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Cirrhosis Regression is Associated with Improved Clinical Outcomes in Patients with Nonalcoholic Steatohepatitis

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Keywords: Enhanced Liver Fibrosis score; machine learning; nonalcoholic fatty liver disease; NAFLD Activity Score; FIB-4

Abbreviations: ANCOVA, analysis of covariance; α -SMA, alpha-smooth muscle actin; CPT, Child-Pugh-Turcotte; CRN, Clinical Research Network; CSPH, clinically significant portal hypertension; ELF, Enhanced Liver Fibrosis; FHVP, free hepatic vein pressure; FIB-4, Fibrosis-4 index; HCC, hepatocellular carcinoma; HVPG, hepatic venous pressure gradient; MELD, Model for End-Stage Liver Disease; ML, machine learning; NAS, NAFLD Activity Score; NASH, nonalcoholic steatohepatitis; NFS, NAFLD fibrosis score; NIT, noninvasive test; VCTE, vibration-controlled transient elastography; WHVP, wedged hepatic venous pressure

ABSTRACT

Background & Aims: Surrogate endpoints that predict complications are necessary for assessment and approval of NASH therapies. We assessed associations between histologic and noninvasive tests of fibrosis (NITs) with liver-related complications in patients with NASH cirrhosis.

Approach & Results: Patients with compensated cirrhosis due to NASH were enrolled in two placebo-controlled trials of simtuzumab and selonsertib. Liver fibrosis at baseline and week 48 (W48) was staged by NASH CRN and Ishak classifications and a machine learning (ML) approach, hepatic collagen and α -SMA expression were quantified by morphometry, liver stiffness (LS) was measured by transient elastography, and serum NITs (ELF, NAFLD Fibrosis Score [NFS], and FIB-4) were calculated. Cox regression determined associations between these parameters at baseline and their changes over time with adjudicated liver-related clinical events. Among 1135 patients, 709 (62%) had Ishak stage 6 fibrosis, and median ELF and LS were 10.66 and 21.1 kPa, respectively. During a median follow-up of 16.6 months, 71 (6.3%) had a liver-related event; associated baseline factors included Ishak stage 6 fibrosis, and higher hepatic collagen, α -SMA expression, ML-based fibrosis parameters, LS, ELF, NFS, and FIB-4. Cirrhosis regression observed in 16% (176/1135) between BL and W48 was associated with a lower risk of events vs non-regression (1.1% [2/176] vs 7.2% [69/957]; HR: 0.16; 95%CI 0.04,0.65 [p=0.0104]). Conversely, after adjustment for baseline values, increases in hepatic collagen, α -SMA, ML-based fibrosis parameters, NFS, and LS were associated with an increased risk of events.

Conclusions: In patients with compensated cirrhosis due to NASH, regression of fibrosis is associated with a reduction in liver-related complications. These data support the utility of histologic fibrosis regression and NITs as clinical trial endpoints for NASH cirrhosis.

INTRODUCTION

Cirrhosis is the end-stage of chronic liver diseases associated with hepatocyte injury and inflammation, including nonalcoholic steatohepatitis (NASH). Histologically, cirrhosis is characterized by diffuse nodular regeneration surrounded by fibrotic septa.(1) The resultant architectural distortion leads to portal hypertension, which along with hepatic synthetic dysfunction and hepatocellular carcinoma (HCC), accounts for the majority of complications of cirrhosis.(2-4) Over time, approximately 10% to 20% of NASH patients will progress to cirrhosis.(5) Due to rising rates of obesity and insulin resistance and time-dependent fibrosis progression among affected patients, the prevalence of NASH cirrhosis is rising exponentially.(6, 7) In the United States, the prevalence of compensated NASH cirrhosis is expected to increase from approximately 1.2 million in 2015 to 3.1 million in 2030. Corresponding rates of decompensated cirrhosis, liver transplantation, HCC, and liver-related death are expected to rise 168%, 59%, 137%, and 178%, respectively, a phenomenon that is mirrored internationally.(6, 8) Commensurate with these increases in clinical complications, the economic costs attributable to cirrhosis □ which have been estimated at ~\$9 billion in the United States alone □ will also grow.(7)

The evidence currently required for approval of therapeutic agents targeting patients with NASH cirrhosis includes event-based clinical trials rather than histological endpoints, which are accepted in non-cirrhotic NASH.(9, 10) The rationale for this recommendation is based on uncertainty regarding the feasibility of histologic cirrhosis regression; specifically, whether observed cases in clinical trials are real or simply reflect sampling variability in histological assessment. A clear solution to this question would be to determine if cases of cirrhosis regression are accompanied by a reduction in the risk of liver-related complications and mortality. While regression of cirrhosis with effective therapy is associated with improved outcomes in multiple disorders including chronic hepatitis B and C virus infection and autoimmune hepatitis(11-13), the clinical benefits of cirrhosis regression in NASH have not been adequately studied.

In order to address these uncertainties, we analyzed data from two large placebo-controlled trials of simtuzumab and selonsertib in patients with compensated cirrhosis due to NASH.(11, 12) Although

these therapies were not effective, the comprehensive dataset from these trials provides an unprecedented opportunity to describe the natural history of NASH cirrhosis, including the frequency of cirrhosis regression, in a well-characterized patient population. Trial assessments included serial liver biopsies with centralized assessments of fibrosis stage, hepatic collagen content and alpha-smooth muscle actin (α -SMA) expression by morphometry, and noninvasive tests of fibrosis (NITs), including serum markers and liver stiffness by vibration-controlled transient elastography (VCTE). In addition, biopsies were evaluated using a novel machine learning (ML) approach that has been validated for the assessment of NASH-related histology.⁽¹³⁾ Finally, clinical outcomes including hepatic decompensation were formally adjudicated.

