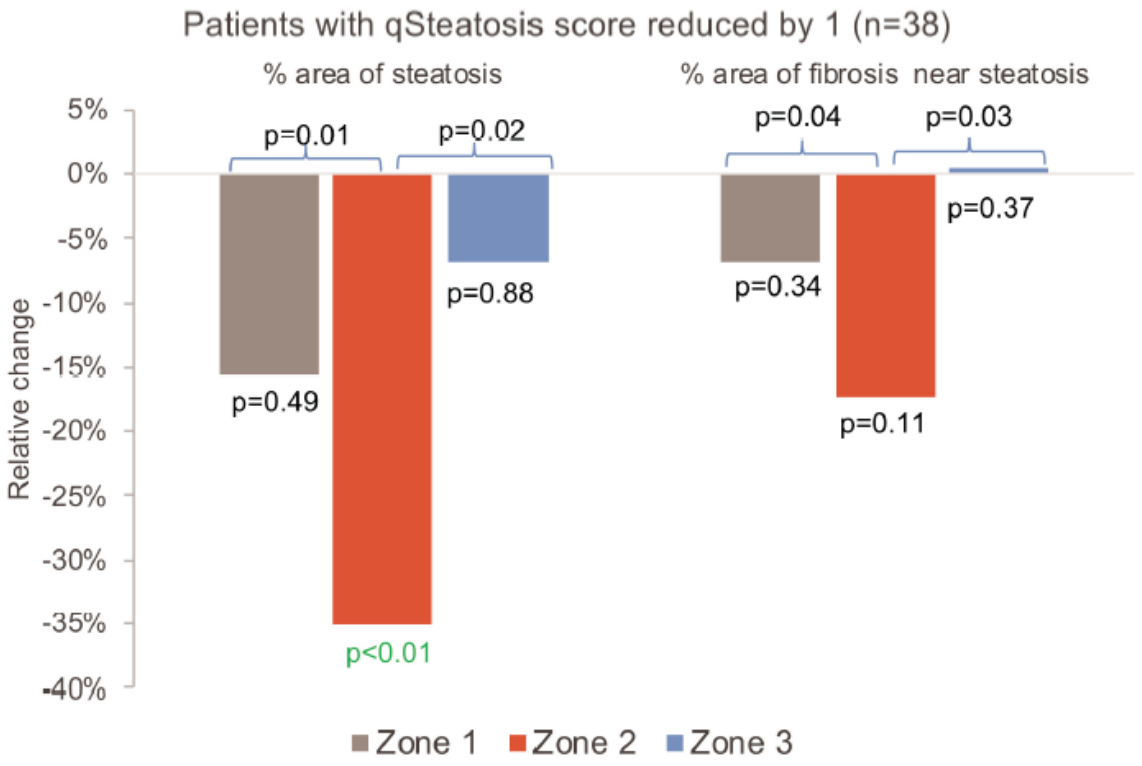


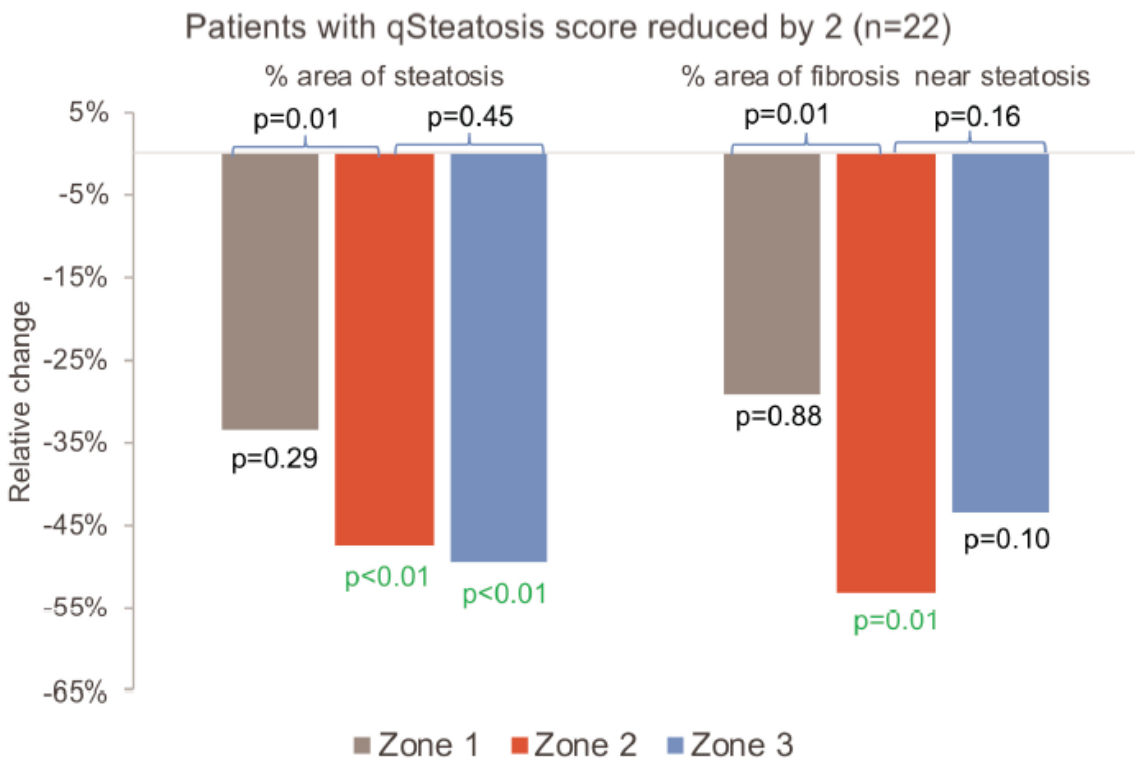
Change of fibrosis near steatosis in patients with reduced qSteatosis score (n=60)

