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Clinical Trial: A Phase 2b Study to Evaluate the Efficacy and Safety of MK-3655 in Individuals With Pre-Cirrhotic MASH

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ABSTRACT

Background: Fibroblast growth factor 21 (FGF21) is a metabolic regulator with demonstrated efficacy for the treatment of metabolic dysfunction-associated steatohepatitis (MASH). FGF21 signals through 'c' isoforms of the FGF receptors (FGFR) 1–3 and the co-receptor β-klotho.

Aims: We report the safety and efficacy of MK-3655, a monoclonal antibody that binds β-klotho and selectively activates the FGFR1c/β-klotho co-receptor complex, in patients with pre-cirrhotic MASH.

Methods: Phase 2b, randomised, multicenter, double-blind, placebo-controlled, parallel-group study in patients with pre-cirrhotic MASH (NAS ≥ 4 and MASH CRN fibrosis score Stage 2 or 3). Participants were randomised 1:1:1:1 to receive MK-3655 50 mg, 100 mg, 300 mg, or matching placebo subcutaneously every 4 weeks. The primary endpoint was MASH resolution without worsening of fibrosis by histology at Week 52. An interim analysis (IA) of liver fat content (LFC) was planned once ≥ 25 participants per treatment group completed an MRI-PDFF assessment at Week 24.

Results: Among 183 participants, mean BMI was 33.4 kg/m^2, mean LFC was 18.1%, and 52.5% had type 2 diabetes. At the IA, the differences from placebo in relative reduction from baseline in LFC were assessed as insufficient for continuation of the trial. Among participants with Week 24 LFC assessment, percent relative reductions from baseline (LS mean difference vs. placebo) for MK-3655 50 mg ($N = 33$), 100 mg ($N = 36$), and 300 mg ($N = 31$), were 19.1%, 19.0%, and 26.1%, respectively. MK-3655 was generally well tolerated.

Conclusions: In patients with pre-cirrhotic MASH, treatment with MK-3655 resulted in a modest reduction in LFC at 24 weeks.

Clinical Trial Number: EudraCT: 2019-003048-63; NCT: 04583423.

1 | Introduction

Metabolic dysfunction-associated steatohepatitis (MASH) is a form of metabolic dysfunction-associated steatotic liver disease (MASLD) characterised by liver steatosis, inflammation, and hepatocellular damage. The pathophysiology of MASH involves over-accumulation of fat in the liver (steatosis) that can lead to the production of cytotoxic lipid oxidation products (lipotoxicity),

which incite a necro-inflammatory process that leads to liver fibrosis and cirrhosis [1]. It is generally accepted that reducing liver fat content (LFC) is a key driver for MASH resolution and reversal of fibrosis. Data suggest that a 30% relative reduction in LFC as assessed by magnetic resonance imaging-proton density fat fraction (MRI-PDFF) is associated with histologic improvement in MASH [2, 3].

Preclinical studies in rodents and nonhuman primates identified the hepatokine fibroblast growth factor 21 (FGF21) as an important metabolic regulator that improves glucose and lipid metabolism and reduces body weight [4]. Over the past several decades, those observations have led to FGF21 being considered a promising therapeutic agent for the treatment of obesity, type 2 diabetes, and MASH.

FGF21 is thought to signal through a ternary complex comprised of FGF21, β-klotho (KLB) and the 'c' splice isoforms of fibroblast growth factor receptors (FGFR) 1, 2, or 3 [5]. According to this ternary complex model, KLB functions as a primary high-affinity co-receptor for FGF21, whereas the FGFR mediates receptor dimerisation and intracellular signalling.

Recently, the FGF21 analogs efruxifermin and pegozafermin demonstrated clinical efficacy in the treatment of MASH [6, 7]. Those compounds can be considered nonselective FGFR agonists in that, like native FGF21, they signal through multiple 'c' isoforms of the FGFR 1-3/KLB complex. However, the physiological effects of activation of the FGFR2c/KLB and FGFR3c/KLB are incompletely understood [8, 9].

Another approach taken to FGF21 drug development has been to target the components of the FGFR1c/KLB co-receptor complex (FGFR1c and KLB or KLB alone). The rationale for this approach is based on what is known about the tissue distributions of KLB and FGFRs 1–3 in both preclinical species and humans, and genetic experiments defining the physiological role of FGFR1 in mice [10–16]. Quantitative assessment of the mRNA distribution of KLB and FGFR isoforms in mice indicates that KLB is highly expressed in the liver, adipose tissue and pancreas, whereas FGFR1c, FGFR2c and FGFR3c have broader tissue distributions [10–12]. FGFR1 was also shown to be highly expressed in human adipocytes [13, 14]. Importantly, conditional deletion of FGFR1 in adipose tissue in mice demonstrated the importance of FGFR1 and adipose tissue in the metabolic actions of FGF21 [11, 12, 15, 16]. Taken together, these data suggest that selectively targeting components of the FGFR1c/KLB co-receptor complex could provide the metabolic effects of FGF21 required for the treatment of MASH while avoiding potential side effects associated with broader activation of FGF21 signalling pathways.

Bispecific monoclonal antibodies (mAbs) that bind both KLB and the FGFR1c, leading to the formation of an activated FGFR1c/KLB complex, have been described [11, 12]. In this study, MK-3655 (formerly NGM313), a humanised mAb that binds KLB and activates the FGFR1c/KLB complex, was administered to patients with pre-cirrhotic MASH.

2 | Methods

2.1 | Participant Selection

This study enrolled males and females aged 18 to 80 years (aged 20 to 80 years in Japan and Taiwan). Inclusion criteria included histological confirmation of MASH based on a non-alcoholic fatty liver disease activity score (NAS) ≥ 4 with a score ≥ 1 point in each component (steatosis, ballooning and lobular inflammation) and a MASH Clinical Research Network (CRN) fibrosis score of Stage 2 or 3, an LFC of ≥ 8% as assessed by MRI-PDFF, body mass index (BMI) ≥ 25 kg/m^2 and ≤ 50 kg/m^2, and either no history of type 2 diabetes mellitus (T2DM) or a history of T2DM with a haemoglobin A1C ≤ 9.5% at screening and controlled by diet and/or stable doses of antihyperglycemic agents.