The objectives of this analysis were to: 1) evaluate the incidence of cirrhosis regression in NASH clinical trials; 2) assess associations between cirrhosis regression and changes in NITs; and 3) evaluate associations between fibrosis assessed histologically and with NITs—at baseline and their changes over time—with liver-related complications in patients with cirrhosis due to NASH.

METHODS

Study Designs and Participants

This analysis used data from two large, randomized, placebo-controlled studies of simtuzumab (NCT01672879) and selonsertib (STELLAR-4, NCT03053063) in patients with compensated cirrhosis due to NASH. The primary results of these studies are reported elsewhere, where the methods are fully described.(11, 12) Briefly, the simtuzumab phase 2b study enrolled 258 patients with histologically confirmed NASH and compensated cirrhosis (modified Ishak fibrosis stage 5-6, equivalent to NASH Clinical Research Network [CRN] stage 4) between 22 January 2013 and 20 October 2014.(11) Patients were randomized in a 1:1:1 ratio to receive 200 mg of simtuzumab, 700 mg of simtuzumab, or placebo by intravenous infusion every 2 weeks. Patients with cryptogenic cirrhosis (i.e., grade 0 steatosis according to the NAFLD Activity Score [NAS]) were eligible if at least one clinical feature suggestive of underlying NASH (e.g., diabetes, insulin resistance, obesity, hypercholesterolemia, hyperlipidemia, or hypertension) was present. Randomization was stratified by the presence or absence of diabetes and clinically significant portal hypertension (CSPH), defined as a hepatic venous pressure gradient (HVPG) ≥ 10 mmHg.

In the STELLAR-4 phase 3 study, 877 patients with compensated cirrhosis due to NASH (NASH CRN stage 4) were enrolled between 16 February 2017 and 31 January 2018.(12) All patients had at least one point for each of the three NAS components (steatosis, lobular inflammation, and hepatocellular ballooning). Patients were randomly assigned in a 2:2:1 ratio to receive 18 mg of selonsertib, 6 mg of selonsertib, or placebo administered orally once daily. Randomization was stratified by the presence or absence of diabetes and Enhanced Liver Fibrosis (ELF) score (Siemens, Tarrytown, NY) ≥ 11.3 .

In both studies, patients were excluded if they had liver disease of other etiologies (e.g., alcoholic liver disease, hepatitis B or C virus infection, and autoimmune disorders), a history of hepatic decompensation, HCC or solid organ transplantation, a Model for End-Stage Liver Disease (MELD) score >12 , or a Child-Pugh-Turcotte (CPT) score >7 . In STELLAR-4, a platelet count of at least 100,000 per μL was required. In both studies, the planned duration of treatment was 240 weeks. However, the studies were halted after pre-planned interim analyses conducted after all patients had

completed at least 48 weeks (STELLAR-4) or 96 weeks (sintuzumab) of treatment found no meaningful differences between the active treatment groups or placebo in any efficacy endpoint.(11, 12) Therefore, for the purposes of this analyses, treatment groups were combined.

Study Assessments

Liver Histology

In both studies, core liver biopsies were obtained at screening and week 48. In the sintuzumab study, an additional biopsy was collected at week 96. Biopsies were read by a single central reader (ZG) who was blinded to treatment assignment, but not biopsy sequence. As previously described, (3, 11, 12) histological assessments included the adequacy of the biopsy specimen, confirmation of the diagnosis, fibrosis staged according to a modified Ishak classification and the NASH CRN classification, and grading of steatosis, lobular inflammation, and hepatocellular ballooning according to the NAS. Morphometric quantification of hepatic collagen content and α -smooth muscle actin (α -SMA) expression were also performed, as previously described.

In addition, we utilized a ML approach (PathAI; Boston, MA) that has been validated for the assessment of liver histology in NASH.(13) Briefly, an end-to-end model was trained using digitized images of Masson's trichrome-stained liver biopsies and pathologist annotations to predict the stage of fibrosis within fibrotic regions in the tissue. Slide-level ML parameters were generated by computing proportionate areas of each histologic feature, including fibrosis patterns consistent with each NASH CRN fibrosis stage. In addition, we calculated the weighted mean of these predictions to generate a single, slide-level, continuous score referred to as the ML NASH CRN fibrosis score, that summarizes the underlying heterogeneity of fibrosis in the slide.

HVPG Measurement, Serum Markers and Liver Stiffness

HVPG measurements were performed according to a standardized protocol during the screening period and at weeks 48 and 96 of the sintuzumab study only. As previously described, measurements of wedged (occluded) hepatic venous pressure (WHVP) and free hepatic vein pressure (FHVP) were made in triplicate.(3, 11) Permanent tracings for each measurement were obtained and the mean value was recorded for that visit. HVPG was calculated as the difference between the mean WHVP and

mean FHVP. All tracings were evaluated centrally by a single reader (JB); the intra-class correlation coefficients at each time point were 0.97 or greater.