PBO, placebo; TXR, tropifexor

N Naoumov et al. EASL/ILC 2021; Poster #889

Liver fibrosis dynamics in the perisinusoidal area after treatment with Resmetirom (MGL-3196) – Results from Phase 2b NASH Study



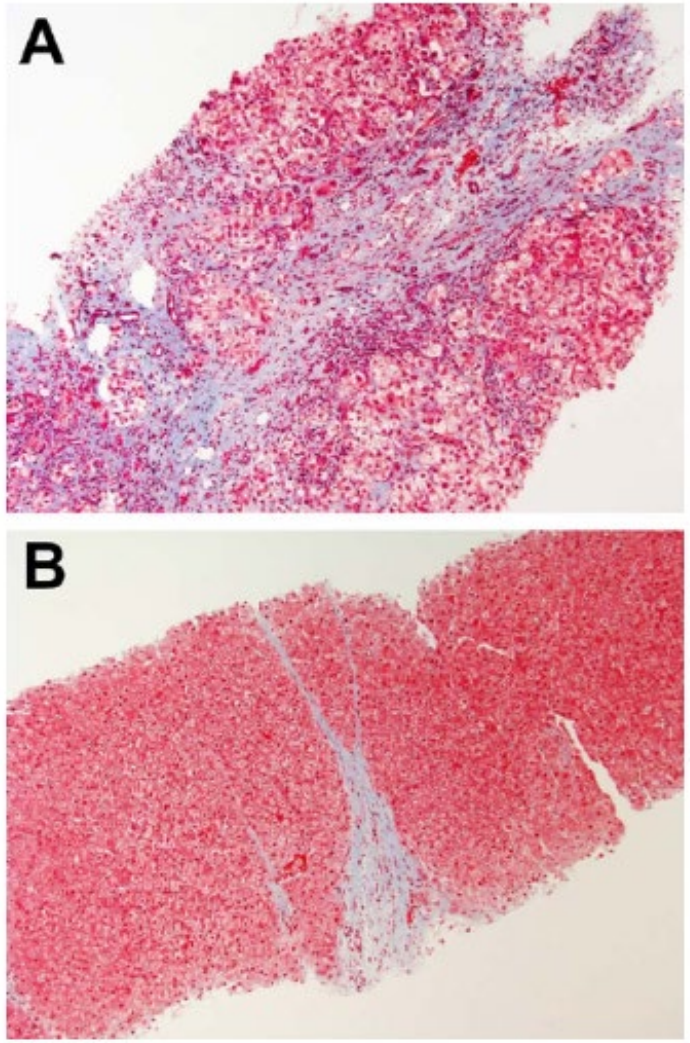


Stephen Harrison et. al. "Steatosis and Fibrosis Measured as Continuous Variables on Paired, Serial Liver Biopsies in the Resmetirom (MGL-3196) 36-Week Phase 2 NASH Study", AASLD 2019, Poster #2133.

Beyond Staging and Grading: Liver Biopsy Evaluation in a Post-treatment World

- There is an inherent danger to assume that a chronic liver disease can be completely characterized by its grade and stage.

- All grading and staging systems used in chronic liver disease were developed based on histological changes in untreated individuals and so do not necessarily account for changes that occur after successful therapy.



Examples of **progressive (A) and regressive (B) fibrosis phenotypes** in two cases with cirrhosis (**Ishak stage 6**).

(A), the fibrous band is wide, with uneven staining, and shows disruption of the adjacent parenchyma.

(B), the fibrous band is thin, with homogeneous staining and sharp borders. (Masson trichrome)

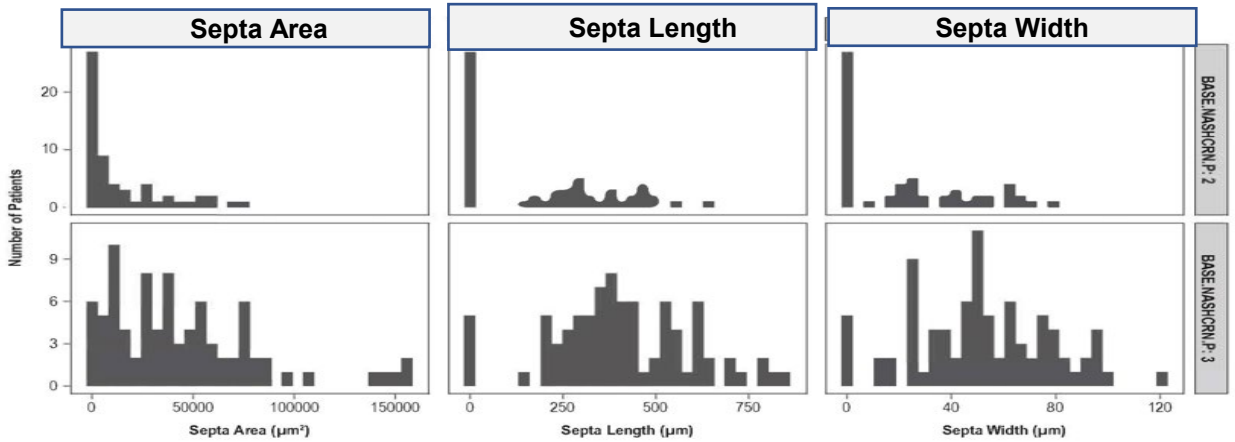
D. Kleiner. Editorial, Hepatology 2017;65:1432-4

