FRI003

# Machine Learning Models Identify Novel Histologic Features Predictive of Clinical Disease Progression in Patients With Advanced Fibrosis Due to Nonalcoholic Steatohepatitis

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## Introduction

- Fibrosis is the primary determinant of disease progression in patients with nonalcoholic steatohepatitis (NASH), but the prognostic value of other histologic features is unclear[1,2]
- Human pathologist staging of fibrosis and NAFLD Activity Score (NAS) are limited by sampling variability, and intra- and inter-reader variability[3-6]
- Machine learning (ML) approaches to interpretation of liver histology may enable more reliable and quantitative assessment of both traditional and novel histologic features, with potential prognostic relevance in NASH[7,8]

## Objective

- To evaluate the relationship between ML-derived histologic features and disease progression in patients with advanced fibrosis due to NASH

## Methods

- Study population:
  - Adults with bridging fibrosis (NASH Clinical Research Network [CRN] F3) or compensated cirrhosis (F4) due to NASH (NAS ≥3) were enrolled in the Phase 3, placebo-controlled STELLAR trials of selonsertib (ClinicalTrials.gov NCT03053050 and NCT03053063)[9]
  - The trials were discontinued after 48 wk due to lack of efficacy; thus treatment groups were combined for this analysis
- Conventional liver histology:
  - Central pathologist review of liver biopsies at baseline (BL) and Week 48
  - Fibrosis staged according to NASH CRN and Ishak classifications
  - NAS parameters (steatosis, lobular inflammation, and hepatocellular ballooning) graded according to NASH CRN classification
- ML assessment of liver histology (PathAI, Inc., Boston, Massachusetts, USA)[7,8]:
  - For quantification of fibrosis, an "end-to-end" model was trained using slide-level pathologist scores to recognize unique patterns associated with each stage within fibrotic regions of images of trichrome (TC)–stained biopsies
  - For quantification of NAS and other features, a deep convolutional neural network was trained based on annotations collected from 75 board-certified pathologists on images of hematoxylin and eosin (H&E)–stained slides to produce pixel-level predictions of each feature
- Outcome measures (clinical disease progression):
  - Histologic progression to cirrhosis on Week 48 biopsy in patients with F3 at BL
  - Adjudication-confirmed, liver-related clinical events (ie, ascites, hepatic encephalopathy Grade ≥2, gastrointestinal bleeding due to portal hypertension, liver transplantation, qualification for transplantation [Model for End-stage Liver Disease ≥15], and death) in all patients
- Statistical analyses:
  - Associations between ML parameters (at BL and change from BL) and clinical disease progression through the end of follow-up determined using Kaplan-Meier and Cox proportional hazards regression analysis
  - Optimal cutoffs for ML parameters selected from time-dependent receiver operating characteristic curves

## Results

### Baseline Demographics and Clinical Characteristics of Patients With Bridging Fibrosis (F3) and Cirrhosis (F4)*

| | | | Bridging Fibrosis (F3) n=755 | Compensated Cirrhosis (F4) n=838 |
|---|---|---|---|---|
| Demographics | Age, y | | 59 (51, 64) | 59 (53, 65) |
| | Women, n (%) | | 427 (57) | 525 (63) |
| | White, n (%) | | 522 (69) | 642 (77) |
| | Weight, kg | | 90.5 (76.4, 105.0) | 90.8 (76.9, 106.6) |
| | BMI, kg/m$^2$ | | 32.4 (28.7, 36.7) | 32.9 (28.9, 37.5) |
| | Diabetes, n (%) | | 528 (70) | 643 (77) |
| Liver Biochemistry | ALT, U/L | | 55 (36, 80) | 43 (32, 61) |
| | AST, U/L | | 46 (33, 67) | 45 (34, 61) |
| | GGT, U/L | | 57 (37, 94) | 83 (49, 143) |
| | Total bilirubin, mg/dL | | 0.6 (0.4, 0.8) | 0.6 (0.5, 0.9) |
| | Platelets, 10$^3$/µL | | 204 (164, 255) | 159 (125, 204) |
| Conventional Liver Histology (central reader) | Ishak stage, n (%) | 3 | 430 (57) | 0 |
| | | 4 | 325 (43) | 1 (0.1) |
| | | 5 | 0 | 329 (39) |
| | | 6 | 0 | 508 (61) |
| | NAS ≥4, n (%) | | 726 (96) | 799 (95) |
| | Steatosis grades 2–3, n (%) | | 51 (7) | 33 (4) |
| | Lobular inflammation grade 3, n (%) | | 401 (53) | 454 (54) |
| | Hepatocellular ballooning grade 2, n (%) | | 604 (80) | 682 (81) |
| Noninvasive Tests of Fibrosis | ELF™ score | | 10.0 (9.4, 10.6) | 10.6 (10.0, 11.3) |
| | FIB-4 | | 1.70 (1.28, 2.59) | 2.49 (1.76, 3.59) |
| | Liver stiffness by transient elastography, kPa | | 12.6 (9.6, 17.3) | 21.0 (14.2, 28.8) |

*Continuous data are median (interquartile range [IQR]). ALT, alanine aminotransferase; AST, aspartate aminotransferase; BMI, body mass index; ELF, Enhanced Liver Fibrosis test (Siemens Healthcare GmbH, Erlangen, Germany); FIB-4, Fibrosis-4; GGT, γ-glutamyltransferase.