Laboratory assessments, including liver biochemistry and serum NITs including ELF, NAFLD fibrosis score (NFS), and the Fibrosis-4 index (FIB-4), were measured during screening and at least every 3 months during the studies. Where available, liver stiffness was measured during screening and every 6 months thereafter by trained operators using vibration-controlled transient elastography (VCTE; FibroScan, Echosens, Paris, France), as previously described.(12)

Outcome Measures

The primary histologic outcome of interest for this analysis was cirrhosis regression, defined as a ≥ 1 -stage improvement in fibrosis according to the NASH CRN classification from baseline to the last available biopsy. We also evaluated fibrosis regression, defined as a ≥ 1 -stage improvement in fibrosis according to the modified Ishak classification. Finally, we evaluated time to first liver-related clinical event, defined as hepatic decompensation (clinically apparent ascites requiring treatment, hepatic encephalopathy of Grade 2 or above according to the West Haven criteria requiring treatment, and portal hypertension-related gastrointestinal bleeding), liver transplantation, qualification for transplantation (MELD ≥ 15), or all-cause mortality, as confirmed by an independent Hepatic Events Adjudication Committee. Cases of HCC, which were not officially adjudicated, were also recorded.

Statistical Analyses

We analyzed data from all patients who were enrolled and treated in both trials through the end of follow-up. The primary goal was to evaluate associations between histological parameters of fibrosis and NITs (at baseline and their change) with clinical disease progression, as indicated by the occurrence of liver-related clinical events (defined above). Associations between cirrhosis regression and clinical parameters (e.g., NITs) were evaluated using Fisher's exact and Wilcoxon rank sum tests for baseline parameters and analysis of covariance (ANCOVA) with adjustment for baseline value and study for parameters of change. Associations with time to clinical disease progression were evaluated using Kaplan-Meier and Cox proportional hazards regression analyses. Only the first clinical event per patient was included. We implemented a last observation carried forward approach,

including baseline, to impute missing post-baseline values. Univariate models were used for baseline predictors, while all models for change from baseline adjusted for baseline values. Since some patients experienced a clinical event prior to their week 48 liver biopsy, we conducted a sensitivity analysis excluding these patients and considering the start of follow-up for survival analysis (time zero) in the remaining patients as the date of the week 48 liver biopsy. SAS v9.4 software (SAS Institute, Inc.; Cary, NC) was used for all analyses.

RESULTS

Demographic and Clinical Characteristics

The demographic and clinical characteristics of patients in both trials (n=1135) are included in **Table 1**. The median age was 59 years (IQR 53, 64), and most patients (81%) were white, 63% were female, and approximately three-quarters had diabetes. The majority of patients had Ishak stage 6 fibrosis (63%) and median (IQR) ELF and liver stiffness by VCTE were 10.66 (10.00, 11.37) and 21.1 kPa (14.2, 29.3), respectively. In the simtuzumab study, 68% of patients (175/256) had CSPH. Although there were no differences in patient characteristics between treatment groups within the individual studies (data not shown), notable differences were observed between studies. Specifically, patients in the simtuzumab study had evidence of more advanced cirrhosis than those in STELLAR-4, as demonstrated by lower platelets, greater hepatic collagen content, α -SMA expression, and NITs, and a higher prevalence of cryptogenic cirrhosis (42% vs 0%). On the contrary, patients in STELLAR-4 had evidence of more active NASH, as supported by a higher prevalence of NAS ≥ 4 (95% vs 64%) and greater proportions with grade 2 or 3 lobular inflammation (53% vs 31%) and grade 2 hepatocellular ballooning (82% vs 44%; **Table 1**).

Liver-Related Clinical Events

During a median follow-up of 16.6 months (IQR 14.1, 21.0), 71 of the 1135 patients (6.3%) with compensated cirrhosis at baseline experienced liver-related clinical events; 6 patients (<1%) were diagnosed with HCC. The first events to occur in patients with events were ascites in 34 patients (3%), hepatic encephalopathy in 20 (2%), portal hypertension-related gastrointestinal hemorrhage in 11 (<1%), MELD ≥ 15 or liver transplantation in 5 (<1%), and death (due to multiorgan failure) in 1 (<1%). Patients in the simtuzumab study had an increased risk of liver-related events compared with

those in STELLAR-4 (log-rank $p < 0.0001$; **Supplementary Figure 1**). At 12, 24, and 36 months, estimated event-free survival (95% CI) in the combined cirrhosis cohort were 96.0% (94.7%, 97.0%), 89.7% (86.3%, 92.4%), and 87.7% (83.3%, 91.0%), respectively.

Associations Between Clinical Events and Fibrosis-Related Parameters

Associations between fibrosis-related histological parameters and NITs with time to first liver-related clinical event are outlined in **Figure 1**. The presence of Ishak fibrosis stage 6 versus ≤ 5 at baseline was associated with an approximately 2-fold risk of clinical events (hazard ratio [HR] 2.28; 95% CI 1.29, 4.04) (**Figure 2**). On the other hand, an improvement in Ishak fibrosis stage was associated with a greater than 10-fold reduction in the risk of events (HR 0.08; 95% CI 0.02, 0.32) (**Figure 3**).

Clinical events were observed in 8.3% (69/834) of patients without fibrosis regression compared with 0.7% (2/300) in those with fibrosis regression ($p = 0.004$). Changes in fibrosis according to baseline Ishak fibrosis stage are shown in **Supplementary Table 1**.

Higher hepatic collagen content and α -SMA expression at baseline and greater increases in these parameters over time were associated with an increased risk of clinical events (**Figure 1**). Similar findings were observed for ML-based histological parameters. For example, higher ML NASH CRN fibrosis score at baseline (HR per unit: 5.09; 95% CI 2.23, 11.60) and greater increases during follow-up (HR per unit: 3.05; 95% CI 1.51, 6.18) were associated with an increased risk of disease progression.

