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# Safety, Tolerability, and Biologic Activity of AXA1125 and AXA1957 in Subjects With Nonalcoholic Fatty Liver Disease

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**INTRODUCTION:** AXA1125 and AXA1957 are novel, orally administered endogenous metabolic modulator compositions, specifically designed to simultaneously support multiple metabolic and fibroinflammatory pathways associated with nonalcoholic fatty liver disease (NAFLD). This study assessed safety, tolerability, and biologic activity of AXA1125 and AXA1957 in NAFLD.

**METHODS:** In this multicenter, 16-week, placebo-controlled, single-blind, randomized clinical study in subjects with NAFLD stratified by type 2 diabetes, AXA1125 24 g, AXA1957 13.5 g or 20.3 g, or placebo was administered twice daily. Key metabolism (MRI-proton density fat fraction [MRI-PDFF] and homeostasis model assessment of insulin resistance [HOMA-IR]) and fibroinflammation markers (alanine aminotransferase [ALT], corrected T1 [cT1], keratin-18 [K-18] M65, and N-terminal type III collagen propeptide [Pro-C3]) were evaluated. Safety outcomes included adverse events and standard laboratory assessments.

**RESULTS:** Baseline characteristics of the 102 enrolled subjects, including 40 with type 2 diabetes, were consistent with presumed nonalcoholic steatohepatitis. AXA1125 showed consistently greater biologic activity than AXA1957 or placebo. Week 16 changes from baseline with AXA1125 vs placebo: MRI-PDFF  $-22.9\%$  vs  $-5.7\%$ , HOMA-IR  $-4.4$  vs  $+0.7$ , ALT  $-21.9\%$  vs  $-7.2\%$ , K-18 M65  $-13.6\%$  vs  $+20.1\%$ , cT1  $-69.6$  vs  $+18.3$  ms ( $P < 0.05$ ), and Pro-C3  $-13.6\%$  vs  $-3.6\%$ . Week 16 changes from baseline with AXA1957 20.3 g: MRI-PDFF  $-8.1\%$ , HOMA-IR  $+8.4$ , ALT  $-20.7\%$ , K-18 M65  $6.6\%$ , cT1  $-34.7$  ms, and Pro-C3  $-15.6\%$ . A greater proportion of subjects treated with AXA1125 achieved clinically relevant thresholds:  $\geq 30\%$  MRI-PDFF,  $\geq 17$ -IU/L ALT, and  $\geq 80$ -ms cT1 reductions at week 16. Study products were safe and well tolerated with stable lipid and weight profiles.

**DISCUSSION:** Both compositions showed multitargeted activity on relevant NAFLD pathways. AXA1125 demonstrated the greatest activity over 16 weeks, warranting continued clinical investigation in nonalcoholic steatohepatitis subjects.

**SUPPLEMENTARY MATERIAL** accompanies this paper at <http://links.lww.com/AJG/C99> and <http://links.lww.com/AJG/C100>.

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## INTRODUCTION

Nonalcoholic fatty liver disease (NAFLD) is associated with a spectrum of hepatic histologic manifestations, including steatosis, nonalcoholic steatohepatitis (NASH), fibrosis, and cirrhosis (1). Although global prevalence of NAFLD is greater than 25% (2), it is 55% in patients with type 2 diabetes (T2D) (3). Current standard of care for patients with NAFLD or NASH remains dietary changes and increased physical activity (4); there is no approved pharmacologic treatment in the United States for these patients.

Because multiple metabolic and fibroinflammatory pathways are dysregulated in NAFLD and NASH, often simultaneously, patients may benefit from approaches supporting restoration of these processes to normal health (5). A strategy aimed at complex mechanisms affecting multiple organ systems that would be inherently safe could possibly be achieved with the use of endogenous metabolic modulators (EMMs) (6). EMMs are a broad set of molecular families that include amino acids (AAs), fatty acids and other lipids, bile acids, ketone bodies, hormones, and other

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physiologically intrinsic molecules. EMMs can be combined to form novel compositions that simultaneously target multiple metabolic nodes and pathways key to liver health and complex multifactorial diseases. AA supplementation (e.g., branched-chain AA [BCAA], L-carnitine, arginine, and serine) has been shown to have potential benefits in a broad range of liver conditions, from NAFLD to cirrhosis (7–10).

AXA1125 and AXA1957 are novel, orally administered EMM compositions that were developed to simultaneously target pathways related to liver metabolism, inflammation, and fibrosis. AXA1125 includes specific ratios of 5 AAs (leucine, isoleucine, valine, arginine, and glutamine) and an AA precursor (*N*-acetylcysteine [NAC]). In an open-label, 12-week pilot study (AXA1125-002) in approximately 32 subjects with NAFLD and T2D, AXA1125 demonstrated positive trends in biomarkers related to liver structure (steatosis and fibrosis) and function (insulin sensitivity and inflammation) (11). In addition, data from primary human cell systems and NASH rodent models confirmed multifactorial activity with AXA1125 on core NASH pathophysiologic pathways, including regulation of key metabolic and fibroinflammatory nodes (11,12).

These findings prompted the present evaluation of AXA1125 in an isocaloric, placebo-controlled, randomized clinical study over a longer duration (16 weeks) in subjects with NAFLD with and without T2D. To elucidate potentially differential EMM-dependent biologic activity, another EMM composition, AXA1957, comprising 5 AAs (leucine, isoleucine, arginine, glutamine, and serine), an AA metabolite (carnitine), and NAC, was included as an isonitrogenous control to AXA1125. The clinical study described herein elucidates AXA1125 and AXA1957 safety, tolerability, and biologic activity on liver structure and function. The findings demonstrate differential activity on key biologic nodes relevant to NASH pathophysiology, indicating that specific compositions of EMMs are necessary to elicit consistent and clinically relevant effects.

## METHODS

### Study design

This 16-week, multicenter, randomized, single-blind, placebo-controlled study (AXA1125-003; NCT04073368) was performed under US Food and Drug Administration regulations and guidance in support of research with food, and in accordance with the tenets of the Declaration of Helsinki, and complied with International Council for Harmonisation Harmonised Tripartite Guideline for Good Clinical Practice and all applicable local regulations. The protocol was approved by a central institutional review board (IntegReview) before study initiation. All subjects provided written informed consent.