Key exclusion criteria included presence of cirrhosis on liver biopsy; diagnosis of type 1 diabetes mellitus; history or evidence of chronic liver disease other than MASH; history of malignancy (unless cancer free ≥ 5 years) or under evaluation for active or suspected malignancy (except for adequately treated basal cell or squamous cell skin cancer or in situ cervical cancer); history of bariatric surgery ≤ 5 years before study participation; and significant systemic or major illnesses other than liver disease, including recent events (≤ 6 months before study entry) of congestive heart failure, unstable coronary artery disease, arterial revascularisation, pulmonary disease, renal failure, stroke, transient ischemic attack, or organ transplantation.

2.2 | Study Design

This was a Phase 2b, randomised, multicenter, double-blind, placebo-controlled, parallel-group, interventional study to evaluate the safety and efficacy of MK-3655 in adult patients with pre-cirrhotic MASH (protocol P001; EudraCT: 2019-003048-63; NCT: 04583423). The study protocol was approved by the institutional review board or independent ethics committee at each investigational site and conducted in accordance with applicable regulations and the ethical principles of Good Clinical Practice as defined by the International Conference on Harmonisation and Declaration of Helsinki. Written informed consent was obtained from all participants.

The study design is shown in Figure 1. This included a 6-week screening period, a 2-week single-blind placebo run-in period, a 52-week double-blind, placebo-controlled treatment period, and a post-treatment follow-up visit. Participants who met entry criteria were randomised 1:1:1:1 to receive either 50 mg, 100 mg, or 300 mg of MK-3655 s.c., or matching placebo s.c., self-administered once every 4 weeks. Randomisation was stratified by concurrent diagnosis of T2DM (Yes or No) at the time of randomisation, fibrosis score (Stage 2 or 3), and region (Japan, East Asia excluding Japan or Other).

Patients with a liver biopsy performed within 6 months before screening were eligible to participate if histology from the biopsy met entry criteria, LFC by MRI-PDFF was ≥ 8%, and all other eligibility criteria were met and they proceeded

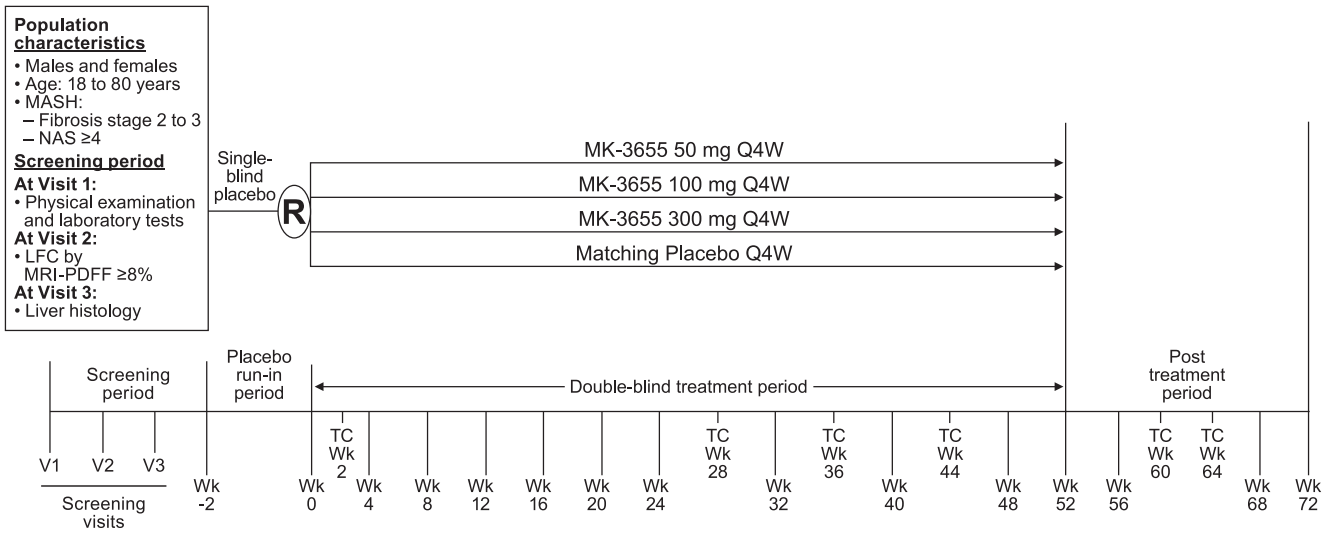

FIGURE 1 | Study design. For participants in Japan and Taiwan, the population age was from 20 to 80 years. All participants had a liver biopsy performed within 6 months of screening or conducted during the screening period that was read centrally by a pathologist to confirm eligibility for the study. The duration of the screening period was approximately 6 weeks. LFC, liver fat content; MRI-PDFF, magnetic resonance imaging-proton density fat fraction; MASH, metabolic dysfunction-associated steatohepatitis; NAS, nonalcoholic fatty liver disease activity score; R, randomisation; Q4W, once every 4 weeks; TC, telephone contact; V, visit; Wk, week.

to the placebo run-in. Patients without a liver biopsy within 6 months before screening in whom LFC by MRI-PDFF was ≥ 8% and all other eligibility criteria were met underwent a liver biopsy and proceeded to the placebo run-in if liver histology met eligibility criteria.

All participants received dietary and activity guidance sheets at the beginning of the placebo run-in. At subsequent visits, site staff reviewed the diet and activity guidance sheets with the participants. Also, at the start of the placebo run-in, participants received training/instructions on preparation and self-administration of the investigational product (or placebo) and were witnessed in the self-administration of two 1.5 mL SC injections of single-blind placebo. Participants also received a diary that was to be used throughout the study to record experiences with self-injection and any immediate/delayed injection reactions.

2.3 | Dose Selection

The chosen doses and dosing regimen were informed by pharmacokinetic/pharmacodynamic (PK/PD) modelling of Phase 1 data to achieve a range of responses and inform the dose–response relationship. Data available for modelling included the biomarkers adiponectin, triglycerides, HDL-C, and homeostatic model assessment of insulin resistance (HOMA-IR) from single and multiple ascending dose studies; pharmacokinetic data from these same studies to determine when saturation of target-mediated drug disposition (TMDD) occurs; and reductions in liver fat as determined by MRI-PDFF in a Phase 1 proof of concept study. TMDD is a nonlinear PK phenomenon that is caused by high-affinity and high-specificity binding of drug to its low-capacity pharmacologic targets, resulting in linear PK (dose proportionality) at high doses but non-linear PK at low, non-saturating doses. Together, these assessments demonstrated that 50 mg Q4W was projected to achieve a demonstrative, but sub-maximal, response, while 300 mg Q4W was projected to be at the plateau of the dose–response relationship.