- In all, 1593 NASH patients with F3–4 fibrosis were included
- Median age was 59 y, 74% had diabetes, and 53% had cirrhosis (F4) as determined by the central reader

### ML-Based Histologic Features According to Centrally Read Fibrosis Stage at Baseline*

| | | | Bridging Fibrosis (F3) n=755 | Compensated Cirrhosis (F4) n=838 |
|---|---|---|---|---|
| Fibrosis-Related Parameters† | Average ML NASH CRN fibrosis score | | 2.40 (1.91, 2.77) | 3.18 (2.79, 3.46) |
| | NASH CRN area, % | F4 | 13 (4, 28) | 50 (32, 66) |
| | | ≤F3 | 87 (72, 96) | 50 (34, 68) |
| | | ≤F2 | 49 (32, 69) | 21 (12, 34) |
| | | F1 | 15 (7, 26) | 6 (3, 11) |
| | Average ML Ishak fibrosis score | | 3.00 (2.39, 3.53) | 4.28 (3.75, 4.74) |
| | Ishak area, % | 6 | 0 (0, 1) | 11 (2, 29) |
| | | ≤4 | 87 (72, 96) | 43 (28, 61) |
| | | ≤2 | 34 (19, 54) | 12 (5, 21) |
| | | 1 | 13 (6, 23) | 5 (2, 10) |
| NAS-Related and Other Parameters | Steatosis area, % | | 7 (4, 14) | 5 (2, 9) |
| | Lobular inflammation area, % | | 0.6 (0.3, 0.9) | 0.4 (0.2, 0.6) |
| | Hepatocellular ballooning area, % | | 3 (2, 5) | 3 (2, 5) |
| | Portal inflammation area, % | | 4 (3, 6) | 10 (7, 14) |
| | Bile duct area, mm$^2$ | | 0.10 (0.06, 0.17) | 0.22 (0.12, 0.40) |

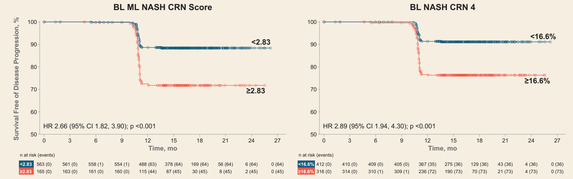
*All data are median (IQR); average ML NASH CRN and Ishak fibrosis scores reflect slide-level weighted averages of pixel-level predictions of each fibrosis stage; NASH CRN and Ishak area parameters reflect proportionate areas of specified fibrosis stage over total area scored as fibrosis; bile duct area refers to area of pixels consistent with bile ducts; steatosis area includes both macro- and microvesicular steatosis; †ML parameters evaluated on images of TC-stained slides; other parameters evaluated on images of H&E slides.

- Both F3 and F4 patients had heterogeneous fibrosis patterns, including features of F1–4 fibrosis within individual biopsies
- As defined by ML, proportionate areas of NASH CRN F4 fibrosis were 13% and 50% in F3 and F4 patients, respectively
- Compared with patients with bridging fibrosis (F3), those with cirrhosis (F4) had lower areas of steatosis (5% vs 7%), but greater areas of portal inflammation (10% vs 4%) and bile ducts (0.22 vs 0.10 mm$^2$)
- Proportionate areas of lobular inflammation and ballooning were similar between groups

### ML-Based Histologic Features Predicted Disease Progression in Patients With Bridging Fibrosis (F3)

- During median follow-up of 16.5 mo, 15% of patients with F3 fibrosis progressed to cirrhosis (n=112) or experienced a liver decompensation event (n=1)

| | Bridging Fibrosis (clinical disease progression; n=113/755) | | |
|---|---|---|---|
| | HR | 95% CI | p-Value |
| **ML NASH CRN fibrosis score*** | | | |
| Average score, per 1 unit | 2.18 | 1.56, 3.06 | **<0.001** |
| NASH CRN F4 area | 1.33 | 1.21, 1.45 | **<0.001** |
| NASH CRN ≤F2 area | 0.83 | 0.76, 0.90 | **<0.001** |
| NASH CRN F1 area | 0.77 | 0.65, 0.91 | **0.002** |
| **ML Ishak fibrosis score*** | | | |
| Average score, per 1 unit | 1.78 | 1.39, 2.26 | **<0.001** |
| Ishak 6 area | 1.49 | 1.22, 1.82 | **<0.001** |
| Ishak ≤4 area | 0.79 | 0.73, 0.87 | **<0.001** |
| Ishak ≤2 area | 0.83 | 0.75, 0.91 | **0.001** |
| Ishak 1 area | 0.77 | 0.64, 0.93 | **0.006** |
| **NAS-related and other parameters†** | | | |
| Steatosis area | 0.98 | 0.95, 1.01 | 0.13 |
| Lobular inflammation area | 0.82 | 0.57, 1.19 | 0.30 |
| Hepatocellular ballooning area | 1.06 | 1.00, 1.12 | 0.051 |
| Portal inflammation area | 1.11 | 1.06, 1.16 | **<0.001** |
| Bile duct area, per mm$^2$ | 0.94 | 0.60, 1.47 | 0.78 |




