Digital Pathology assistance tool in NASH clinical trials

HistoIndex's Approach

Liver Forum 15, Paris
6th September 2023

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Chief Scientific Officer

Disclosure

Dr. Dean Tai

I disclose the following financial relationship(s) with a commercial interest:

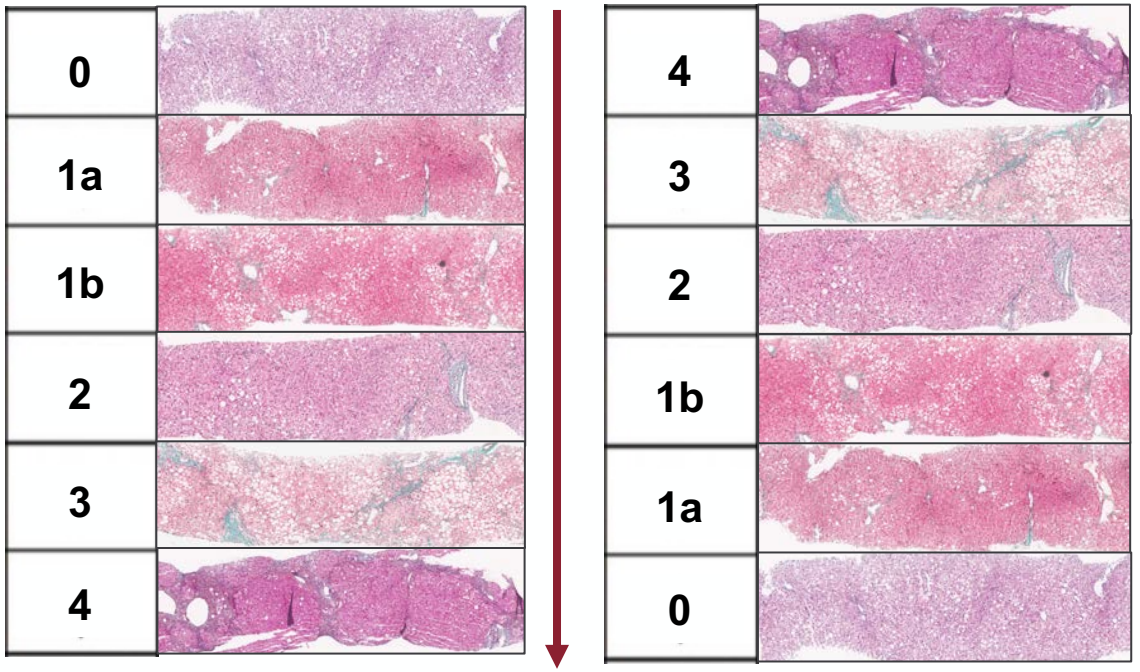
Employee and shareholder of HistoIndex

Agenda

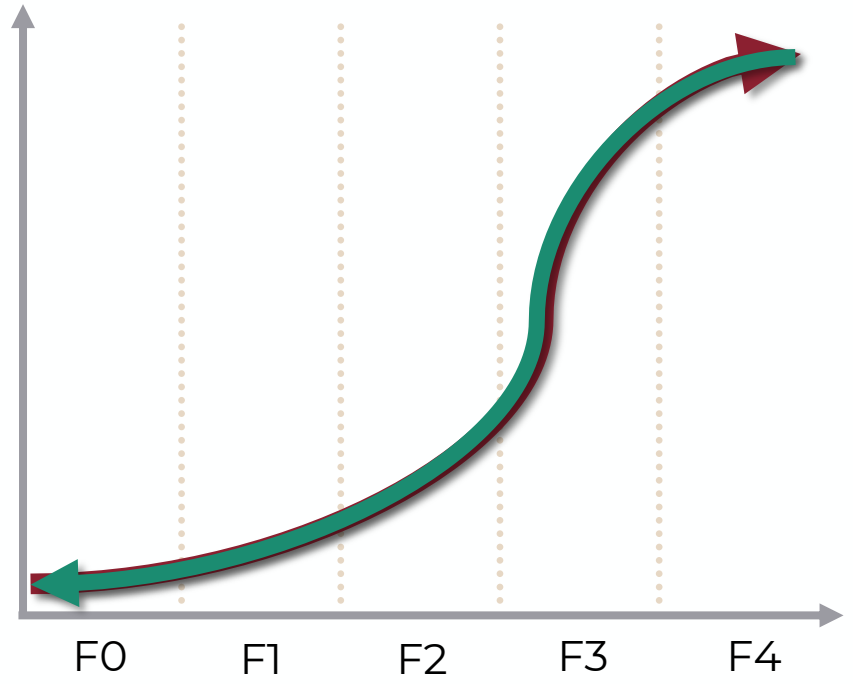
- **Common limitations in recent clinical trials :**
  - Inter/intra observer discrepancies
  - Ordinal staging system describes progression only, not designed to quantitate fibrosis regression
- **Digital pathology is increasingly used to provide:**
  - Fully quantitative assessment
  - Highly reproducible assessment
- **In addition, stain-free imaging technical reveals further insights to:**
  - Understanding mechanism of action specific histology changes
  - Better design clinical trials
  - Post-approval patient management strategies

Fibrosis regression follows the same stages of progression in reverse

NASH CRN



- Fibrosis staging system: Assumes a one-directional track with progression, with regression to follow the opposite route
- Quantification and a high kappa are key for the evaluation of fibrosis changes in a single track



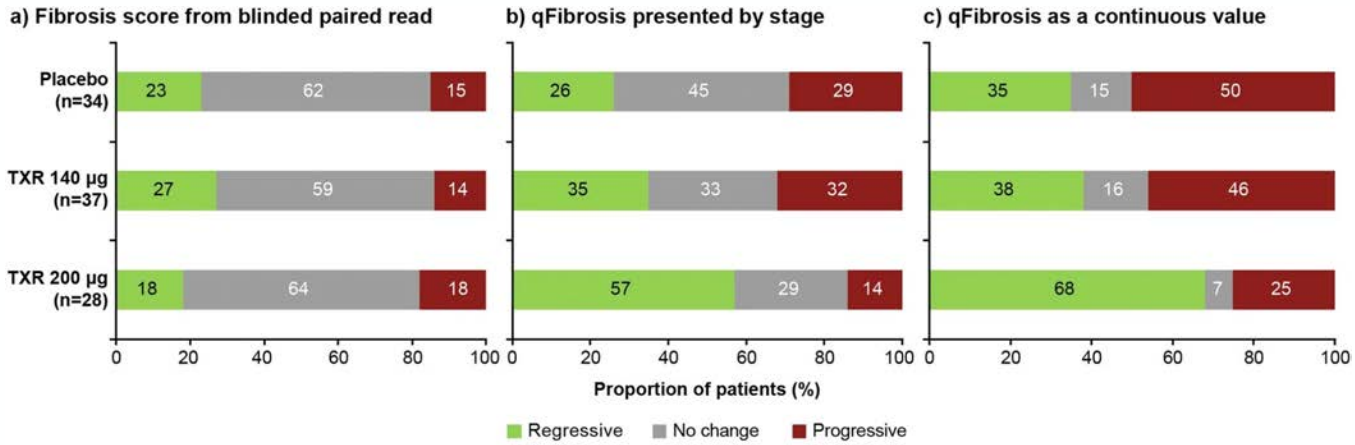
Advantage of a fully quantitative assessment

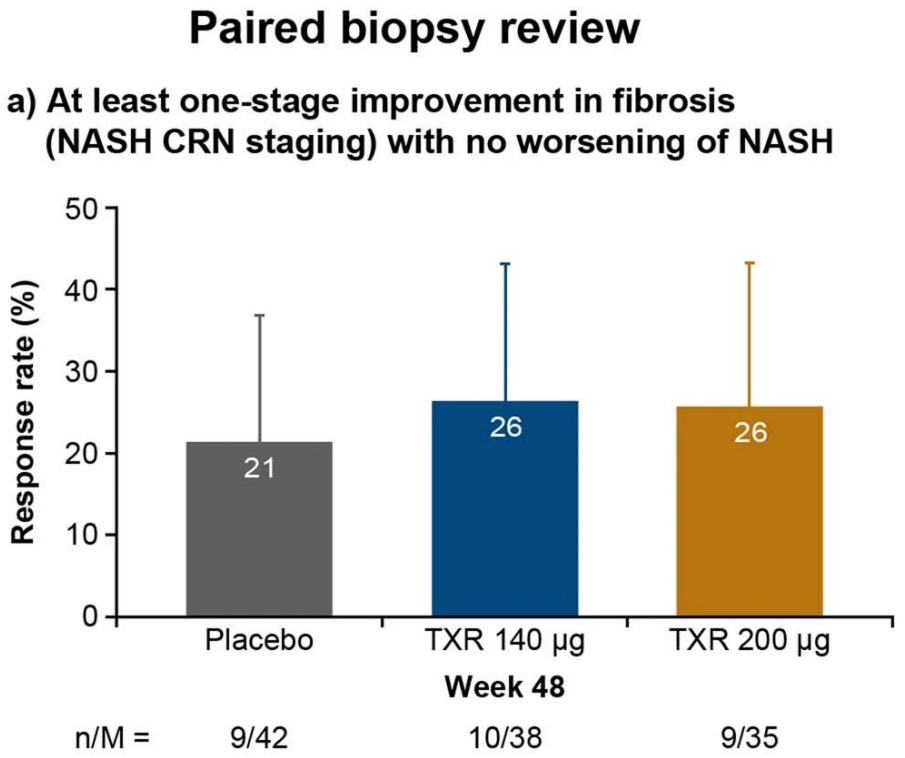
nature medicine

Article

https://doi.org/10.1038/s41591-022-02200-8

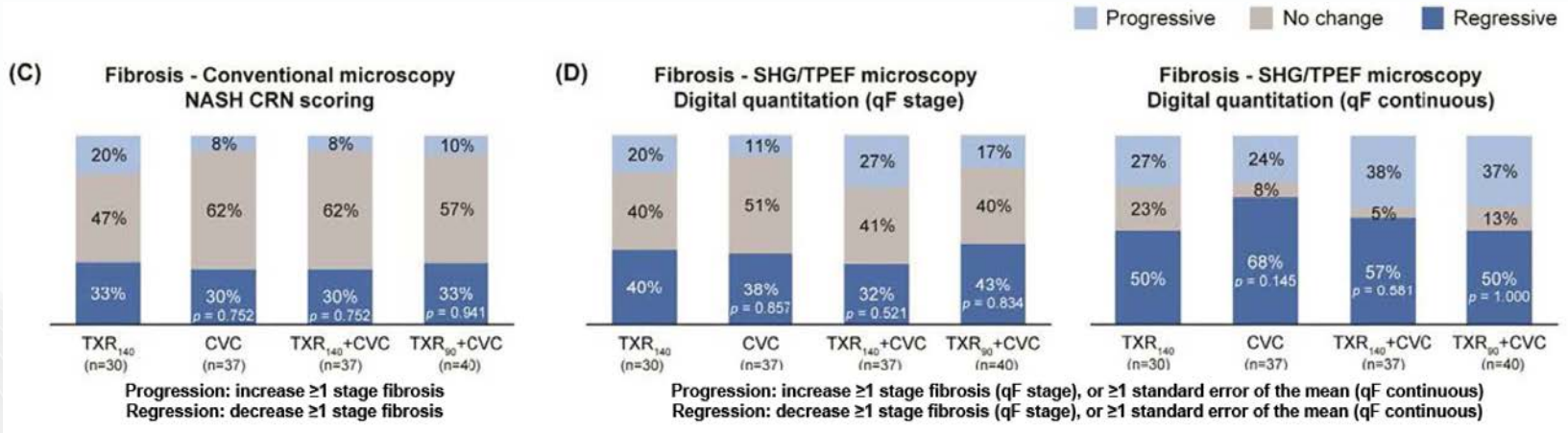
Tropifexor for nonalcoholic steatohepatitis: an adaptive, randomized, placebo-controlled phase 2a/b trial