### Study population

Male and female subjects aged 18 years and older with NAFLD were recruited from outpatient clinics in the Summit Clinical Research network if they had proton density fat fraction (PDFF)  $\geq 10\%$ , corrected T1 [cT1]  $\geq 830$  ms by multiparametric MRI, and fasting aspartate aminotransferase  $>20$  IU/L. Body weight (BW) was required to be stable ( $\pm 5\%$  within 3 months preceding the screening visit with the expectation that subjects would not engage in lifestyle interventions/changes during the study). Subjects with T2D were required to have stable glycemic control on their existing medications (thiazolidinediones, glucagon-like peptide-1 analogs/glucagon-like peptide-1 receptor agonists, and prandial/short-

acting insulins were exclusionary), with a screening hemoglobin A1c  $<9.5\%$ . Key exclusion criteria were current or history of significant alcohol consumption, liver disease (other than NAFLD or NASH) and/or hepatic decompensation, current or planned use of dietary supplements containing proteins or AAs, ketones, or fish oils, and instability in chronic conditions. Detailed eligibility criteria are provided in the Supplementary Table (see Supplementary Digital Content 1, <http://links.lww.com/AJG/C99>).

### Intervention

Subjects were randomized 2:2:2:1 to receive twice-daily AXA1125 24 g (22.6-g free AA); AXA1957 (13.5 g or 20.3 g, the latter isocaloric and isonitrogenous to AXA1125); or a calorie-, excipient-, and color-matched placebo 24 g orally (Figure 1a). Randomization occurred through an interactive Web response system using a stratified design in blocks of 7 to ensure even allocation of T2D subjects across all arms. Subjects were blinded to treatment assignment; personnel dispensing study product were unblinded to package AXA1957 into high or low doses and to instruct subjects on the number of packages to consume daily based on dose. Subjects maintained their usual dietary and physical activity patterns/regimens for the study duration.

The study products were packaged in dry powder stick packs. Each AXA1125 stick pack was composed of leucine 1.00 g, isoleucine 0.50 g, valine 0.50 g, arginine HCl 1.81 g, glutamine 2.00 g, and NAC 0.15 g (5.65-g free AA/stick pack), and each AXA1957 stick pack was composed of leucine 1.00 g, isoleucine 0.50 g, arginine HCl 1.61 g, glutamine 0.67 g, serine 2.50 g, carnitine 0.33 g, and NAC 0.43 g (6.76-g free AA/stick pack). Each dose (2–4 stick packs) was to be reconstituted as an orange-flavored suspension in 8 oz ( $\sim 240$  mL) of water and administered 30 minutes before a meal. The initial dose was administered at the day 1 (baseline) visit.

### Assessments

Demographics and clinical characteristics were collected on day 1. Subsequent study visits were scheduled at weeks 1, 2, 4, 8, 12, and 16 and a safety follow-up at week 18 (Figure 1a). Multiparametric MRI examinations and oral glucose tolerance tests were performed at baseline (day 1), week 8, and week 16 to characterize liver fat content (MRI-PDFF) and fibroinflammation (cT1) (13), and to assess glucose homeostasis, respectively. Overnight fasted blood samples for key markers of metabolism (homeostatic model assessment of insulin resistance [HOMA-IR]), inflammation (alanine aminotransferase [ALT] and keratin-18 M65 [K-18 M65]), and fibrosis (*N*-terminal type III collagen propeptide [Pro-C3]) were obtained throughout the study and analyzed in a central laboratory. Adverse events (AEs), vital signs, electrocardiograms, safety laboratory tests (including fasting lipid profiles), and physical examinations (including BW) were collected.

### Statistical analysis

All analyses were performed at the significance level of 2-sided 0.05 and considered exploratory. The safety population included all subjects receiving  $\geq 1$  dose of study product, and subjects were analyzed according to the product/dose received on day 1. The per-protocol population included all randomized subjects who had  $\geq 1$  postbaseline MRI, received  $\geq 80\%$  and  $\leq 120\%$  of study product, and had no major protocol deviations.

ANCOVA for continuous endpoints and the Cochran–Mantel–Haenszel test for binary endpoints were applied (both

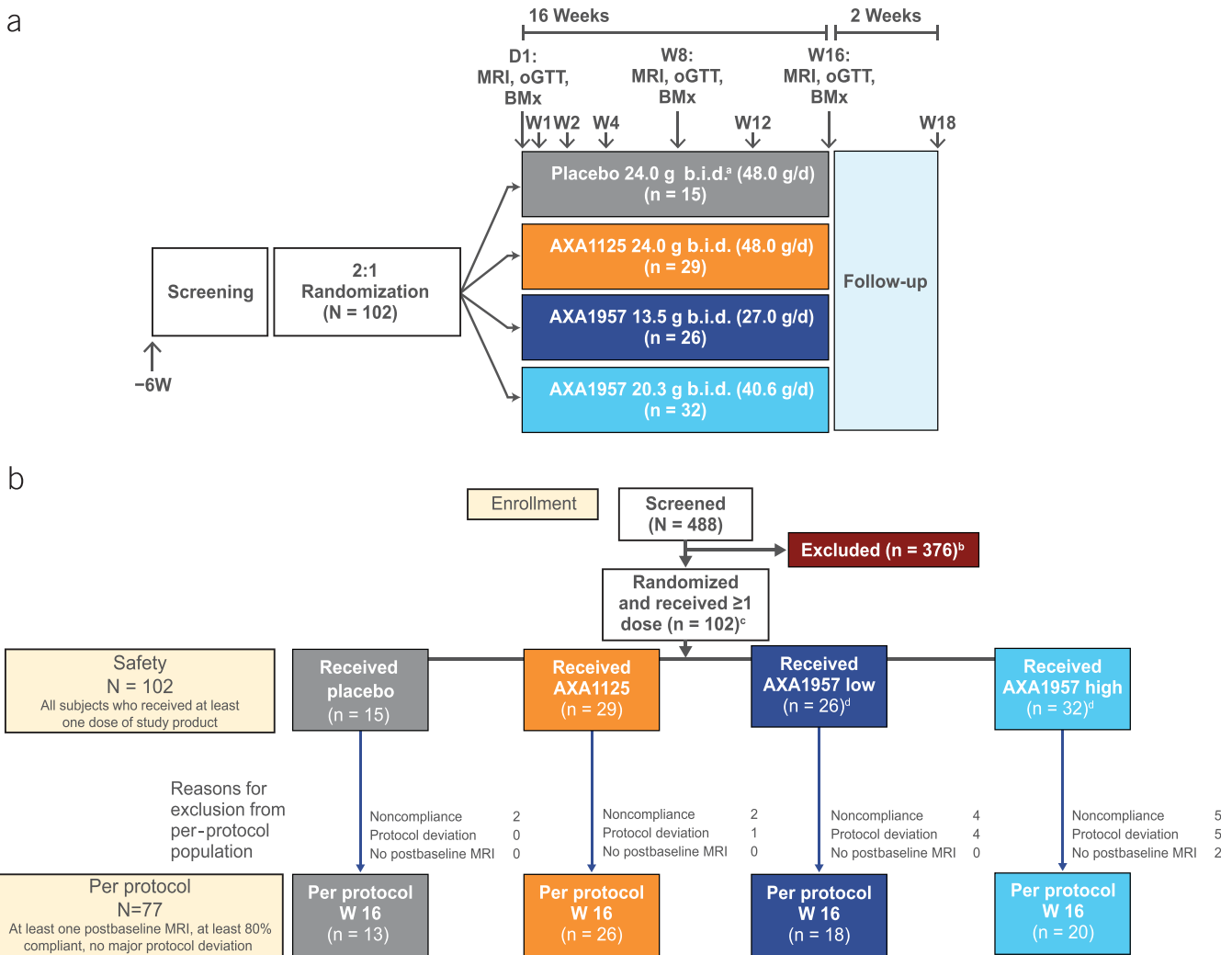
adjusted for baseline T2D status); summary statistics were reported based on observed data collected at each visit. Absolute or relative change from baseline at weeks 8 and 16 of various biomarkers, lipid profiles, and other clinical parameters was summarized, and pairwise comparisons with placebo were performed. Least squares means were estimated through ANCOVA models adjusted by baseline value and T2D status. Responder analyses of clinically relevant thresholds of biologic activity correlated to histologic improvements in the NAFLD activity score and fibrosis were also performed (e.g., percentage of subjects at week 16 achieving reductions of  $\geq 30\%$  in MRI-PDFF,  $\geq 17$  IU/L in ALT, and  $\geq 80$  ms in cT1) (14–16).