2.4 | Efficacy Endpoints

The primary efficacy endpoint was MASH resolution (defined as a score of 0–1 for lobular inflammation, 0 for ballooning, and any grade of steatosis) without worsening of fibrosis at Week 52, based on histological assessments of liver biopsies centrally read by the blinded independent central review (BICR) using the MASH CRN scoring system. Secondary efficacy endpoints included mean percent relative reduction from baseline in LFC measured by MRI-PDFF (evaluated by BICR) at Week 24 and the proportion of participants with ≥ 1 stage improvement in fibrosis without worsening of steatohepatitis (defined as no increase in the NAS for ballooning, inflammation, or steatosis) at Week 52. For participants who discontinued study intervention but had completed at least 12 weeks of treatment, obtaining an end-of-study liver biopsy was encouraged as part of discontinuation visit procedures.

2.5 | Pharmacokinetic Assessment

MK-3655 serum concentrations were determined from samples collected before each dose administration at Weeks 0, 4, 8, 12, 24, and 52. The PK endpoint for this study was the MK-3655 serum trough concentration (C_{trough}) taken before each dose administration.

2.6 | Pharmacodynamic Assessments

PD endpoints at Week 52 included changes from baseline in total adiponectin measured by ELISA (Covance) and body composition and bone mineral density assessed by dual-energy X-ray absorptiometry (DXA).

2.7 | Safety Assessments

Safety and tolerability were monitored throughout the study by clinical evaluation of adverse events and assessment of other study parameters including vital signs, body weight, physical examination, laboratory safety tests, measures of hypothalamic–pituitary function (adrenocorticotropic hormone, insulin-like growth factor-1, thyrotropin, free thyroxine, salivary cortisol), 12-lead electrocardiograms, and DXA. Prespecified safety events of clinical interest included drug-induced liver injury and suicidality as assessed by the Columbia-Suicide Severity Rating Scale.

2.8 | Statistical Analysis

The efficacy analysis population included all randomised participants who received at least one dose of the study intervention and had at least one assessment for the efficacy endpoint being analysed.

Participants with missing post-baseline biopsy data were considered not to have achieved Week 52 histologic efficacy endpoints. Participants without an end-of-study biopsy because of early study termination by the Sponsor were excluded from histologic efficacy analyses.

The primary efficacy analysis compared the efficacy of MK-3655 to placebo in the proportion of individuals with MASH resolution (defined as a score of 0–1 for lobular inflammation, 0 for ballooning, and any grade of steatosis) without worsening of fibrosis by histology at Week 52. The difference (MK-3655 minus placebo; three pair-wise comparisons) in proportions, and the associated 95% CIs and *p*-values, used the stratified Miettinen and Nurminen (M&N) method and Cochran–Mantel–Haenszel weighting scheme.

The secondary endpoint of mean percent relative reduction from baseline in LFC for each of the three pair-wise comparisons of MK-3655 versus placebo was analysed using the Constrained Longitudinal Data Analysis (cLDA) method of Liang and Zeger [17].

The secondary histological endpoint of the proportion of participants achieving ≥1 stage improvement in fibrosis without worsening of steatohepatitis for each of the three pair-wise comparisons of MK-3655 versus placebo was analysed using the M&N method used for the primary efficacy endpoint.

Mean body weight over time was summarised descriptively.

The safety analysis population consisted of all randomised participants who received at least one dose of the study intervention.

Study sample size was chosen based on the proportion of participants with MASH resolution without worsening in fibrosis after 52 weeks. Assuming the true proportion is 35% for each MK-3655 dose versus 12% for placebo and the proportion using a non-completer (failure) approach is 30% for each MK-3655 dose versus 10% for placebo, a sample size of 82 participants per group provided 90% power to establish that MK-3655 was superior to placebo with respect to each of the three pair-wise treatment comparisons, with a 1-sided $\alpha = 0.025$. For the secondary endpoint of percent relative reduction from baseline in LFC after 24 weeks, a sample size of 82 participants per group provided > 99% power, assuming a standard deviation of 20% and mean difference over placebo ≥ 40%, with a 1-sided $\alpha = 0.025$.

A prespecified interim analysis (IA) was performed for administrative purposes and to assess futility after at least 25 participants per treatment group completed the Week 24 LFC assessment. IA results were reviewed by an external data monitoring committee (eDMC) and subsequently by the Sponsor's internal Executive Oversight Committee (EOC). Key endpoints evaluated included LFC, body weight, adverse events, and laboratory parameters. The study was to be stopped for futility if the posterior probability was < 5% that the true mean difference (MK-3655 minus placebo) in the percent reduction from baseline in LFC was ≥ 40% for all MK-3655 groups.

3 | Results

This study was performed from November 11, 2020, to April 13, 2023, across 180 centers in 23 countries. Participant disposition is shown in Figure 2. A total of 183 participants were randomised to treatment, of which 37 completed the study. The majority of participants (136 [74.3%]) discontinued the study due to early trial termination by the Sponsor. The proportions of participants who discontinued from the study were similar across the treatment groups.

3.1 | Demographics and Baseline Characteristics

The demographics and baseline characteristics of the randomised participants were generally comparable across the treatment groups (Table 1). Among the 183 randomised subjects (MK-3655 50 mg, $N = 45$; MK-3655 100 mg, $N = 47$; MK-3655 300 mg, $N = 46$; placebo, $N = 44$), all participants had a primary diagnosis of MASH, the majority (52.5%) were male, the mean age was 56.5 years, the mean body mass index was 33.4 kg/m^2, the mean body weight was 92.2 kg, 52.5% had T2DM, 53.6% had a fibrosis score of Stage 3, and the mean LFC was 18.1%.

3.2 | Efficacy

At the IA, futility criteria were not met; however, the between-group differences (MK-3655 relative to placebo) in the least squares (LS) mean percent relative reductions in LFC with MK-3655 at Week 24 were assessed by the EOC as insufficient for further clinical development.