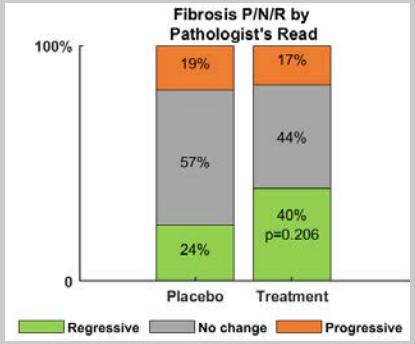
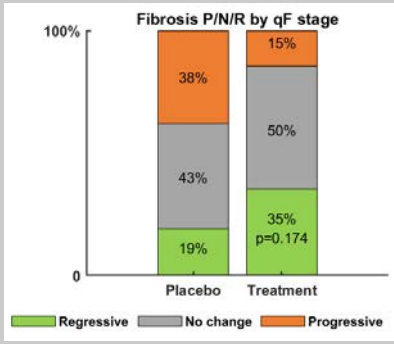
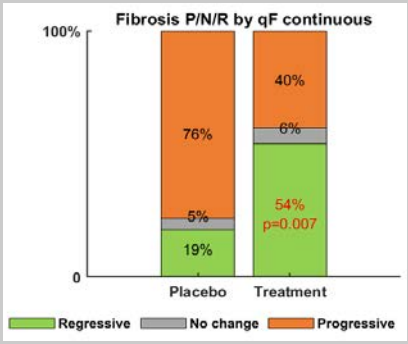
Sanyal et. al. Nature Medicine 2023

Results from TANDEM trial: Combination of tropifexor and cenicriviroc in pre-cirrhotic MASH



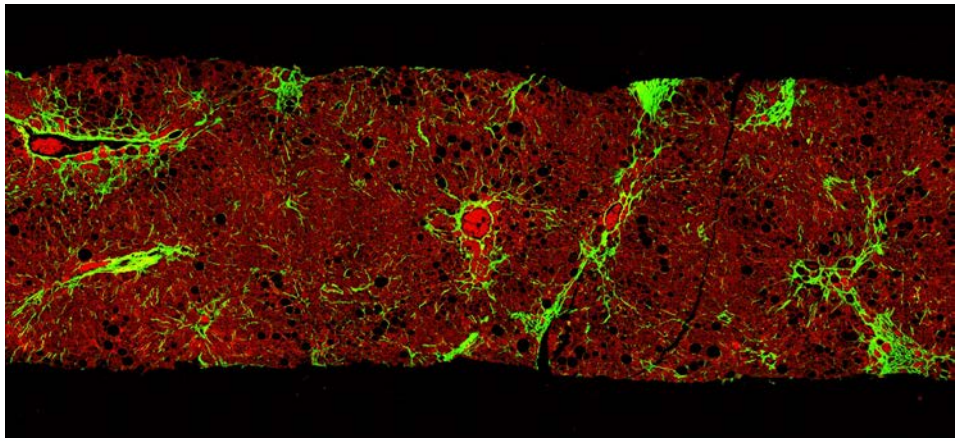
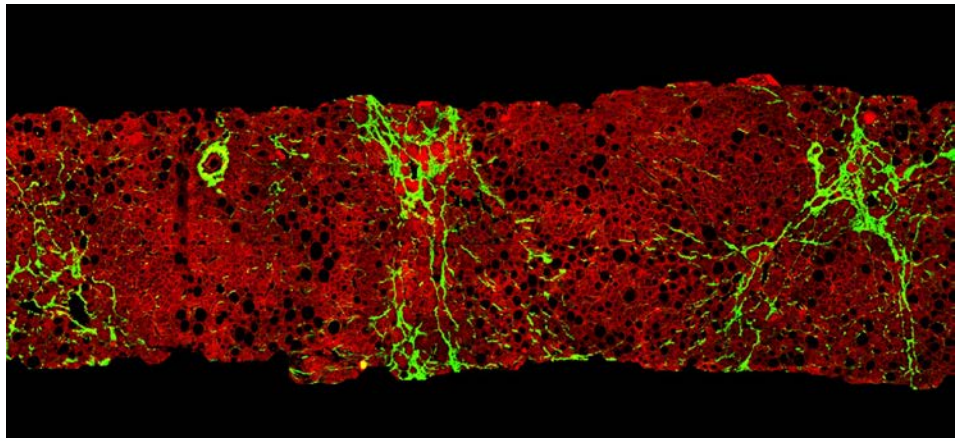
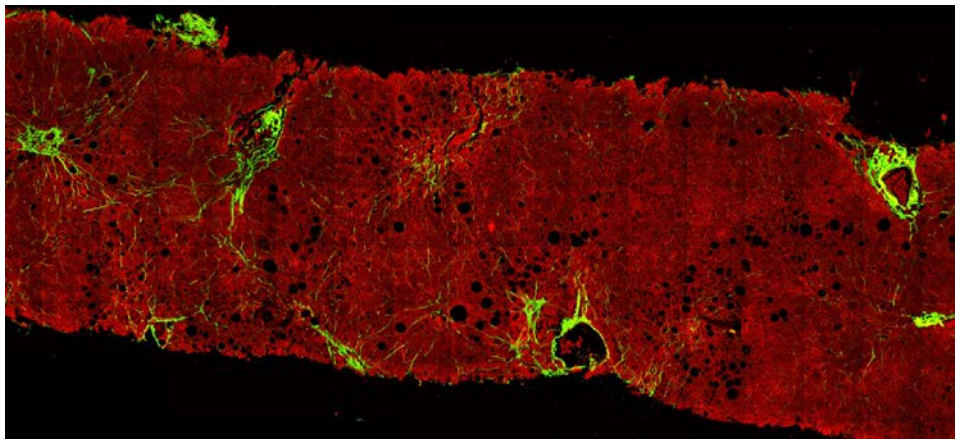
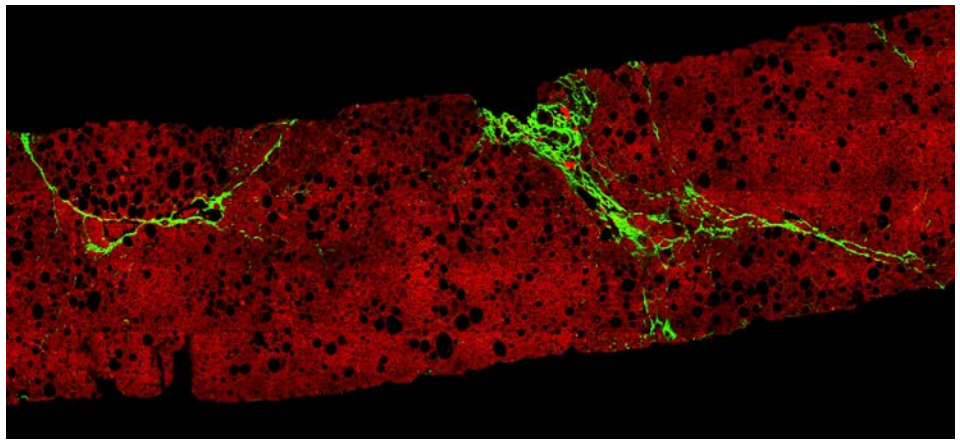
Anstee et. al. Hepatology 2023, in press

Results from COHORT 4 trial: Aldafermin in pre-cirrhotic MASH

	Pathologist's Read	*qF/qS Stage/Grade*	*qF/qS continuous value*
Fibrosis			

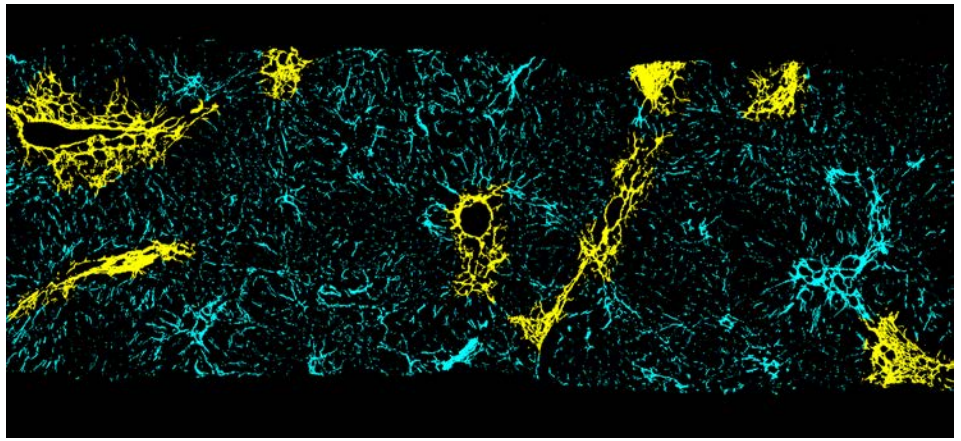
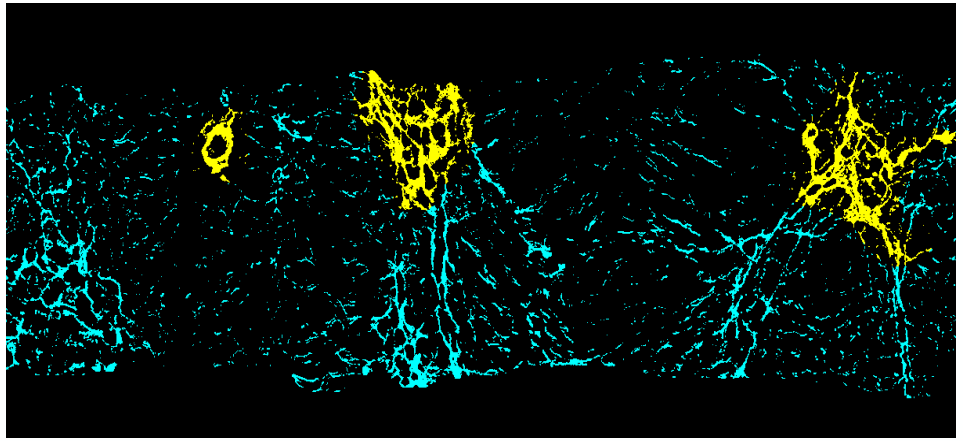
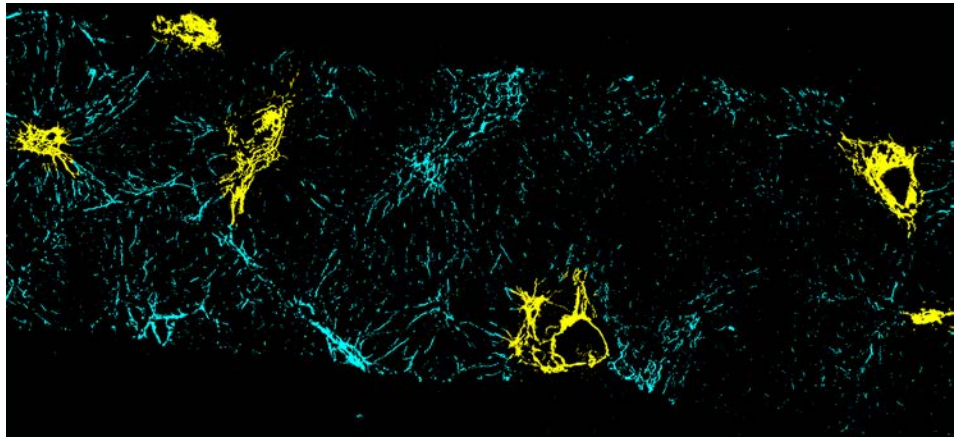
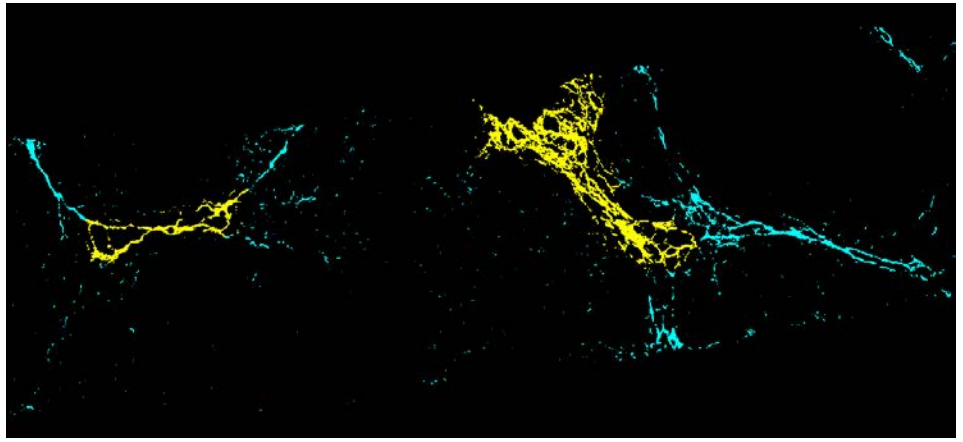
Manuscript in preparation

More fibrosis in F2 than F3 ???

