Antisense oligonucleotide DGAT-2 inhibitor, ION224, for metabolic dysfunction-associated steatohepatitis (ION224-CS2): results of a 51-week, multicentre, randomised, double-blind, placebo-controlled, phase 2 trial

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Summary

Background ION224, a liver-directed antisense inhibitor of diacylglycerol O-acyltransferase 2 (DGAT2), suppresses de novo lipogenesis, an important metabolic pathway associated with lipotoxicity and the underlying inflammation, hepatocellular injury, and fibrosis in metabolic dysfunction-associated steatohepatitis (MASH). This study aimed to prospectively assess the safety and efficacy of ION224 in patients with MASH and fibrosis.

Methods ION224-CS2 was an adaptive, two-part, multicentre, randomised, double-blind, placebo-controlled, phase 2 trial conducted at 43 clinical sites in the USA and Puerto Rico in patients aged 18–75 years with biopsy-confirmed MASH and fibrosis (stages F1, F2, and F3) and baseline liver steatosis ≥10%. In part 1, participants were randomly assigned (1:1:1) to subcutaneous injections of ION224 60 mg, 90 mg, or 120 mg, or placebo, once per month. In part 2, participants were randomly assigned (2:1) to ION224 90 mg and 120 mg or placebo after a pre-specified interim analysis of safety and efficacy (liver steatosis). The primary endpoint was ≥2-point reduction in Non-Alcoholic Fatty Liver Disease Activity Score Activity Score (NAS) with ≥1-point improvement in hepatocellular ballooning or lobular inflammation, and without worsening of fibrosis at week 51. The primary analysis was in a predefined per-protocol set that included patients who received at least ten of 13 doses of the study drug without missing three consecutive doses and completed the final liver biopsy at the end of treatment. ION224-CS2 was registered at ClinicalTrials.gov (NCT04932512) and is closed.

Findings Between June 8, 2021, and Dec 27, 2022, 160 participants were randomly assigned to receive ION224 60 mg (n=23), 90 mg (n=45), or 120 mg (n=46), or placebo (n=46), of whom 123 were included in the per-protocol set. The primary endpoint was met in 18 (46%) of 39 participants in the 90-mg group (predicted risk 46·2% [95% CI 30·5–61·8]; risk difference 27·4% [95% CI 6·7–48·1], p=0·0094) and 20 (59%) of 34 in the 120-mg group (58·8% [42·3–75·4]; 40·1% [18·7– 61·4], p=0·0002) compared with six (19%) of 32 in the placebo group (predicted risk 18·7% [95% CI 5·2–32·3]). ION224 was safe and well tolerated. Adverse events were reported in 107 (94%) of participants treated with ION224 and 41 (89%) of 46 participants treated with placebo. There were no deaths and no treatment-related serious adverse events.

Interpretation This study provides the first clinical evidence that antisense-mediated inhibition of DGAT2 with ION224 could be a safe and efficacious strategy for the treatment of MASH. The observed histological improvements were independent of changes in bodyweight, suggesting potential to combine with other therapies such as GLP-1 based treatments.

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*ION224-CS2 Investigators are listed in the appendix (pp 3–7)

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See **Online** for appendix

Introduction

Metabolic dysfunction-associated steatohepatitis (MASH), formerly known as non-alcoholic steatohepatitis, is the more severe form of metabolic dysfunction-associated steatotic liver disease and can lead to liver fibrosis and cirrhosis, as well as liver-related and other adverse health outcomes.[1] The prevalence of MASH is estimated to be 5%, is globally increasing in parallel with obesity and type 2 diabetes, and is expected to affect 27 million individuals in the USA by 2030.[2,3] Up to 20% of patients with MASH are reported to develop cirrhosis,[3] and a third of patients with MASH cirrhosis will have a liver-related death.[4,5]

Developing novel therapeutic strategies for treating MASH and the associated fibrosis represents an important unmet clinical need. Several investigational therapeutic candidates with various mechanisms of action are in development to address the multifactorial pathology of MASH.[6] In 2024, the US Food and Drug Administration (FDA) approved resmetirom, an oral

Research in context

Evidence before this study

Metabolic dysfunction-associated steatohepatitis (MASH), the severe form of metabolic dysfunction-associated steatotic liver disease, is a growing global health concern, affecting an estimated 5% of the global population. The condition is characterised by excess accumulation of liver fat, hepatic inflammation, cellular injury, and fibrosis, which can progress to cirrhosis and liver-related mortality. Despite ongoing efforts to develop pharmacological treatments, only one drug, resmetirom, has received approval from the US Food and Drug Administration.

One potential therapeutic approach is to inhibit de novo lipogenesis, thereby reducing the progression of inflammation and fibrosis in MASH. Diacylglycerol O-acyltransferase 2 (DGAT2) is a rate-limiting enzyme in triglyceride synthesis. We previously demonstrated antisense-mediated DGAT2 inhibition as a potential therapeutic approach in a 13-week study in patients with type 2 diabetes and liver steatosis, in whom treatment with an unconjugated DGAT2 antisense oligonucleotide significantly (clinically and statistically) reduced hepatic steatosis. These findings provided a strong rationale for further investigation of DGAT2 inhibition as a targeted therapy for MASH and fibrosis.

We searched PubMed from database inception to April 21, 2025, for articles published in English on DGAT2 inhibition in MASH. We used the following search terms without any further restrictions: ("DGAT2 inhibition" [All Fields] AND "NASH" [All Fields] OR "DGAT2 inhibition" [All Fields] AND "MASH" [All Fields]). The search yielded 38 articles. No biopsy-anchored randomised trials were found.

Added value of this study

This multicentre, randomised, placebo-controlled trial evaluated ION224, a ligand-conjugated antisense oligonucleotide inhibitor of DGAT2, in patients with biopsy-confirmed MASH and liver fibrosis. Subcutaneous ION224 once per month for 1 year resulted in significant histological improvements, including resolution of MASH and improved fibrosis, and the effects were maintained in participants with advanced fibrosis (stage F3). ION224 was well tolerated, with no treatment-related serious adverse events or adverse effects on lipid profiles, including no hypertriglyceridemia—an issue observed with other investigational therapies targeting de novo lipogenesis. These findings support DGAT2 inhibition via a liver-directed antisense oligonucleotide as a promising therapeutic strategy for MASH.

