Supplementary Appendix

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The ASK1 Inhibitor Selonsertib in Patients with Nonalcoholic Steatohepatitis:

A Randomized, Phase 2 Trial

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Inclusion Criteria

Subjects must meet all of the following inclusion criteria to be eligible for participation in this study.

1) Males and non-pregnant, non-lactating females between 18-70 years of age; inclusive based on the date of the screening visit;

2) Willing and able to give informed consent prior to any study specific procedures being performed;

3) Meets the following conditions:

a) Presence of at least 3 of the following criteria for Metabolic syndrome: abdominal (central) obesity, elevated blood pressure or treatment of previously diagnosed hypertension, elevated fasting glucose or treatment for previously diagnosed type 2 diabetes, high serum triglycerides or specific treatment for this lipid abnormality, and low high-density lipoprotein (HDL) levels or specific treatment for this lipid abnormality AND

b) ALT $\leq 5 \times$ ULN, AND

c) Fibroscan: $\geq$7 kPa, except in subjects meeting criteria d by historical biopsy, AND

Note: For subjects who will have a liver biopsy performed during the screening period-criteria a, b and c have to be met before evaluating criteria d.

For subjects with a historical biopsy within 3 months of the screening visit- criteria a and b have to be met. Criteria c need not be met for these subjects but the Fibroscan must still be performed.

d) Liver biopsy consistent with NASH (NAS $\geq$5 with each component $\geq$1 (steatosis, ballooning, inflammation)) with fibrosis stage 2 to 3. A historical biopsy within 3 months of the screening visit may be accepted as the screening biopsy upon pre-approval by the Medical Monitor;

4) Platelet count $\geq$120,000/mm^3;

5) Serum creatinine <2.0 mg/dL;

6) Female subjects of childbearing potential must have a negative serum pregnancy test prior to starting study treatment;

7) All female subjects of childbearing potential who engage in heterosexual intercourse must agree to use a highly effective method of contraception during intercourse from the screening visit throughout the study period and for 90 days following the last dose of study drug. If females utilize hormonal agents as one of their contraceptive methods, the same hormonal methods must have been used for at least 1 month before study dosing. Females on hormonal methods must also utilize a barrier method as another form of contraception;

8) Male subjects must agree to use condoms during intercourse from the screening through the study completion and for 90 days following the last dose of study drug;

9) Male subjects must refrain from sperm donation from screening through at least 90 days following the last dose of study drug;

10) Female subjects must refrain from egg donation or harvest for 90 days after last dose of study drug;

11) Willing and able to comply with scheduled visits, drug administration plan, laboratory tests, liver biopsies, other study procedures, and study restrictions.

12) Must be able to read and complete Quality of Life questionnaires independently;

13) Adequate liver biopsy sample for evaluation by the central reader

Exclusion Criteria

Subjects who meet *any* of the following exclusion criteria are not to be enrolled in this study.

1) Pregnant or lactating females;

2) Cirrhosis of the liver (e.g. Brunt/Kleiner score of F4);

3) Other causes of liver disease including viral hepatitis and alcoholic liver disease;

4) Any history of decompensated liver disease, including ascites, hepatic encephalopathy or variceal bleeding;

5) History of liver transplantation;

6) Weight reduction surgery in the past;

7) BMI <18 kg/m^2;

8) INR >1.2 not due to therapeutic anticoagulation;

9) Total bilirubin >1 × ULN, except in confirmed cases of Gilbert's syndrome;

10) Chronic hepatitis B (HBsAg positive);

11) Chronic hepatitis C (HCV RNA positive);

12) HIV Ab positive;

13) Alcohol consumption greater than 21 oz/week for males or 14 oz/week for females (1oz/30mL of alcohol is present in one 12oz/360mL beer, one 4oz/120mL glass of wine, and a 1 oz/30mL measure of 40% proof alcohol);

14) Positive urine screen for amphetamines, cocaine or opiates (i.e. heroin, morphine) at screening. Subjects on stable methadone or buprenorphine maintenance treatment for at least 6 months prior to screening may be included in the study. Subjects with a positive urine drug screen due to prescription opioid-based medication are eligible if the prescription and diagnosis are reviewed and approved by the investigator;