	Baseline F2 → End of Treatment F2	Baseline F3 → End of Treatment F3
Before Treatment		
End of Treatment		

Ratziu et. al. Journal of Hepatology 2022

Fibrosis stage ⇩ does not always mean Collagen Proportionate area ⇩

	Baseline F2 → End of Treatment F2	Baseline F3 → End of Treatment F3
Before Treatment		
End of Treatment		

PT/CV collagen

PS collagen

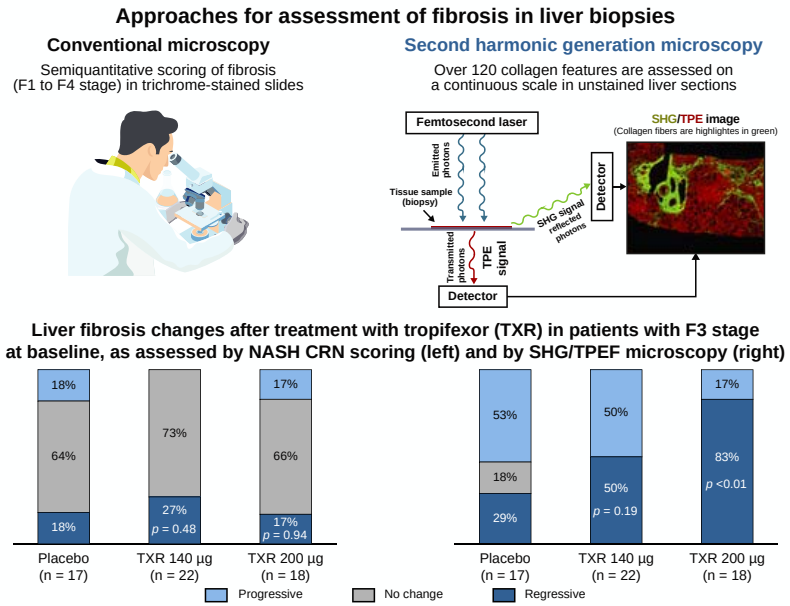
Ratziu et. al. Journal of Hepatology 2022

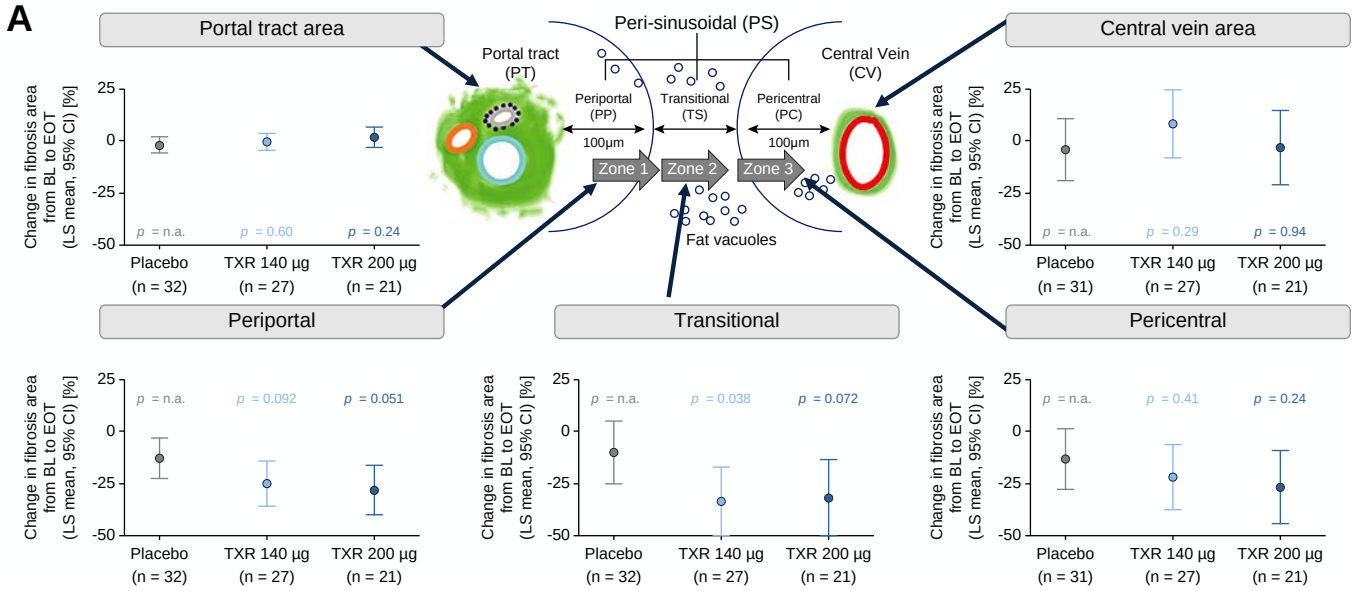
AI reveals where the activities are taking place

Research Article
Innovative Diagnostics, Modelling and Digital Hepatology

JOURNAL OF HEPATOLOGY

Digital pathology with artificial intelligence analyses provides greater insights into treatment-induced fibrosis regression in NASH





Naoumov et. al. Journal of Hepatology 2022

Beyond standard zonal analysis: septa parameters

Comparison of regressive septa versus progressive septa

A) Septa Parameters and Comparison between Regressive septa and Progressive septa from F3 biopsies in FLIGHT_FXR study

No.	Septa parameters	Progressive septa N = 43, mean	Regressive septa N=50, mean	p value
1	Septa Area	234638.21	27002.33	<0.001
2	Cellular/acellular	0.75	0.56	0.082
3	Cellular/Collagen	1.27	0.93	0.169
4	Septa length	947.27	543.95	<0.001
5	Septa width	167.45	40.88	<0.001
6	Intersection Septa	2475.00	262.00	<0.001
7	Number of Thick Fiber Septa	64.00	5.00	<0.001
8	Number of Thin Fiber Septa	3016.00	344.50	<0.001
9	Thick/Thin Septa ratio	0.02	0.02	0.420
10	Aggregated Septa	80490.42	8730.77	<0.001
11	Distributed collagen within septa	2218.02	407.71	<0.001
12	Aggregated/Distributed collagen within septa	36.09	26.11	0.228

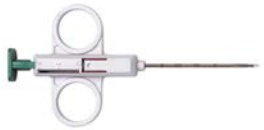
Naoumov et al. EASL 2023, Vienna, Poster No 1504

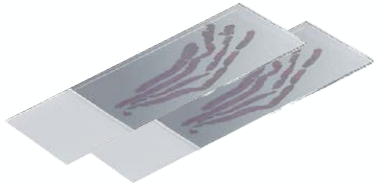
Stain-free imaging approach

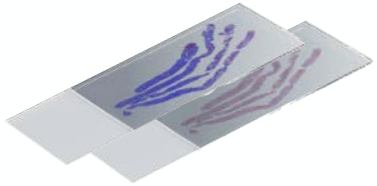
- **Stain-free imaging: second harmonic generation microscopy**
  - Rationale and advantages
- **Performance data**
  - Improvement on inter/intra observer discrepancies
  - Differences between glass vs digital slides
- **Visualization for pathologists**
  - Easier to see
  - AI annotation

Conventional staining vs unstained qFibrosis approach

Staining





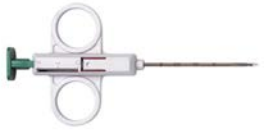


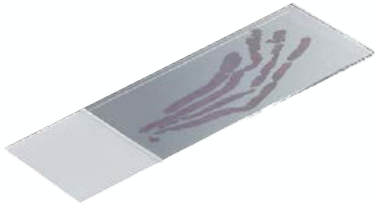
Liver biopsy → **2 consecutive unstained cuts** → **H&E and MT staining** → **Microscopy**

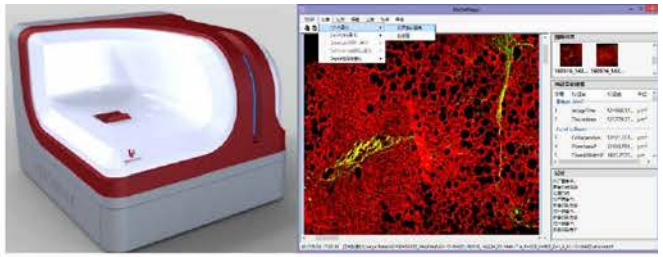
Pathological assessment using microscopy