**RESULTS**

**Subject disposition and baseline characteristics**

Four hundred eighty-eight subjects signed consent and were screened between December 2018 and September 2019 at 17

study centers. Of these, 102 subjects were randomized and received  $\geq 1$  dose of study product (placebo,  $n = 15$ ; AXA1125 24 g b.i.d.,  $n = 29$ ; AXA1957 13.5 g b.i.d.,  $n = 26$ ; and AXA1957 20.3 g b.i.d.,  $n = 32$ ) (Table 1; Figure 1b; see Supplementary Table, Supplementary Digital Content 2, <http://links.lww.com/AJG/C100> for exclusions). Mean cohort age was 50.2 years (Table 1). Mean (SE) BW in the AXA1125 and AXA1957 groups ranged between 102.3 (4.6) and 106.2 (4.6) kg, and the mean (SE) body mass index ranged between 36.8 (1.36) and 38.5 (1.50) kg/m<sup>2</sup>. Baseline demographics were generally well balanced across groups with the exception of higher BW in the placebo group (Table 1). Baseline characteristics were phenotypically consistent with presumed NASH (mean ALT 54.4 IU/L, FibroScan 13.0 kPa, and Pro-C3 16.80 ng/mL) and insulin resistance (mean HOMA-IR 10.9) and were similar among groups except for lower HOMA-IR in the placebo group. Forty subjects (39.2%) had comorbid T2D. Mean baseline hemoglobin A1c was 7.43% in T2D subjects.



**Figure 1.** Study design (a) and subject disposition (b). <sup>a</sup>Calorie-matched placebo control; <sup>b</sup>reasons for exclusion are presented in Supplementary Table (see Supplementary Digital Content 2, <http://links.lww.com/AJG/C100>); <sup>c</sup>10 subjects were randomized but not dosed and thus were not part of the safety population; and <sup>d</sup>3 subjects who received AXA1957 high dose in error on day 1 (randomized to AXA1957 low dose) were included in the AXA1957 high-dose safety analysis, considered part of the AXA1957 low-dose completer population, and excluded from both arms of the per-protocol analysis. b.i.d., 2 times a day; BMx, biomarkers; D, day; cT1, corrected T1; MRI-PDFF, MRI-proton density fat fraction; OGTT, oral glucose tolerance test; T2D, type 2 diabetes; W, week.

### Biologic activity of AXA1125 and AXA1957

On a background of stable lifestyle regimen and despite the provision of ~210 kcal/d with the study products, BW remained stable over the 16-week duration across all treatment arms (mean % BW [SE] change: -0.6% [0.28]) at week 16 vs baseline (Table 2).

Compared with placebo, although both AXA1125 and AXA1957 reduced liver fat content at weeks 8 and 16, reductions with AXA1125 were more pronounced (Figure 2a). Relative reduction in MRI-PDFF with AXA1125 was significantly greater than the corresponding isonitrogenous composition (AXA1957 20.3 g); at week 8: -20.7% vs -8.8%, week 16: -22.9% vs -8.1%,  $P < 0.05$  (Figure 2b). Compared with both calorie-matched (placebo) and isonitrogenous (AXA1957) compositions, AXA1125 demonstrated greater absolute mean reductions from baseline to weeks 8 and 16 in HOMA-IR (Figure 2c), reflecting improved insulin sensitivity. HOMA-IR at week 8 was significantly reduced in the AXA1125 vs AXA1957 20.3-g group (-3.8 vs +6.9;  $P < 0.05$ ; Figure 2c).

Compared with placebo, larger reductions from baseline to weeks 8 and 16 in ALT were seen across all active arms (Figure 3a), with changes observed as early as week 2 and sustained to week 16, and a trend toward larger ALT reduction in AXA1125 vs AXA1957 groups (Figure 3b). Changes in ALT were significantly correlated to changes in MRI-PDFF for AXA1125 ( $r = 0.62$ ;  $P < 0.001$ ) and placebo ( $r = 0.69$ ;  $P = 0.012$ ). K-18 M65, a marker of apoptosis (17), was significantly reduced from

baseline with AXA1125 vs placebo at week 8 (-22.4% vs +29.6%;  $P < 0.05$ ), and remained suppressed at week 16, whereas it tended to increase in the isonitrogenous AXA1957 20.3-g arm (Figure 3c).