SHG/TPEF Microscopy and Quantification of Fibrous Septa

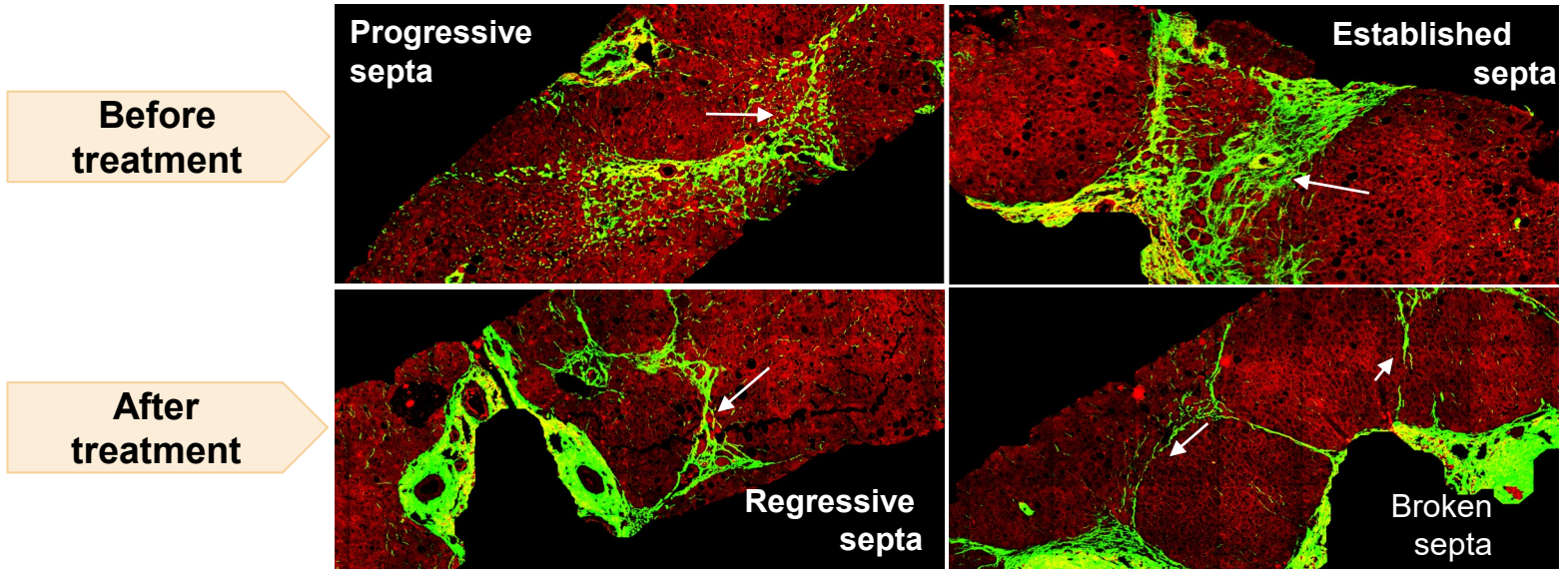
Septa parameters

No	Parameters
1	Septa Area
2	Cellular/Acellular
3	Cellular/Collagen
4	Septa Length
5	Septa Width
6	Intersection Septa
7	# Thick Fiber Septa
8	# Thin Fiber Septa
9	Thick/Thin Ratio
10	Aggregated Fibers in Septa
11	Distributed Fibers in Septa
12	Aggregated/Distributed Fiber Ratio

Digital quantitation of septa area, length, and width in the subgroups of F2 or F3 fibrosis at Baseline