Liver-related clinical events were also more frequent in patients with higher HVPG and baseline levels of all NITs (**Figure 1**). For example, the relative risk of events increased 68% per 0.5-unit increase in ELF score (HR 1.68; 95% CI 1.50, 1.88) and 13% per 2-kPa increase in liver stiffness by VCTE (HR 1.13; 95% CI 1.09, 1.17) at baseline. After adjustment for baseline values, changes in NFS, liver stiffness by VCTE, and tissue inhibitor of metalloproteinase-1 (TIMP-1) were associated with clinical events; changes in FIB-4 and ELF did not reach statistical significance. Relationships between clinical events and changes in ELF, FIB-4, NFS, liver stiffness by VCTE, and hepatic collagen after adjustment for baseline values, are illustrated graphically in **Supplementary Figure 2**.

Baseline levels and changes in body weight, BMI, glucose, and HbA1c were not associated with clinical events (data not shown).

Associations Between Cirrhosis Regression, Clinical Events, and Other Parameters

As with Ishak fibrosis stage improvement, regression of cirrhosis (decrease in NASH CRN fibrosis stage from 4 to <4), which occurred in 16% (176/1135) of patients during follow-up, was associated with a greater than 6-fold reduction in the risk of liver-related events (HR 0.16; 95% CI 0.04, 0.65) (**Figure 3**). Clinical events occurred in 1.1% (2/176) of patients with cirrhosis regression compared with 7.2% (69/957) among those without regression ($p=0.0104$). In a sensitivity analysis excluding 38 patients with clinical events prior to the week 48 liver biopsy, fewer events were observed in patients with versus without cirrhosis regression, but the results did not reach statistical significance (**Supplementary Figure 3**). The relationship between cirrhosis regression and clinical events was consistent after adjustment for measures of fibrosis severity at baseline (**Supplementary Figure 4**). All 6 cases of HCC occurred in patients without cirrhosis regression.

Demographics and clinical characteristics at baseline and their changes during the study according to cirrhosis regression are outlined in **Table 2**. Compared with non-regressors, those with cirrhosis regression had lower BMI, fasting glucose, and HbA1c; higher platelets; a lower prevalence of grade 0 steatosis and Ishak stage 6 fibrosis; and lower hepatic collagen content, α -SMA expression, ML-based parameters of fibrosis, HVPG, and all NITs at baseline. Regression of cirrhosis was more common in STELLAR-4 than in the simtuzumab study (18% [154/877] vs 8.6% [22/256]; $p=0.0004$) and in patients with ELF <11.3 at baseline (19% [157/824] vs 6.3% [19/302] with ELF \geq 11.3; $p<0.0001$). Compared with non-regressors, patients with cirrhosis regression also had greater reductions during follow-up in hepatic collagen content and α -SMA expression by morphometry, ML-based parameters of fibrosis, ELF (including procollagen III amino terminal propeptide [PIIINP] and TIMP-1), and liver stiffness by VCTE. Patients with cirrhosis regression were also more likely to experience a \geq 2-point reduction in NAS (20% [35/174] vs 12% [115/949]; $p=0.0072$) and improvements in lobular inflammation (37% [65/174] vs 18% [172/948]; $p<0.0001$) and hepatocellular ballooning (26% [45/170] vs 17% [152/897]; $p=0.0049$), but not steatosis (12% [20/165] vs 15% [131/856]; $p=0.34$). In the simutuzumab study, a greater reduction in HVPG was

observed in patients with versus without cirrhosis regression (LSmean change from baseline: -1.4 vs 0.2 mm Hg; $p=0.0454$). While baseline liver biopsy length did not differ between patients with and without cirrhosis regression (median [IQR]: 2.0 [1.5, 2.8] vs 2.1 [1.5, 3.0] cm; $p=0.184$), those with regression had shorter biopsies at week 48 (1.9 [1.3, 2.6] vs 2.2 [1.5, 3.0] cm; $p=0.0028$). Changes in body weight, BMI, glucose, and HbA1c did not differ between groups (**Table 2**).

DISCUSSION

The progression of fibrosis to cirrhosis is widely considered an important milestone in the natural history of all chronic liver diseases including NASH, following which liver-related complications occur at an annual incidence of 3-4%. However, it was unknown whether cirrhosis could actually regress in this population, how frequently this occurs, whether documented cases of cirrhosis regression simply reflect an artifact of how fibrosis was assessed, and ultimately, if such regression had any clinical relevance. The current study provides the evidence base to answer these critical gaps in our knowledge regarding the natural history of NASH cirrhosis.

In this study, fibrosis regression was observed in 16% of cirrhotic patients enrolled in two large placebo-controlled trials that included protocol liver biopsies 48 weeks apart. In a previous study from the NASH CRN of a cohort with the full histological spectrum of NAFLD, 34% of patients experienced fibrosis regression on standard of care follow-up biopsies after a median duration of 4.9 years.⁽¹⁴⁾ Importantly, 6 of 18 patients (33%) with cirrhosis on the first biopsy in this study had stage 3 fibrosis on a follow-up biopsy. Theoretically, the higher proportion of patients with cirrhosis regression reported by Kleiner and colleagues may reflect the smaller number of cirrhotic patients, longer follow-up, or an artifact due to sampling error of liver biopsy and/or inter-observer variability in histological interpretation. However, data from the current study argue against the latter hypotheses.