Prior to study completion, 137 participants had baseline and Week 24 assessments of LFC. At Week 24, the LS mean relative reductions in LFC from baseline were 11.0%, 30.1%, 30.0%, and 37.2% in the placebo, 50-mg, 100-mg, and 300-mg groups, respectively. The differences in the LS mean relative reduction from baseline in LFC at Week 24 in the MK-3655 50-mg, 100-mg, and 300-mg groups compared to placebo were 19.1% (95% CI: 1.7, 36.4), 19.0% (2.2, 35.8), and 26.1% (9.5, 42.8), respectively

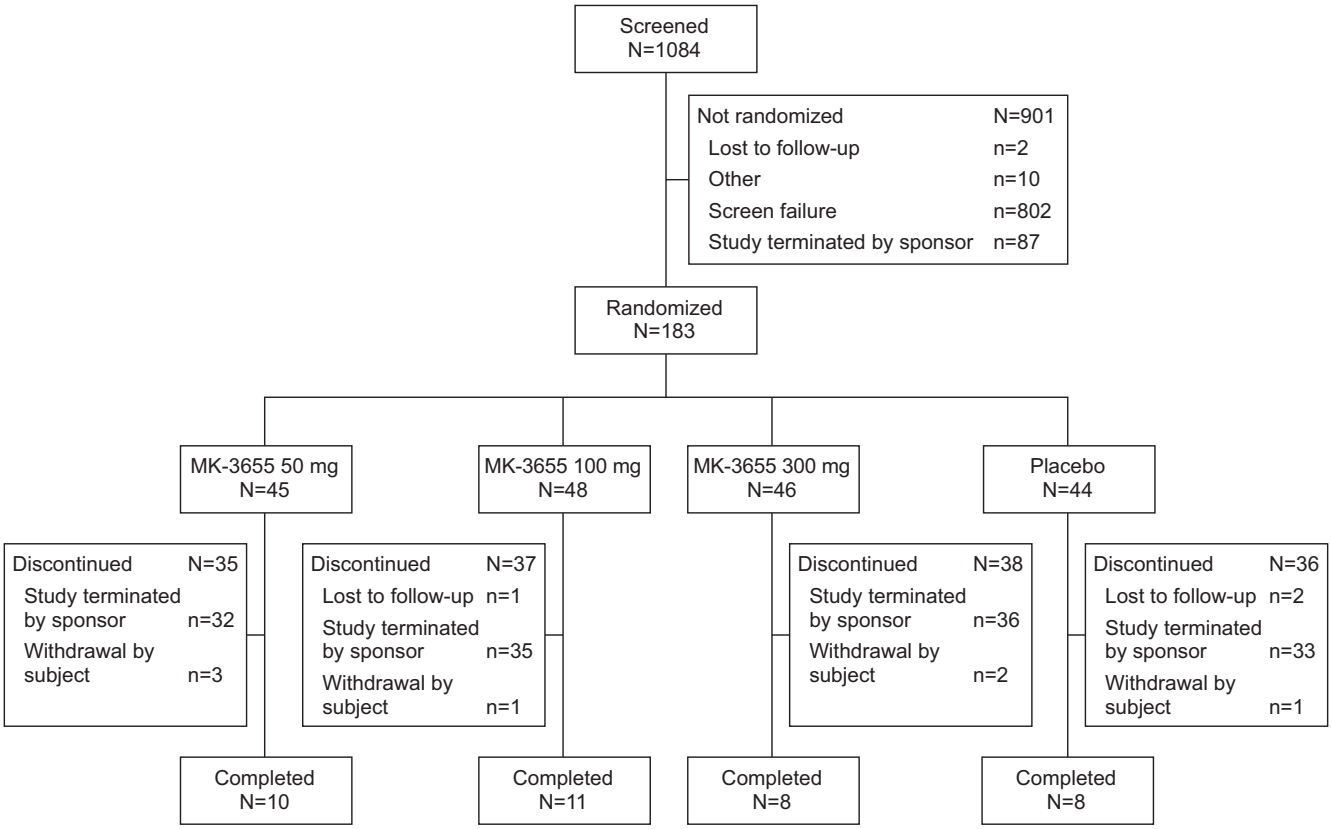

FIGURE 2 | Disposition of participants.

(Figure 3). The results of this assessment were consistent with the results of the IA, which included MRI-PDFF Week 24 data from 110 participants.

Of the 183 randomised participants, 73 were included in the Week 52 histologic efficacy assessments; 62 of these 73 participants had an end-of-study biopsy at Week 52 (Table 2). The proportions of individuals with MASH resolution without worsening of fibrosis at Week 52 were 5.9%, 16.7%, 14.3%, and 17.6% in the placebo, 50-mg, 100-mg, and 300-mg groups, respectively (Table 2). The proportions of participants who had a ≥ 1 stage improvement in fibrosis without worsening of steatohepatitis at Week 52 were 17.6%, 22.2%, 38.1%, and 29.4% in the placebo, 50-mg, 100-mg, and 300-mg groups, respectively (Table 2).

3.3 | Pharmacokinetics

MK-3655 serum C_{trough} concentrations generally increased in a greater-than-dose-proportional manner, consistent with previous observations and the presence of target-mediated drug disposition (TMDD) (Table S1). The saturation of TMDD occurs at the higher end of the dose range. Variability in PK was higher at the lower doses.

3.4 | Pharmacodynamics

Significant mean increases from baseline in adiponectin were observed in all MK-3655 groups compared with placebo at Week 24 (Figure 4). Although the mean adiponectin levels increased with increasing doses of MK-3655, the 95% CIs across doses overlapped (Table S2). Adiponectin levels remained higher in the MK-3655 100-mg and 300-mg groups compared with placebo at Week 52 (Figure 4, Table S2).

3.5 | Anti-Drug Antibodies

Of the 141 participants who received MK-3655, 24 (17.0%) displayed a positive anti-drug antibody (ADA) response to MK-3655 (22 treatment-emergent positive [negative at baseline and positive post dose] and two treatment-boosted positive [positive at baseline and post-dose titre increased by ≥ 2-fold relative to the baseline titre]), with no apparent trend of ADA incidence with dose level. A neutralising antibody assay was not available at the time of study termination.

3.6 | Safety

Table 3 summarises the cumulative safety data at the time of study termination. The incidences of adverse events overall were similar across treatment groups. Incidences of adverse events considered related to the study intervention by the investigator were higher in the MK-3655 groups compared with placebo. There were otherwise no meaningful differences between the MK-3655 and placebo groups in the incidence of serious adverse events, adverse events that led to discontinuation, or safety events of clinical interest (drug-induced liver injury or suicidality). No serious adverse events were considered related to the study intervention. No deaths were reported.

TABLE 1 | Demographics and baseline characteristics.