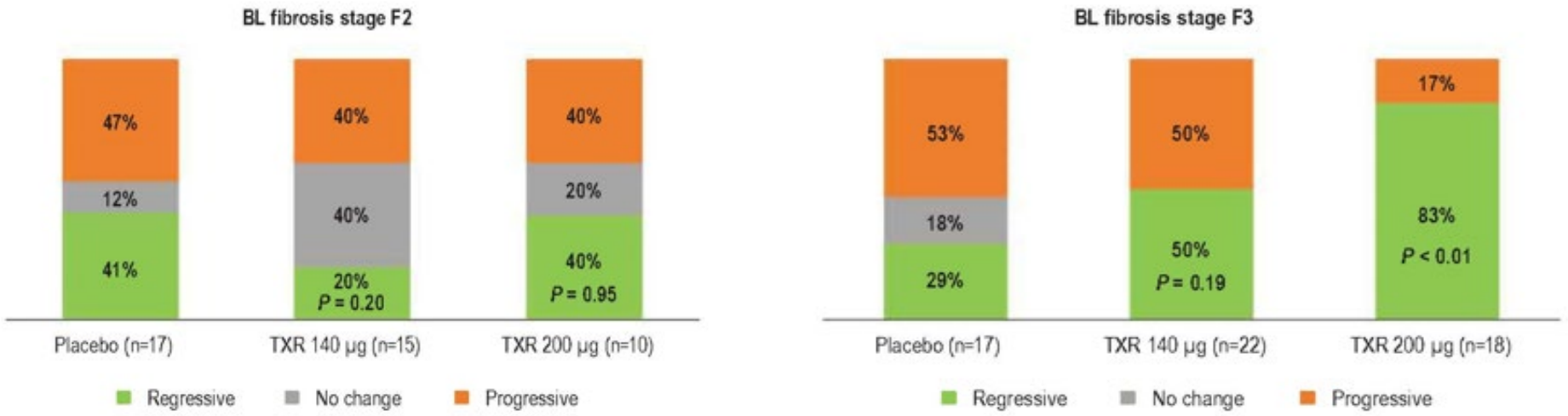


Morphological Changes of Fibrous Septa



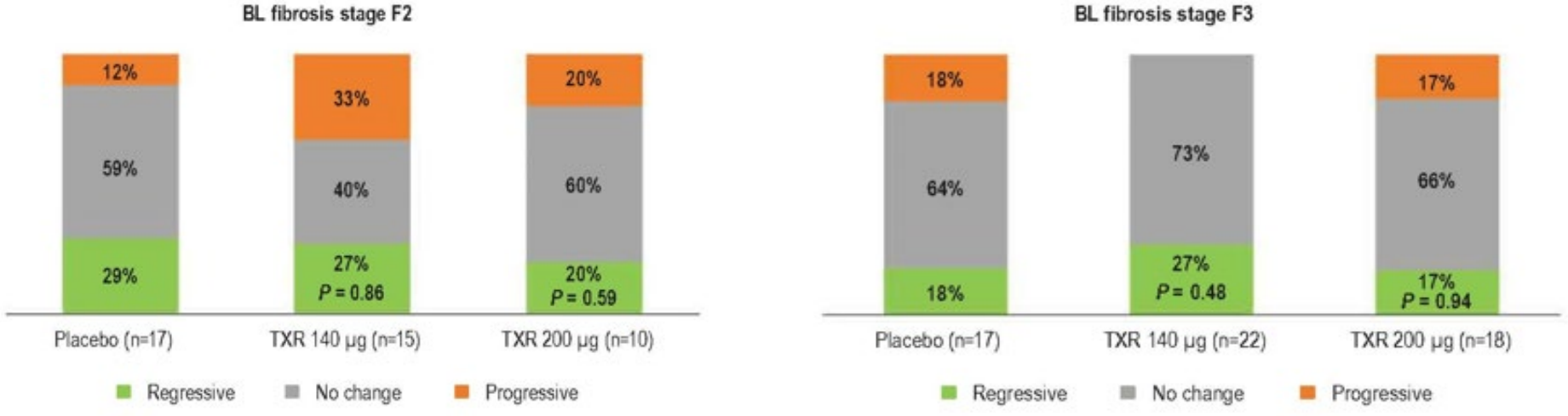
Treatment-related fibrosis dynamics in patients with F2 or F3 stage at Baseline as assessed by NASH CRN and by AI Digital Pathology

P/N/R analysis based on qFibrosis assessment as a continuous value



Progressive: increase by ≥1 Standard Error of Measurement (SEm);
Regressive: decrease by ≥1 Standard Error of Measurement (SEm)

P/N/R analysis based on the NASH CRN fibrosis stage



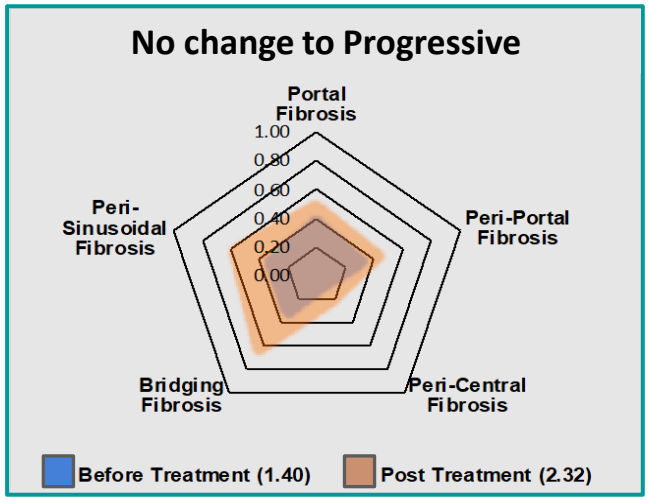
Progressive: increase by ≥1 fibrosis stage;
Regressive: decrease by ≥1 fibrosis stage)