- Fibrosis stage
- Steatosis grade
- Hepatocyte ballooning
- Inflammation

Unstained





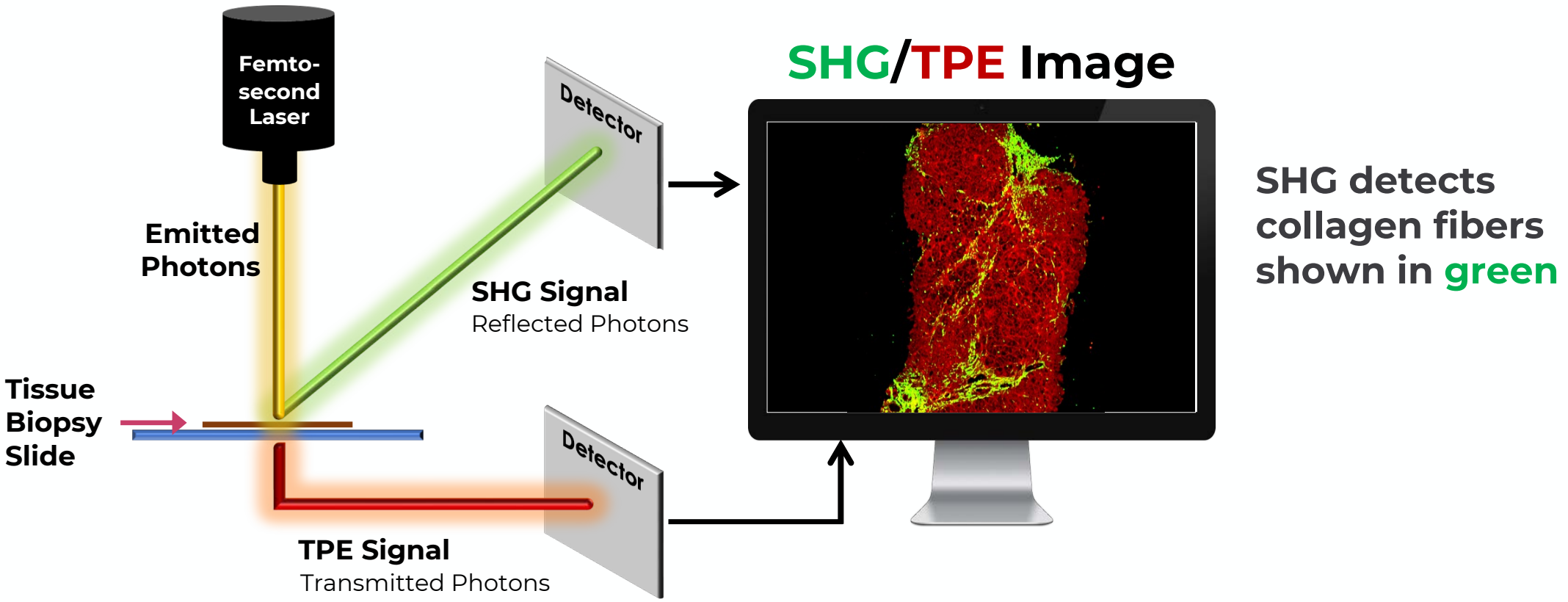


Liver biopsy → **1 unstained cut** → **SHG/TPE imaging + AI Analysis**

Quantitative assessment using SHG/TPE images and AI

- qFibrosis
- qSteatosis
- qBallooning
- qInflammation

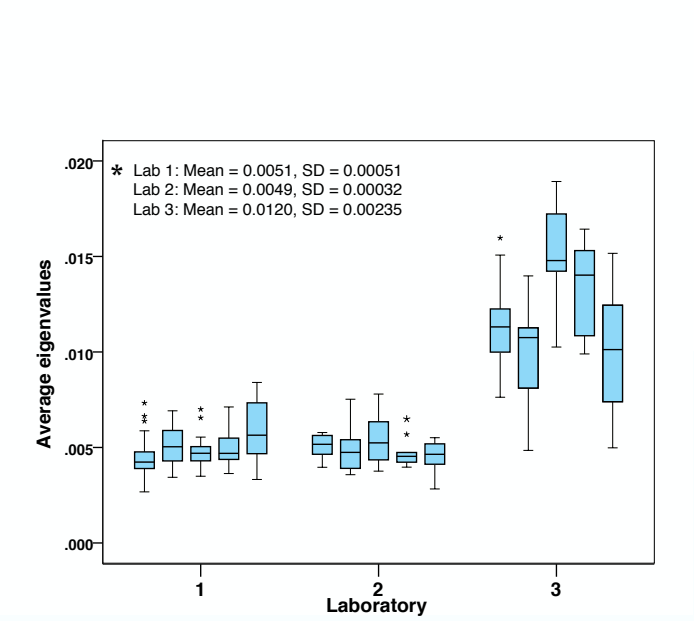
Genesis®200 unstained imaging system

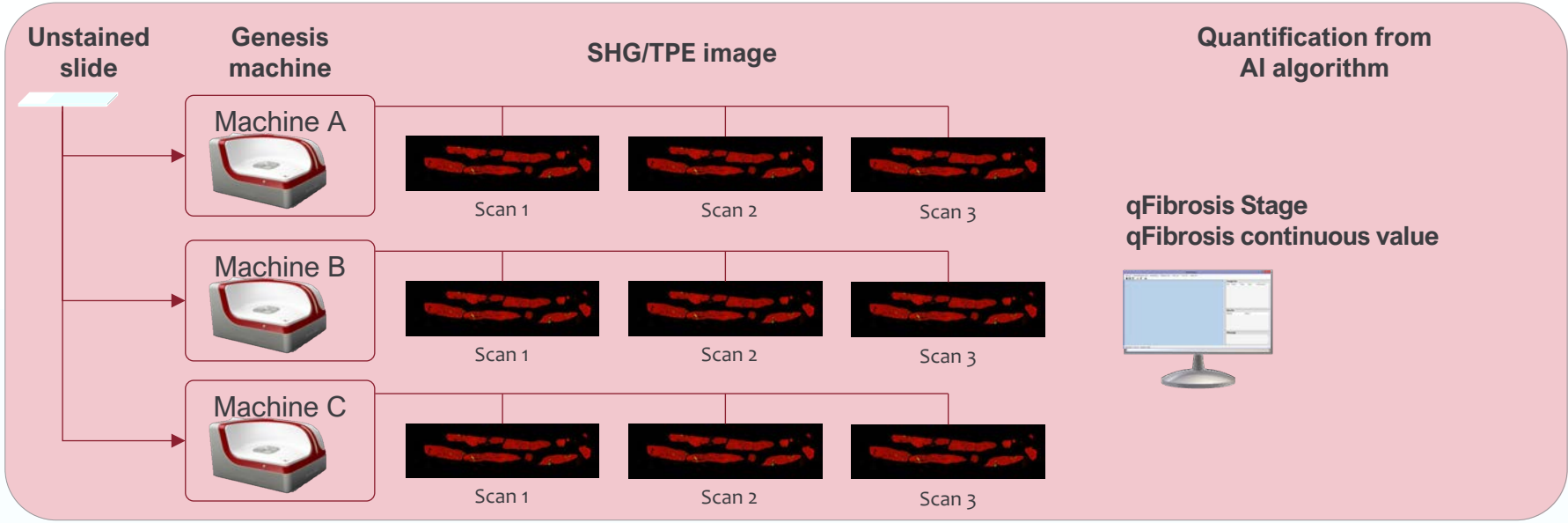


SHG detects collagen fibers shown in green

Xu S, et al. J Hepatol. 2014;61(2):260-269

Non-stain approach improves quantification consistency from 66% → 93%





Chang et al. EASL 2023, Vienna, Poster No 1947

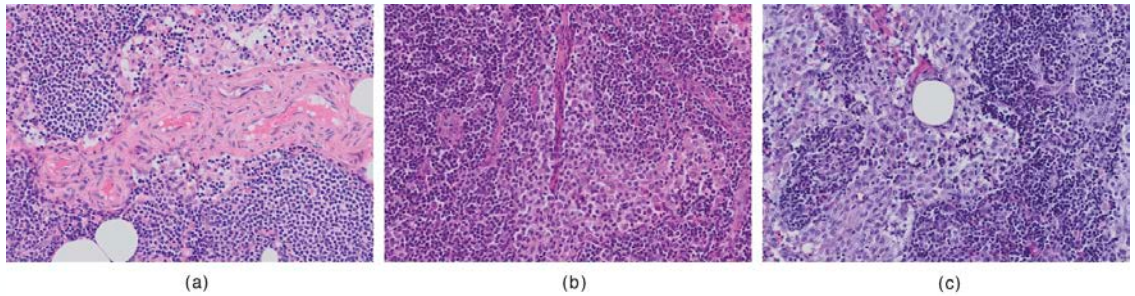


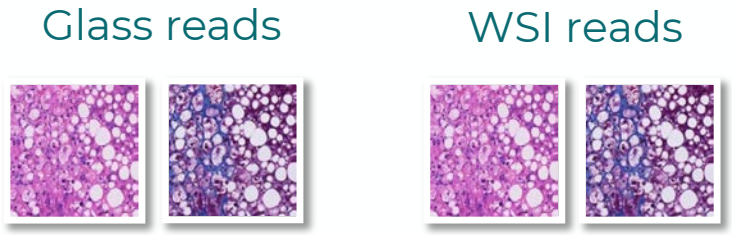
Figure 2: Sample images from the three laboratories. (a), (b), and (c) are three sample ROI images stained by laboratory 1, 2 and 3 respectively.

Bejnordi et al, SPIE 2014

- Quantification results consistency with staining ~ 66%
- Quantification results consistency with no staining ~ 93%

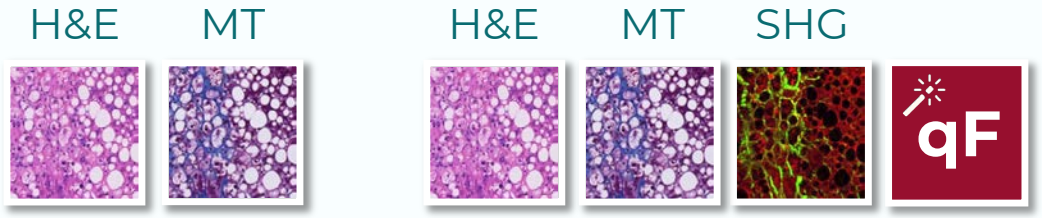
Stepwise approach: To investigate utility of AI components

1. Pooled data from AI DP companies



2. **HistoIndex approach using WSI and SHG-based digital reads:**

   Value of SHG + Quantitative Assessment

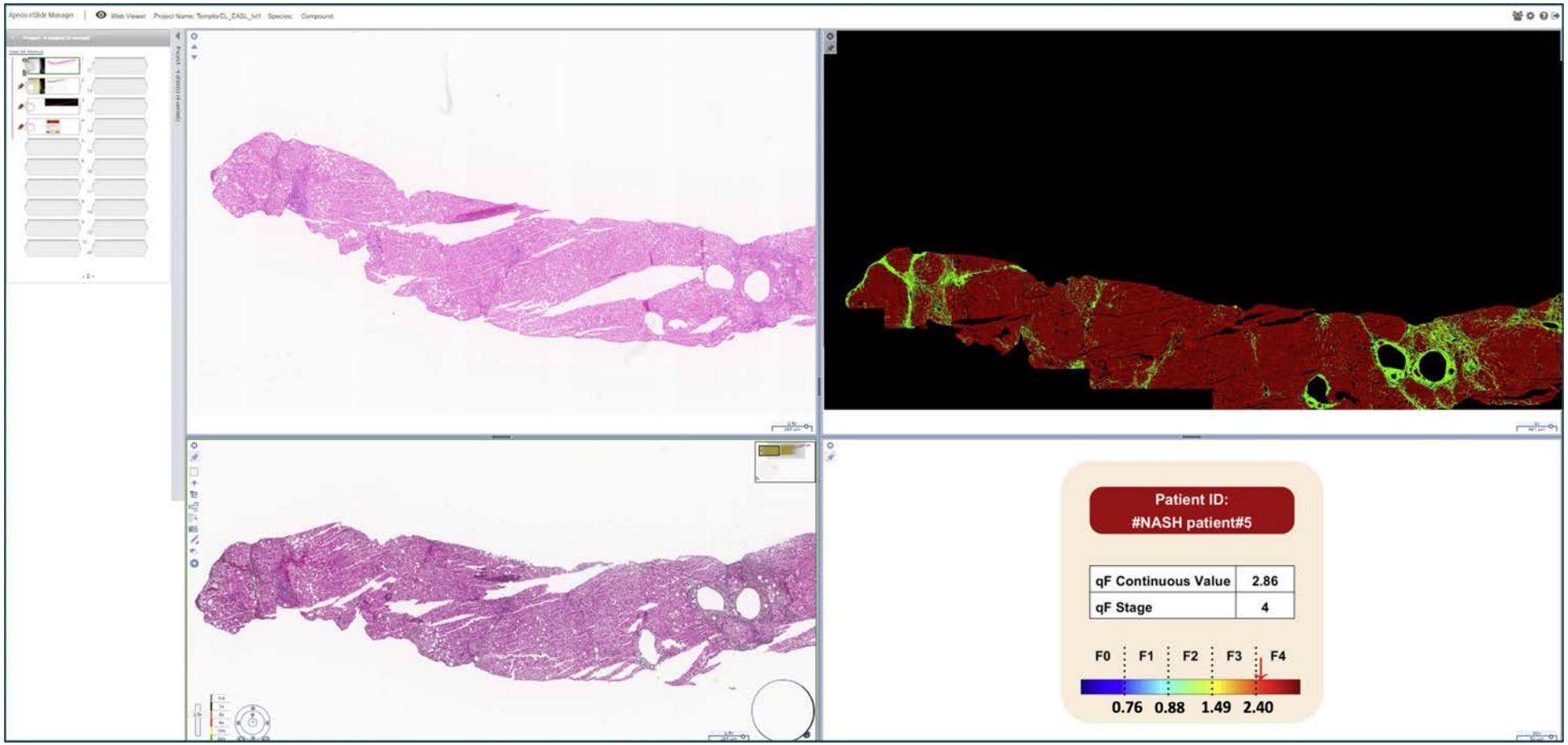


AI-aiding tools as primary end points

Pilot Study Details	
Profile of pathologists	• 3 pathologists with 40, 10 and 5 years of experience
Modalities	• Un-aided read: H&E, MT images • Aided read: H&E, MT and SHG images, qFibrosis readout
Sample set	• 40 adult NASH, non-treated liver biopsies • 10 **F0**, 10 **F1**, 10 **F2**, 5 **F3**, 5 **F4**
Data collected	• Fibrosis assessment for un-aided and aided reads