Implications of all the available evidence

This study provides the first clinical evidence that antisense-mediated inhibition of DGAT2 leads to MASH resolution and fibrosis improvement without worsening of hepatic function or hyperlipidaemia and without any changes in bodyweight. These results validate liver-targeted DGAT2 inhibition as a potential new treatment approach for MASH and support further clinical development of ION224 for MASH as single-agent and combination therapy.

thyroid hormone receptor-beta agonist, as the first pharmacological treatment for adults with MASH.[7,8]

MASH is characterised by excess liver fat accumulation and consequent hepatic inflammation, hepatic cell injury and death, and eventually fibrosis.[9–12] The imbalance between hepatic triglyceride accumulation and elimination leads to hepatic steatosis. Hence, decreasing endogenous fat synthesis represents a viable therapeutic option to prevent or reduce the progression of inflammation and fibrosis in MASH. Diacylglycerol O-acyltransferase 2 (DGAT2) is a rate-limiting enzyme that catalyses the final step in the synthesis of triglycerides. In preclinical models, DGAT2 reduction using an antisense oligonucleotide reduced hepatic triglyceride levels, improved hepatic steatosis, and increased fatty acid oxidation and oxidative gene expression.[13] We previously demonstrated the potential of DGAT2 inhibition as a therapeutic target for the reduction of liver fat accumulation in a 13-week study with 44 individuals with type 2 diabetes and liver steatosis treated with once per week subcutaneous injections of an unconjugated DGAT2 antisense oligonucleotide (ISIS 484137) or placebo.[14] This proof-of-concept study met its primary endpoint, and treatment with unconjugated DGAT2 antisense oligonucleotide resulted in a statistically significant absolute and relative reduction from baseline in hepatic steatosis, as quantified by MRI-derived proton density fat fraction (MRI-PDFF), at week 13.

ION224 is a ligand-conjugated antisense oligonucleotide against DGAT2 that has the same nucleotide sequence as ISIS 484137, the unconjugated predecessor tested previously. The sequence is conjugated to a triantennary N-acetyl-galactosamine ($GalNAc_3$) moiety, which provides targeted delivery of ION224 to the liver via uptake through surface hepatocyte asialoglycoprotein receptors, resulting in an up to 30-fold increase in potency.[15] A phase 1, randomised, double-blind, placebo-controlled, dose-escalation study in healthy volunteers evaluated the safety, tolerability, and pharmacokinetics of single subcutaneous doses of ION224 at 40 mg, 80 mg, 120 mg, and 160 mg (appendix p 2). Overall, ION224 was well tolerated at all dose levels, indicating that liver antisense DGAT2 inhibition by ION224 could provide a novel and safe strategy for the treatment of MASH. Here, we present the results of the ION224-CS2 trial, in which the efficacy and safety of ION224 were assessed in patients with biopsy-confirmed MASH with moderate or severe liver fibrosis.

Methods

Study design

This study was an adaptive, two-part, multicentre, randomised, double-blind, placebo-controlled, phase 2 trial conducted at 43 clinical sites in the USA and Puerto Rico (appendix pp 3–7). The protocol, including the statistical analysis plan, was developed by the sponsor, Ionis Pharmaceuticals (appendix p 35). The trial was approved by a central institutional review board, Advarra, on May 11, 2021 (IRB PRO00053842). The trial was conducted in accordance with the principles of the Declaration of Helsinki, the International Council for Harmonisation Good Clinical Practice guidelines, and applicable national drug and data protection laws and other applicable regulatory requirements. ION224-CS2 was registered at ClinicalTrials.gov (NCT04932512) and is closed.

Participants

Eligible participants were adults aged 18–75 years with a BMI ≥25 kg/m², liver steatosis ≥10% as assessed by MRI-PDFF, glycated haemoglobin (HbA_{1c}) <9·5%, and alanine transferase and aspartate transferase levels ≤200 U/L. Histologically confirmed inclusion criteria included a biopsy-confirmed diagnosis of MASH, characterised by a Non-Alcoholic Fatty Liver Disease Activity Score (NAS) ≥4 (on a scale of 0–8, with higher scores indicating more severe disease), with at least 1 point for each subcomponent: steatosis, ballooning, and lobular inflammation; and a fibrosis score of F1 to F3 (on a scale of F0 [no fibrosis] to F4 [cirrhosis]) for part 1 (dose selection), and F2 to F3 for part 2 (expanded cohorts). Liver biopsies were obtained at screening and evaluated by two independent central pathologists. In patients for whom eligibility scores differed, the two pathologists met to reach consensus. Key exclusion criteria included a known history or evidence of liver disease other than MASH, fibrosis score of F4 at screening, clinical evidence of liver decompensation, a history of or current use of illicit drugs (excluding recreational marijuana) or misuse of alcohol (defined as more than seven drinks per week for female patients and >14 drinks per week for male patients for more than 3 consecutive months within the 2 years before screening), active HIV or hepatitis B or C infection, and the use of confounding concomitant medications. The full eligibility criteria are listed in the protocol. All participants provided written and informed consent.

Randomisation and masking

Using a centralised, web-based, real-time interactive response technology system, participants were randomly assigned (1:1:1) in part 1 (dose selection) to three dose cohorts: cohort A (ION224 60 mg or placebo), cohort B (ION224 90 mg or placebo), and cohort C (ION224 120 mg or placebo). Within each dose cohort, participants were randomly assigned (3:1) to receive ION224 or placebo (block size of 4). All cohorts received ION224 or placebo once every 4 weeks. As part of the adaptive design in this proof-of-concept study, after the week-13 dose of study drug and the week-15 assessments (MRI-PDFF) were completed in the first 50 participants, the effects of treatment on safety, liver steatosis, and liver transaminases were assessed in a planned, unmasked interim analysis as specified in the protocol by an independent data safety and monitoring board. Based on this analysis (appendix p 26), cohorts B and C were selected by the data safety and monitoring board for expanded enrolment. In part 2 (expanded cohorts), additional participants were randomly assigned (1:1) to cohort B or cohort C and then randomly assigned (2:1) to receive ION224 90 mg or 120 mg or placebo (block size of 3). Additional participants were not enrolled in cohort A, and existing participants continued to be dosed at the same level to the end of the study. The sponsor, participants, research monitors, and study centre personnel were masked throughout the study. The data safety and monitoring board received unmasked safety outputs from the unmasked statistician at prespecified intervals per the dedicated charter for safety reviews. The board received unmasked week-13 MRI-PDFF data from the first 50 randomly assigned participants only at the planned interim analysis to guide cohort selection for expanded enrolment in part 2. Pathologists were masked to treatment assignment and visit. When pathologists' readings differed, they met to reach a consensus on all scores.