	MK-3655 50 mg *N* = 45	MK-3655 100 mg *N* = 48	MK-3655 300 mg *N* = 46	Placebo *N* = 44	Total *N* = 183
Sex, *n* (%)					
Male	20 (44.4)	28 (58.3)	23 (50.0)	25 (56.8)	96 (52.5)
Female	25 (55.6)	20 (41.7)	23 (50.0)	19 (43.2)	87 (47.5)
Mean (SD) Age, years	59.6 (8.9)	54.3 (12.3)	56.6 (11.3)	55.8 (10.1)	56.5 (10.8)
Race, *n* (%)					
American Indian Or Alaska Native	0	0	0	1 (2.3)	1 (0.5)
Asian	15 (33.3)	18 (37.5)	16 (34.8)	16 (36.4)	65 (35.5)
Black Or African American	3 (6.7)	2 (4.2)	1 (2.2)	1 (2.3)	7 (3.8)
Multiple	0	0	1 (2.2)	0	1 (0.5)
Black Or African American, White	0	0	1 (2.2)	0	1 (0.5)
Native Hawaiian Or Other Pacific Islander	1 (2.2)	0	1 (2.2)	0	2 (1.1)
White	26 (57.8)	28 (58.3)	27 (58.7)	26 (59.1)	107 (58.5)
Ethnicity, *n* (%)					
Hispanic Or Latino	12 (26.7)	13 (27.1)	17 (37.0)	18 (40.9)	60 (32.8)
Not Hispanic Or Latino	33 (73.3)	34 (70.8)	28 (60.9)	25 (56.8)	120 (65.6)
Unknown	0	1 (2.1)	1 (2.2)	1 (2.3)	3 (1.6)
Primary diagnosis of MASH, *n* (%)	45 (100)	48 (100)	46 (100)	44 (100)	183 (100)
Mean (SD) BMI, kg/m^2	33.8 (6.5)	33.6 (6.5)	32.2 (4.5)	33.7 (5.9)	33.4 (5.9)
Mean (SD) Body weight, kg	92.2 (22.2)	95.2 (23.3)	88.6 (17.7)	92.8 (19.8)	92.2 (20.9)
T2DM (stratification), *n* (%)					
Yes	22 (48.9)	25 (52.1)	25 (54.3)	24 (54.5)	96 (52.5)
No	23 (51.1)	23 (47.9)	21 (45.7)	20 (45.5)	87 (47.5)
Fibrosis Score (stratification), *n* (%)					
Stage 2	21 (46.7)	22 (45.8)	21 (45.7)	21 (47.7)	85 (46.4)
Stage 3	24 (53.3)	26 (54.2)	25 (54.3)	23 (52.3)	98 (53.6)
Region (stratification), *n* (%)					
Japan	11 (24.4)	12 (25.0)	11 (23.9)	11 (25.0)	45 (24.6)
East Asia excluding Japan	6 (13.3)	5 (10.4)	5 (10.9)	5 (11.4)	21 (11.5)
Other	28 (62.2)	31 (64.6)	30 (65.2)	28 (63.6)	117 (63.9)
Mean (SD) LFC, %	18.0 (7.9)	20.1 (7.3)	17.4 (6.0)	16.9 (7.5)	18.1 (7.2)

Abbreviations: BMI, body mass index; LFC, liver fat content; MASH, metabolic-associated steatohepatitis; T2DM, type 2 diabetes mellitus.

The number of participants meeting predefined limits of change in liver function tests (two in each of the MK-3655 100-mg, MK-3655 300-mg, and placebo groups) is shown in Table 3.

There were small mean percent increases from baseline in body weight over time in the three MK-3655 groups compared with placebo (Figure 5).

Small increases in mean systolic blood pressure in the MK-3655 groups compared with placebo were observed at most time points, with no other notable changes in vital signs (data not shown).

DXA analysis showed no notable differences in mean changes from baseline over time in body composition or bone mineral

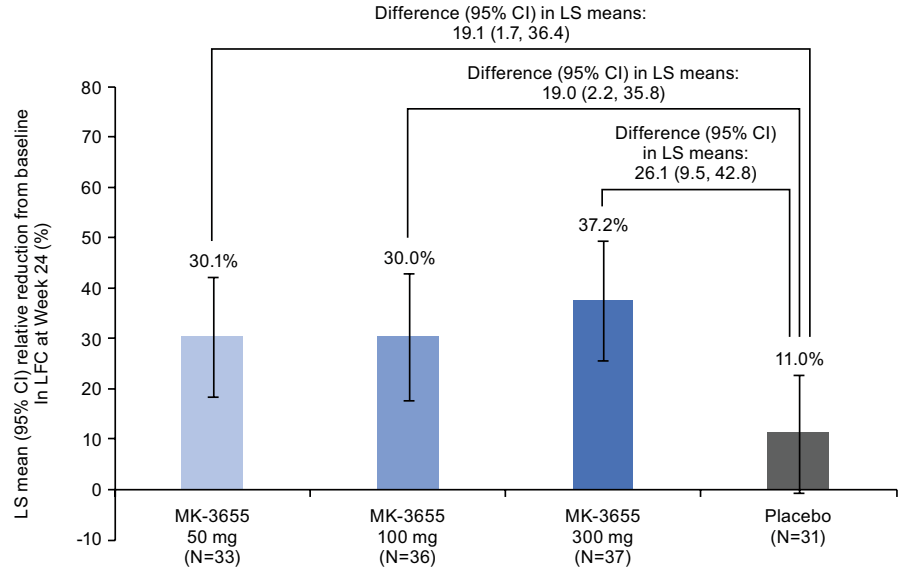

FIGURE 3 | Least squares (LS) mean percent relative reduction from baseline in liver fat content (LFC) at Week 24.

TABLE 2 | Histologic efficacy results.

			Treatment versus placebo at 52 weeks	
Treatment	***N*[a]**	***n* (%)**	**Difference in % (95% CI)[b]**	***p*-value**
Proportion of participants with MASH resolution without worsening of fibrosis by histology				
MK-3655 50 mg	18	3 (16.7)	10.4 (−13.4, 35.1)	0.3504
MK-3655 100 mg	21	3 (14.3)	9.2 (−15.6, 32.1)	0.3730
MK-3655 300 mg	17	3 (17.6)	15.4 (−8.5, 42.3)	0.1428
Placebo	17	1 (5.9)		
Proportion of participants with ≥1 Stage improvement in fibrosis without worsening of steatohepatitis				
MK-3655 50 mg	18	4 (22.2)	1.6 (−26.5, 30.3)	0.8978
MK-3655 100 mg	21	8 (38.1)	20.0 (−10.6, 46.4)	0.1869
MK-3655 300 mg	17	5 (29.4)	8.5 (−22.1, 38.5)	0.5636
Placebo	17	3 (17.6)		

Note: MASH resolution is defined as a score of 0–1 for inflammation, 0 for ballooning, and any grade of steatosis.
Abbreviation: MASH, metabolic dysfunction-associated steatohepatitis.
[a]Includes participants who underwent an end-of-study biopsy at Week 52, those who completed Week 52 but had missing end-of-study biopsy data, and those who discontinued due to reasons other than early termination of the study and who had at least 12 weeks of treatment but had missing end-of-study biopsy data. Excludes participants who did not have an end-of-study biopsy assessment and who discontinued due to early termination of the study. Of the 73 participants included in the histologic efficacy analyses, 62 had an end-of-study biopsy at Week 52 and 11 had no end-of-study biopsy.
[b]Based on Miettinen and Nurminen's method stratified by concurrent diagnosis of type 2 diabetes mellitus at the time of randomisation (yes, no) and fibrosis score (Stage 2 or Stage 3) and using the Cochran–Mantel–Haenszel weighting scheme.

density (hip, spine, femoral neck, and whole body) across the treatment groups (data not shown).