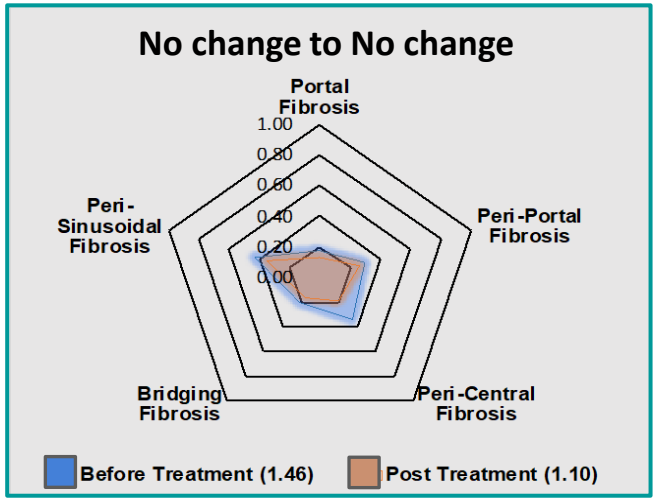
qFibrosis provides greater granularity of fibrosis dynamics in comparison to the NASH CRN staging

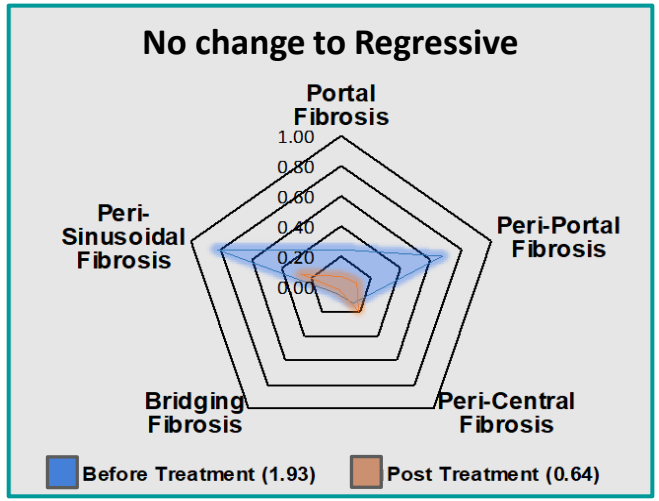
Examples of 3 patients with F3 stage (NASH CRN) at Baseline and End of Treatment (EOT)

	qFibrosis increased		qFibrosis no-change		qFibrosis decreased	
	Baseline	EOT	Baseline	EOT	Baseline	EOT
Portal Fibrosis	0.37	0.48	0.17	0.13	0.25	0.07
Peri-Portal Fibrosis	0.31	0.43	0.30	0.26	0.68	0.10
Peri-Central Fibrosis	0.08	0.20	0.35	0.20	0.13	0.19
Bridging Fibrosis	0.31	0.64	0.21	0.17	0.05	0.02
Peri-Sinusoidal Fibrosis	0.32	0.57	0.43	0.35	0.82	0.25
Total Weighted Score	**1.40**	**2.32**	**1.46**	**1.10**	**1.93**	**0.64**

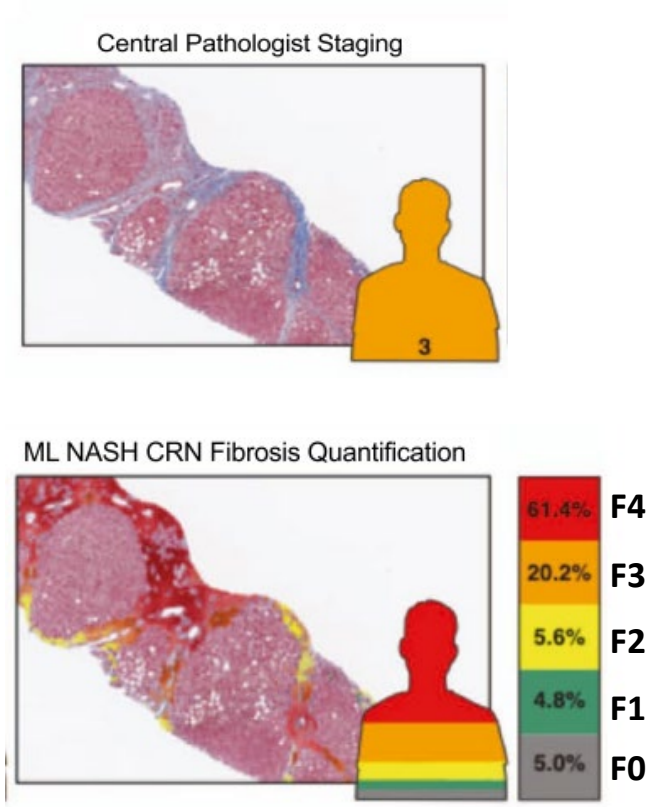
Radar maps of qFibrosis readouts provide graphical view of fibrosis changes in 5 dimensions