Procedures

All enrolled participants received ION224 or placebo every 4 weeks over 12 months for 13 doses, followed by a 13-week period of post-treatment follow-up. A liver biopsy was completed at screening to ascertain eligibility and at the end of the treatment period (week 51) for efficacy assessment. According to the protocol, participants who received at least eight doses of the ION224 or placebo were qualified for the final study assessments, MRI, elastography, and liver biopsy at week 51 or early termination visit. For the final analysis, baseline biopsies were re-read alongside end-of-treatment biopsies by the same two independent central pathologists from the screening assessments. Additional details on the liver biopsy assessment procedure are provided in the appendix (pp 9–10). Liver steatosis was measured by serial MRI-PDFF, and hepatic fat content was calculated by an independent, central reader masked to treatment. Demographic data were collected using an electronic case report form; options provided for sex were male or female.

Outcomes

The primary endpoint was the improvement of MASH at week 51 without worsening fibrosis. Improvement was measured by a ≥2-point reduction in NAS score, with

a ≥1-point improvement in hepatocellular ballooning or lobular inflammation. Safety assessments included adverse events, vital signs, and clinical laboratory assessments.

Secondary endpoints were assessment of changes in liver steatosis by MRI-PDFF on days 99, 194, and 351; the proportion of participants having MASH resolution, as defined by score of 0 for ballooning and 0–1 for lobular inflammation, and without worsening of fibrosis at week 51; the proportion of participants having reduction of at least one stage in the fibrosis score, and without worsening of steatohepatitis by NAS (no increase in NAS for either ballooning, inflammation, or steatosis) at week 51; the proportion of participants with alanine aminotransferase (ALT) and aspartate aminotransferase (AST) ≤1·5 x upper limit of normal (ULN) at the end of treatment; and changes from baseline in liver-related laboratory tests, such as ALT, AST, bilirubin, and gamma-glutamyl transferase, and changes in plasma fasting lipid profile. The safety and tolerability of ION224 was assessed by determining the incidence, severity, and dose-relationship of adverse events and changes in laboratory parameters. Safety results in participants dosed with ION224 was compared with those from participants dosed with placebo. Exploratory endpoints are listed in the appendix (p 8).

Statistical analysis

The sample size was pre-planned based on different assumptions for part 1 and part 2. In part 1, we estimated that 12 participants in each treatment group would provide at least 80% power to detect a 25% relative difference in the percentage of liver fat fraction reduction assessed by MRI-PDFF between the active and pooled placebo groups at week 13 with a significance level of 0·05, assuming an SD of 20%. In part 2, we estimated that 39 participants in each treatment group would provide 80% power to show a 30% difference in the proportion of responders between ION224 and placebo with respect to the primary endpoint. The sample sizes were based on a 15% withdrawal rate. The study was powered for the two cohorts selected for expanded enrolment in part 2 (90 mg and 120 mg).

All efficacy analyses were conducted on the prespecified per-protocol set, which included all participants who were randomly assigned and received at least ten doses of ION224 or placebo, without missing three consecutive doses, and who had no major protocol deviations (eg, participant started using a disallowed concomitant medication). Efficacy analyses were conducted for each ION224 dose group versus the pooled placebo group, regardless of randomisation during part 1 or part 2. Before the database lock, a masked review of protocol deviations pertaining to compliance with the study drug and other protocol-defined requirements (eg, restricted concomitant medications and dietary and lifestyle modifications) was completed and documented to determine exclusions from the prespecified per-protocol set. Post-hoc analyses of the primary and key secondary histology endpoints were performed on all randomly assigned participants (intention-to-treat population; appendix). Safety analyses were conducted in the safety set, which was defined as all participants who were randomly assigned and received at least one dose of the study drug.

The primary efficacy analysis compared the proportion of responders between groups using the Chi squared test (or Fisher's exact test if the expected cell counts in either group was <5). Point estimates of the odds ratio (OR), 95% CIs, and p values for the comparison of proportions between each ION224 group and the pooled placebo group are provided. Additionally, modified Poisson regression and binomial regression were also conducted to obtain the estimated risk ratio (RR) and risk difference. For continuous secondary endpoints, the values, absolute change, and percentage change from baseline at each visit were summarised using descriptive statistics by the treatment group. Comparisons between treatment groups for continuous variables assessed over time were analysed with a linear mixed-effect model with repeated measures. The same method use for the primary efficacy variable was used for the other categorical secondary efficacy variables.

Post-hoc responder analyses were performed for the primary and key secondary histology endpoints in the intention-to-treat population. Missing liver biopsy data at the end of treatment were handled using two approaches: the Jump to Reference multiple imputation approach that assumes the data followed the pattern of the placebo group, and by imputing patients with missing biopsy as non-responders to assess the robustness of the findings. The estimated ORs, RRs, risk differences, 95% CIs and p values for comparisons between each ION224 group and pooled placebo were obtained.

All statistical analyses were performed using SAS, version 9.4. Further details are provided in the statistical analysis plan, available with the protocol (appendix p 35).

Role of the funding source

The funder had a role in the study design, data analysis, data interpretation, and writing of the report.

Results

Between June 18, 2021, and Dec 27, 2022, 1072 patients were screened and 160 were randomly assigned to ION224 60 mg (n=23), 90 mg (n=45), or 120 mg (n=46), or placebo (n=46; figure 1). 131 (82%) of 160 completed the treatment, and 145 (91%) completed post-treatment follow-up. 132 participants had an evaluable final liver biopsy at the end of the study. Before the database lock, a thorough masked review of patient profiles resulted in the exclusion of nine participants from the per-protocol set (figure 1). The per-protocol set included 123 participants; 32 received placebo, and 18 received

