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The ASK1 Inhibitor Selonsertib in Patients With Nonalcoholic Steatohepatitis: A Randomized, Phase 2 Trial

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Inhibition of apoptosis signal–regulating kinase 1, a serine/threonine kinase, leads to improvement in inflammation and fibrosis in animal models of nonalcoholic steatohepatitis. We evaluated the safety and efficacy of selonsertib, a selective inhibitor of apoptosis signal–regulating kinase 1, alone or in combination with simtuzumab, in patients with nonalcoholic steatohepatitis and stage 2 or 3 liver fibrosis. In this multicenter phase 2 trial, 72 patients were randomized to receive 24 weeks of open-label treatment with either 6 or 18 mg of selonsertib orally once daily with or without once-weekly injections of 125 mg of simtuzumab or simtuzumab alone. The effect of treatment was assessed by paired pretreatment and posttreatment liver biopsies, magnetic resonance elastography, magnetic resonance imaging–estimated proton density fat fraction, quantitative collagen content, and noninvasive markers of liver injury. Due to the lack of effect of simtuzumab on histology or selonsertib pharmacokinetics, selonsertib groups with and without simtuzumab were pooled. After 24 weeks of treatment, the proportion of patients with a one or more stage reduction in fibrosis in the 18-mg selonsertib group was 13 of 30 (43%; 95% confidence interval, 26-63); in the 6-mg selonsertib group, 8 of 27 (30%; 95% confidence interval, 14-50); and in the simtuzumab-alone group, 2 of 10 (20%; 95% confidence interval, 3-56). Improvement in fibrosis was associated with reductions in liver stiffness on magnetic resonance elastography, collagen content and lobular inflammation on liver biopsy, as well as improvements in serum biomarkers of apoptosis and necrosis. There were no significant differences in adverse events between the treatment groups. *Conclusion:* These findings suggest that selonsertib may reduce liver fibrosis in patients with nonalcoholic steatohepatitis and stage 2-3 fibrosis. (HEPATOLOGY 2018;67:549-559).

Nonalcoholic steatohepatitis (NASH) is a progressive liver disease characterized by hepatic steatosis, lobular inflammation, ballooning, and perisinusoidal fibrosis in individuals who consume little or no alcohol and who do not have a secondary cause of steatosis.[1,2] NASH is estimated to affect up to 5% of the population in the United States.[3-5] Between 30% and 50% of patients with NASH develop progressive fibrosis,

Abbreviations: ALT, alanine aminotransferase; ASK1, apoptosis signal–regulating kinase 1; AST, aspartate aminotransferase; CI, confidence interval; CRN, Clinical Research Network; JNK, c-Jun N-terminal kinase; MRE, magnetic resonance elastography; MRI-PDFF, magnetic resonance imaging–estimated proton density fat fraction; NAS, nonalcoholic fatty liver disease activity score; NASH, nonalcoholic steatohepatitis; α-SMA, α-smooth muscle actin.

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which increases the risk for cirrhosis, liver decompensation, and hepatocellular carcinoma.[6-9] In recent years, NASH has become a leading indication for liver transplantation.[10] Emerging data suggest that fibrosis is the most important predictor of all-cause and liver-related mortality in NASH; the risk of liver-related mortality increases with increase in the stage of fibrosis.[11-14] An effective and safe pharmacologic treatment to halt or reverse fibrosis in NASH would address an important unmet medical need.[15-19]

Ballooned hepatocytes, representing the activation of apoptosis-related pathways, are a hallmark of NASH and fibrosis progression.[3,20] In the setting of oxidative stress, activation of apoptosis signal–regulating kinase 1 (ASK1), a serine/threonine signaling kinase, can lead to phosphorylation of p38 mitogen-activated kinase and c-Jun N-terminal kinase (JNK), leading in turn to activation of stress response pathways that worsen hepatic inflammation, apoptosis, and fibrosis.[21-23] Inhibition of ASK1 has therefore been proposed as a target for the treatment of NASH. In a murine model of NASH, selonsertib (formerly GS-4997), a selective inhibitor of ASK1, significantly improved not only metabolic parameters associated with NASH but also reduced hepatic steatosis, inflammation, and fibrosis.[21] Simtuzumab is a humanized monoclonal antibody directed against lysyl oxidase-like molecule 2, an enzyme that catalyzes the crosslinkage of collagen and elastin, leading to remodeling of the extracellular matrix.[24] When the current study was designed, it was hypothesized that inhibition of lysyl oxidase-like molecule 2 with simtuzumab could have an antifibrotic effect in NASH. Moreover, preclinical data from a murine model of advanced fibrosis suggested that simtuzumab has an additive effect when combined with an ASK1 inhibitor.[25] However, results from phase 2 studies of simtuzumab monotherapy—one in patients with F3 fibrosis (NCT01672866) and another in patients with cirrhosis (NCT01672879)—which became available after the start of the current trial, indicated that simtuzumab was ineffective at reducing hepatic fibrosis.[26]