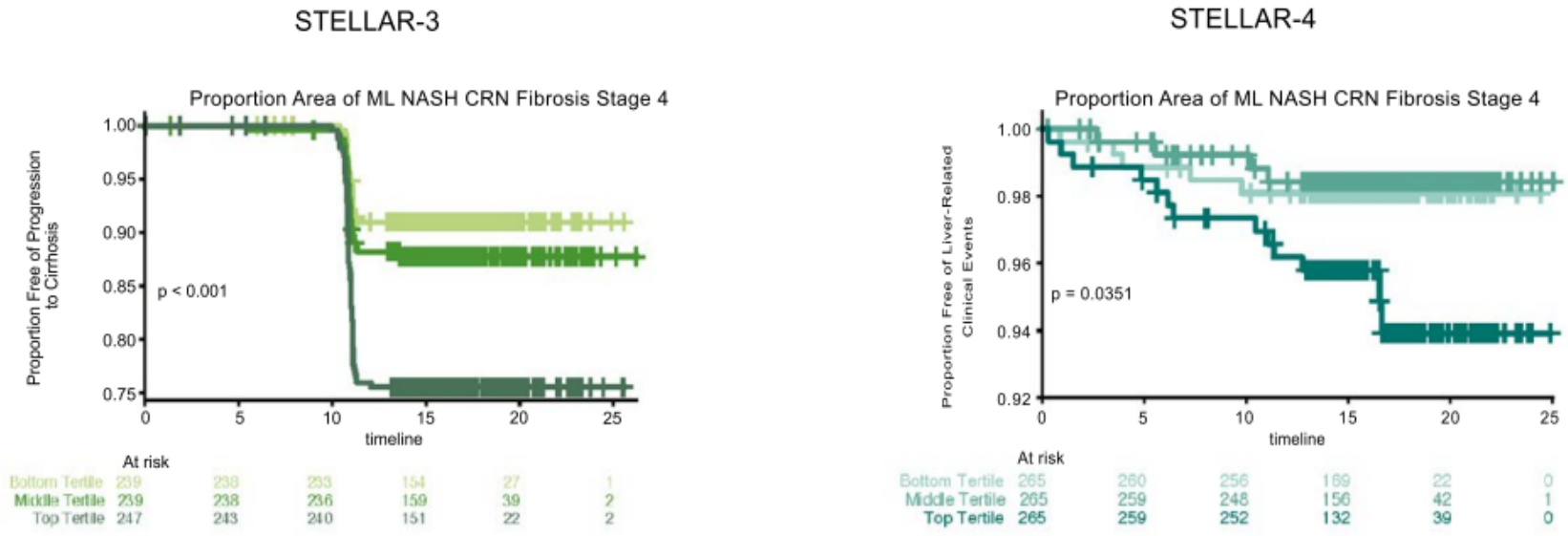




Machine Learning (ML)-based Fibrosis Assessment from Trichrome Images Predicts Clinical Outcomes in NASH



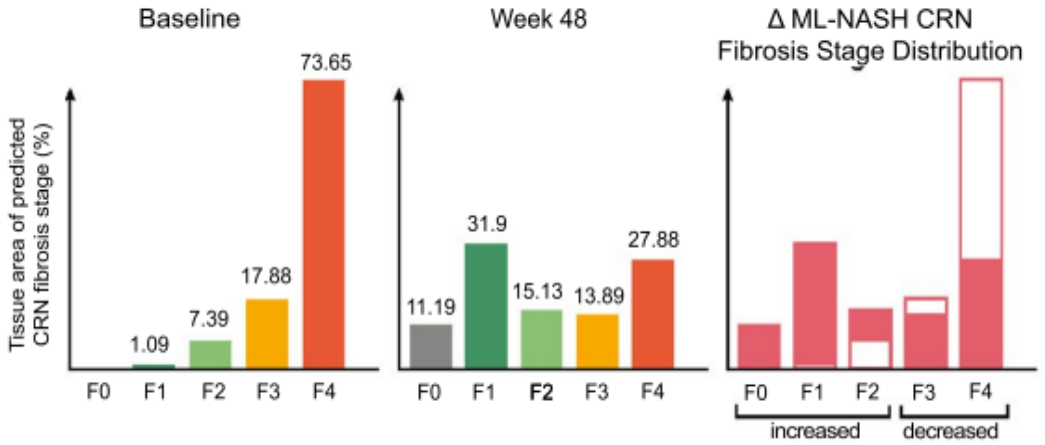
The proportionate area of fibrosis stage 4 (NASH CRN) was associated with clinical disease progression



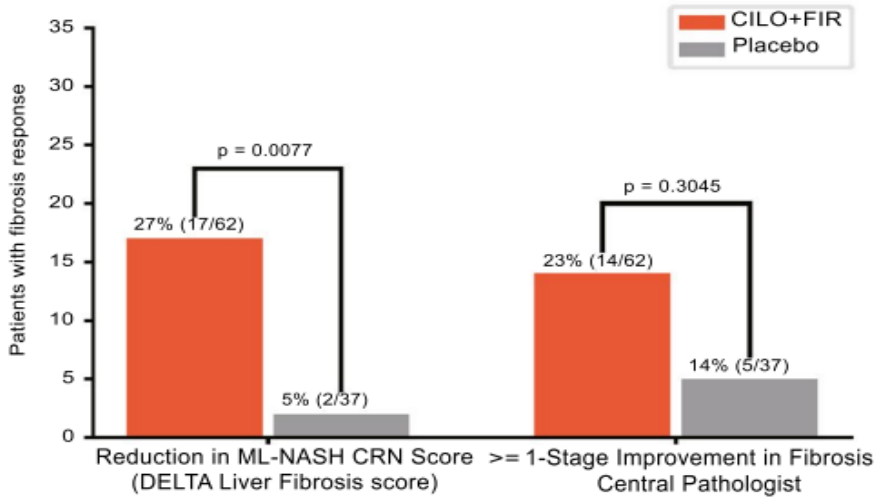
Kaplan-Meier curves showing proportions of patients with bridging fibrosis (F3) without progression to cirrhosis (left panel, STELLAR-3) or patients with cirrhosis (F4) without liver-related clinical events (right panel, STELLAR-4) over time. **Patients are categorized into subgroups by tertile of percentage of area predicted to be NASH CRN stage 4.**

Taylor-Weiner et al. Hepatology 2021;74:133-147.