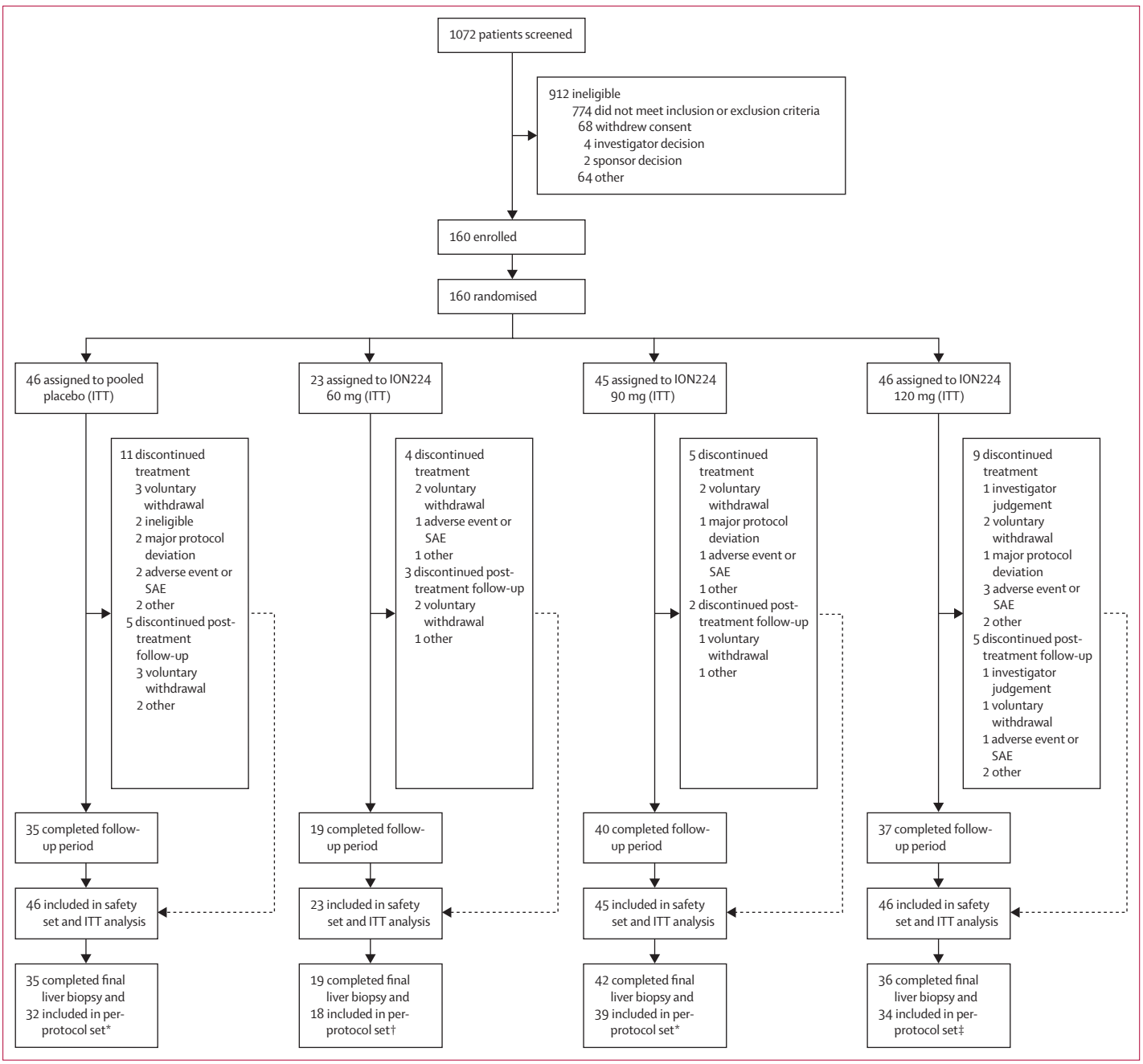

Figure 1: **Trial profile**

ITT=intention-to-treat. SAE=serious adverse event. *Three patients were excluded from the per-protocol set; two deviated from the protocol because of antidiabetic-related medications and one had a final liver biopsy ≥12 weeks from last dose. †One patient was excluded from the per-protocol set due to having a final liver biopsy ≥12 weeks from last dose. ‡Two patients were excluded from the per-protocol set; one had unevaluable liver biopsy and one had a final liver biopsy ≥12 weeks from last dose.

ION224 60 mg, 39 received ION224 90 mg, and 34 received ION224 120 mg. The baseline demographic and clinical characteristics were similar across the groups (table 1). Participants' mean age was 53 years (SD 12), and BMI was 37·8 kg/m² (SD 7·1). 114 (71%) of 160 participants were female and 46 (29%) were male. 145 (91%) of 160 participants were White, and 70 (44%) participants identified as Hispanic or Latino. The overall population was consistent with expectations for adults with confirmed MASH (appendix p 27): 81 (51%) of 160 had type 2 diabetes, 102 (64%) had dyslipidaemia, 96 (60%) had hypertension, 65 (41%) had stage F2 fibrosis, and 85 (53%) had stage F3 fibrosis. Baseline liver biopsy assessments are provided in the appendix (pp 28–29).

	Pooled placebo (n=46)	60-mg group (n=23)	90-mg group (n=45)	120-mg group (n=46)
Age, years				
Mean (SD)	54 (12)	53 (10)	54 (13)	52 (10)
Median (IQR)	56 (46–63)	53 (48–62)	58 (46–64)	54 (45–59)
Sex				
Female	36 (78%)	18 (78%)	28 (62%)	32 (70%)
Male	10 (22%)	5 (22%)	17 (38%)	14 (30%)
Ethnicity				
Hispanic or Latino	14 (30%)	10 (43%)	26 (58%)	20 (43%)
Not Hispanic or Latino	32 (70%)	13 (57%)	19 (42%)	26 (57%)
Race				
White	42 (91%)	21 (91%)	39 (87%)	43 (93%)
Black	1 (2%)	1 (4%)	1 (2%)	2 (4%)
Asian	1 (2%)	1 (4%)	2 (4%)	1 (2%)
American Indian or Alaskan Native	0	0	1 (2%)	0
Other	2 (4%)	0	2 (4%)	0
Bodyweight, kg				
Mean (SD)	106·1 (23·6)	104·9 (18·3)	99·3 (20·8)	106·0 (21·5)
Median (IQR)	100·5 (88·5–115·1)	104·0 (94·9–115·2)	96·5 (84·5–113·5)	102·3 (90·2–123·3)
BMI, kg/m²				
Mean (SD)	39·1 (8·2)	37·9 (5·8)	36·6 (6·7)	37·7 (6·7)
Median (IQR)	37·2 (33·1–43·6)	37·7 (33·4–43·3)	34·7 (31·1–41·5)	38·2 (33·7–41·1)
Healthy (18·5–24·9 kg/m²)	0	1 (4%)	0	0
Overweight (25–29·9 kg/m²)	2 (4%)	1 (4%)	8 (18%)	6 (13%)
Obese (≥30 kg/m²)	44 (96%)	21 (91%)	37 (82%)	40 (87%)
Liver fat content*	21·9% (7·6)	21·6% (7·7)	23·5% (7·9)	22·3% (6·8)
NAS: eligibility screening read†	6 (3–7)	5 (4–7)	5 (4–7)	6 (4–8)
Liver fibrosis stage: eligibility screening read‡				
F1	3 (7%)	1 (4%)	3 (7%)	3 (7%)
F2	17 (37%)	11 (48%)	20 (44%)	17 (37%)
F3	26 (57%)	11 (48%)	22 (49%)	26 (57%)
Alanine aminotransferase, U/L	58 (34)	57 (34)	58 (42)	59 (32)
>1·5 × ULN	11 (24%)	6 (26%)	9 (20%)	9 (20%)
Aspartate aminotransferase, U/L	43 (25)	43 (34)	37 (22)	40 (17)
>1·5 × ULN	7 (15%)	3 (13%)	5 (11%)	5 (11%)
Bilirubin, mg/dL	0·48 (0·14)	0·47 (0·13)	0·53 (0·25)	0·54 (0·23)
Gamma glutamyl transferase, U/L	69 (60)	53 (40)	59 (66)	63 (53)
Total cholesterol, mg/dL	192·7 (45·7)	175·8 (45·6)	176·8 (41·8)	185·2 (40·1)
LDL cholesterol, mg/dL	108·9 (39·2)	98·7 (41·9)	101·1 (40·3)	103·7 (35·1)
HDL cholesterol, mg/dL	45·3 (10·2)	41·7 (14·1)	45·0 (12·2)	44·6 (10·1)
Triglycerides, mg/dL	200·4 (104·5)	177·5 (64·6)	152·9 (60·1)	183·8 (72·2)
Liver stiffness, kPa§	13·7 (8·61)	12·6 (6·1)	11·3 (4·0)	12·3 (5·4)
Enhanced Liver Fibrosis test score¶	9·7 (0·9)	9·7 (0·9)	9·7 (0·7)	9·8 (0·9)