15) Unstable cardiovascular disease as defined by any of the following:

a) Unstable angina within 6 months prior to screening

b) Myocardial infarction, coronary artery bypass graft surgery or coronary angioplasty within 6 months prior to screening

c) Transient ischemic attack or cerebrovascular accident within 6 months prior to screening

d) Obstructive valvular heart disease or hypertrophic cardiomyopathy

e) Congestive heart failure;

16) Immunosuppressive drugs including systemic corticosteroids, tacrolimus, sirolimus, cyclosporine, azathioprine, mycophenolate mofetil, and methotrexate (intra-articular, topical, nasal, or inhaled steroids are allowed) within 30 days of screening;

17) Use of the following CYP3A4 inhibitors: clarithromycin, conivaptan, grapefruit juice, itraconazole, ketoconazole, nefazodone, posaconazole, suboxone, telithromycin, voriconazole within 2 weeks of baseline;

18) Use of the following CYP3A4 inducers: carbamazepine, phenytoin, rifampin, St. John's Wort within 2 weeks of baseline;

19) History of a malignancy within 5 years of screening with the following exceptions:

Exclusion Criteria (continued)

a) Adequately treated carcinoma in situ of the cervix

b) Adequately treated basal or squamous cell cancer or other localized non-melanoma skin cancer;

20) Major surgical procedure within 30 days prior to screening or the presence of an open wound;

21) History of bleeding diathesis within 6 months of screening;

22) Any laboratory abnormality or condition that, in the investigator's opinion, could adversely affect the safety of the subject or impair the assessment of study results;

23) Participation in another investigational study of a drug or device within 1 month prior or within 5 half-lives of the prior investigational agent (whichever is longer) prior to screening;

24) Concurrent participation in another therapeutic clinical study;

25) Known hypersensitivity to the study drugs (GS-4997/SIM), the metabolites, or formulation excipient;

26) Presence of any condition that could, in the opinion of the investigator, compromise the subject's ability to participate in the study, such as history of substance abuse or a psychiatric or medical condition;

27) Unavailable for follow-up assessment or concern for subject's compliance with the protocol procedures;

28) Contraindications to MRI scanning (e.g. presence of permanent pacemakers, implanted cardiac devices, etc.)