Soon, et. al., Clin. Gastroenterol. Hepatol., 2022

AI-aiding tools: SHG image and qFibrosis readout

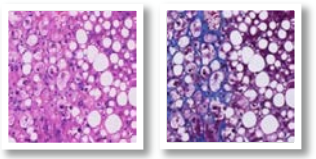


Soon, et. al., Clin. Gastroenterol. Hepatol., 2022

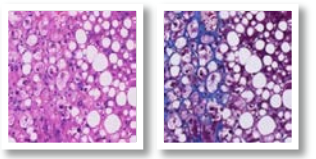
1. Glass vs WSI reads

1. Pooled data from AI DP companies

Glass reads



WSI reads



	Glass vs WSI	Glass vs WSI + SHG, qF
Mean Intra-observer variability	91.8%	92.5%

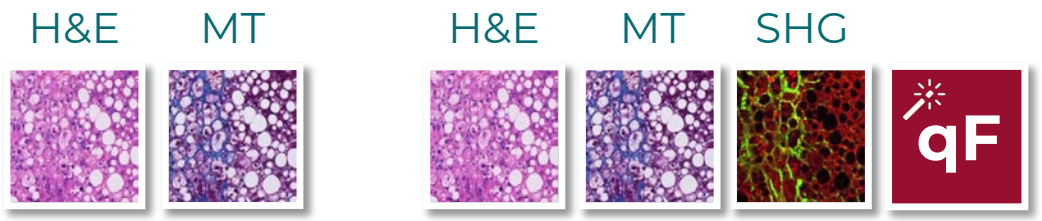
- 240 reads for each set across 3 pathologists with varying years of experience

Soon, et. al., Clin. Gastroenterol. Hepatol., 2022

2. Value of SHG and Quantitative assessment

HistoIndex approach using WSI and SHG-based digital reads:

Value of SHG + Quantitative Assessment



Fibrosis assessment		Un-aided	Aided
Inter-observer	Mean Percentage Agreement	89.4%	92.9%
	Mean Weighted Kappa (Linear)	0.72	0.82
Intra-observer	Mean Percentage Agreement	92.1%	96.5%
	Mean Weighted Kappa (Linear)	0.79	0.91

AI-aiding improved mean linearly weighted kappa for fibrosis assessment

Soon, et. al., Clin. Gastroenterol. Hepatol., 2022

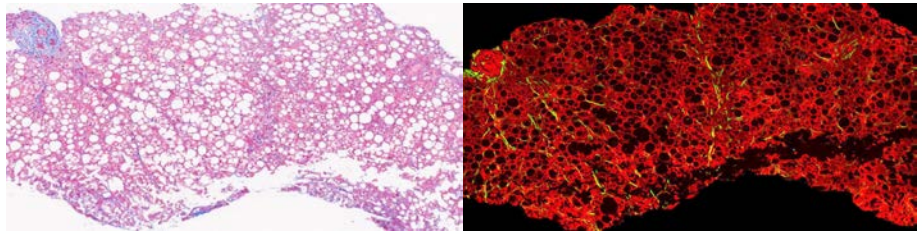
qFibrosis: high level of repeatability and reproducibility that matches (or even exceeds) repeated reads by multiple pathologists

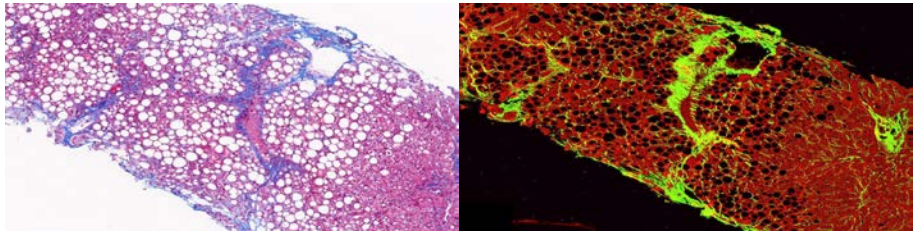
	qFibrosis*	Kleiner *et al* 2005	Kleiner *et al* 2019	Davison *et al* 2020
Inter-rater Kappa	0.85	0.84	0.75	0.484
Intra-rater Kappa	0.88	0.85	NA	0.854

Soon, et. al., Clin. Gastroenterol. Hepatol., 2022
Kleiner et al. Hepatology. 2005; 41(6):1313-2;
Kleiner et al. JAMA Netw Open 2019;2(10):e1912565;
Davison et al. J Hepatol. 2020; 73(6):1322-32;

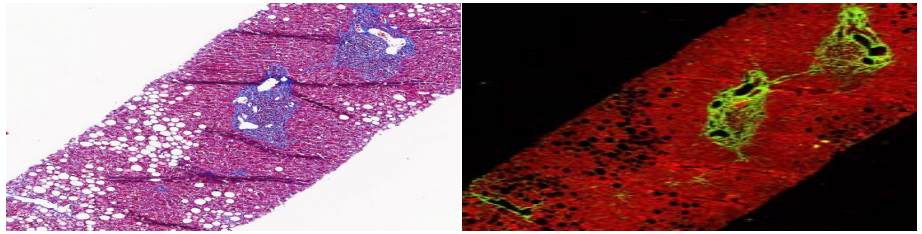
Stained vs Unstained (easier to see)

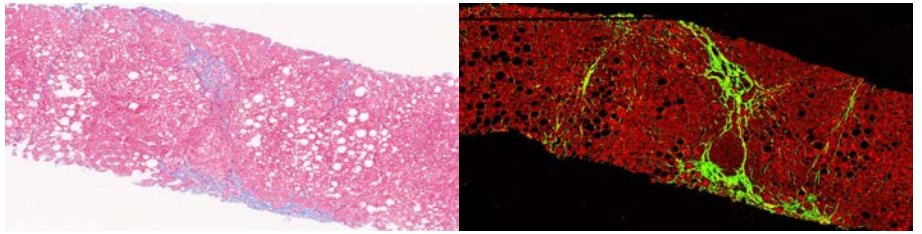
NASH CRN F1



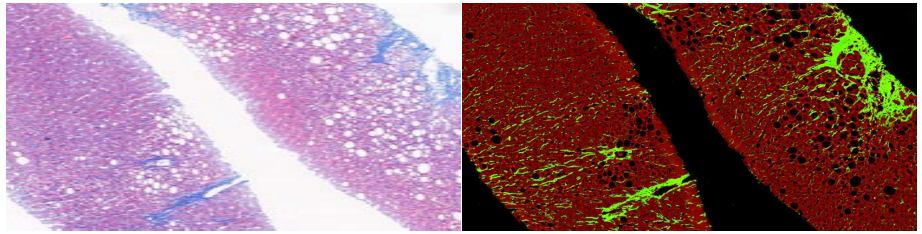


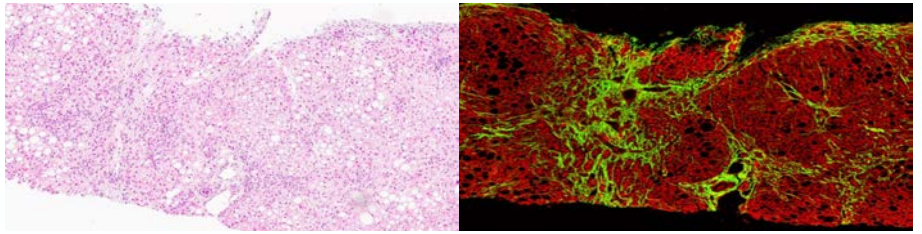
NASH CRN F2



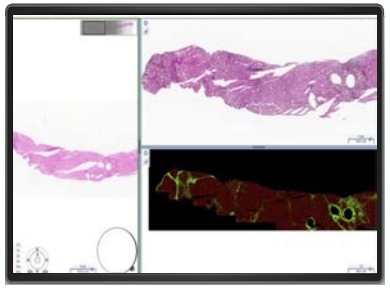


NASH CRN F3

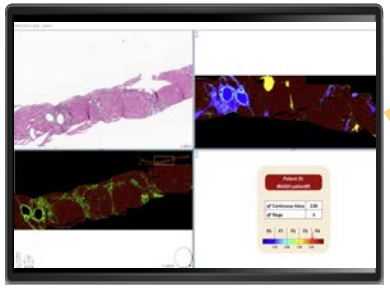




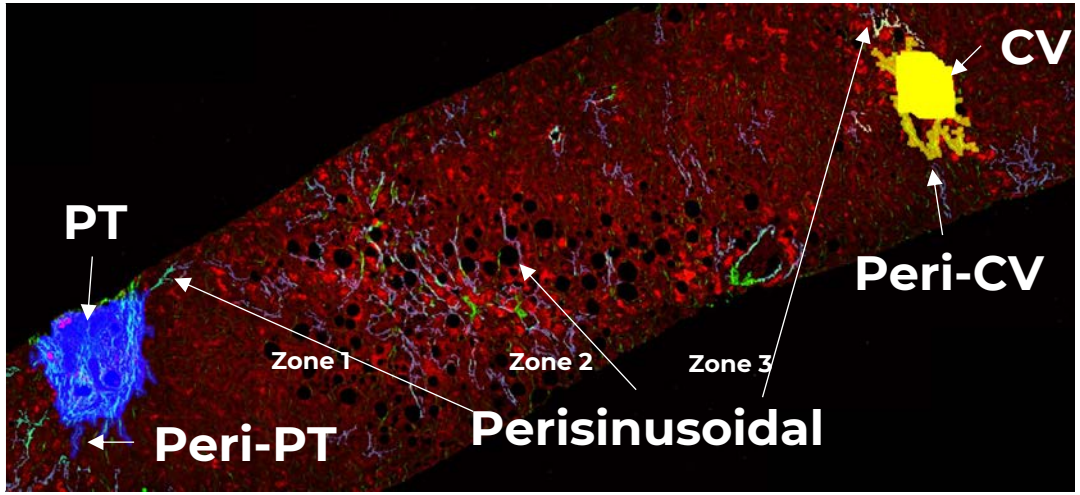
Further improvement in inter-observer agreement for fibrosis assessment



AI-aided Level I Tools



AI-aided Level II Tools



		Un-aided	Aided With Level I tools	Aided With Level II tools
Inter-observer	Mean Percentage Agreement	89.4%	92.9%	93.8%
	Mean Weighted Kappa (Linear)	0.72	0.82	0.84
Intra-observer	Mean Percentage Agreement	92.1%	96.5%	95.63%
	Mean Weighted Kappa (Linear)	0.79	0.91	0.88

Manuscript in preparation

Additional insights provided by SHG-based AI

In Resmetirom

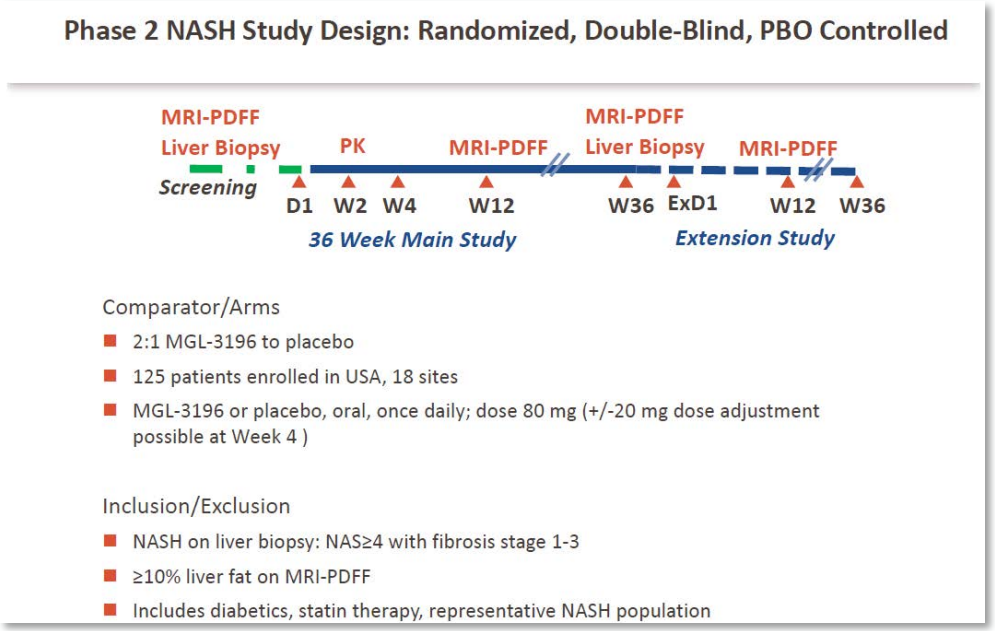
- Phase 3 patient stratification based on phase 2 data
- Results from phase 3 study, with qFibrosis assessment