(Table 1 continues on next page)

The primary endpoint of at least a 2-point reduction in NAS, with at least 1-point improvement in hepatocellular ballooning or lobular inflammation, and without worsening fibrosis stage at week 51 was met in 20 (59%) of 34 participants in the 120-mg group (predicted risk 58·8% [95% CI 42·3–75·4]; risk difference 40·1% [95% CI 18·7–61·4], p=0·0002), 18 (46%) of 39 in the 90-mg group (predicted risk 46·2% [95% CI 30·5–61·8]; risk difference 27·4% [95% CI 6·7–48·1], p=0·0094), and six (19%) of 32 in the placebo group (predicted risk 18·7% [95% CI 5·2–32·3]; figure 2A). In the post-hoc analysis in the intention-to-treat population, based on the observed data, the primary endpoint was met by 22 (48%) of 46 participants in the 120-mg group (predicted risk 52·8% [95% CI 37·5–68·2]; risk difference 35·9% [95% CI 16·7–55·1]; p=0·0002) and 20 (44%) of 45 in the 90-mg group (predicted risk 45·0 [95% CI 30·3–59·6]; risk difference 28·0% [95% CI 8·8–47·3]; p=0·0043) versus six (13%) of 46 in the placebo group (predicted risk 16·9% [95% CI 4·5–29·3]; appendix pp 13, 30–31). Under both methods for handling missing data at the end of treatment, the 120-mg group and 90-mg group showed consistent results with a statistically significantly greater proportion of responders than the placebo group (appendix pp 30–31).

Resolution of MASH without worsening of fibrosis occurred in 12 (35%) of 34 participants in the 120-mg group and 14 (36%) of 39 in the 90-mg group, compared with five (16%) of 32 in the placebo group (per-protocol population; figure 2B). In the post-hoc ITT analysis, MASH resolution occurred in 13 (28%) of 46 participants in the 120-mg group (predicted risk: 29·3% [95% CI 15·8–42·8]; risk difference: 16·9 [95% CI 0–33·8], p=0·050), 15 (33%) of 45 in the 90-mg group (predicted risk: 33·8% [95% CI 19·9–47·8]; risk difference: 21·5 [95% CI 4·2–38·7], p=0·015), and five (11%) of 46 in the placebo group (predicted risk: 12·4% [95% CI 2·2–22·5]; appendix pp 13, 30–31). Risk difference estimates indicated a significantly greater proportion of responders in the 120-mg group and 90-mg group (appendix pp 30–31).

A reduction of at least 1 stage in fibrosis score without worsening steatohepatitis occurred in 11 (32%) of 34 participants in the 120-mg group, ten (26%) of 39 participants in the 90-mg group, and four (13%) of 32 in the placebo group (per-protocol population; figure 2C). In the post-hoc intention-to-treat population, fibrosis improvement occurred in 12 (26%) of 46 in the 120-mg group (predicted risk: 30·3% [95% CI 16·1 to 44·6]; risk difference: 16·3 [95% CI –1·5 to 34·2], p=0·073) and 11 (24%) of 45 in the 90-mg group (predicted risk: 24·8% [95% CI 12·1 to 37·6]; risk difference: 10·8 [95% CI –6·2 to 27·9], p=0·21), compared with four (9%) of 46 in the placebo group (predicted risk: 14·0% [95% CI 2·6 to 25·4]; appendix pp 13, 30–31). When patients with missing biopsy results were imputed as non-responders, the 120-mg and 90-mg groups showed a statistically significantly higher proportion of patients who responded

than the placebo group (appendix pp 30–31). Results from the placebo-based J2R multiple imputation were similar to the per-protocol set.

Post-hoc subgroup analyses were completed to further explore the effects of ION224 in participants with advanced fibrosis and inform future studies. In participants with stage F3 fibrosis, the primary endpoint was met, and MASH resolution and fibrosis improvement was observed in more patients treated with ION224 90 mg and 120 mg than with placebo (appendix p 14).

Treatment with ION224 improved all components of NAS at week 51 (appendix pp 15–16). Notably, 13 (33%) of 39 participants in the 90-mg group (p=0·041) and 16 (47%) of 34 in the 120-mg group (p=0·0020) had at least a 1-point reduction in both hepatocellular ballooning and lobular inflammation, respectively, compared with four (13%) of 32 in the placebo group.

A key secondary endpoint was the relative change from baseline in hepatic fat content as evaluated by MRI-PDFF. Participants treated with ION224 showed statistically significant reduction in hepatic fat content measured by MRI-PDFF, which was dose-dependent and time-dependent and did not exhibit a plateau within 1 year of treatment (figure 3; appendix p 17). In the 120-mg group, ≥30% and ≥50% relative reduction from baseline at day 351 occurred in 25 (74%) of 34 participants (11 [34%] of 32 in the placebo group; p=0·0010; appendix p 17), and 15 (44%) of 34 (one [3%] of 32 in the placebo group; p<0·0001; figure 3B; appendix p 17), respectively. In participants with stage F3 fibrosis, a greater proportion of patients treated with ION224 reached ≥30% and ≥50% reduction in liver fat than with placebo (appendix p 18).