Currently, the diagnosis of NASH requires a liver biopsy demonstrating the histologic features of steatosis, lobular inflammation, ballooning, and variable degrees of fibrosis.[1] Recent studies have demonstrated that the presence of fibrosis is the only independent factor associated with mortality and liver-related clinical complications in patients with NASH.[13] Although liver biopsy is the current gold standard for NASH diagnosis, its limitations include sampling error due to variable distribution of fibrosis and the invasive nature of the procedure. Noninvasive approaches, including imaging and serum tests, are being explored.[27,28] Estimations of liver stiffness by magnetic resonance elastography (MRE) and of the quantity of hepatic fat by magnetic resonance imaging-estimated proton density fat fraction (MRI-PDFF) have shown strong correlations with histology and low interobserver variability, suggesting that these noninvasive methods may be reliable, accurate, and precise tools for clinical assessment of the extent of liver fibrosis.[29-34]

We conducted a phase 2, randomized, open-label study to assess the safety and efficacy of selonsertib with and without simtuzumab or simtuzumab alone in patients with moderate to severe fibrosis resulting from NASH. This trial included longitudinal paired assessment with advanced MRI-derived parameters including MRI-PDFF and MRE along with liver biopsy, all assessed centrally by blinded reviewers in a multicenter setting.

Patients and Methods

PATIENTS

Patients 18-70 years of age were enrolled at 23 sites in the United States and Canada from June 8, 2015, to

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March 31, 2016. All patients provided written informed consent, and the protocol was approved by individual institutional review boards.

The patients included in the trial must have had a liver biopsy within 3 months of screening consistent with a diagnosis of NASH, with presence of either stage 2 or stage 3 fibrosis based upon the NASH Clinical Research Network (CRN) Histologic Scoring System and a nonalcoholic fatty liver disease activity score (NAS) of 5 or higher, with a score of at least 1 for each of the three components (steatosis, hepatocellular ballooning, and lobular inflammation).[1] Patients were also required to have at least three of the following features of the metabolic syndrome: abdominal obesity, hypertension, elevated fasting glucose, elevated levels of serum triglycerides, or low levels of high-density lipoprotein cholesterol (see Supporting Information for ranges). Eligible patients had levels of alanine aminotransferase (ALT) no more than 5 times the upper limit of normal and a liver stiffness by transient elastography (FibroScan; Echosens, Paris, France) of at least 7 kPa. The FibroScan requirement did not apply to patients who qualified on the basis of a historical biopsy.

Key exclusion criteria were presence of cirrhosis or history of decompensated liver disease, as well as positive tests for hepatitis B surface antigen or human immunodeficiency virus RNA. Patients with other causes of chronic liver disease or secondary causes of hepatic steatosis were also excluded. Alcohol consumption in excess of 21 ounces per week for men or 14 ounces per week for women was excluded.

STUDY DESIGN

In this multicenter, open-label trial, patients were randomly assigned in a 2:2:1:1:1 ratio to receive 24 weeks of treatment with 6 mg or 18 mg of selonsertib alone, 6 mg or 18 mg of selonsertib with 125 mg of simtuzumab, or 125 mg of simtuzumab alone. Selonsertib was administered orally once daily with or without food. Simtuzumab was self-administered by subcutaneous injection once a week. Randomization and treatment assignment were managed with an Interactive Web Response System using sequentially numbered containers (Bracket, San Francisco, CA) and a block size of 7. Randomization was stratified by presence or absence of diabetes mellitus. Screening assessments included medical history and physical examination, as well as standard laboratory and clinical testing.

ASSESSMENTS AND OUTCOMES

Histology

The presence of NASH was assessed in a blinded fashion by a central pathologist (Z.D.G.) with extensive experience in assessing treatment response in NASH clinical trials. Biopsy specimens were graded according to the NAS, a standardized grading system for steatosis (on a scale of 0-3), lobular inflammation (on a scale of 0-3), and hepatocellular ballooning (on a scale of 0-2), with higher scores indicating increasing disease activity. Improvement in liver fibrosis was defined as a reduction of one or more stage using the NASH CRN Histologic Scoring System. Additional histologic outcomes included progression to cirrhosis and improvement in the NAS and its individual components. Computer-assisted morphometry was also used to quantify hepatic collagen and fat content using picrosirius red–stained liver sections, as well as α-smooth muscle actin (α-SMA) expression.[35]