There were no notable differences in mean changes from baseline over time in measures of the hypothalamic–pituitary function across the treatment groups (data not shown).

4 | Discussion

MK-3655 is a humanised mAb that binds KLB with high affinity, which leads to the formation of an active FGFR1c/KLB complex. MK-3655 was designed to stimulate the metabolic effects of FGF21 in tissues that co-express FGFR1c and KLB, especially adipose tissue. The results of this study showed that in participants with pre-cirrhotic MASH (fibrosis stages F2 and F3), the placebo-adjusted relative reduction in LFC from baseline at Week 24 with MK-3655 was less than 30% in all active treatment groups, suggesting insufficient reduction in liver fat. The study was stopped early, after the prespecified interim analysis, because hepatic fat content impact was predicted to yield insufficient improvements in MASH histology.

Recently, the nonselective FGF21 analogs efruxifermin (a bivalent FGF21-Fc fusion protein) and pegozafermin (a glycopegylated FGF21) have been demonstrated in clinical studies

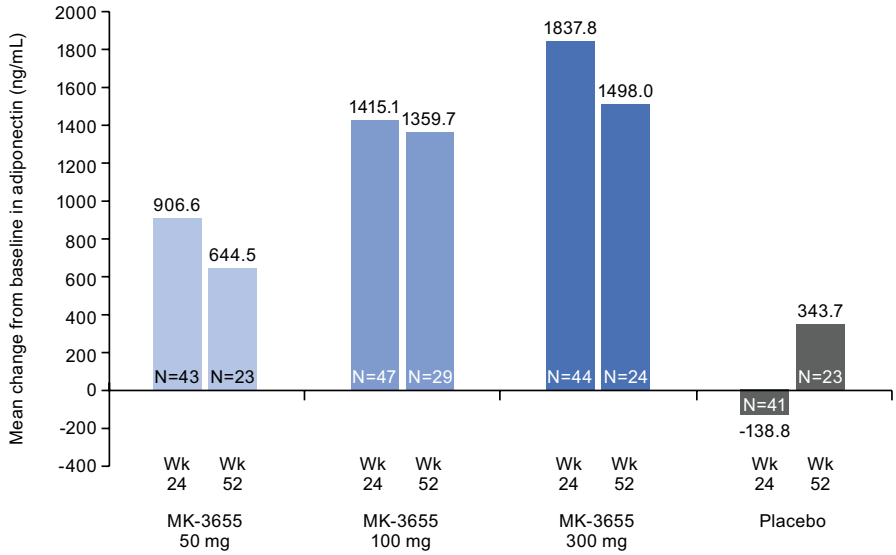

FIGURE 4 | Mean change from baseline in adiponectin (ng/mL) at Week 24 and Week 52.

to have efficacy in MASH resolution and improving fibrosis [6, 7]. In those studies, greater relative reductions in LFC were observed at a Week 24 timepoint than that observed in this MK-3655 study.

In the Phase 2b HARMONY study, treatment of participants with pre-cirrhotic MASH (fibrosis stages F2 and F3) with efruxifermin resulted in an LS mean relative percentage reduction in LFC (as assessed by MRI-PDFF) from baseline at Week 24, of −51.6% and −63.7% in the efruxifermin 28-mg and 50-mg dose groups, respectively, compared with −6.0% with placebo [6]. In the Phase 2b ENLIVEN study, treatment of participants with pre-cirrhotic MASH (fibrosis stages F2 and F3) with pegozafermin resulted in an LS mean relative percentage reduction in LFC (as assessed by MRI-PDFF) from baseline at Week 24 of −27.1%, −48.2%, and −41.9%, in the pegozafermin 15-mg, 30-mg, and 44-mg dose groups, respectively, compared with −5.0% with placebo [7].

However, nonselective FGF21 analogs have not uniformly been successful in reducing LFC and resolving MASH. In the Phase 2b FALCON 1 study, participants with pre-cirrhotic MASH (fibrosis stage F3) were treated with the pegylated FGF21 analog pegbelfermin. In that study, the relative reductions in LFC compared to placebo were not reported; however, the reported percentage of participants with > 30% relative reduction in hepatic fat fraction (assessed by MRI-PDFF) suggests the relative reductions in LFC were not robust [18]. The percentage of participants with > 30% reduction in LFC was 22%–36% in the pegbelfermin groups compared with 6% for placebo at Week 24 (between-group significance not reported), and 20%–23.1% in the pegbelfermin groups compared to 8.8% for placebo at Week 48 (between-group differences from placebo did not reach statistical significance). Consistent with this observation, the primary endpoint of ≥ 1 point decrease in fibrosis score without MASH worsening or MASH improvement without fibrosis worsening was not met in the FALCON 1 study.

MK-3655 PK from this Phase 2b study confirmed the presence of TMDD behaviour observed from previous Phase 1 studies, with saturation of TMDD at the 300 mg Q4W dose, suggesting saturation of the target and concentrations on the plateau of the dose–response relationship. Assessment of data from Phase 1 studies indicated that saturation of TMDD occurred at serum concentrations of approximately 1–3 μg/mL, and serum C_{trough} levels at the 300 mg Q4W dose were well in excess of this saturating level. Projections of the dose–response relationship also appear to be consistent with results from this study, as a linear increase in LFC reduction was not observed across the 50–300 mg Q4W dose range, suggesting that doses higher than 300 mg Q4W would not likely achieve a greater response.

Adiponectin measurements in this study are of interest. Preclinical studies suggest adiponectin is an important mediator of the metabolic effects of FGF21 on glucose homeostasis and insulin sensitivity in mice [19]; therefore, adiponectin may serve as a biomarker for FGF21 target engagement in humans. While adiponectin measurements showed a dose–response increase from baseline in the current study, the increase was not commensurate with differences in dose or PK, further suggesting limited benefit of dose increases above 300 mg Q4W.