At the end of treatment, most participants had an ALT and AST <1·5×ULN: 34 (87%) of 39 in the 90-mg group, 32 (94%) of 34 in the 120-mg group, and 29 (91%) of 32 in the placebo group. ALT and AST levels did not worsen with ION224 treatment and remained near baseline levels (appendix pp 19–20). In a subgroup analysis of participants with a baseline ALT or AST >1·5×ULN, the percentage change from baseline was numerically reduced for ALT and statistically significantly reduced for AST in the 120-mg group compared with the placebo group (appendix p 21). Total bilirubin levels remained within the normal range and gamma glutamyl transferase levels were numerically lower in participants treated with ION224 than with placebo (appendix pp 19–20). No hypertriglyceridemia or worsening of plasma lipids was observed with ION224 treatment (appendix pp 22, 33).

Participants in the 90-mg and 120-mg groups had statistically significant reductions in liver stiffness at the end of treatment (appendix p 32). 38 (52%) of 73 participants in the 90-mg and 120-mg groups had more than 25% relative reduction in liver stiffness compared with nine (28%) of 32 in the placebo group.

The mean HbA_{1c} level numerically increased from baseline by 0·3% in the placebo group but was unchanged in the 90-mg group (p=0·15) and had decreased by 0·2% (p=0·059) in the 120-mg group (appendix 32). The absolute change in enhanced liver fibrosis scores showed a statistically significant reduction from baseline in the 120-mg group on day 386, with a difference in least squares mean of –0·41 (p=0·013). These changes correlated with reductions in ALT and liver steatosis by MRI-PDFF (appendix p 23). Bodyweight and BMI over time were generally unchanged over the course of the study (appendix p 24).

Adverse events were reported in 107 (94%) of 114 participants treated with ION224, including 21 (91%) in the 60-mg group, 43 (96%) in the 90-mg group, and 43 (94%) in the 120-mg group, and 41 (89%) of 46 in the placebo group (table 2). Of all treatment-emergent adverse events, most adverse events were mild (404 [64%] of 634 in the ION224 group and 159 [64%] of 249 in the placebo group) or moderate (224 [35%] of 634 and 85 [34%] of 249). The most common adverse events related to the study drug were injection site erythema (nine [8%] of 114 in the ION224 group and none of 46 in the placebo group), injection site pain (eight [7%] and two [4%]), and nausea (16 [14%] and nine [20%]). Serious adverse events were reported in seven (6%) participants in the ION224 group and four (9%) participants in the placebo group, but none were considered related to the study drug. A list of serious adverse events is provided in the appendix (p 34). One participant who received 120 mg ION224 had

	Pooled placebo (n=46)	60-mg group (n=23)	90-mg group (n=45)	120-mg group (n=46)
(Continued from previous page)				
HbA_{1c}	6·5% (1·0)	6·3% (0·8)	6·6% (1·4)	6·9% (1·3)
≤5·6%	10 (22%)	5 (22%)	8 (18%)	8 (17%)
5·7–6·4%	15 (33%)	9 (39%)	20 (44%)	13 (28%)
≥6·5%	21 (46%)	9 (39%)	17 (38%)	25 (54%)
Type 2 diabetes	26 (57%)	11 (48%)	19 (42%)	25 (54%)
Hypertension	30 (65%)	14 (61%)	26 (58%)	26 (57%)
Dyslipidaemia	36 (78%)	13 (57%)	27 (60%)	26 (57%)
Baseline statin use	18 (39%)	6 (26%)	18 (40%)	13 (28%)
Baseline GLP-1 receptor agonist use	8 (17%)	2 (9%)	5 (11%)	7 (15%)
Baseline SGLT-2 inhibitor use	1 (2%)	3 (13%)	3 (7%)	2 (4%)

Data are n (%), mean (SD), or median (IQR), unless otherwise specified. Percentages might not total 100 because of rounding. HbA_{1c}=glycated haemoglobin. NAS=Non-Alcoholic Fatty Liver Disease Activity Score. ULN=upper limit of normal. *Liver fat content was measured using MRI-derived proton density fat fraction. †NAS is the unweighted sum of scores for steatosis (scale 0–3), lobular inflammation (0–3), and hepatocellular ballooning (0–2) and ranges from 0 to 8 on the basis of the non-alcoholic steatohepatitis (NASH) Clinical Research Network scoring system. Higher scores indicate more severe disease. Data are median (range). ‡The fibrosis stages according to the NASH Clinical Research Network are as follows: F0, no fibrosis; F1, mild (perisinusoidal or periportal) fibrosis; F2, moderate (perisinusoidal and portal or periportal) fibrosis; F3 severe (bridging) fibrosis; and F4, cirrhosis.[16] §Liver stiffness was assessed by means of vibration-controlled transient elastography (FibroScan). Higher values indicate more severe fibrosis. Advanced fibrosis is considered unlikely if the value is below 8 kPa and likely if the value is 12 kPa or higher. ¶The Enhanced Liver Fibrosis test consists of a panel of three serum biomarkers associated with matrix turnover: hyaluronic acid, tissue inhibitor of metalloproteinase 1, and procollagen type III N-terminal peptide. Advanced fibrosis is considered unlikely if the value is below 7·7 and likely if the value is 9·8 or higher. A score of 9·8 or higher indicates an increased risk of progression to cirrhosis and liver-related clinical events.