Imaging Assessments

In this trial, longitudinal changes in liver fat and liver stiffness from baseline to week 12 and end of study were assessed using MRI-PDFF and two-dimensional MRE (60 Hz), respectively. Assessments were performed by an experienced central reader (University of California San Diego Radiology Reading Center) blinded to treatment assignments and clinical and histologic data.[35,37] The methodology and assessments of changes in these parameters in colocalized regions of interest were performed as described.[29-34,37,38] All sites underwent a quality-assessment process prior to study initiation, and all images were approved by the central reader. We determined the proportion of patients in each treatment group who had at least a 30% decrease between baseline and week 24 in MRI-PDFF[40] and at least a 15% reduction in MRE.[41] Liver stiffness was also measured by FibroScan.

Serum Biomarkers

Changes from baseline in noninvasive markers of fibrosis, including the Enhanced Liver Fibrosis test (Siemens, Tarrytown, NY) and FibroSure/FibroTest (LabCorp, Burlington, NC), were assessed, as well as changes in markers of liver injury and function, including serum ALT, aspartate aminotransferase (AST), bilirubin, gamma-glutamyltransferase, and alkaline

phosphatase. Changes from baseline in serum levels of cytokeratin-18 M30 and M65 fractions were measured as indicators of hepatocellular apoptosis and necrosis, respectively, using the M30 Apoptosense and M65 EpiDeath enzyme-linked immunosorbent assays (Diapharma, West Chester, OH).

Safety

Safety was assessed by the incidence of treatment-emergent adverse events, including serious adverse events, clinical laboratory tests, and vital signs collected at every visit from first dose to 30 days after the last dose of study drug. The number and percentage of patients who prematurely discontinued the study drug or the study owing to adverse events were calculated.

STATISTICAL ANALYSIS

The sample size assessment was based on clinical experience with other proof-of-concept studies to assess directionality across multiple outcomes rather than statistical significance. Descriptive statistics were calculated for continuous exploratory parameters by treatment group. For categorical variables, descriptive statistics with count, percentage of patients in each category, and 95% confidence intervals (CIs) for the percentages within each treatment group based on Clopper-Pearson were calculated.

Safety was assessed in all patients who received at least one dose of study drug. The effect of treatment on histologic endpoints was assessed in all patients with evaluable liver biopsy results at baseline and week 24. Given the demonstrated lack of efficacy of simtuzumab,[26] we conducted an analysis pooling the results from selonsertib groups with and without simtuzumab. Results by the original unpooled dose groups are provided in the Supporting Information.

We also conducted exploratory logistic regression analyses to assess associations between fibrosis improvement and changes from baseline at week 24 in select factors (after adjustment for baseline values).

STUDY OVERSIGHT

This study was approved by the institutional review board or independent ethics committees at all participating sites and conducted in compliance with the Declaration of Helsinki, Good Clinical Practice guidelines, and local regulatory requirements. The study was designed and conducted according to protocol by the sponsor (Gilead) in collaboration with the principal investigators. The sponsor collected the data, monitored study conduct, and performed the statistical analyses. An independent data-monitoring committee reviewed the progress and provided oversight of the study. The investigators, participating institutions, and sponsor agreed to maintain confidentiality of the data. All authors had access to the data and assumed responsibility for the integrity and completeness of the reported data. A professional writer employed by Gilead Sciences helped prepare the manuscript with input from the authors, and all authors approved the final manuscript. The study protocol is available with this article.

Results

STUDY PATIENTS

Of the 242 patients who were screened, 72 with NASH and stage 2 or 3 fibrosis on biopsy were randomized into this multicenter trial (reasons for screen failure are provided in Supporting Table S1). The majority (65%) of patients had stage 3 fibrosis and a NAS $\geq$6 (71%) at baseline. Overall, 86% had severe hepatocellular ballooning. Detailed characteristics of the study cohort are provided in Table 1. Approximately two thirds (69%) of patients were female, the median age was 56 years, and 71% of patients had diabetes mellitus. The overall median body mass index was 33 kg/m^2; the median body mass index in the simtuzumab-alone group was nonsignificantly higher at 37 kg/m^2. Otherwise, there were no substantial differences between the study cohorts across the treatment groups at baseline.