Although cT1, an integrative imaging marker of inflammation and fibrosis (18), was reduced more in active arms vs placebo (Figure 4a), approximately, 2-fold greater reductions in cT1 were observed after AXA1125 administration vs the isonitrogenous AXA1957 dose (Figure 4b). At week 16, cT1 was significantly reduced by AXA1125 vs placebo (-69.6 ms vs +18.3 ms;  $P < 0.05$ ; Figure 4b). These imaging findings were corroborated by changes in plasma Pro-C3, a soluble marker of hepatic fibrogenesis (19), also reduced by both AXA1125 and AXA1957 vs placebo (Figure 4c).

At week 16, responder analysis showed a greater proportion of subjects treated with AXA1125 vs placebo achieved clinically relevant thresholds of biologic activity across several markers, each of which has been correlated to histologic improvements in the NAFLD activity score and fibrosis (14–16): 38.5% vs 8.3% of subjects had  $\geq 30\%$  relative reduction in MRI-PDFF (Figure 5a), 38.5% vs 25% had  $\geq 17$ -IU/L reduction in ALT (Figure 5b), and 34.6% vs 16.7% had  $\geq 80$ -ms reduction in cT1 (Figure 5c). Proportion of subjects who achieved these clinically relevant thresholds in 2 or more biomarkers (i.e.,  $\geq 30\%$   $\downarrow$ PDFF +  $\geq 17$ -IU/L  $\downarrow$ ALT) was 23.1% with AXA1125 vs 0.0% with placebo; similarly, a higher

**Table 1. Subject demographics and baseline characteristics in the overall safety population**

	Placebo (n = 15)	AXA1125 24 g b.i.d. (n = 29)	AXA1957 13.5 g b.i.d. (n = 26)	AXA1957 20.3 g b.i.d. (n = 32)
Age, yr	53.2 (2.48)	49.2 (2.38)	49.6 (2.11)	50.1 (2.26)
Sex				
Female, n (%)	10 (66.7)	17 (58.6)	16 (61.5)	19 (59.4)
Male, n (%)	5 (33.3)	12 (41.4)	10 (38.5)	13 (40.6)
Weight, kg	118.25 (8.20)	102.86 (4.42)	106.24 (4.61)	102.31 (4.62)
BMI, kg/m <sup>2</sup>	42.0 (2.42)	36.8 (1.36)	37.4 (1.20)	38.5 (1.50)
Type 2 diabetes, n (%)	6 (40.0)	12 (41.4)	10 (38.5)	12 (37.5)
HbA1c, %	6.9	7.8	7.3	7.5
Metabolism				
MRI-PDFF, %	21.19 (1.51)	22.35 (0.93)	21.01 (1.16)	22.27 (0.93)
HOMA-IR	8.59 (1.26)	13.51 (3.39)	9.62 (1.35)	10.82 (1.78)
Inflammation				
ALT, IU/L	50.5 (8.73)	55.2 (4.89)	60.6 (5.66)	50.5 (4.11)
AST, IU/L	41.3 (34.29)	37.3 (22.69)	34.9 (16.6)	40.6 (21.00)
cT1, ms	1022.3 (41.35)	960.6 (16.84)	959.5 (21.67)	1017.2 (24.30)
K-18 M65, U/L	900.5 (369.71)	450.8 (99.39)	594.8 (97.98)	491.0 (102.74)
Fibrosis				
FibroScan score, kPa	13.51 (1.47)	11.73 (1.24)	16.31 (3.09)	11.18 (0.68)
Pro-C3, ng/mL	15.85 (1.81)	17.07 (1.56)	17.02 (1.90)	16.76 (1.73)
ELF score	9.42 (0.32)	9.24 (0.17)	9.18 (0.17)	9.30 (0.16)
FIB-4	1.42 (0.25)	1.05 (0.12)	1.17 (0.13)	1.0 (0.10)

All values are mean (SE).

ALT, alanine aminotransferase; AST, aspartate aminotransferase; b.i.d., twice daily; BMI, body mass index; cT1, corrected T1; ELF, enhanced liver fibrosis; FIB-4, fibrosis-4; HOMA-IR, homeostasis model assessment of insulin resistance; K-18 M65, keratin-18 measured using M65 enzyme-linked immunosorbent assay; MRI-PDFF, MRI-proton density fat fraction; Pro-C3, N-terminal type III collagen propeptide.

**Table 2.** Summary of body weight and serum lipid profile changes in the overall safety population at week 16

	Placebo (n = 15)	AXA1125 24 g b.i.d. (n = 29)	AXA1957 13.5 g b.i.d. (n = 26)	AXA1957 20.3 g b.i.d. (n = 32)
<b>Body weight</b>				
Absolute change, kg	-0.6 (0.54)	-0.5 (0.51)	-0.5 (0.42)	-1.1 (0.77)
Percentage change	-0.66 (0.54)	-0.46 (0.50)	-0.38 (0.49)	-0.83 (0.62)
<b>Lipid profile</b>				
<b>Triglycerides</b>				
Absolute change, mg/dL	-15.9 (9.21)	-22.1 (12.93)	-5.3 (9.30)	-4.4 (10.10)
Percentage change	-6.47 (6.08)	-6.02 (5.58)	-1.79 (4.74)	-1.79 (5.73)
<b>Total cholesterol</b>				
Absolute change, mg/dL	-18.79 (6.45)	-5.26 (5.79)	-3.79 (3.52)	-7.12 (4.29)
Percentage change	-9.52 (2.89)	-1.02 (3.37)	-1.60 (1.85)	-2.87 (2.15)
<b>LDL</b>				
Absolute change, mg/dL	-15.78 (6.48)	-2.71 (4.62)	-1.20 (2.65)	-4.91 (4.20)
Percentage change	-13.24 (5.26)	0.73 (4.88)	0.63 (3.73)	-1.93 (2.79)
<b>HDL</b>				
Absolute change, mg/dL	0.12 (1.89)	1.43 (1.45)	-1.43 (1.57)	-1.24 (1.18)
Percentage change	2.06 (3.90)	6.50 (3.70)	-3.49 (3.35)	-2.64 (2.49)

All values are mean (SE).  
HDL, high-density lipoprotein; LDL, low-density lipoprotein.