In the present study, strong concordance was observed between cirrhosis regression and changes in other measures used to assess fibrosis, including NITs such as the ELF score and liver stiffness by VCTE. While FIB-4 tended to increase in both patients with and without fibrosis regression, which is expected given the inclusion of age in its computation, increases in FIB-4 were lower in those with

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regression and differences did not reach statistical significance. Differences between changes in other histological parameters of fibrosis (e.g., hepatic collagen content, α -SMA expression, and ML-based parameters) were also observed between patients with and without cirrhosis regression. We speculate that a decrease in α -SMA expression in patients with cirrhosis regression reflects reduced fibrogenic drive in these patients. While sampling variability could explain the observed improvements in other histological findings among patients with cirrhosis regression, consistency with changes in NITs argues against this hypothesis. Moreover, regression of cirrhosis was associated with a reduction in portal pressure assessed by HVPG, which is the key driver of cirrhotic complications.(15, 16) Indeed, a striking 6-fold reduced risk of cirrhosis-related events was observed in patients with cirrhosis regression. Together, these data indicate that the changes in fibrosis observed in this study are real and clinically relevant.

The potential for NASH cirrhosis to regress, and this regression to be associated with a dramatic ~85% reduction in the risk of liver-related events, has several important implications. From a clinical perspective, histological cirrhosis regression in NASH should no longer be considered unattainable, particularly if accompanied by improvements in NITs or portal pressure. These findings, which complement studies showing regression of NASH cirrhosis following bariatric surgery,(17, 18) are reassuring, but we caution against altering follow-up of cirrhotic patients in whom fibrosis regression is demonstrated (e.g., surveillance for HCC or esophageal varices) pending additional confirmation. From a clinical trial perspective, data from this study may inform the design of trials of therapies for this patient population. The observed incidence of cirrhosis regression may be used to estimate the placebo response and coupled with the impact of cirrhosis regression on the incidence of liver-related events, be useful for sample size estimation. From a patient selection standpoint, this study provides information regarding factors associated with cirrhosis regression in the absence of effective therapeutic intervention. To increase the likelihood of successful clinical trial outcomes, enrichment of studies with subjects who have the lowest likelihood of cirrhosis regression (i.e., minimize the placebo response) may be an effective strategy. In this regard, our data show that patients with a lower propensity to regress cirrhosis have lower platelet counts, and higher BMI, glycemic parameters, AST, NITs, histological fibrosis parameters (e.g., Ishak stage 6 fibrosis, hepatic collagen, α -SMA expression), and HVPG. It is however unclear from these data if the positive predictive value

for cirrhosis regression of any of these parameters is sufficiently high to provide specific guidance, and this remains an area for future research.

Finally, from a regulatory perspective, the data from our study confirm the link between fibrosis regression demonstrated histologically with “hard” liver-related outcomes and support the validity of liver histology as a surrogate endpoint in clinical trials of therapies for NASH cirrhosis. While histological endpoints are considered acceptable for the accelerated approval of therapies for patients with non-cirrhotic NASH, current regulatory guidance advocates event-based trials and approval based on the traditional (full) approval pathway in NASH patients with cirrhosis.(9, 10) However, the feasibility of event-based trials for approval of therapies in cirrhotic NASH is challenging due to the estimated sample sizes, event rates, and study durations that will be necessary to demonstrate an effect of treatment on clinical outcomes. Therefore, the potential for accelerated drug approval based on a histologic endpoint in this population with high unmet medical need is an attractive option that warrants discussion between regulators and pharmaceutical sponsors.

Several additional findings of our study warrant discussion. First, our data highlight the direct impact of not only fibrosis stage, but also fibrosis burden, on clinical outcomes. Regardless of the method of assessment—routine histology, ML-based histology, or NITs—a greater burden of fibrosis at baseline, and greater increases over time, were associated with an increased risk of events. Along these lines, the current study informs the use of NITs in routine clinical practice. The associations between baseline FIB-4, NFS, ELF score, and liver stiffness by VCTE to fibrosis burden and risk of clinical events support their use for risk stratification. Moreover, the relationship between changes of these parameters with risk of events provide proof of concept that these tools provide may serve as useful disease monitoring tests. Future focused studies to confirm these observations are needed to develop specific guidance on their application for this context of use, including definitions of clinically important changes. Finally, our data confirm the prognostic significance of portal pressure as measured by HVPG, extending observations from cohorts with predominantly viral or alcoholic cirrhosis to those with NASH.(15, 16) These data support HVPG as an additional potential surrogate endpoint for evaluation of therapies for NASH cirrhosis.(11, 19, 20)

The current study has several notable strengths that support the validity of the results and related conclusions. These include the large size of the cohort, rigorous characterization of patients with serial NITs and centrally read liver biopsies, and the adjudication of liver-related clinical events by a committee of experts. However, our study has several limitations. Most importantly, the relatively small number of events and short follow-up in the trials warrant validation of our observations in additional cohorts. Generalizability of our findings from clinical trial subjects to the broader population of patients with NASH cirrhosis also requires confirmation. Moreover, while neither simtuzumab nor selonsertib demonstrated obvious evidence for efficacy, we cannot exclude minor effects on the natural course of the disease in these trials. In addition, based on the available data, we are unable to identify the underlying contributors to cirrhosis regression in our cohort. While changes in body weight and glycemic parameters were not associated with cirrhosis regression or clinical events, an impact of other lifestyle factors (e.g., alcohol intake, exercise, concomitant medications) cannot be excluded. This hypothesis is supported by the observed associations between improvements in lobular inflammation and hepatocellular ballooning with cirrhosis regression. Finally, we observed a low incidence of HCC in these trials (n=6, <1%), potentially due to selection bias and/or incomplete case ascertainment. HCC was not included in the composite liver-related event endpoint based on guidance from regulatory agencies during the design of these trials. Regardless of these limitations, the data from the current study provide valuable new information on regression of cirrhosis in NASH that have important implications for both drug development and clinical practice.