EFFICACY

Histologic Outcomes

The proportion of patients with at least a one-stage reduction in fibrosis after 24 weeks of treatment in the 18-mg selonsertib group was 13 of 30 (43%; 95% CI, 26-63); in the 6-mg selonsertib group, 8 of 27 (30%; 95% CI, 14-50); and in the simtuzumab-alone group, 2 of 10 (20%; 95% CI, 3-56) (Fig. 1). The proportion with progression to cirrhosis at week 24 in the 18-mg selonsertib group was 1 of 30 (3%; 95% CI, 0-17); in the 6-mg selonsertib group, 2 of 27 (7%; 95% CI, 1-24); and in the simtuzumab-alone group, 2 of 10 (20%; 95% CI, 3-56). Fibrosis responses according to

TABLE 1. Baseline Demographic and Clinical Characteristics

Characteristic	Selonsertib 18 mg ± Simtuzumab (n = 32)	Selonsertib 6 mg ± Simtuzumab (n = 30)	Simtuzumab (n = 10)
Demographic factors			
Age, years	55 (49-61)	54 (46-62)	57 (56-59)
Female sex	22 (69)	22 (73)	6 (60)
White race	28 (88)	27 (90)	10 (100)
Diabetes mellitus	21 (66)	22 (73)	8 (80)
Serum biochemical levels			
ALT, U/L	69 (52-93)	56 (46-91)	61 (46-83)
AST, U/L	48 (37-71)	50 (36-82)	56 (37-76)
Gamma-glutamyl transferase, U/L	52 (38-83)	62 (40-78)	48 (35-86)
Alkaline phosphatase, U/L	86 (73-100)	88 (64-101)	66 (53-97)
Total bilirubin, mg/dL	0.5 (0.4-0.6)	0.4 (0.3-0.5)	0.5 (0.4-0.6)
Metabolic factors			
Body mass index, kg/m^2	33 (30-37)	33 (29-41)	37 (31-37)
Lipids			
Triglycerides, mg/dL	181 (138-271)	176 (125-232)	178 (111-238)
Total cholesterol, mg/dL	187 (151-211)	201 (170-213)	183 (168-196)
High-density lipoprotein, mg/dL	40 (33-49)	43 (36-52)	42 (36-44)
Low-density lipoprotein, mg/dL	111 (80-135)	122 (109-143)	110 (100-123)
Noninvasive measures			
MRI-PDFF, %	18 (11-24)	17 (11-22)	16 (9-20)
MRE, kPa	3.7 (2.9-4.8)	3.7 (3.1-4.5)	3.4 (3.0-4.3)
FibroSure/FibroTest	0.35 (0.16-0.49)	0.29 (0.20-0.46)	0.38 (0.28-0.61)
FibroScan, kPa	10.4 (8.5-14.9)	11.0 (8.3-11.3)	13.5 (9.4-15.0)
Liver histologic findings			
NASH CRN fibrosis stage 3	21 (66)	20 (67)	6 (60)
NAS 6-8	22 (69)	24 (80)	5 (50)
Steatosis grade 2-3	13 (41)	9 (30)	2 (20)
Lobular inflammation grade 3	21 (66)	23 (77)	5 (50)
Hepatocyte ballooning grade 2	26 (81)	27 (90)	9 (90)
Hepatic collagen content, %	3.8 (2.6-4.8)	4.3 (2.8-5.6)	3.5 (2.4-5.8)
α-SMA, %	3.2 (2.0-6.4)	3.8 (2.3-7.2)	4.9 (2.3-9.9)

Values are either n (%) or median (interquartile range).

the original five unpooled treatment groups are provided in Supporting Fig. S2.

Median percent change from baseline at week 24 in morphometric collagen content for patients receiving 18 and 6 mg of selonsertib was −8.7% and −8.2%, respectively, compared with an increase of 2.1% in those receiving simtuzumab alone. Changes in fibrosis stage correlated with changes in hepatic collagen content by morphometry ($r = 0.54$, $P < 0.001$).

The proportion of patients with improvement of ≥1 point in the NAS in the 18-mg selonsertib group was 16 of 31 patients (52%); in the 6-mg selonsertib group, 11 of 27 patients (41%); and in the simtuzumab group, 6 of 10 (60%). Improvements were noted in all three components of the NAS—steatosis, lobular inflammation, and hepatocellular ballooning (Table 2)—but did not differ substantially by treatment group.