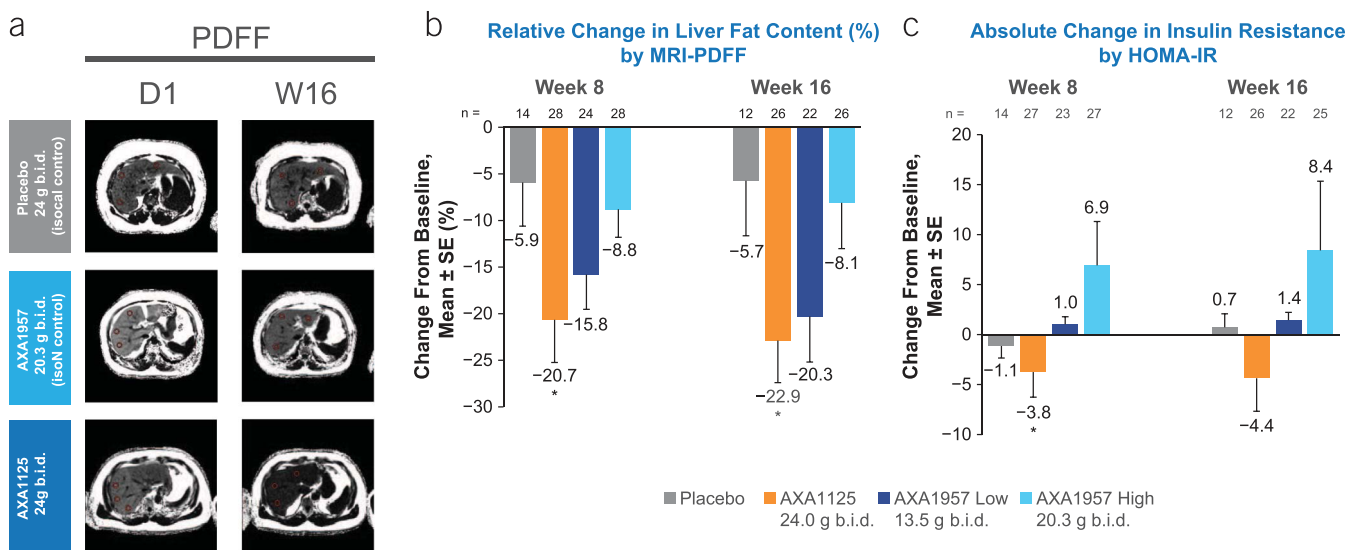
proportion of subjects receiving AXA1125 had changes in 2 or more biomarkers when cT1 was also considered (data not shown).

Per-protocol population findings showed similar trends to the safety population, with more notable and consistent improvement in metabolic and fibroinflammatory biomarkers after AXA1125 treatment vs placebo or AXA1957 (Table 3). Biologic activity with AXA1125 tended to be more pronounced in those with comorbid T2D than in the overall population; AXA1125 showed greater reductions in MRI-PDFF (-31.2% vs -8.3%), ALT (-34.6% vs

-13.9%), and cT1 (-105.1% vs -42.7 ms) than placebo. Detailed analysis including the positive changes on glucose homeostasis induced by AXA1125 in the T2D subgroup will be reported in a subsequent publication.

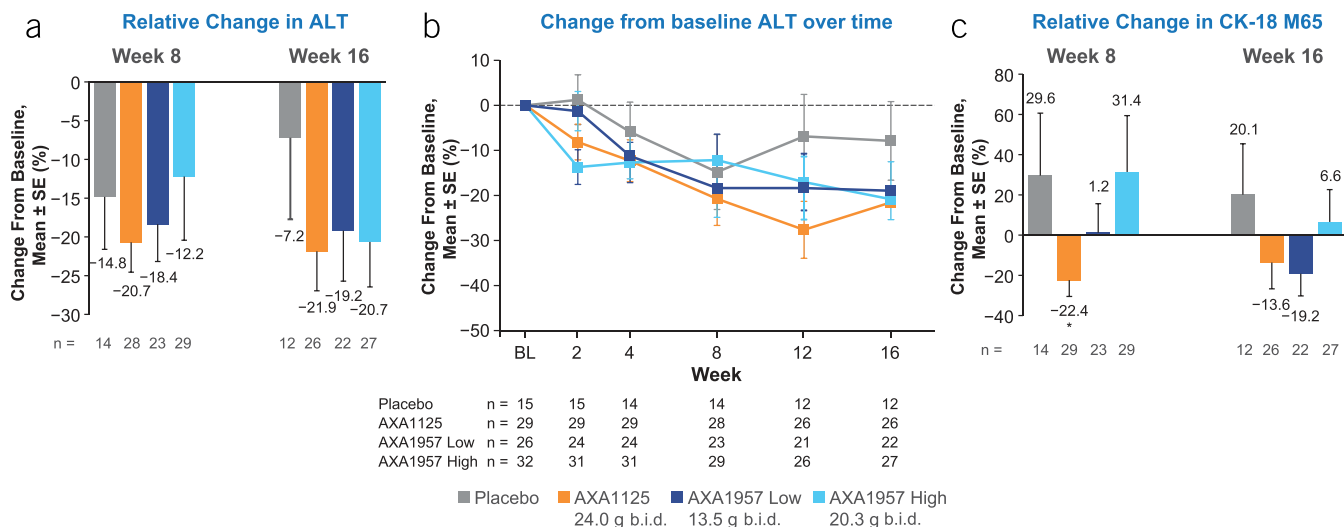
**Safety and tolerability**

Product-emergent AEs (PEAEs) reported by subjects receiving AXA1125 and AXA1957 were mild or moderate (Table 4). The only PEAEs reported by ≥ 10% of subjects in any arm were



**Figure 2.** Change from baseline in liver fat content (MRI-PDFF) (a and b) and insulin resistance (HOMA-IR) (c) in the overall safety population. \*P < 0.05 vs AXA1957 high dose. b.i.d., 2 times a day; D, day; HOMA-IR, homeostatic model assessment of insulin resistance; isocal, isocaloric; isoN, isonitrogenous; MRI-PDFF, MRI-proton density fat fraction; W, week.





**Figure 3.** Change from baseline in ALT (a and b) and K-18 M65 (c) in the overall safety population. \**P* < 0.05 vs placebo. ALT, alanine aminotransferase; K-18 M65, keratin-18 measured using M65 enzyme-linked immunosorbent assay.