In summary, the current study shifts the paradigm that NASH-related cirrhosis is irreversible by demonstrating a strong concordance between histologic evidence of cirrhosis regression with decreases in noninvasive measures of fibrosis burden and histological markers of fibrogenesis. The clinical relevance of these findings is underscored by a 6-fold decrease in risk of liver-related complications in patients with cirrhosis regression. Furthermore, the study provides a profile of individuals most likely to experience cirrhosis regression. Finally, these data support the regulatory acceptance of cirrhosis regression as a surrogate endpoint for drug approval, as well as the use of NITs for risk stratification and disease monitoring, in clinical practice and in clinical trials.

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- Accepted Article
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Table 1. Baseline demographics and clinical characteristics of NASH patients with cirrhosis

	Simtuzumab Study (N=258)	STELLAR-4 (N=877)	Overall (N=1135)
Demographics and Clinical Characteristics			
Age, y	57 (51, 61)	59 (53, 65)	59 (53, 64)
Female, n (%)	163 (63)	547 (62)	710 (63)
United States, n (%)	211 (82)	520 (59)	731 (64)
White, n (%)	238 (92)	676 (77)	914 (81)
Hispanic/Latino, n (%)	39 (15)	122 (14)	161 (14)
BMI, kg/m ²	33.6 (29.7, 38.2)	33.0 (28.8, 37.7)	33.1 (29.0, 37.8)
Body weight, kg	95.2 (81.7, 108.8)	91.0 (76.9, 106.8)	92.5 (78.3, 107.2)
Diabetes, n (%)	175 (68)	674 (77)	849 (75)
ALT, U/L	35 (25, 50)	43 (31, 60)	41 (30, 58)
AST, U/L	41 (31, 54)	45 (34, 61)	45 (33, 60)
GGT, U/L	84 (49, 163)	82 (49, 144)	83 (49, 147)
Bilirubin, mg/dL	0.6 (0.5, 1.0)	0.6 (0.5, 0.9)	0.6 (0.5, 0.9)
INR	1.1 (1.0, 1.2)	1.1 (1.0, 1.1)	1.1 (1.0, 1.1)
Platelets, x10 ³ /μL	130 (91, 175)	157 (124, 203)	151 (116, 198)
MELD	7 (6, 8)	7 (6, 8)	7 (6, 8)
NITs			
ELF score	10.74 (9.94, 11.48)	10.64 (10.03, 11.32)	10.66 (10.00, 11.37)
FIB-4	3.15 (1.95, 4.70)	2.50 (1.76, 3.64)	2.57 (1.80, 3.89)
NFS	1.28 (0.30, 2.20)	0.66 (-0.20, 1.53)	0.78 (-0.119, 1.717)
Liver stiffness by VCTE, kPa*	22.0 (12.3, 37.3)	21.1 (14.3, 28.8)	21.1 (14.2, 29.3)
Standard Histologic Parameters			
NAS ≥4, n (%)	159/247 (64)	837/877 (95)	996/1124 (89)
Steatosis grade 2-3, n (%)	25/247 (10)	34/877 (4)	59/1124 (5)
Steatosis grade 0, n (%)	103/247 (42)	0/877 (0)	103/1124 (9)
Lobular inflammation grade 3, n (%)	76/247 (31)	469/877 (53)	545/1124 (48)
Hepatocellular ballooning grade 2, n (%)	108/247 (44)	717/877 (82)	825/1124 (73)
Hepatic collagen content, %	12.5 (8.2, 19.3)	10.6 (7.4, 14.6)	11.0 (7.7, 15.5)
α-SMA expression, %	18.2 (12.0, 26.4)	13.1 (8.6, 19.1)	14.0 (9.2, 20.8)
Ishak stage 6 fibrosis, n (%)	171/257 (67)	538/877 (61)	709 (63)

Machine Learning (ML) Fibrosis Parameters[†]			
ML NASH CRN fibrosis score	3.4 (3.1, 3.6)	3.2 (2.8, 3.5)	3.2 (2.8, 3.5)
Proportionate area of F4, %	58.0 (43.5, 70.6)	49.8 (32.4, 66.3)	51.9 (33.9, 67.0)
Proportionate area of F3, %	26.0 (18.6, 36.1)	25.2 (17.9, 34.0)	25.3 (18.1, 34.4)
Proportionate area of F2, %	7.7 (4.5, 12.9)	10.1 (6.4, 16.4)	9.7 (5.9, 15.6)
Proportionate area of F1, %	2.6 (0.9, 6.9)	5.8 (2.8, 11.2)	5.3 (2.4, 10.6)

Data are n (%) or median (IQR).

* Liver stiffness by VCTE at baseline available in 40 patients in SIM study and 694 patients in STELLAR-4.

[†] ML histologic parameters available in 169 patients in SIM study and 796 patients in STELLAR-4.