Factors Associated With Fibrosis Improvement

To identify factors associated with improvement in fibrosis at week 24, we performed exploratory logistic regression analyses of changes from baseline to week 24 in several factors (see Fig. 2). Changes in body weight were not associated with changes in fibrosis. After adjustment for baseline values, an improvement in the NAS, as well as relative reductions in hepatic collagen content, α-SMA expression, liver stiffness by FibroScan, gamma-glutamyltransferase, and the M30 and M65 fractions of cytokeratin-18 were significantly associated with fibrosis improvement (Fig. 2). Fibrosis responders also had greater reductions in liver stiffness by MRE and were more likely to have improvement or no change in the lobular inflammation component of

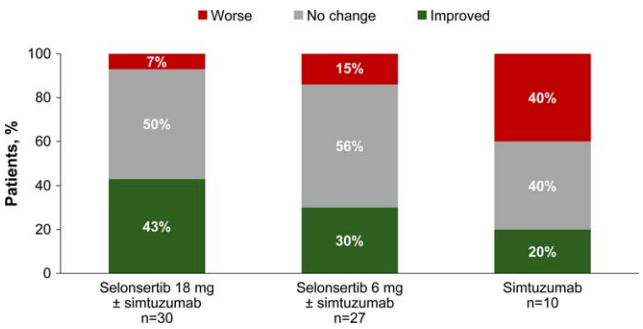

FIG. 1. Proportions of patients with improved, unchanged, or worse fibrosis from baseline to week 24. Fibrosis improvement is defined as a decrease of at least one stage in fibrosis according to the NASH CRN Histologic Scoring System. Data are shown for the 67 patients with liver biopsies evaluable for fibrosis at baseline and week 24.

the NAS compared with worsening (odds ratio, 4.80; 95% CI, 0.68-33.80; $P = 0.12$), but these differences were not statistically significant.

Imaging Outcomes

The proportion of patients who achieved ≥15% relative reductions in liver stiffness by MRE from baseline to week 24 in the 18-mg selonsertib group was 4 of 26 (15%); in the 6-mg selonsertib group, 7 of 22 (32%); and in the simtuzumab-alone group, 0 of 10. Compared with fibrosis nonresponders, fibrosis responders had nonsignificantly greater reductions in liver stiffness by MRE (median –2.3% versus 3.0%; $P = 0.17$; Fig. 3A). Changes in liver stiffness by FibroScan were not associated with selonsertib treatment (Table 3) or with fibrosis improvement. The proportion of patients who achieved ≥30% relative reductions in steatosis by MRI-PDFF from baseline to week 24 in the 18-mg selonsertib group was 8 of 31 (26%); in the 6-mg selonsertib group, 3 of 24 (13%); and in the simtuzumab-alone group, 1 of 10 (10%). Patients with improved steatosis on biopsy had greater reductions in MRI-PDFF compared to those without steatosis improvement (median –20.2% versus –0.8%; $P = 0.01$; Fig. 3B).

Percentage change from baseline in MRE and MRI-PDFF, as well as proportions of patients achieving a ≥15% reduction in MRE and a ≥30% reduction in MRI-PDFF by week 12 of treatment, is shown in Supporting Table S3.

LIVER BIOCHEMISTRY

Across all groups, decreases from baseline in median ALT and gamma-glutamyltransferase were observed at week 24 (Table 3). Greater median relative decreases in cytokeratin-18 M30 and M65 fractions were observed in patients receiving 18 mg of selonsertib (−31% and −44%, respectively) and in those receiving 6 mg of selonsertib (−6% and −35%, respectively) than among patients receiving simtuzumab alone (22% and −4%, respectively). Changes from baseline in Enhanced Liver Fibrosis scores were minimal in all treatment groups (Table 3).

TABLE 2. Change in Histology and Imaging From Baseline to Week 24

	Selonsertib 18 mg ± Simtuzumab (n = 32)	Selonsertib 6 mg ± Simtuzumab (n = 30)	Simtuzumab (n = 10)
Histology			
Patients with improvement in fibrosis (CRN)	13/30 (43)	8/27 (30)	2/10 (20)
Patients with progression to cirrhosis	1/30 (3)	2/27 (7)	2/10 (20)
Hepatic collagen content (% change from baseline)	−8.7 (−48.7 to 29.6)	−8.2 (−48.1 to 40.0)	2.1 (−27.3 to 37.9)
Patients with ≥1 point reduction in NAS	16/31 (52)	11/27 (41)	6/10 (60)
Patients with ≥2 point reduction in NAS	7/31 (23)	5/27 (19)	2/10 (20)
Steatosis ≥1 point reduction	10/31 (32)	8/27 (30)	2/10 (20)
Lobular inflammation ≥1 point reduction	10/31 (32)	6/27 (22)	2/10 (20)
Ballooning ≥1 point reduction	5/31 (16)	9/27 (33)	3/10 (30)
Imaging			
MRI-PDFF, % change from baseline	−4.55 (−32.72 to 20.87)	−6.67 (−21.66 to 15.25)	−12.72 (−24.62 to −6.53)
Patients with ≥30% reduction	8/31 (26)	3/24 (13)	1/10 (10)
MRE, % change from baseline	1.79 (−10.96 to 11.19)	−0.09 (−19.00 to 8.10)	2.06 (−8.65 to 22.33)
Patients with ≥15% reduction	4/26 (15)	7/22 (32)	0
FibroScan, kPa	0.20 (−3.50 to 1.40)	−0.80 (−1.90 to 2.30)	−0.50 (−3.80 to 3.40)

Values are either n/N (%) or median (interquartile range).