Machine Learning (ML)-based Fibrosis Assessment from Trichrome Images Measures Treatment Response in NASH

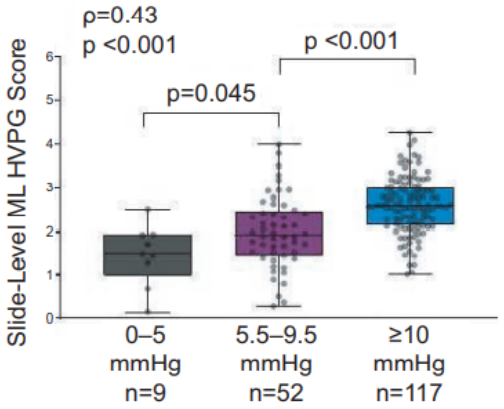


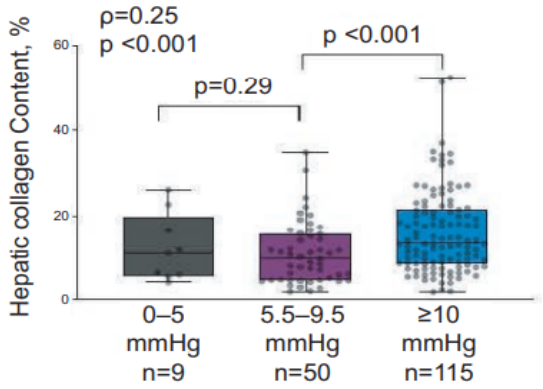
Proportion of patients in the placebo and CILO + FIR arms of the ATLAS study with a reduction in fibrosis as assessed by the DELTA Liver Fibrosis score and according to the CP using the NASH CRN classification

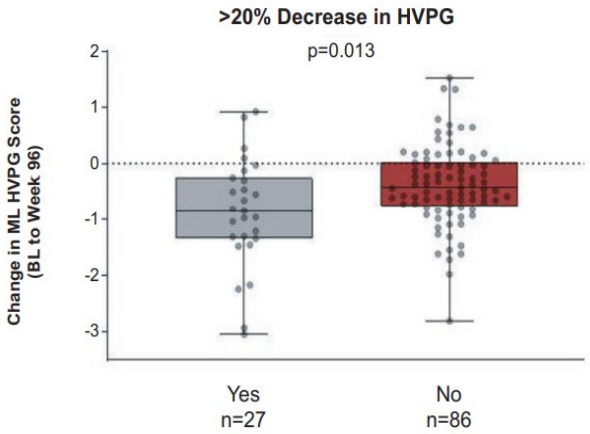


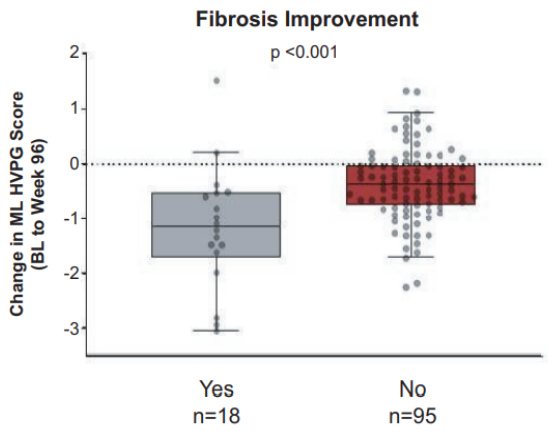
Taylor-Weiner et al. Hepatology 2021;74:133-147.

Machine Learning Evaluation of Liver Histology Predicts Clinically Significant Portal Hypertension in NASH Cirrhosis