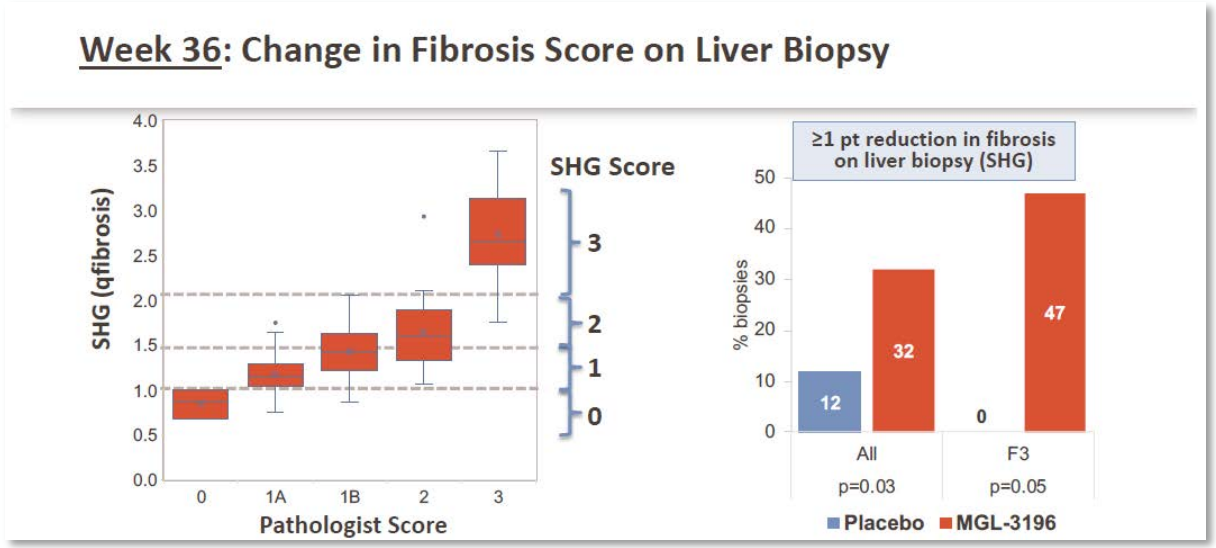
Mechanism of action (MOA) specific fibrosis changes

- Better visualization of multi-dimensional data
- Results from different MOA drugs

With Madrigal's Resmetirom phase 2 trials

- There is no statistical significance using conventional pathologists' assessment
- Using HistoIndex's qFibrosis, there is now clear statistical significant positive results
- Futuremore, it identified patients with F3 fibrosis is the best responders
- **Change of primary end point for phase 3 studies to include >51% F3 patients**





Madrigal Pharmaceuticals, AASLD 2018

Change of clinical trial protocol to include more F3 patients

In previous phase 3 NASH clinical trials:

Current Primary Outcome Measures ICMJE **(submitted: January 29, 2019)**	• To evaluate the effect of Obeticholic Acid compared to placebo on liver histology in non-cirrhotic nonalcoholic steatohepatitis (NASH) subjects with stage 2 or 3 fibrosis by assessing the following primary endpoints [Time Frame: Measurements at Baseline and 18 months] Primary endpoints include: • The proportion of Obeticholic Acid treated patients relative to placebo achieving at least one stage of liver fibrosis improvement with no worsening of NASH, or • The proportion of Obeticholic Acid treated patients relative to placebo achieving NASH resolution with no worsening of liver fibrosis.

https://clinicaltrials.gov/ct2/show/record/NCT02548351?term=Intercept&cond=NASH&draw=2&rank=1

But in Madrigal's phase 3 NASH trials:

Original Primary Outcome Measures ICMJE **(submitted: March 30, 2019)**	• To evaluate the effect of MGL-3196 80 mg or 100 mg compared to placebo to achieve NASH resolution on liver histology in non-cirrhotic NASH patients with stage 2 or 3 fibrosis [Time Frame: Measurements at Baseline and 52 weeks] Primary endpoint: • Assessment will be in the first 900 patients, **at least 450 F3**, and be based on the proportion of MGL-3196 80 mg or 100 mg treated patients relative to placebo achieving NASH resolution (NASH Activity Score (NAS), ballooning =0; lobular inflammation =0,1) with at least a 2-point reduction in NAS and no worsening of fibrosis.

What drove the success of Resmetirom?

Implications for Successful Phase 3 Program

Data from Phase 2

- Drug exposure levels and histopathologic response evaluated in phase 2 helped understand the likelihood of effect of 80mg and 100mg doses in phase 3
- Half of F3 patients on IP showed ≥ 1-point improvement in fibrosis, compared to no placebo F3 patients, using Second Harmonic Generation (SHG)
- 56% of patients who resolved NASH also resolved fibrosis, 61% of NASH resolvers achieved ≥ 1 point improvement in fibrosis
- Statistically significant reductions by resmetirom in multiple fibrosis biomarkers including PRO-C3, ELF, most pronounced in patients with advanced fibrosis at baseline (F2 / F3)

Screening Criteria

- 3 metabolic syndrome risk factors, prescreening criteria (AST and fibroscan cut-off value of 8.5kPa) and MRI-PDFF prior to biopsy

Histopathology Reading

- Core biopsy size of 16 gauge
- Same pathologists as phase 2
- NASH Resolution definition included having a 2-point improvement in NAS

Courtesy of Stephen Harrison and Madrigal Pharmaceuticals

qFibrosis: primary end-point in NASHGEN-2 trial

NIH U.S. National Library of Medicine

ClinicalTrials.gov

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A Study to Evaluate the Efficacy and Safety of ALN-HSD in Adult Participants With Non-alcoholic Steatohepatitis (NASH) With Fibrosis With Genetic Risk Factors (NASHGEN-2)

ClinicalTrials.gov Identifier: NCT05519475

Outcome Measures Go to

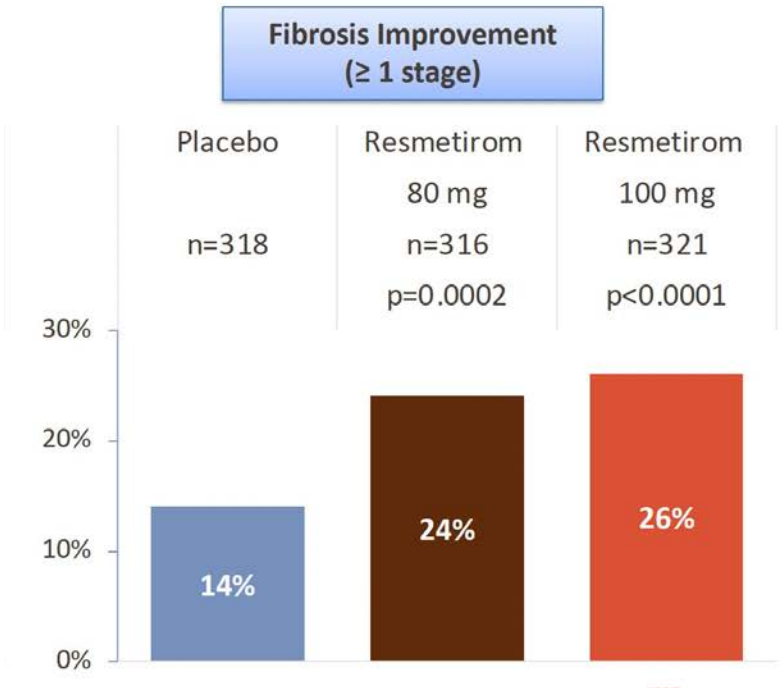
Primary Outcome Measures :

1. Change from baseline in qFibrosis [Time Frame: Baseline to week 52]

   Study 1 and Study 2

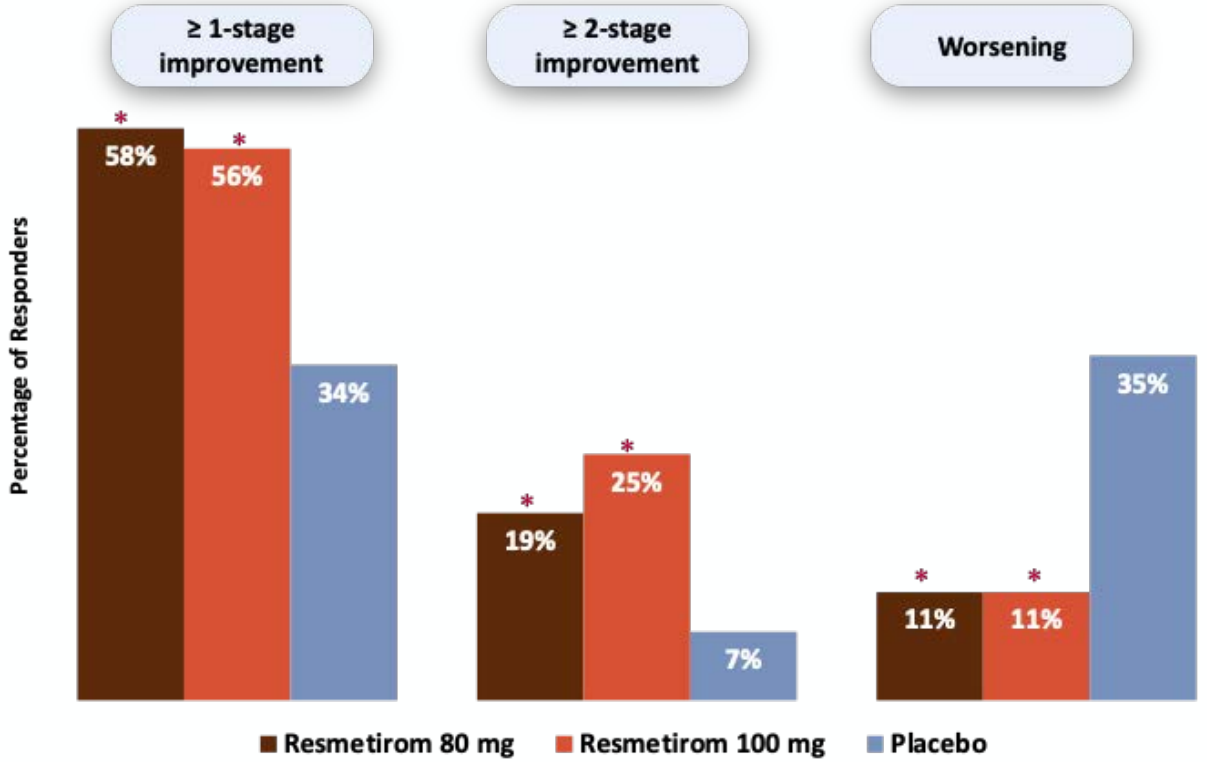
   Change in the continuous quantitative liver fibrosis (qFibrosis) score measured by second harmonic generation/two-photon excitation microscopy

NASH-CRN



Data Source: Madrigal Pharmaceuticals December 19, 2022

qFibrosis



* p-value <0.0001 (vs placebo)