Table 1: **Baseline demographic and clinical characteristics**

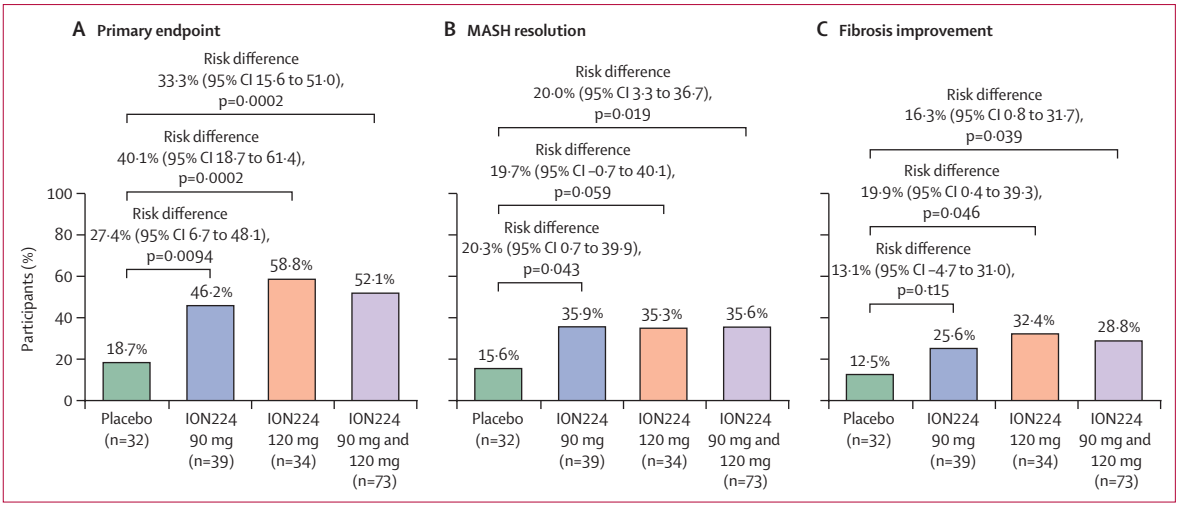

Figure 2: **Primary and key secondary biopsy endpoints in the pre-specified per-protocol set**
(A) The proportion of participants with at least a 2-point reduction in NAS, at least a 1-point improvement in hepatocellular ballooning or lobular inflammation, and without worsening in fibrosis stage at week 51 (primary endpoint). (B) Proportion of participants with MASH resolution without worsening fibrosis at week 51. MASH resolution was defined as a score of 0–1 for lobular inflammation and a score of 0 for hepatocellular ballooning. (C) Proportion of participants with fibrosis improvement (ie, reduction of ≥1 stage in fibrosis) without worsening steatohepatitis by Non-Alcoholic Fatty Liver Disease Activity Score Activity score at week 51. Predicted risks, risk differences, 95% CIs, and p values for the risk differences in percentage between each ION224 group and pooled placebo group were estimated using a binomial regression model with an identity link. The proportions displayed in the bar plot represent the predicted rates. MASH=metabolic dysfunction associated steatohepatitis. NAS=Non-Alcoholic Fatty Liver Disease Activity Score Activity Score.

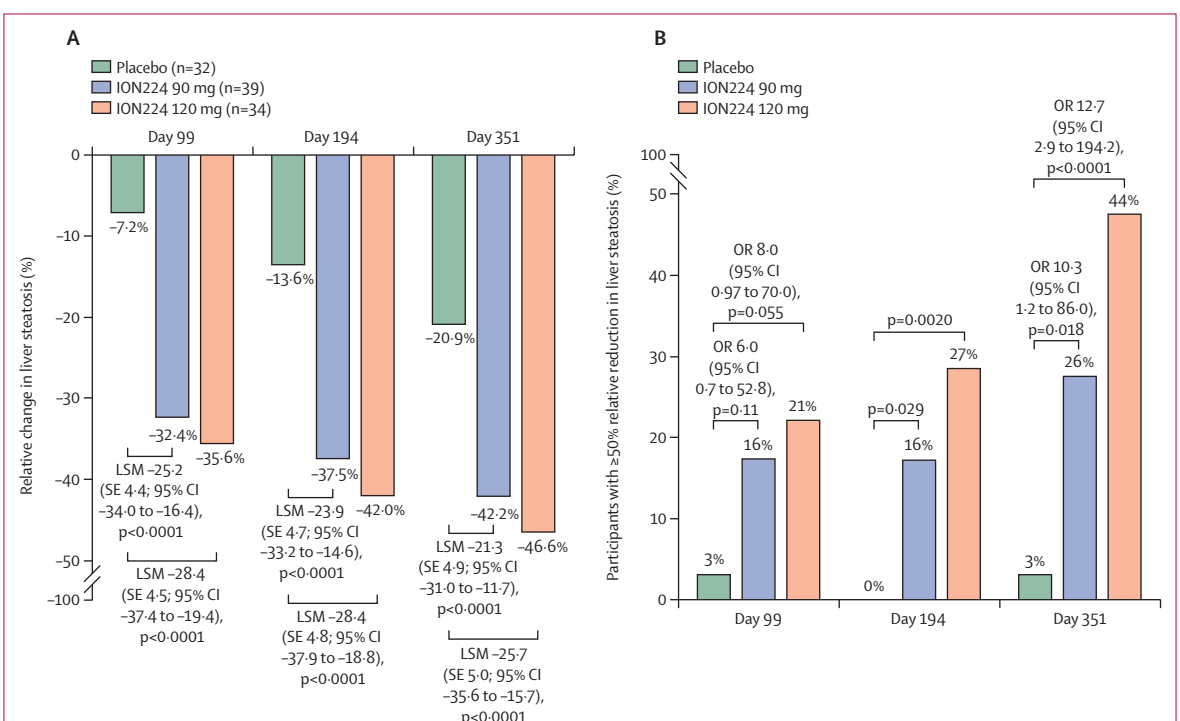

Figure 3: **Liver steatosis evaluated by MRI-PDFF over time**
(A) The key secondary endpoint of relative reduction in liver steatosis from baseline over time as evaluated by MRI-PDFF. Baseline was defined as the last non-missing measurement taken before the first administration of study drug. p values were obtained using the mixed-effect model with repeated measures. (B) Post-hoc analysis of the proportion of participants with ≥50% relative reduction in liver steatosis from baseline. p values were determined using the Chi square test or Fisher's exact test if the expected cell counts were less than 5 in either group. The denominator for percentage calculation is the number of participants in each treatment group with non-missing MRI-PDFF results (31 in the placebo group; 39 in the 90-mg group, 34 in the 120-mg group). For Day 194, the odds ratio and 95% CIs could not be estimated because the number of participants with ≥50% relative reduction in liver steatosis was 0 in the placebo group. Data are from the prespecified per-protocol set. LSM=least squares mean. MRI-PDFF=MRI-derived proton density fat fraction. OR=odds ratio.