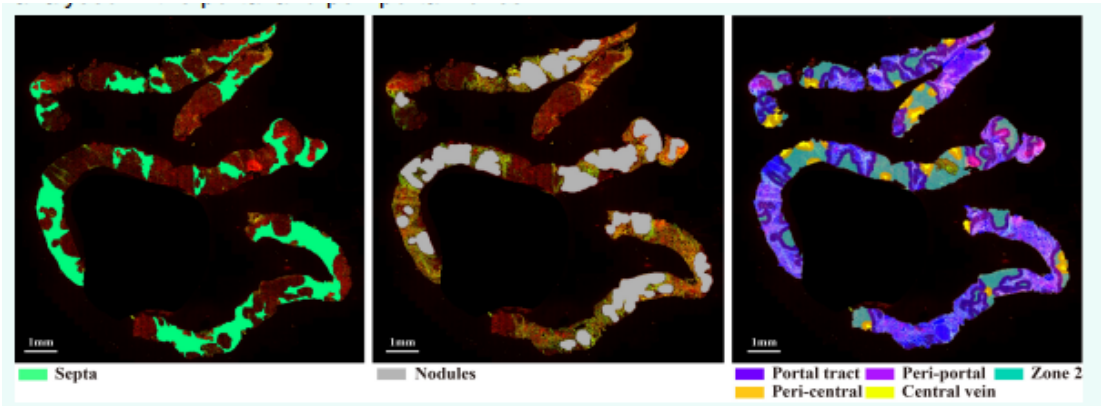




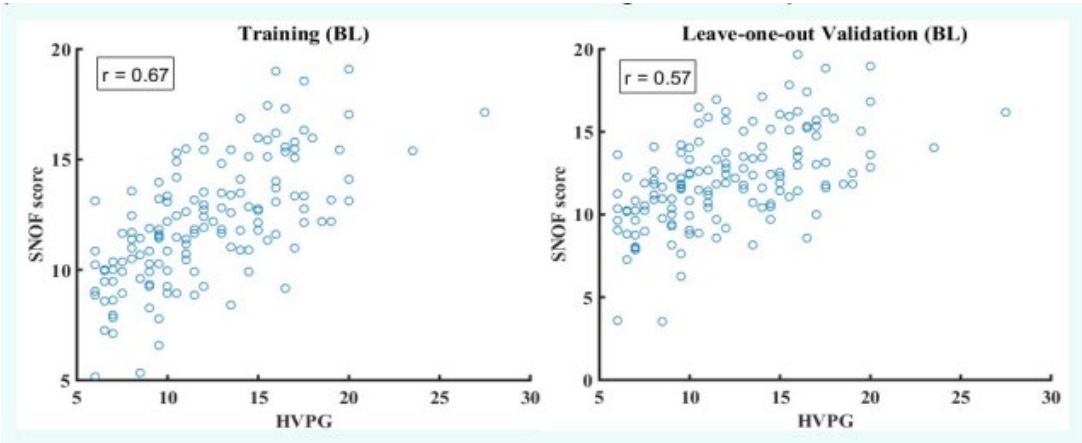




J. Bosch et al. Hepatology 2021;74:3146-3160



(SHG/TPE) imaging-based model provides quantitative assessment of septa, nodules, and fibrosis. (SNOF)



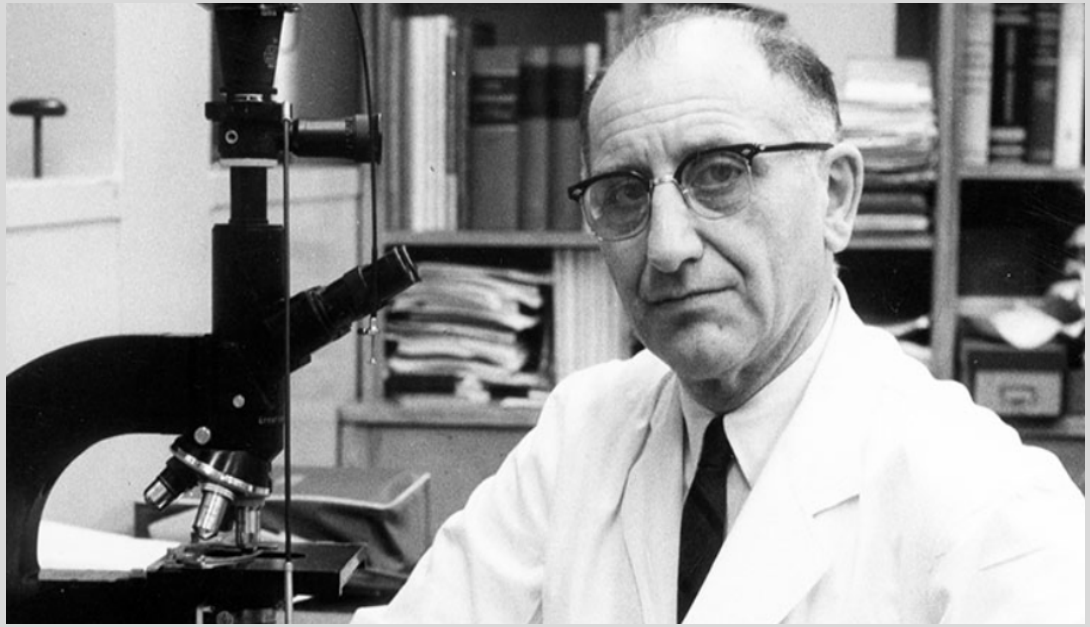
Training and validation plots of SNOF score vs HVPG at baseline (BL)

M. Noureddin et al. AASLD 2021; Abstract No 1591

Digital Pathology with AI analyses of tissue slides is already used in clinical practice and trials: recent examples

➢ **Prostate Cancer diagnosis**	- Paige Prostate AI software - Paige (FDA approved) - Galen Prostate AI software – Ibex (approved in EU)
➢ **Breast Cancer diagnosis**	- Galen Breast Solution AI model – Ibex (approved in EU)
➢ **Detection of Breast Cancer metastases in lymph nodes**	- Paige Breast Lymph Node, AI digital tool *Paige - Press Release 2022*
➢ **PD-L1 tumour expression**	- Digital quantification with AI tool, BMS/PathAI, *AACR June 22-24 2020, Poster 2017*
➢ **Cardiac Allograft rejection**	- Diagnosis and grading in of endomyocardial biopsies *J Lipkova, T Chen et al. Nature Medicine 2022:28;575–582*

Digital Pathology – Future Outlook



"The greatest research tool in hepatology is a microscope connected to a brain"
Hans Popper

Courtesy of Prof. John Vierling

Future Outlook: The greatest research tool in hepatology is a microscope, enhanced by AI-based digital pathology platforms, and connected to a brain.