Table 2. Baseline factors and changes in clinical parameters associated with cirrhosis regression

	Baseline (Median [Q1, Q3])			LSmean Change from Baseline (95% CI)*		
	Cirrhosis Regression (N=176)	No Regression (N=957)	P-value	Cirrhosis Regression (N=176)	No Regression (N=957)	P-value
Demographics and Clinical Characteristics						
Age, yrs	59 (52, 66)	59 (53, 64)	0.58	--	--	--
Female, n (%)	109 (62)	600 (63)	0.87	--	--	--
BMI, kg/m ²	32.4 (27.7, 37.2)	33.3 (29.3, 38.0)	0.032	0.10 (-0.21, 0.41)	0.06 (-0.09, 0.20)	0.80
Body weight, kg	87.6 (73.8, 103.7)	93.0 (79.0, 107.6)	0.0146	0.31 (-0.57, 1.20)	0.19 (-0.22, 0.61)	0.79
Diabetes, n (%)	123 (70)	726 (76)	0.11	--	--	--
Fasting glucose, mg/dL	110 (96, 140)	121 (101, 155)	0.0012	4 (-4, 11)	7 (4, 11)	0.35
HbA1c, %	6.2 (5.5, 7.1)	6.6 (5.7, 7.7)	<0.0001	0.1 (0, 0.3)	0.1 (0, 0.2)	0.72
ALT, U/L	42 (30, 59)	41 (30, 58)	0.70	-3 (-6, 0)	-4 (-6, -3)	0.32
AST, U/L	38 (31, 50)	46 (34, 61)	<0.0001	-5 (-8, -1)	-2 (-3, 0)	0.075
Bilirubin, mg/dL	0.6 (0.5, 0.8)	0.6 (0.5, 0.9)	0.0036	0.0 (-0.1, 0.1)	0.1 (0.0, 0.1)	0.076
INR	1.0 (1.0, 1.1)	1.1 (1.0, 1.1)	<0.0001	0.0 (0.0, 0.0)	0.0 (0.0, 0.0)	0.15
Platelets, 10 x10 ³ /μL	175 (139, 230)	147 (113, 193)	<0.0001	-3 (-8, 3)	-6 (-9, -4)	0.21
MELD	7 (6, 7)	7 (6, 8)	<0.0001	0 (0, 0)	1 (0, 1)	0.026
NITs						

ELF score	10.06 (9.40, 10.74)	10.77 (10.13, 11.45)	<0.0001	0.12 (0.00, 0.24)	0.29 (0.23, 0.34)	0.0076
PIIINP	9.86 (7.86, 13.82)	13.21 (9.78, 17.49)	<0.0001	0.66 (-0.34, 1.66)	1.40 (0.94, 1.85)	0.1601
TIMP-1	263.3 (223.2, 307.2)	311.2 (260.3, 388.9)	<0.0001	-8.2 (-22.4, 6.1)	11.6 (5.0, 18.2)	0.0087
Hyaluronic acid	90.41 (51.57, 159.59)	154.65 (90.08, 272.78)	<0.0001	64.15 (14.71, 113.58)	121.56 (98.43, 144.70)	0.0275
FIB-4	2.03 (1.28, 2.69)	2.70 (1.90, 4.00)	<0.0001	0.23 (-0.01, 0.46)	0.45 (0.34, 0.56)	0.0645
NFS	0.26 (-0.74, 1.15)	0.90 (0.02, 1.83)	<0.0001	0.24 (0.12, 0.35)	0.31 (0.26, 0.36)	0.2188
Liver stiffness by VCTE, kPa [†]	14.0 (10.9, 20.2)	21.8 (15.7, 31.6)	<0.0001	-3.9 (-6.5, -1.4)	0.4 (-1.3, 2.2)	<0.0001
Standard Histologic Parameters and HVPG						
Ishak stage 6 fibrosis, n (%)	80 (45)	628 (66)	<0.0001	--	--	--
Hepatic collagen content, %	9.6 (6.8, 12.7)	11.2 (7.8, 15.9)	0.0002	-6.3 (-7.4, -5.3)	-0.1 (-0.6, 0.4)	<0.0001
α -SMA expression, %	10.7 (7.8, 15.2)	14.6 (9.8, 22.2)	<0.0001	-5.9 (-7.2, -4.5)	0.6 (0.0, 1.2)	<0.0001
Steatosis grade 1-3	165/174 (95)	856/950 (90)	0.0459	--	--	--
Lobular inflammation grade 3	80/174 (46)	465/950 (49)	0.51	--	--	--
Hepatocellular ballooning 2	120/174 (69)	705/950 (74)	0.16	--	--	--
HVPG, mm Hg [§]	8.3 (6.0, 10.0)	12.5 (9.5, 17.0)	<0.0001	-1.4 (-3.0, 0.1)	0.2 (-0.2, 0.7)	0.0454
Machine Learning (ML) Fibrosis Parameters[‡]						
ML NASH CRN fibrosis score	3.0 (2.5, 3.3)	3.2 (2.9, 3.5)	<0.0001	-0.36 (-0.45, -0.27)	0.04 (0, 0.090)	<0.0001
Proportionate area of F4, %	38.6 (18.8, 54.9)	53.8 (36.4, 68.0)	<0.0001	-14.45 (-17.95, -10.96)	3.05 (1.32, 4.79)	<0.0001
Proportionate area of F3, %	27.9 (20.1, 35.7)	25.0 (17.9, 33.8)	0.0270	2.84 (0.95, 4.73)	-2.22 (-3.16, -1.29)	<0.0001

Proportionate area of F2, %	12.9 (8.0, 21.2)	9.3 (5.6, 14.7)	<0.0001	4.73 (3.21, 6.26)	-1.08 (-1.84, -0.32)	<0.0001
Proportionate area of F1, %	7.9 (3.8, 15.8)	4.8 (2.2, 9.7)	<0.0001	5.57 (3.94, 7.20)	-0.03 (-0.85, 0.78)	<0.0001

* LSmeans, 95% CI, and p-values by ANCOVA with adjustment for baseline value and study. Change from baseline up to clinical event in patients with events or last available value.