- Progression to cirrhosis was associated with higher ML NASH CRN and Ishak fibrosis scores, higher proportionate areas of NASH CRN F4 and Ishak stage 6 fibrosis, and lower proportionate areas of mild fibrosis (all p <0.05)
- Among nonfibrosis-related parameters, higher proportionate areas of hepatocellular ballooning and portal inflammation were associated with progression to cirrhosis

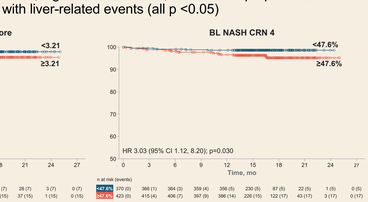



*Unless otherwise specified, hazard ratio (HR) reflects per 10% difference in parameter; †Unless otherwise specified, HR reflects per 1% difference in parameter. CI, confidence interval.

### ML-Based Histologic Features Predicted Disease Progression in Patients With Cirrhosis (F4)

- During median follow-up of 15.8 mo, 3% (22/838) of F4 patients had liver-related clinical events

| | Cirrhosis (clinical disease progression; n=22/838) | | |
|---|---|---|---|
| | HR | 95% CI | p-Value |
| **ML NASH CRN fibrosis score*** | | | |
| Average score, per 1 unit | 2.94 | 1.04, 8.33 | **0.043** |
| NASH CRN F4 area | 1.23 | 1.00, 1.51 | 0.050 |
| NASH CRN ≤F2 area | 0.69 | 0.49, 0.97 | **0.034** |
| NASH CRN F1 area | 0.46 | 0.20, 1.08 | 0.08 |
| **ML Ishak fibrosis score*** | | | |
| Average score, per 1 unit | 3.36 | 1.61, 7.02 | **0.001** |
| Ishak 6 area | 1.31 | 1.10, 1.55 | **0.002** |
| Ishak ≤4 area | 0.64 | 0.49, 0.82 | **<0.001** |
| Ishak ≤2 area | 0.55 | 0.32, 0.94 | **0.029** |
| Ishak 1 area | 0.39 | 0.15, 1.02 | 0.06 |
| **NAS-related and other parameters†** | | | |
| Steatosis area | 0.84 | 0.73, 0.97 | **0.017** |
| Lobular inflammation area | 0.56 | 0.15, 2.16 | 0.40 |
| Hepatocellular ballooning area | 1.11 | 1.02, 1.21 | **0.016** |
| Portal inflammation area | 1.10 | 1.04, 1.17 | **<0.001** |
| Bile duct area, per mm$^2$ | 1.98 | 1.15, 3.41 | **0.014** |




- Liver-related events were associated with higher ML NASH CRN and Ishak fibrosis scores, higher proportionate areas of NASH CRN F4 and Ishak stage 6 fibrosis, and lower proportionate areas of mild fibrosis
- Among nonfibrosis-related parameters, higher proportionate areas of hepatocellular ballooning and portal inflammation, higher bile duct area, and a lower proportionate area of steatosis were associated with liver-related events (all p <0.05)




*Unless otherwise specified, HR reflects per 10% difference in parameter; †Unless otherwise specified, HR reflects per 1% difference in parameter.

## Conclusions

- Liver histologic evaluation using this automated, ML-based approach identified novel features associated with clinical disease progression in NASH patients with advanced fibrosis
- Higher proportionate areas of more advanced fibrosis patterns, portal inflammation, and ballooning, as well as lower areas of steatosis, were associated with increased risk of disease progression
- These data support the utility of ML-based assessment of liver histology for risk stratification of patients with NASH and, potentially, as endpoints in NASH clinical trials

**References:** **1.** Angulo P, et al. Gastroenterology 2015;149:389-97; **2.** Sanyal AJ, et al. Hepatology 2019;70:1913-27; **3.** Gawrieh S, et al. Ann Diagn Pathol 2011;15:19-24; **4.** Jung ES, et al. J Pathol Transl Med 2016;50:190-6; **5.** Ratziu V, et al. Gastroenterology 2005;128:1898-906; **6.** Rockey DC, et al. Hepatology 2009;49:1017-44; **7.** Pokkalla H, et al. AASLD 2019, abstr 0187; **8.** Younossi ZM, et al. AASLD 2019, abstr 1718; **9.** Harrison SA, et al. J Hepatol 2020;73:26-39. **Acknowledgments:** We extend our thanks to the patients, their families, and all participating investigators. These studies were funded by Gilead Sciences, Inc.

Presented at The Digital International Liver Congress, 27–29 August 2020