Data Source: Madrigal Pharmaceuticals, EASL 2023 late-breaker, LBP-017

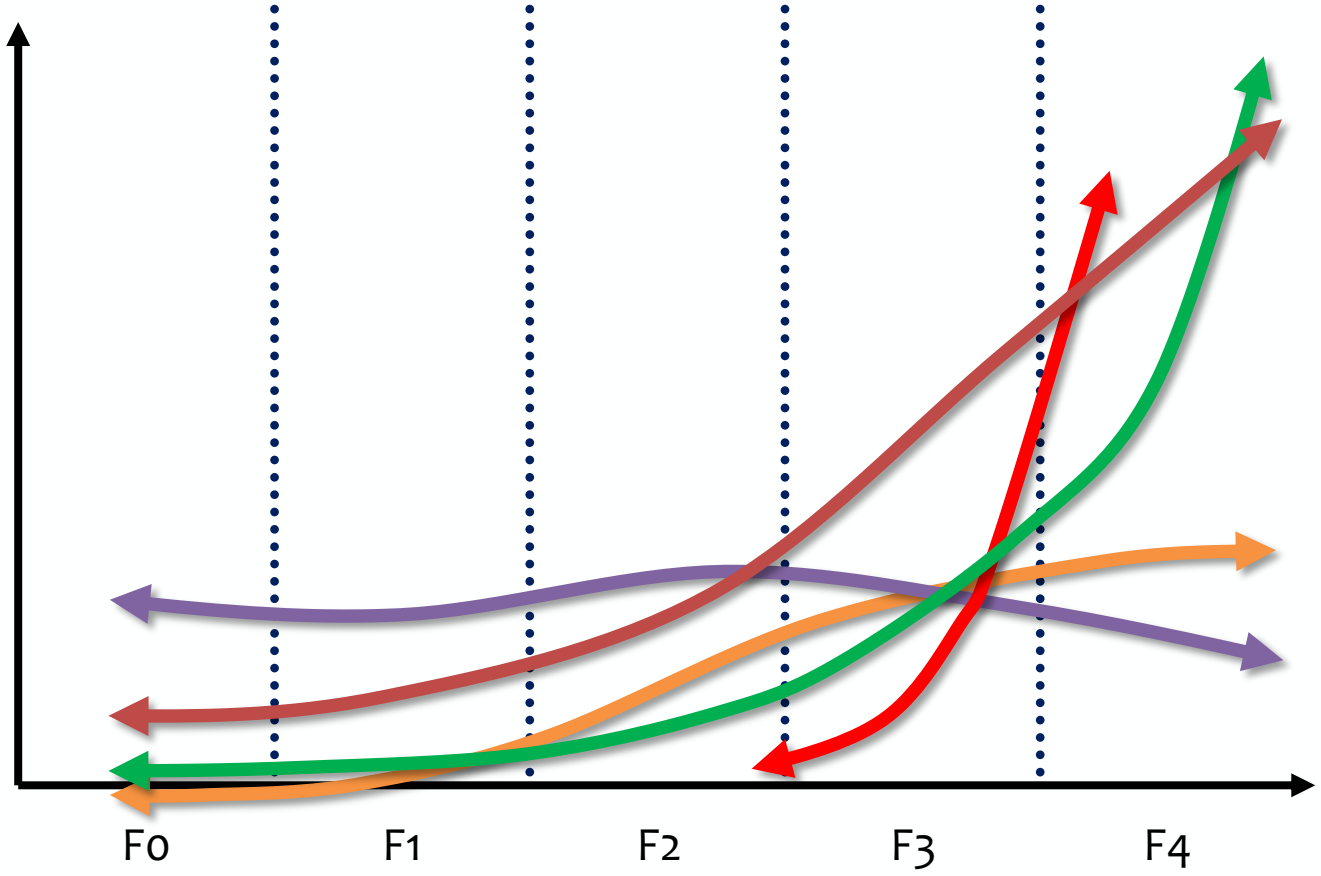
qFibrosis is a knowledge-based AI developed using NASH-CRN system

Stages	Description
0	None
1a	Mild (delicate) zone 3 **perisinusoidal** fibrosis
1b	Moderate (dense) zone 3 **perisinusoidal** fibrosis
1c*	Portal/periportal fibrosis only
2	Zone 3 **perisinusoidal** fibrosis with **portal/ periportal** fibrosis
3	**Bridging** fibrosis
4	**Cirrhosis**

F1c will not be covered within this presentation

Portal fibrosis, Peri-portal fibrosis, Perisinusoidal fibrosis, Bridging fibrosis, Cirrhosis (nodule)

Each of these parameters changes independently with different trends



Portal Fibrosis

Peri Portal Fibrosis

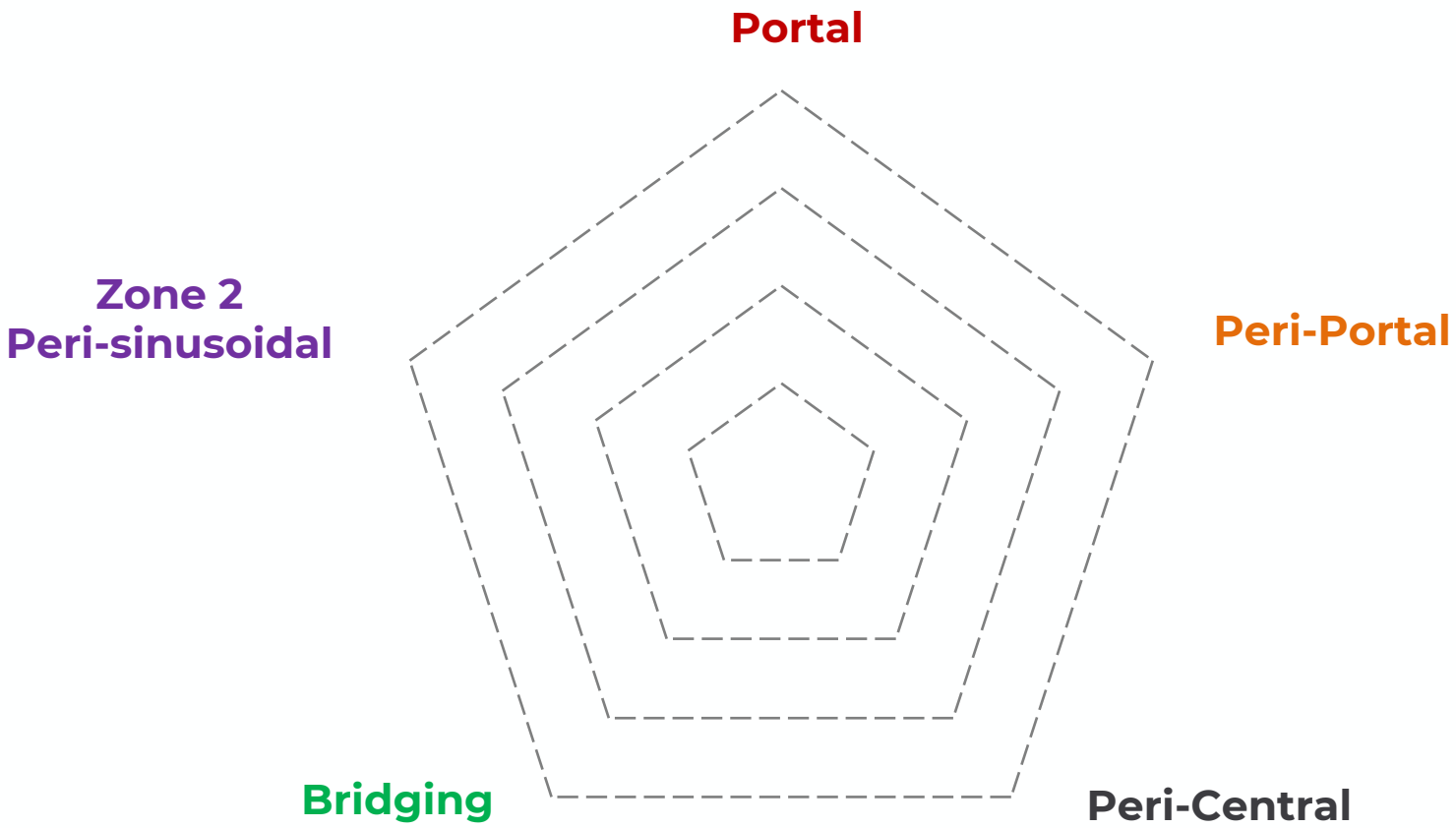
Bridging Fibrosis

Peri-sinusoidal Fibrosis

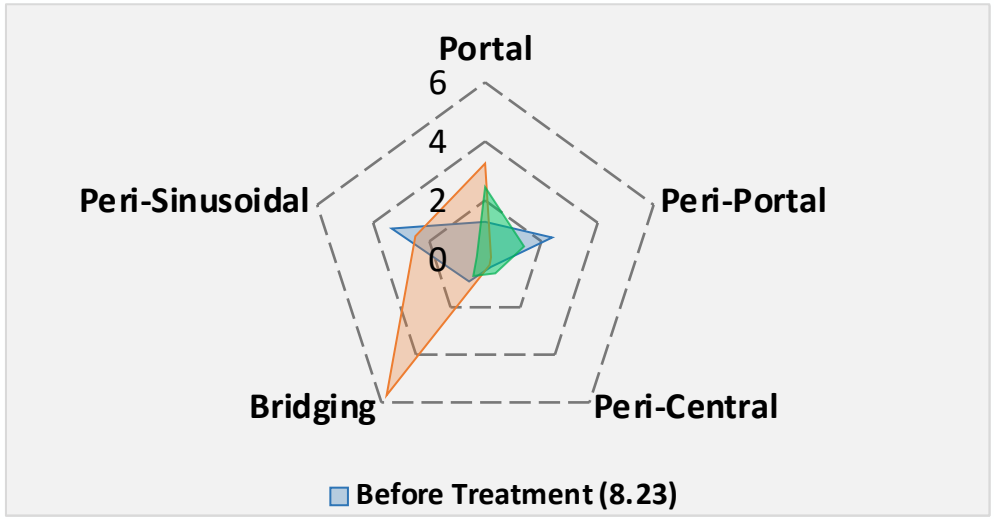
Nodule

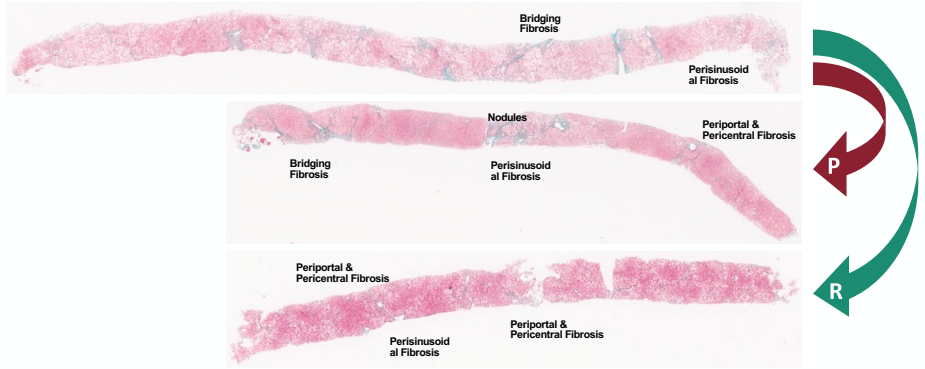
Graph is plotted based on data obtained from clinical projects

qFibrosis provides fully quantitative assessment to capture all changes



Review the liver biopsies using radar map to better record multi-dimensional nature of fibrosis