The reason(s) for the modest reduction in LFC observed in the present study are unclear, but several possibilities can be postulated. Unlike FGF21 analogs, which bind both components of the FGFR/KLB co-receptor complex, MK-3655 binds only KLB. One possibility is that the MK-3655 and KLB interaction does not lead to a fully activated FGFR1/KLB co-receptor complex. Another possibility is that the receptor complex that is formed has altered downstream signalling.

Another possibility is that broader activation of FGFR subtypes, including FGFR2 and 3, are required to achieve optimal metabolic effects in humans for the treatment of MASH. Interspecies differences in the physiological effects of the FGF21 pathway exist, and preclinical findings indicating that activation of FGFR1c/KLB in adipose tissue is predominantly responsible for the metabolic effects of FGF21 (and by inference might be sufficient for treating MASH) may not translate to humans. One notable example of interspecies differences is the effect of FGF21 on body weight, where profound weight loss is observed in mice and non-human

TABLE 3 | Adverse events and safety events of clinical interest.

	MK-3655 50 mg		MK-3655 100 mg		MK-3655 300 mg		Placebo	
	n	(%)	*n*	(%)	*n*	(%)	*n*	(%)
Participants in population	45		47		46		44	
With one or more adverse events	33	(73.3)	33	(70.2)	35	(76.1)	34	(77.3)
With drug-related adverse events[a]	9	(20.0)	16	(34.0)	12	(26.1)	4	(9.1)
With adverse events reflecting abuse potential	1	(2.2)	0		1	(2.2)	1	(2.3)
With serious adverse events	1	(2.2)	1	(2.1)	1	(2.2)	8	(18.2)
With serious drug-related adverse events	0		0		0		0	
Who died	0		0		0		0	
Discontinued drug due to an adverse event	1	(2.2)	0		0		0	
Discontinued drug due to a drug-related adverse event	1	(2.2)	0		0		0	
Discontinued drug due to a serious adverse event	0		0		0		0	
With diarrhoea	4	(8.9)	7	(14.9)	5	(10.9)	3	(6.8)
With nausea	2	(4.4)	3	(6.4)	1	(2.2)	4	(9.1)
With vomiting	2	(4.4)	0		0		1	(2.3)
With decreased appetite	3	(6.7)	1	(2.1)	0		0	
With ALT and AST ≤ ULN at baseline who later met:	0		0		0		0	
ALT or AST > 3 × ULN or								
Total bilirubin > 2 × ULN (if direct bilirubin was elevated) or								
Alkaline phosphatase > 3 × ULN or								
INR > 1.5 × ULN or[b]								
Clinical signs or symptoms that were consistent with hepatitis[c]								
With ALT and/or AST > ULN at baseline who later met:	0		2	(4.3)	2	(4.3)	2	(4.5)
ALT and/or AST > 2 × baseline or[d]								
Total bilirubin > 1.5 × baseline AND > ULN (if direct bilirubin was elevated) or								
Alkaline phosphatase > 3 × ULN or								
INR > 1.5 × ULN or[b]								
Clinical signs or symptoms that were consistent with hepatitis[c]								

Abbreviations: ALT, alanine aminotransferase; AST, aspartate aminotransferase; INR, international normalised ratio; ULN, upper limit of normal range.
[a]Considered by the investigator to be related to the drug.
[b]For participants who initiated anticoagulant therapy after study initiation, the INR threshold does not apply.
[c]In the opinion of the investigator, such as right upper quadrant discomfort, fever, nausea, vomiting, jaundice, rash or eosinophilia > 5%.
[d]The criterion of '> 2× baseline' only applied to the analyte (i.e., ALT or AST) that was > ULN at baseline. Otherwise, if the analyte was ≤ ULN at baseline, the criterion of '> 3× ULN' applied.

primates, whereas very limited weight loss and even weight gain are observed with FGF21 agonists in humans [6, 7, 14, 20].

Regarding selective mechanisms of engagement of the FGFR1c/KLB complex for the treatment of MASH, the results of BFKB8488A (Genentech) are potentially relevant. BFKB8488A is a bispecific mAb that binds KLB and FGFR1c [21]. In a Phase 1 study, mean relative reductions in LFC from baseline of −13.0%, −34.5%, and −49.0% in the low- ($N = 12$), medium- ($N = 20$), and high- ($N = 10$) exposure tertiles, respectively, compared with 0.1% with placebo ($N = 14$), were observed in the subset of participants with MASLD at Day 85. The small sample size, short treatment period, and post hoc analysis by tertiles limit interpretation of the data; nonetheless, the results of the study are potentially supportive of the concept of selectively targeting the FGFR1c/KLB complex for the treatment of MASH. A subsequent Phase 2 study in patients with MASH with the same compound (NCT04171765) was prematurely terminated (reasons not disclosed); in that study, reductions in LFC (11 participants in each BFKB8488A dose group and 13 in the placebo group) at Week 16 were less than