† Liver stiffness by VCTE available at baseline in 40 patients in SIM study and 694 patients in STELLAR-4.

§ HVPG measured only in the simtuzumab study.

‡ Machine learning histological parameters available at baseline in 169 patients in SIM study and 796 patients in STELLAR-4.

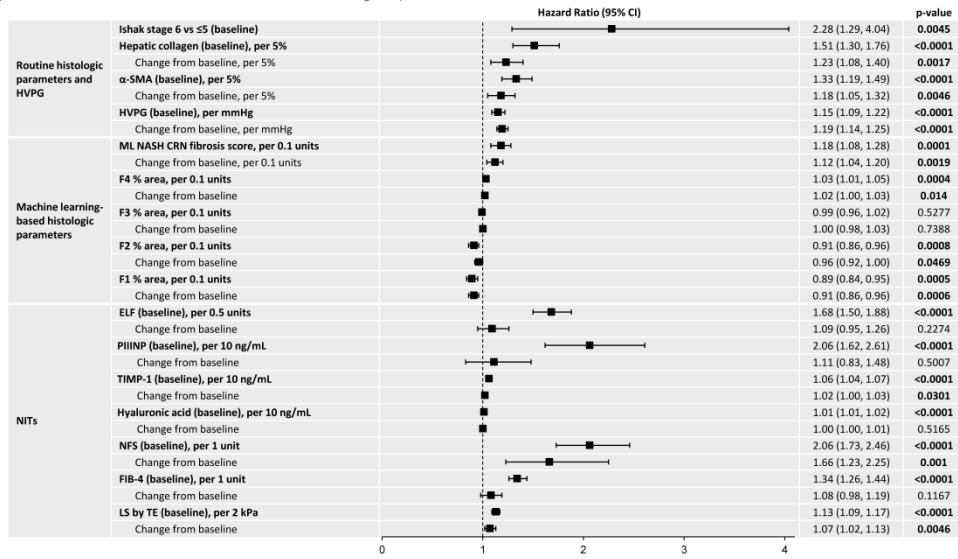
FIGURE LEGENDS

Figure 1. Associations between fibrosis-related histological parameters and NITs with time to first liver-related clinical event. Separate multivariate models run with baseline and change from baseline for each variable. Models for change adjusted for baseline value.

Figure 2. Liver-related clinical events according to baseline Ishak fibrosis stage

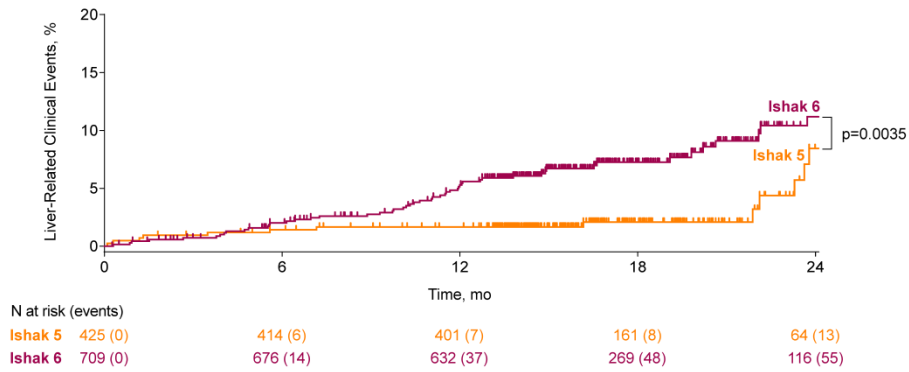
Figure 3. Association between fibrosis regression and liver-related clinical events. Hazard ratios (HR) for clinical events with fibrosis regression vs no fibrosis regression (reference). p-values by Fisher's exact test.

Figure 1. Associations between fibrosis-related histological parameters and NITs with time to first liver-related clinical event



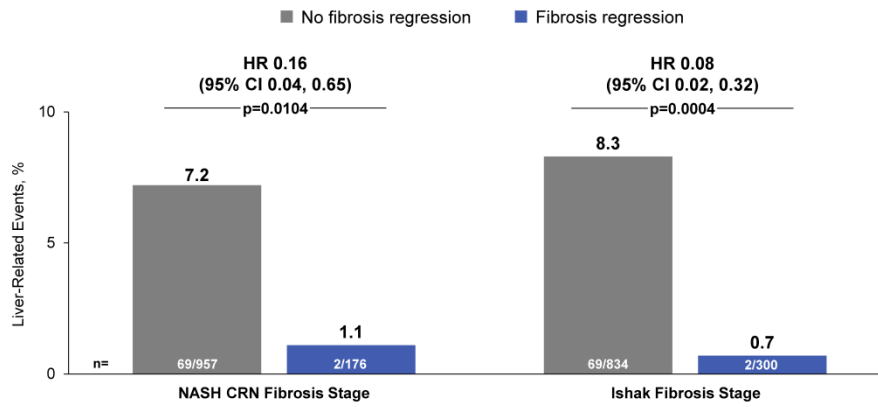
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Figure 2. Liver-related clinical events according to baseline Ishak fibrosis stage



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Figure 3. Association between fibrosis regression and liver-related clinical events



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