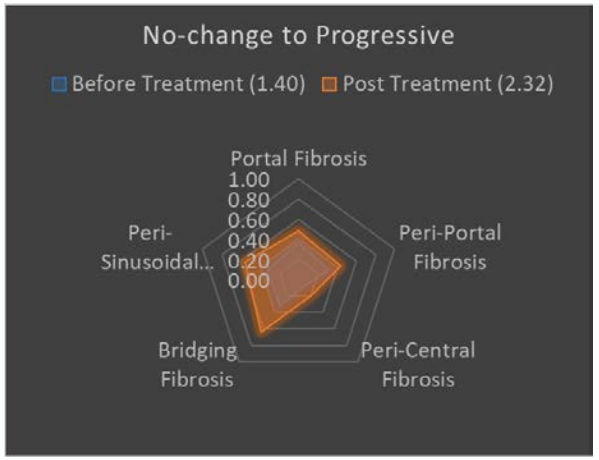


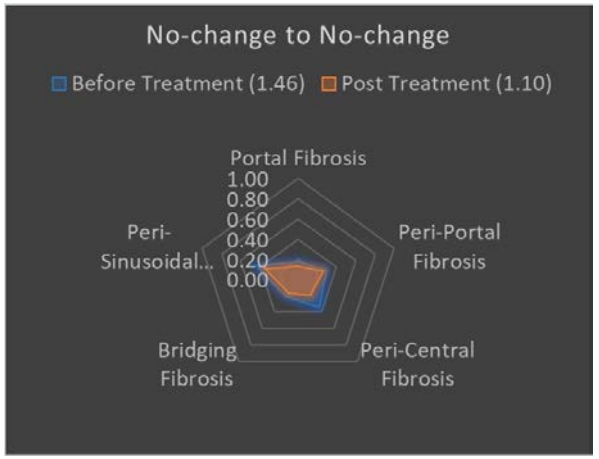
	Before Treatment	Post Treatment (Progressive)	Post Treatment (Regressive)
Portal Fibrosis	1.27	3.25	2.45
Peri-Portal Fibrosis	2.38	0.2	1.38
Peri-Central Fibrosis	0.31	0.25	0.58
Bridging Fibrosis	0.93	5.7	0.7
Peri-Sinusoidal Fibrosis	3.34	2.5	0.3
Total Weighted Score	**8.23**	**11.9**	**5.41**

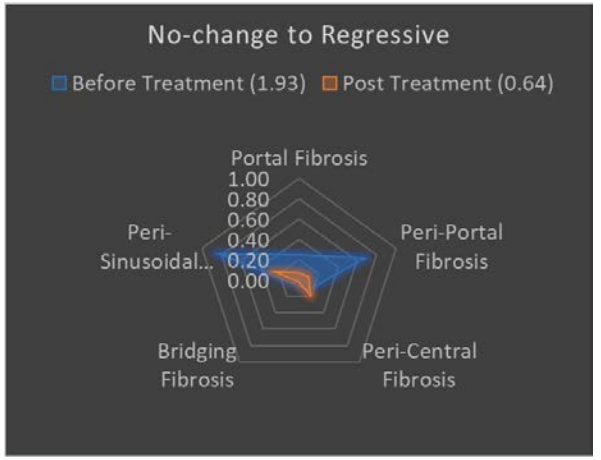
Use of qFibrosis to evaluate dynamic changes

- For patients who are F3 at baseline and after treatment
- Radar provides intuitive view of collagen changes

	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**







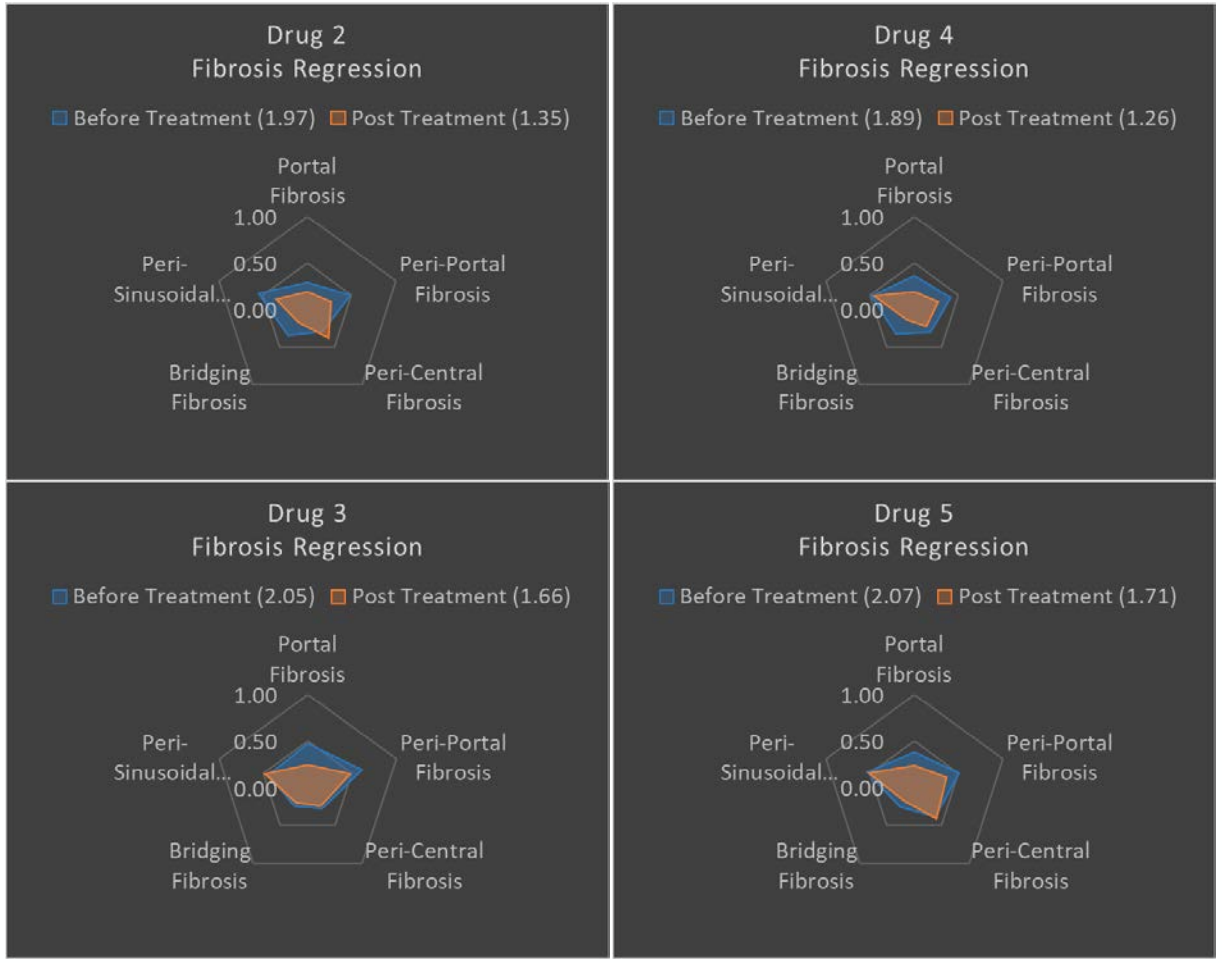
Naoumov et al., EASL 2023 Poster No 1504

qFibrosis radar map also reveals MOA specific change patterns

For patients who were staged as F3 at baseline and F2 at the end of treatment by trial pathologist

All showed similar effects on
- Portal fibrosis and
- Peri-portal fibrosis and
- Bridging fibrosis

Different effects on
- Perisinusoidal fibrosis and
- Peri-central fibrosis



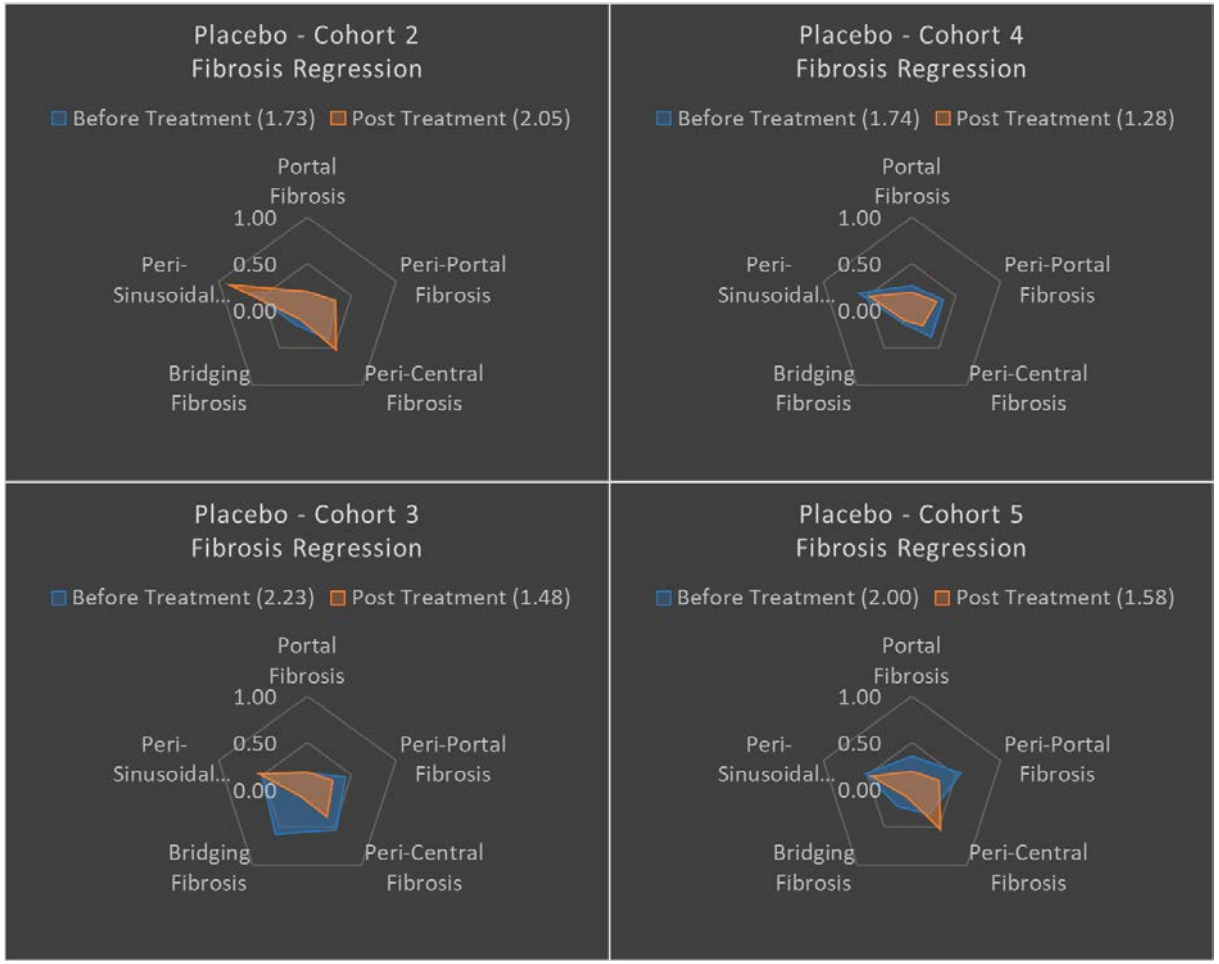
Regression patterns in placebo patients – reduction in bridging fibrosis

For patients who were staged as F3 at baseline and F2 at the end of treatment by trial pathologist

Same regression patterns in all cohorts:

- Low bridging fibrosis

Different what MOA related patterns observed previously



Conclusions

- **Fibrosis changes are dynamic and heterogeneous**
  - Progression and regression features can exist at the same time (in the same liver specimen?)
  - Progression path is different from regression path (suggest to reverse this sentence – Regression path is different from Progression path)
  - Current gold-standard was not designed to record (assess?) treatment efficacy
- **qFibrosis has been extensively validated:**
  - High repeatability and reproducibility
  - Improves inter/intra observer discrepancies
  - Reveals additional insights – changes in different areas of liver lobule; septa analyses, (e.g. steatosis correction)
- **qFibrosis (stain-free methodology) provides**
  - Designed and validated for treatment efficacy evaluation
  - Data based drug development pathway, from phase 2 to phase 3
  - Post drug approval treatment strategy design

Thank you!

Liver Forum 15, Paris
6th September 2023

By Dr Dean Tai
Chief Scientific Officer

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