Table S1. Reasons for Screen Failure

	Total
Screened Subjects	242
Screen Failure Subjects	170/242 (70.2%)
Screen Failure Subjects Who Did Not Meet Eligibility Criteria	166/170 (97.6%)
Inclusion Criterion 03: Liver biopsy consistent with NASH (NAS >= 5 with each component >=1 with fibrosis stage 2 to 3.	47/166 (28.3%)
Inclusion Criterion 03: Liver biopsy consistent with NASH (NAS >= 5 with each component >=1 (steatosis, ballooning, inflammation)) with fibrosis stage 2 to 3.	22/166 (13.3%)
Inclusion Criterion 03: ALT >= 1.5 x ULN and <= 5 x ULN	16/166 (9.6%)
Inclusion Criterion 03: Fibroscan: >= 7 kPa and < 12 kPa	16/166 (9.6%)
Inclusion Criterion 03: Fibroscan >= 7 kPa	14/166 (8.4%)
Inclusion Criterion 03: ALT > ULN and <= 5 x ULN	12/166 (7.2%)
Inclusion Criterion 03: Screening liver biopsy consistent with NASH (NAS >= 5 with each component >=1 (steatosis, ballooning, inflammation)) with fibrosis stage 2 to 3	10/166 (6.0%)
Inclusion Criterion 03: Presence at least 3 of following criteria for Metabolic syndrome: abdominal obesity, elevated BP or treat of prev HTN, elevated fasting plasma glucose, high serum	9/166 (5.4%)
Exclusion Criterion 27: Unavailable for follow-up assessment or concern for subjects compliance with the protocol procedures	8/166 (4.8%)
Inclusion Criterion 04: Platelet count >= 120,000/mm3	6/166 (3.6%)
Inclusion Criterion 11: Willing and able to comply with scheduled visits, drug administration plan, laboratory tests, liver biopsies, other study procedures, and study restrictions.	5/166 (3.0%)
Exclusion Criterion 02: Cirrhosis of the liver (e.g. Brunt/Kleiner score of F4)	5/166 (3.0%)
Inclusion Criterion 03: Presence of at least 3 of the following criteria for Metabolic syndrome: abdominal obesity, elevated BP, elevated fasting plasma glucose, high serum triglycerides, and	4/166 (2.4%)
Exclusion Criterion 16: Immunosuppressive drugs including systemic corticosteroids, tacrolimus, sirolimus, cyclosporine, azathioprine, mycophenolate mofetil, and methotrexate	3/166 (1.8%)
Exclusion Criterion 08: INR > 1.2	2/166 (1.2%)
Exclusion Criterion 13: Alcohol consumption greater than 21 oz per week for males or 14 oz per week for females	2/166 (1.2%)
Exclusion Criterion 20: Major surgical procedure within 30 days prior to screening or the presence of an open wound	2/166 (1.2%)
Exclusion Criterion 22: Any laboratory abnormality or condition that, in the investigators opinion, could adversely affect the safety of the subject or impair the assessment of study res	2/166 (1.2%)
Exclusion Criterion 26: Presence of any condition that could, in the opinion of the investigator, compromise the subjects ability to participate in the study	2/166 (1.2%)
Inclusion Criterion 01: Males and non-pregnant, non-lactating females between 18 to 70 years of age; inclusive based on the date of the screening visit	1/166 (0.6%)
Inclusion Criterion 02: Willing and able to give informed consent prior to any study specific procedures being performed	1/166 (0.6%)
Inclusion Criterion 05: Serum creatinine < 2.0 mg/dL	1/166 (0.6%)
Inclusion Criterion 07: All female subjects of childbearing potential who engage in heterosexual intercourse must agree to use a highly effective method of contraception	1/166 (0.6%)
Inclusion Criterion 08: Male subjects are required to use barrier contraception (condom plus spermicide) during intercourse from the screening through the study completion and for 90 days	1/166 (0.6%)
Exclusion Criterion 06: Weight reduction surgery in the past	1/166 (0.6%)
Exclusion Criterion 09: Total bilirubin > 1X ULN, except in confirmed cases of Gilberts syndrome	1/166 (0.6%)
Exclusion Criterion 11: Chronic hepatitis C (HCV RNA positive)	1/166 (0.6%)
Exclusion Criterion 14: Positive urine screen for amphetamines, cocaine or opiates (i.e. heroin, morphine) at screening.	1/166 (0.6%)
Screen Failure Subjects Who Met Eligibility Criteria	4/170 (2.4%)
Withdrew Consent	4/4 (100.0%)

Table S2. Rates of fibrosis improvement and progression to cirrhosis for the original five unpooled treatment groups

	GS-4997 18 mg (N=22)	GS-4997 18 mg + SIM 125 mg (N=10)	GS-4997 6 mg (N=20)	GS-4997 6 mg + SIM 125 mg (N=10)	SIM 125 mg (N=10)
Patients with fibrosis improvement	11/21 (52%)	2/9 (22%)	4/17 (24%)	4/10 (40%)	2/10 (20%)
95% CI	30% to 74%	3% to 60%	7% to 50%	12% to 74%	3% to 56%
Patients with progression to cirrhosis	0/21	1/9 (11%)	2/17 (12%)	0/10	2/10 (20%)
95% CI	0% to 16%	<1% to 48%	2% to 36%	0% to 31%	3% to 56%

Table S3. Reductions in MRI-PDFF and MRE from baseline to week 12 of treatment

Imaging	Selonsertib 18 mg ±simtuzumab (N=32)	Selonsertib 6 mg ±simtuzumab (N=30)	Simtuzumab (N=10)
MRI-PDFF, % change from baseline	-7.28 (-21.12, 0.55)	-12.90 (-23.59, 2.56)	-7.75 (-12.93, -1.24)
Patients with ≥30% reduction	5/31 (16)	2/24 (8)	0/9
MRE, % change from baseline	0.58 (-12.65, 7.95)	-3.23 (-16.40, 9.51)	5.13 (-5.14, 16.60)
Patients with ≥15% reduction	3/27 (11)	7/22 (32)	0/7

Values are median (interquartile range) or n/N (%).
MRI-PDFF, magnetic resonance imaging-estimated proton density fat fraction; MRE, magnetic resonance elastography.