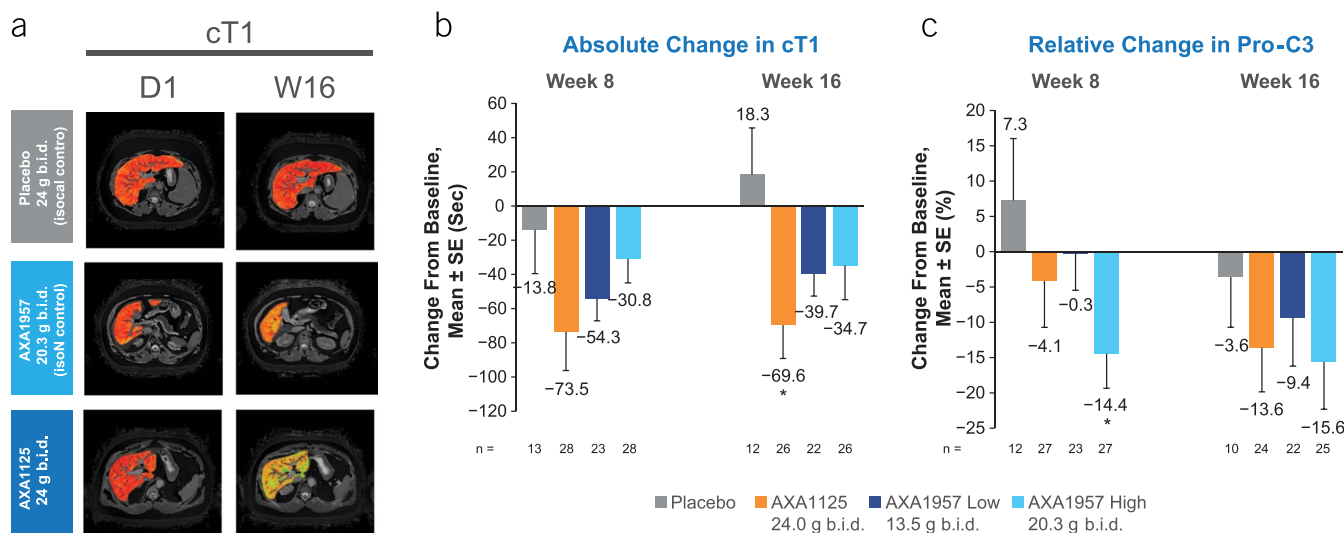
gastrointestinal (diarrhea, nausea, and reduced appetite), upper respiratory tract infection, and headache. Gastrointestinal AEs were generally mild and transient and resolved without intervention (e.g., no antidiarrheal, antiperistaltic, or antiemetic agents required) within 2–3 weeks. Two serious AEs were reported (1 with AXA1125 and 1 with AXA1957 20.3 g); both were determined unrelated to study product. No clinically significant changes in safety laboratory tests including serum lipid levels (Table 2), vital signs, physical examinations, or electrocardiograms were reported. Discontinuations because of AEs were noted in 1 subject each in placebo and AXA1125 arms and 2 in the AXA1957 20.3-g arm (Table 4).

**DISCUSSION**

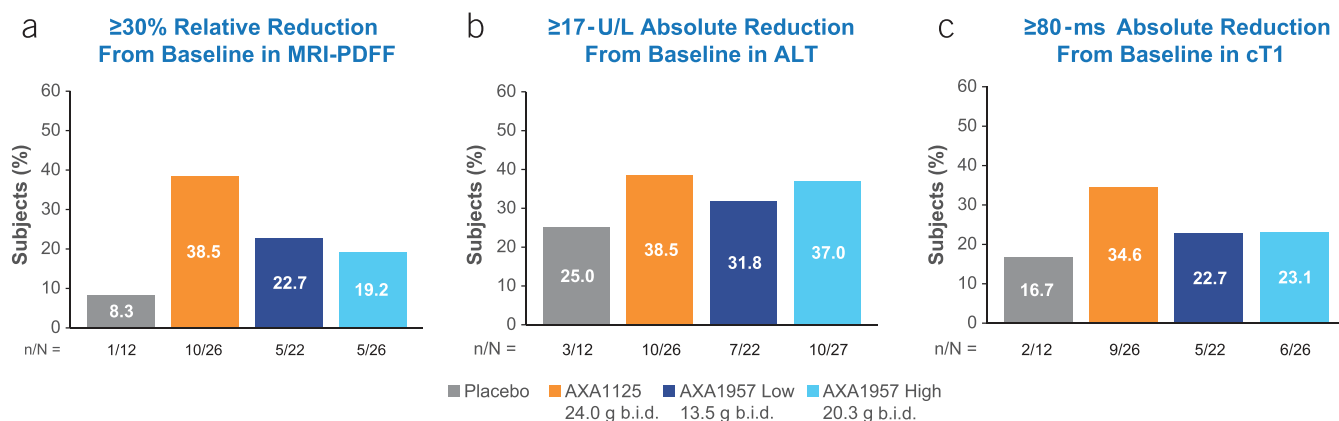
AXA1125 and AXA1957 were safe and well tolerated over 16 weeks and demonstrated clinically relevant multitargeted activity on liver structure and function assessed by biomarkers related to metabolic

and fibroinflammatory pathways in a population of presumed NASH subjects. Notably, these positive findings were observed without confounding from BW or serum lipid changes across treatment arms.

Reductions from baseline in measures of liver fat content (MRI-PDFF), biomarkers of metabolism (HOMA-IR), and fibroinflammation (ALT, CK18-M65, cT1, and Pro-C3) tended to be greater and were more consistently demonstrated with AXA1125 vs isocaloric placebo. By contrast, with isocaloric and isonitrogenous AXA1957, changes from baseline in several of these biomarkers were lower magnitude and inconsistently observed. At week 16, a greater proportion of subjects who received AXA1125 (~35%–40%) vs placebo (~8%–25%) achieved ≥30% ↓MRI-PDFF, ≥17-IU/L ↓ALT, and ≥80-ms ↓cT1; each of these thresholds has been correlated to improved histopathologic outcomes (14–16) and some (e.g., cT1) with treatment response (20).



**Figure 4.** Change from baseline in biomarkers of fibroinflammation (a, b, and c) in the overall safety population. \**P* < 0.05 vs placebo. b.i.d., 2 times a day; cT1, corrected T1; D, day; isocal, isocaloric; isoN, isonitrogenous; Pro-C3, N-terminal type III collagen propeptide; W, week.



**Figure 5.** Proportion of subjects with clinically relevant thresholds of biologic activity (**a**, **b**, and **c**) in the overall safety population. ALT, alanine aminotransferase; cT1, corrected T1; MRI-PDFF, MRI-proton density fat fraction.