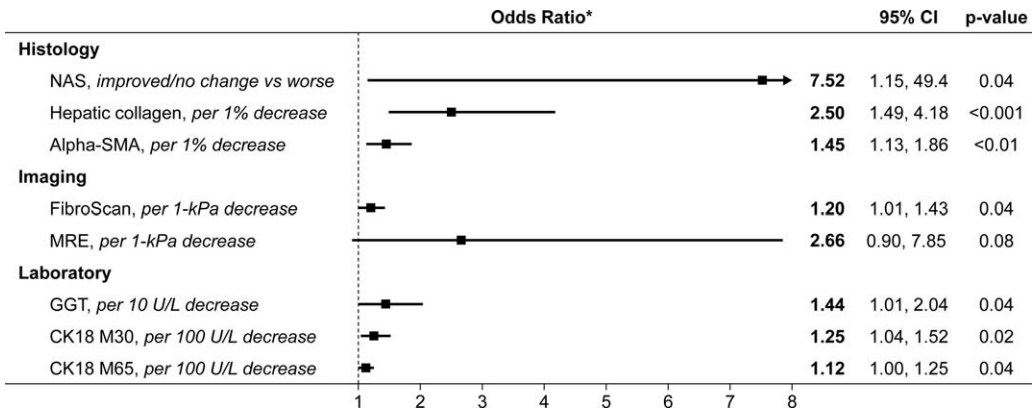

FIG. 2. Factors associated with fibrosis improvement. Changes in body weight, MRI-PDFF, and grades of steatosis and ballooning were not associated with fibrosis improvement. *All odds ratios were adjusted for baseline values. Abbreviations: CK18, cytokeratin-18; GGT, gamma-glutamyltransferase.

SAFETY

Table 4 provides a summary of safety results. The majority of patients in all three treatment groups experienced at least one adverse event, most of which were mild to moderate in severity. Higher proportions of patients in the selonsertib groups experienced headache, nausea, sinusitis, nasopharyngitis, upper abdominal pain, back pain, and fatigue. Three selonsertib-treated patients discontinued treatment owing to adverse events (worsening schizophrenia, numbness of face and upper extremities, and elevated liver enzymes). Five patients experienced serious adverse events (see Table 4). No single serious adverse event was experienced by more than 1 patient.

Treatment-emergent grade 3 and 4 laboratory abnormalities are also provided in Table 4. Four patients developed a transient increase of ALT or AST of at least 2 × baseline and at least 3 × upper limit of normal. Three of these 4 patients had grade 2 elevations at baseline. Only 1 patient (mentioned above) discontinued study treatment owing to elevated liver enzymes.

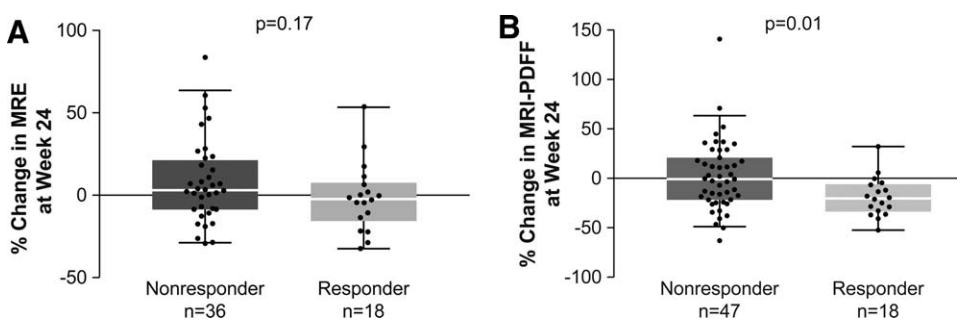

FIG. 3. Change from baseline in MRE and MRI-PDFF from baseline to week 24. Central line represents median value, box represents interquartile range, and whiskers show range not including outliers, which are represented by dots. (A) Change in MRE stiffness from baseline to week 24 in fibrosis responders and nonresponders. Fibrosis response was defined as a reduction of one or more stage in fibrosis. (B) Change in MRI-PDFF from baseline to week 24 in steatosis responders and nonresponders. Steatosis response was defined as a reduction of one or more grade in steatosis.