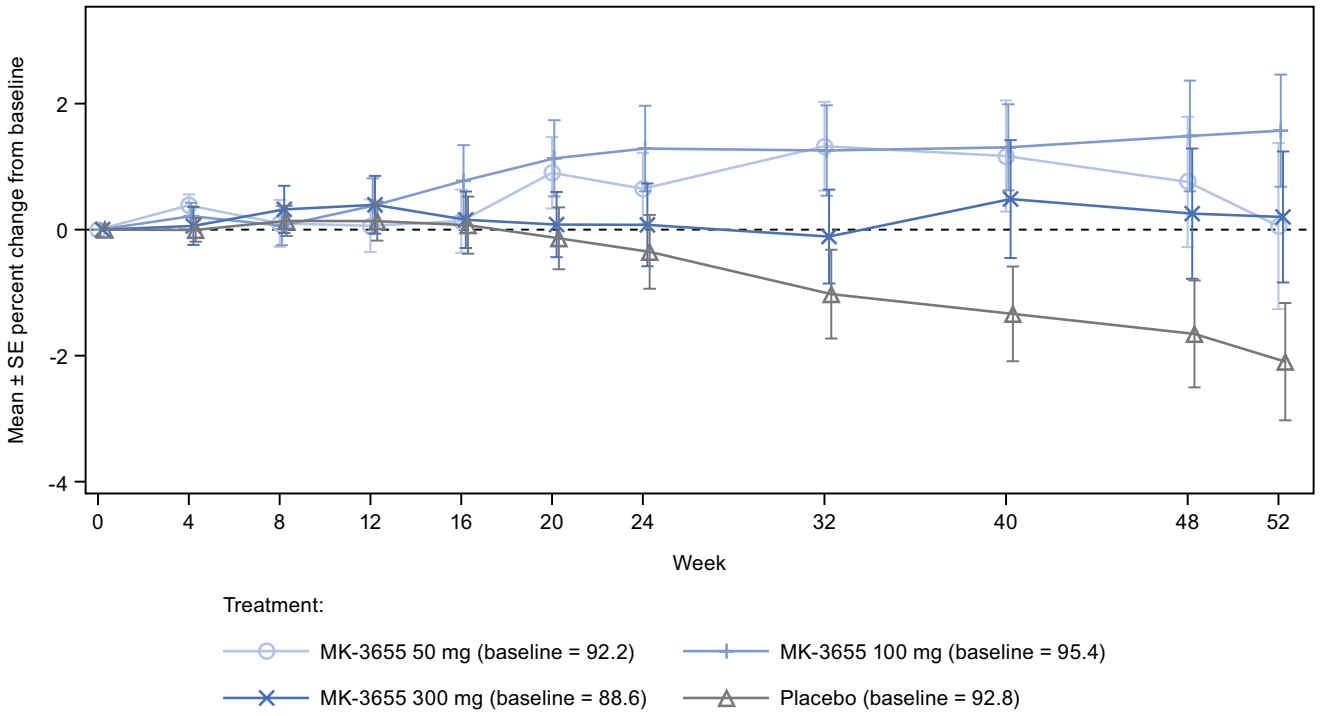

FIGURE 5 | Mean (SE) percent change from baseline over time in body weight. Baseline body weight (kg) is shown in parentheses for each treatment group in the figure legend.

those observed in the Phase 1 study and, as in the Phase 1 study, variability in dose–response was observed [22]. Therefore, based on publicly available data, no conclusions can be drawn about the efficacy of this bifunctional antibody approach.

In the present study, MK-3655 appeared to have a lower incidence of gastrointestinal-related adverse events (nausea, vomiting, and diarrhoea) compared with what has been reported in the efruxifermin HARMONY and pegozafermin ENLIVEN studies [6, 7]. Although cross-study comparisons of adverse events are inherently difficult to make because of multiple factors (e.g., heterogenous populations, different study designs), it is possible that the selectivity of MK-3655 signalling resulted in the improvement of its gastrointestinal profile compared with FGF21 analogs.

The incidences of the adverse event of increased appetite in this study were similar to those observed in the HARMONY and ENLIVEN studies, which suggest a class effect for this adverse event [6, 7]. Consistent with the adverse event of increased appetite, small mean increases in body weight were observed in participants treated with MK-3655 compared with placebo. In the HARMONY study, a small but nonsignificant reduction in body weight was observed with the high dose of efruxifermin (100 mg), while no effect of pegozafermin on body weight was reported in the ENLIVEN study [6, 7].

Small increases in mean systolic blood pressure were observed in the MK-3655 groups compared with placebo at most time points. Transient increases in systolic blood pressure were reported in the HARMONY study in the efruxifermin 28-mg group, but by Week 24 were reported not to be significantly different from placebo [6]. However, 4–7 mmHg increases in systolic blood pressure were observed in the efruxifermin SYMMETRY study (MASH F4 fibrosis population) at Week 36 [23]. This raises the question of whether increases in systolic blood pressure are an FGF21 class effect. Blood pressure changes with pegozafermin were not reported in the ENLIVEN study [7].

In our study, limited histological data at Week 52 did not allow definitive conclusions to be made regarding the impact of MK-3655 on MASH resolution or fibrosis improvement, although a numerically greater proportion of patients in the MK-3655 groups achieved histological endpoints compared to placebo (5.9%, 16.7%, 14.3%, and 17.6% of patients in the placebo, 50-mg, 100-mg, and 300-mg groups, respectively, achieved MASH resolution without worsening of fibrosis; 17.6%, 22.2%, 38.1% and 29.4% of patients in the placebo, 50-mg, 100-mg, and 300-mg groups, respectively, achieved ≥1 stage improvement in fibrosis without worsening of steatohepatitis). It is acknowledged that without statistically significant histological data, a definitive conclusion about the relationship between LFC and MASH resolution or fibrosis improvement cannot be made. The lack of assessment of neutralising antibodies might also be considered a potential limitation.

In summary, treatment with MK-3655 led to a modest reduction in LFC in participants with and without T2DM and MASH and was generally well tolerated. The results of this study should not be interpreted as definitive evidence that selective activation of the FGFR1c/KLB complex is a non-viable strategy for MASH drug development, but the results do contribute to that discussion and contribute to a further scientific understanding of the physiology of FGF21 in humans.

Author Contributions

Annaswamy Raji: conceptualization, methodology, formal analysis, writing – original draft, writing – review and editing. **Ira**

Gantz: methodology, formal analysis, writing – original draft, writing – review and editing. **Michael Crutchlow:** conceptualization, methodology, writing – review and editing. **Heather Flynn:** project administration, writing – review and editing. **Lianzhe Xu:** methodology, formal analysis, writing – original draft, writing – review and editing. **Anthony J. Rodgers:** methodology, formal analysis, writing – review and editing. **Radha Krishnan:** methodology, writing – review and editing. **Matthew L. Rizk:** methodology, writing – review and editing. **Shuai Hu:** methodology, writing – review and editing. **Keith D. Kaufman:** methodology, conceptualization, writing – original draft, writing – review and editing, formal analysis. **Samuel S. Engel:** formal analysis, conceptualization, methodology, writing – original draft, writing – review and editing.

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Conflicts of Interest

A. Raji, I. Gantz, M. Crutchlow, H. Flynn, L. Xu, A.J. Rodgers, R. Krishnan, M.L. Rizk, S. Hu, K.D. Kaufman, and S.S. Engel are current or former employees of Merck Sharp & Dohme LLC, a subsidiary of Merck & Co. Inc., Rahway, NJ, USA, and may own stock/stock options in Merck & Co. Inc., Rahway, NJ, USA.

Data Availability Statement

The data sharing policy, including restrictions, of Merck Sharp & Dohme LLC, a subsidiary of Merck & Co. Inc., Rahway, NJ, USA, is available at http://engagezone.msd.com/ds_documentation.php. Requests for access to the clinical study data can be submitted through the EngageZone site or via email to dataaccess@msd.com.

Protocol Number

P001.

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Supporting Information

Additional supporting information can be found online in the Supporting Information section.