Lipotoxicity and insulin resistance are considered hallmarks in the pathogenesis of NASH (21), with an imbalance in fatty acid biosynthesis and inability of mitochondria to adequately metabolize free fatty acids (22). Reduced liver fat content and improved insulin sensitivity after AXA1125 treatment were observed without BW changes despite ingestion of additional ~210 calories/day and maintenance of prestudy diet/exercise regimens. These findings imply that AXA1125 may promote fatty acid oxidation to induce these key physiological changes. Biochemical data from primary human hepatocytes treated with AXA1125 (23,24) support this hypothesis and are consistent with reported activities of BCAAs to promote fat oxidation and ketogenesis (25). Although BCAAs, when administered individually, have shown mixed effects on glucose homeostasis in previous studies (26,27) that may be related to specific doses (27,28), when provided in the specific stoichiometry and amounts within the context of the overall AXA1125 composition, insulin resistance was improved (Figure 2c).

Concordant and clinically relevant improvements were seen across several fibroinflammatory markers in this study (Figures 3 and 4) and in our 12-week study in NAFLD subjects with T2D (AXA1125-002) (11). Fibrosis is the feature most robustly associated with NASH progression and outcomes (29,30). An absolute reduction of ~2 ng/mL in plasma Pro-C3, a fibrogenesis marker, was associated with 1-stage histologic improvement in fibrosis after 18 months of pioglitazone treatment in NASH subjects (31). With just 4 months of AXA1125 treatment, we observed an absolute reduction of 3.4 ng/mL in Pro-C3 (Figure 4c) in this study, suggesting a potential to favorably impact fibrosis in a subsequent paired-biopsy study.

Our phenotypic and mechanistic data from primary human macrophages and stellate cells suggest that clinical fibroinflammatory effects are likely mediated by direct impact of AXA1125 on these specific cell types: AXA1125 constituents suppressed lipopolysaccharide-induced tumor necrosis factor- $\alpha$  secretion in M1 macrophages, increased anti-inflammatory chemokine (C-C motif) ligand 18 secretion in M2 macrophages, and decreased secretion of Pro-C3 and suppressed expression of the profibrogenic gene, *HSP47* in stellate cells (23,24). We postulate that glutamine and arginine in AXA1125 contributed to its effects on inflammatory pathways by enhancing mucosal

integrity and tight junctions of gut epithelial cells, thereby reducing bacterial endotoxin translocation (32–34), and NAC within AXA1125 likely decreased proinflammatory and profibrotic pathways through suppression of nuclear factor-kappa B, hypoxia-inducible factor 1-alpha, and transforming growth factor beta and stimulated anti-inflammatory pathways by increasing glutathione synthesis and decreasing reactive oxygen species (35–37).

Both EMM compositions were safe and generally well tolerated, consistent with published short- and long-term studies that have long established the safety of AA administration (38–42). However, consistently greater biologic activity was observed with AXA1125 vs AXA1957, although these EMMs were isocaloric and isonitrogenous. Thus, our findings of differential biologic activity in this rigorously controlled study highlight a critical insight: Although administration of AA mixtures may result in comparable safety profiles, biologic activity is highly dependent on the specific EMM composition.

A key strength of this study is the standardization of calorie- and nitrogen-matched study products in the randomized groups including the control (placebo) arm. Other strengths include patient blinding to product allocation and the 16-week administration providing sufficient exposure to adequately assess safety, tolerability, and biologic activity. Although this was an early-stage study, baseline characteristics (Table 1) indicated that subjects enrolled had presumed NASH. Baseline body mass index and HOMA-IR were also indicative of a comorbidly obese, insulin-resistant population, factors associated with NASH onset and progression (43). Body weight was required to be stable for at least 3 months before enrollment, and subjects were not allowed to engage in any new lifestyle interventions during the study. Other strengths included oral administration with high compliance and ease of administration. There are also important limitations to consider. Because this was an early-phase study, it was not powered for statistical significance, although clinically relevant conclusions can be drawn between study groups based on the strong consistency of changes seen across several metabolic and fibroinflammatory markers, many of which correlate with histologic outcomes. Given the relatively small sample size in this study, potential bias-factor(s) might not be balanced at baseline among study groups, which may require further investigation in future studies.

**Table 3. Change from baseline in biomarkers of metabolism and fibroinflammation in the per-protocol population**

	Week 8				Week 16			
	Placebo	AXA1125 24 g b.i.d.	AXA1957 13.5 g b.i.d.	AXA1957 20.3 g b.i.d.	Placebo	AXA1125 24 g b.i.d.	AXA1957 13.5 g b.i.d.	AXA1957 20.3 g b.i.d.
<b>Metabolism</b>								
<b>MRI-PDFF</b>								
n	13	26	18	19	12	25	17	20
Relative change from baseline, %	-4.5 (4.8)	-19.8 (4.6)	-16.6 (4.4)	-8.0 (3.9)	-5.7 (5.9)	-24.6 (4.3) <sup>a,b</sup>	-20.3 (5.9)	-8.8 (6.3)
<b>HOMA-IR</b>								
n	13	25	18	18	12	25	17	19
Absolute change from baseline	-0.2 (0.9)	-4.2 (2.7) <sup>b</sup>	0.6 (0.8)	9.5 (6.5)	0.7 (1.4)	-4.5 (3.4)	1.2 (0.9)	1.6 (2.8)
<b>Inflammation</b>								
<b>ALT</b>								
n	13	25	18	20	12	25	17	20
Relative change from baseline, %	-13.8 (7.2)	-19.5 (4.2)	-17.0 (5.2)	-15.0 (10.6)	-7.2 (10.5)	-22.7 (5.2)	-16.6 (7.9)	-21.3 (6.8)
<b>K-18 M65</b>								
n	13	26	18	20	12	25	17	20
Relative change from baseline, %	37.8 (32.3)	-20.0 (8.7) <sup>a,b</sup>	8.7 (16.8)	50.7 (38.9)	20.1 (25.3)	-12.1 (13.5)	-21.7 (11.6)	-1.7 (16.3)
<b>Fibroinflammation</b>								
<b>cT1</b>								
n	12	26	18	19	12	25	17	20
Absolute change from baseline, ms	4.1 (20.0)	-74.5 (24.2)	-47.1 (11.6) <sup>a</sup>	-30.3 (18.2)	18.3 (27.4)	-74.6 (19.7) <sup>a</sup>	-29.8 (13.2)	-39.4 (25.4)
<b>Fibrosis</b>								
<b>FIB-4</b>								
n	13	24	18	19	11	25	17	20
Relative change from baseline, %	0.45 (8.1)	-8.6 (4.0)	-7.4 (4.6)	-5.2 (3.9)	-1.9 (10.3)	-7.5 (5.0)	-2.7 (6.3)	-6.6 (4.8)
<b>Pro-C3</b>								
n	11	24	18	18	10	23	17	18
Relative change from baseline, %	10.4 (9.0)	-2.9 (7.2)	7.2 (5.3) <sup>b</sup>	-21.5 (4.9) <sup>a,c</sup>	-3.6 (7.1)	-13.5 (6.6)	-1.6 (7.8) <sup>b</sup>	-27.8 (4.6) <sup>a</sup>

All values are mean (SE).  
 ALT, alanine aminotransferase; b.i.d., twice daily; cT1, corrected T1; FIB-4, fibrosis-4 index; HOMA-IR, homeostatic model assessment of insulin resistance; K-18 M65, keratin-18 measured using M65 enzyme-linked immunosorbent assay; MRI-PDFF, MRI-proton density fat fraction; Pro-C3, N-terminal type III collagen propeptide.  
<sup>a</sup>P < 0.05 vs placebo.  
<sup>b</sup>P < 0.05 vs AXA1957 high dose.  
<sup>c</sup>P < 0.05 vs AXA1125.