TABLE 3. Change in Serum Biomarkers and Metabolic Factors From Baseline to Week 24

	Selonsertib 18 mg ± Simtuzumab (n = 32)	Selonsertib 6 mg ± Simtuzumab (n = 30)	Simtuzumab (n = 10)
Enhanced Liver Fibrosis test	0.02 (–0.34 to 0.52)	–0.07 (–0.46 to 0.36)	–0.13 (–0.35 to 0.05)
FibroSure/FibroTest	–0.01 (–0.03 to 0.03)	0.02 (–0.03 to 0.08)	0.01 (–0.04 to 0.05)
ALT, U/L	–8 (–24 to 23)	–6 (–29 to 7)	–3 (–16 to –1)
AST, U/L	–5 (–13 to 13)	–4 (–25 to 17)	–3 (–28 to –1)
Gamma-glutamyltransferase, U/L	–7 (–19 to 5)	–2 (–15 to 11)	–2 (–9 to 2)
Triglycerides, mg/dL	–21 (–41 to 29)	12 (–6 to 32)	–30 (–9 to 28)
Total cholesterol, mg/dL	–10 (–33 to 8)	–5 (–24 to 14)	–13 (–36 to 2)
High-density lipoprotein, mg/dL	–2 (–3 to 1)	1 (–5 to 5)	2 (–4 to 5)
Low-density lipoprotein, mg/dL	–10 (–24 to 6)	–5 (–19 to 9)	–25 (–31 to 0)
HOMA-IR	0.98 (–2.4 to 7.63)	2.17 (0.16-4.77)	–0.22 (–1.90 to 0.12)
Hemoglobin A1c, %	–0.2 (–0.5 to 0.2)	0.2 (0.0-0.5)	–0.2 (–1.1 to 0.6)
CK-18 fractions			
M30, U/L	–110 (–338 to 124)	–34 (–445 to 241)	–89 (–378 to 146)
M65, U/L	–222 (–811 to 238)	–162 (–820 to 341)	–185 (–820 to 251)

Values are median (interquartile range)
Abbreviations: CK, cytokeratin; HOMA-IR, homeostatic model assessment for insulin resistance.

Discussion

In this multicenter, phase 2 trial using paired longitudinal assessment of treatment response with liver biopsy as well as advanced MRI methods including MRE and MRI-PDFF, selonsertib-treated patients had numerically higher rates of fibrosis improvement and lower rates of fibrosis progression than patients

TABLE 4. Safety

	Selonsertib 18 mg ± Simtuzumab (n = 32)	Selonsertib 6 mg ± Simtuzumab (n = 30)	Simtuzumab (n = 10)
Patients with adverse events	24 (75)	26 (87)	7 (70)
Patients with grade 3-4 adverse events	3 (9)	1 (3)	1 (10)
Patients with serious adverse events	3 (9)*	2 (7)†	0
Patients who discontinued study treatment due to adverse event	2 (6)	1 (3)	0
Most common adverse events			
Headache	9 (28)	4 (13)	0
Nausea	6 (19)	4 (13)	0
Sinusitis	4 (13)	3 (10)	1 (10)
Nasopharyngitis	3 (9)	4 (13)	0
Upper abdominal pain	5 (16)	1 (3)	0
Fatigue	5 (16)	1 (3)	0
Grade 3-4 laboratory abnormalities‡	9 (28)	5 (17)	4 (40)
Lymphocytes <500 mm^3	1 (10)	0	0
Hypocalcemia <7.0 mg/dL	0	0	1 (10)
Alkaline phosphatase >5 × ULN	1 (3)	0	0
ALT >5 × ULN	2 (6)	0	0
AST >5 × ULN	2 (6)	0	0
Gamma-glutamyl transferase >5 × ULN	2 (6)	1 (3)	0
Phosphate <1.5 mg/dL	1 (3)	0	0
Serum glucose >250 mg/dL	2 (6)	4 (13)	2 (20)
Glomerular filtration rate <30 mL/minute/1.73 m^2	0	0	1 (10)
INR >2.5 × ULN	1 (3)	0	0
Triglycerides >500 mg/dL	2 (6)	0	1 (10)

*A 59-year-old woman had a transient ischemic attack, a 54-year-old woman had hypoesthesia, and a 52-year-old woman had two serious adverse events: abdominal pain and influenza.
†A 32-year-old woman experienced a serious adverse event of rectal bleeding, and a 57-year-old man experienced seven serious adverse events: chest pain, bronchitis, congestive cardiac failure, pneumonia, sepsis, and two events of dyspnea.
‡Values that were increased at least one toxicity grade from baseline at any time postbaseline, up to and including the last dosing date plus 30 days.
Abbreviations: INR, international normalized ratio; ULN, upper limit of normal.

treated with simtuzumab alone over a 24-week treatment period. These findings suggest that selonsertib may reduce liver fibrosis in patients with NASH and moderate to severe fibrosis. The novelty of this proof-of-concept trial includes its use of standardized assessments of treatment response using MRI-PDFF and MRE with central reading of the images in a colocalized manner. Moreover, this study evaluated a combination of drugs with distinct mechanisms of action in patients with NASH.