In conclusion, our systematic characterization of specific EMM compositions in NAFLD adds to the depth of knowledge on the safety and physiologic activity of EMMs and highlights that specificity of EMM composition matters for optimal effects. Our findings support the potential of a novel EMM composition, AXA1125, to simultaneously address the multifactorial pathogenesis of NAFLD/NASH, their key comorbidities, representing a unique modality with a coordinated multitargeted mechanism of action without major safety or tolerability issues. Future investigations are planned to

assess the histological effects and longer-term safety of AXA1125 in a double-blind, randomized, placebo-controlled, paired-biopsy trial over 48 weeks in individuals with biopsy-confirmed NASH.

#### CONFLICTS OF INTEREST

**Guarantor of the article:** Manu V. Chakravarthy, MD, PhD.

**Specific author contributions:** S.A.H., M.J.K., H.C., J.Z., and M.V.C.: study design and conduct of the study. S.A.H., M.K., H.C.,



**Table 4. Summary safety findings in the overall safety population at week 16**

	Placebo (n = 15)	AXA1125 24 g b.i.d. (n = 29)	AXA1957 13.5 g b.i.d. (n = 26)	AXA1957 20.3 g b.i.d. (n = 32)
Total PEAEs	22	71	43	61
Subjects, n (%) <sup>a,b</sup>				
All PEAEs	10 (66.7)	24 (82.8)	19 (73.1)	19 (59.4)
All PEAEs reported in >10% for any arm				
Diarrhea	1 (6.7)	10 (34.5)	3 (11.5)	6 (18.8)
Nausea	1 (6.7)	4 (13.8)	3 (11.5)	3 (9.4)
Upper respiratory infection	1 (6.7)	4 (13.8)	0	2 (6.3)
Decreased appetite	0	3 (10.3)	2 (7.7)	1 (3.1)
Headache	1 (6.7)	1 (3.4)	4 (15.4)	2 (6.3)
AE leading to discontinuation	1 Dry mouth <sup>c</sup>	1 Upper abdominal pain <sup>d</sup>	0	2 Laryngeal cancer <sup>e,f</sup> Nephrolithiasis <sup>f</sup>
Any serious AE	0	1 (3.4)	0	1 (3.1)
Death	0	0	0	0
Severity of AEs among subjects with product-emergent AE <sup>a,b</sup>				
Mild	6 (40.0)	16 (55.2)	8 (30.8)	6 (18.8)
Moderate	3 (20.0)	8 (27.6)	11 (42.3)	13 (40.6)
Severe	1 (6.7)	0	0	0

AE, adverse event; b.i.d., twice daily; PEA, product-emergent adverse event.

<sup>a</sup>Subjects were counted only once if they had more than 1 event within each category reported during the product administration period.

<sup>b</sup>Safety data presented are based on what subject received on day 1 of dosing.

<sup>c</sup>Related.

<sup>d</sup>Possibly related.

<sup>e</sup>Also, a serious AE.

<sup>f</sup>Unlikely related.

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Esperion, GLG Group, Guidepoint Global, Madrigal, Novartis, Novo Nordisk, Regeneron, and Sanofi; speaking and teaching: Amgen, Boehringer Ingelheim, Eli Lilly, and Esperion; advisory committee or review panel: Akcea, Alexion, Altimmune, Amgen, AstraZeneca, Esperion, Madrigal, Novartis, Regeneron, and Sanofi. N.T.G.: grant/research support: Axcella, Bristol Myers Squibb, CymaBay, Genentech, Genfit, Gilead, HighTide, Madrigal, NGM Bio, NorthSea, and Novo Nordisk; speaking and teaching: AbbVie, Dova, Gilead, Intercept, and Salix. Z.H.Y.: consultant: Gilead; grant/research support: Allergan, AXA, Bristol Myers Squibb, Conatus, Cymabay, Gilead, HighTide, Intercept, Madrigal, NGM Bio, Novartis, and Novo Nordisk; speaking and teaching: Intercept. A.K.: consultant: Gilead, Intercept, and Novartis; grant/research support: Gilead. R.P.: speaking and teaching: Intercept. M.K., H.C., J.Z., and M.V.C.: current employees of Axcella Health and own stock options in the company.

**Clinical trial registry website and trial number:** US National Library of Medicine (<https://clinicaltrials.gov/>); clinical trial number NCT04073368.

**Ethics:** The protocol was approved by a central institutional review board (IntegReview) before study initiation. All subjects provided written informed consent.

## Study Highlights

### WHAT IS KNOWN

- ✓ Nonalcoholic fatty liver disease (NAFLD) is a heterogeneous, systemic metabolic disease associated with a spectrum of hepatic histologic manifestations.
- ✓ The recommended standard of care for patients with NAFLD or nonalcoholic steatohepatitis (NASH) includes diet and exercise.
- ✓ Patients with NAFLD and NASH may benefit from multifactorial approaches that simultaneously address multiple dysregulated metabolic and fibroinflammatory pathways and promote health.
- ✓ AA supplementation has been shown to have potential benefits in liver conditions, although experience in NAFLD and NASH specifically is limited.

### WHAT IS NEW HERE

- ✓ AXA1125 and AXA1957 are novel, orally administered endogenous metabolic modulator compositions designed to support pathways key to liver health.
- ✓ AXA1125 and AXA1957 administered orally for 16 weeks in subjects with NAFLD were safe and well tolerated.
- ✓ AXA1125 showed consistently greater activity vs AXA1957 and placebo on biomarkers related to liver structure and function.
- ✓ AXA1125 has the potential to simultaneously address the multifactorial pathogenesis of NASH and its key comorbidities.

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