Selonsertib is a selective inhibitor of ASK1, a ubiquitously expressed serine/threonine kinase which is activated by oxidative stress to promote hepatocellular apoptosis, inflammation, and fibrosis.[21,41] ASK1 is normally bound and repressed by thiol-containing antioxidant proteins, including thioredoxin 1.[42] Pathological conditions that increase oxidative stress result in ASK1 autoactivation, resulting in downstream phosphorylation of p38 and JNK, which mediate diverse cellular responses by phosphorylating cytosolic substrates and nuclear transcription factors including activating transcription factor 2 and c-Jun.[43] Both p38 and JNK have well-characterized roles in hepatocytes, macrophages, and myofibroblasts to promote lipotoxicity, inflammation, and fibrosis. In preclinical models of NASH, genetic deletion[22,23] or pharmacological inhibition of ASK1[21] reduces p38 and JNK phosphorylation, resulting in reduced hepatic steatosis, inflammation, and fibrosis. Based on the complementary mechanisms of action of selonsertib and simtuzumab, the heterogeneity of patients with NASH, and preclinical data suggesting a benefit of dual therapy over the single agents in a murine model of advanced fibrosis,[25] we hypothesized that a combination of agents would improve efficacy. However, the addition of simtuzumab to selonsertib had no discernible benefit.

Given that fibrosis stage is the most important determinant of outcome in patients with NASH,[11-13] the possible antifibrotic effect of selonsertib may be clinically significant. Patients who responded to selonsertib, i.e., those who had an improvement of one or more stage from baseline to week 24 according to the NASH CRN Histologic Scoring System, had consistent reductions in other markers of fibrosis, including hepatic collagen content, liver stiffness by MRE, and α-SMA expression. The consistency and directionality of these responses suggest that these findings are not likely to be due to sampling error of liver biopsy. The decreased expression of α-SMA suggests that decreased collagen formation through diminished stellate activation may have contributed to the observed attenuation of hepatic fibrosis in the selonsertib-containing treatment groups. Moreover, compared with nonresponders, fibrosis responders demonstrated improvements in liver biochemistry, serum markers of apoptosis and necrosis (cytokeratin-18 M30 and M65), and improvements in lobular inflammation and hepatic steatosis by morphometry. Although changes in liver stiffness by FibroScan were not significant, this may reflect the reduced precision and accuracy of FibroScan in obese patients.[44]

This randomized trial assessed changes in liver fat content by MRI-PDFF and liver stiffness by MRE, along with liver biopsy assessments before and after treatment, with results assessed by central reviewers blinded to treatment assignments. Liver fibrosis on histology was assessed using a comprehensive set of parameters including NASH CRN fibrosis stage, quantitative digital morphometry, and α-SMA immunostaining before and after treatment. The study population included patients with more active NASH and had a higher proportion of patients with advanced fibrosis than in previous phase 2 trials. It likely underscores a phenomenon that inclusion of patients with greater disease severity may help shorten the duration of phase 2 studies because the likelihood of improvement due to a type 2 error is low.

Generalization of the results of this study is limited by its small size and exploratory nature. The pooling of the selonsertib groups with and without simtuzumab was also not prespecified. The open-label design of this trial is unlikely to have affected the interpretation of the histology and imaging assessments because the central readers were blinded to group assignment. Although the trial did not include a placebo group, the simtuzumab-alone group served as a *de facto* placebo group due to lack of additive efficacy to selonsertib in this study and as monotherapy in two large phase 2b studies in patients with NASH and advanced fibrosis.[26]

In this phase 2 exploratory trial, selonsertib appeared to improve liver fibrosis in a substantial proportion of patients with NASH and stage 2 or 3 fibrosis, suggesting that it has the potential to help address an important unmet medical need for an effective antifibrotic therapy for patients with NASH and advanced fibrosis. Because fibrosis is a key predictor of liver mortality in nonalcoholic fatty liver disease, further studies are needed to assess the benefits of selonsertib in improving long-term outcomes associated with NASH-related fibrosis in a larger, randomized controlled trial. Phase 3 studies of selonsertib in patients with NASH and bridging fibrosis

(STELLAR-3; NCT03053050) and compensated cirrhosis (STELLAR-4; NCT03053063) are currently under way.

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