	Placebo (n=46)		60-mg group (n=23)		90-mg group (n=45)		120-mg group (n=46)		Overall (n=160)	
	n (%)	Events	n (%)	Events	n (%)	Events	n (%)	Events	n (%)	Events
Any adverse event	41 (89%)	249	21 (91%)	103	43 (96%)	270	43 (93%)	261	148 (93%)	883
Mild	13 (28%)	159	6 (26%)	58	16 (36%)	174	12 (26%)	172	47 (29%)	563
Moderate	24 (52%)	85	14 (61%)	44	26 (58%)	94	28 (61%)	86	92 (58%)	309
Severe	4 (9%)	5	1 (4%)	1	1 (2%)	2	3 (7%)	3	9 (6%)	11
Any serious adverse event*	4 (9%)	4	2 (9%)	2	1 (2%)	3	4 (9%)	4	11 (7%)	13
Adverse events leading to discontinuation	2 (4%)	2	1 (4%)	1	1 (2%)	1	3 (7%)	5	7 (4%)	9
≥1 TEAE with an incidence rate >5%	31 (67%)	104	18 (78%)	36	35 (78%)	110	37 (80%)	118	121 (76%)	368
COVID-19	8 (17%)	10	7 (30%)	7	12 (27%)	12	11 (24%)	11	38 (24%)	40
Urinary tract infection	8 (17%)	16	2 (9%)	4	8 (18%)	11	11 (24%)	17	29 (18%)	48
Upper respiratory tract infection	2 (4%)	2	3 (13%)	3	5 (11%)	5	10 (22%)	12	20 (13%)	22
Sinusitis	5 (11%)	10	1 (4%)	1	6 (13%)	6	5 (11%)	5	17 (11%)	22
Influenza	3 (7%)	3	1 (4%)	1	3 (7%)	3	2 (4%)	2	9 (6%)	9
Nausea	9 (20%)	10	2 (9%)	2	6 (13%)	9	8 (17%)	8	25 (16%)	29
Abdominal pain upper	2 (4%)	3	2 (9%)	2	4 (9%)	12	3 (7%)	3	11 (7%)	20
Diarrhoea	9 (20%)	10	2 (9%)	2	5 (11%)	7	2 (4%)	2	18 (11%)	21
Vomiting	4 (9%)	4	0	0	5 (11%)	6	1 (2%)	2	10 (6%)	12
Arthralgia	2 (4%)	4	2 (9%)	2	3 (7%)	3	9 (20%)	10	16 (10%)	19
Back pain	4 (9%)	4	2 (9%)	2	7 (16%)	7	5 (11%)	5	18 (11%)	18
Injection site erythema	0	0	1 (4%)	3	1 (2%)	1	7 (15%)	13	9 (6%)	17
Injection site pain	2 (4%)	3	1 (4%)	1	2 (4%)	4	5 (11%)	7	10 (6%)	15
Headache	5 (11%)	5	3 (13%)	3	5 (11%)	7	4 (9%)	4	17 (11%)	19
Procedural pain	3 (7%)	4	2 (9%)	2	6 (13%)	6	2 (4%)	2	13 (8%)	14
Hypertension	3 (7%)	3	1 (4%)	1	5 (11%)	5	4 (9%)	4	13 (8%)	13
Diabetes	2 (4%)	2	0	0	2 (4%)	3	5 (11%)	5	9 (6%)	10

TEAE=treatment-emergent adverse event. *No serious adverse events were deemed by the investigator to be drug-related and there was no trend in the type of serious adverse event.

Table 2: **Treatment-emergent adverse events in the safety set**

six local cutaneous reactions at the injection site, including three of injection site erythema and three of injection site pruritus. The final event led to voluntary withdrawal from the study.

The overall incidence of treatment-emergent antidrug antibodies was five (11%) of 46 in the placebo group and 34 (30%) of 114 in the pooled ION224 group. Three (3%) of 114 participants treated with ION224 had pre-existing antibodies. Demographics and baseline characteristics were similar between participants with negative antidrug antibodies and positive antidrug antibodies between participants with and without pre-existing antibodies. There was no notable effect of antidrug antibody positivity on measure of pharmacokinetics, pharmacodynamics, or safety.

Discussion

In this multicentre, randomised, placebo-controlled, phase 2 study, 1-year treatment of monthly ION224 subcutaneous injections resulted in substantial histological improvements in patients with biopsy-proven MASH and liver fibrosis (F1–F3), supporting DGAT2 inhibition via a liver-targeted antisense oligonucleotide as a promising therapeutic strategy. These results provide the first clinical evidence that antisense inhibition of DGAT2 translates to histological benefit in the context of MASH. The primary endpoint of at least a 2-point reduction in NAS with at least a 1-point improvement in hepatocellular ballooning or lobular inflammation and without worsening of fibrosis was met in the pre-specified primary analysis in the per-protocol set as well as the intention-to-treat population in the expanded dosing groups (90 mg and 120 mg). Moreover, a significantly greater proportion of participants in the ION224-treated groups met the key secondary endpoints of MASH resolution and fibrosis improvement than the placebo groups. MASH resolution and fibrosis endpoints are the two registrational endpoints per the US Food and Drug Administration guidance.[17] In the pre-specified per-protocol set that represents the direct effect of the study drug on these endpoints, ION224 showed a net treatment effect of 20% (placebo adjusted) for MASH resolution and fibrosis improvement, which supports further development of ION224.

Histological benefits of ION224 were supported by parallel improvement in transient elastography-derived

liver stiffness measurement and significant reduction in plasma levels of Enhanced Liver Fibrosis score. Liver stiffness and enhanced liver fibrosis are established, non-invasive tests that are correlated with adverse health outcomes and mortality in patients with MASH.[18–20] Importantly, ION224-treated participants had a placebo-subtracted reduction of up to 0·4% in HbA_{1c} in the 120-mg group; this effect was not statistically significant given the small sample size, but it might indicate the potential of ION224 for improving glycaemic control. This finding is consistent with preclinical data indicating improvement in insulin sensitivity after DGAT2 antisense reduction.[21] Furthermore, subgroup analysis of participants with advanced fibrosis (baseline stage F3) demonstrated that the histological and metabolic changes were maintained. Overall, these findings provide the first clinical evidence that the reduction of hepatic steatosis after DGAT2 inhibition leads to MASH resolution and improvement in fibrosis, and these effects are independent of changes in bodyweight.

Studies show that a reduction of at least 30% in liver steatosis by MRI-PDFF is strongly correlated with histological benefit and MASH resolution.[22] A statistically significantly higher proportion of ION224-treated participants had a reduction of ≥30% or ≥50% in liver steatosis in a dose and time-dependent manner. Indeed, 15 (44%) of 34 participants in the 120-mg groups had ≥50% reduction at the end of treatment compared with only one (3%) of 35 participants in the placebo group, whose reduction could be attributed to weight loss of approximately 20%. The time-dependent reduction in liver steatosis might suggest additional benefit for ION224 with longer-term dosing.

ION224 showed a favourable safety and tolerability profile. There were no deaths, no treatment-related serious adverse events, no safety concerns pertaining to hepatic or renal function, and no thrombocytopenia. There was a low rate of injection site reactions in this study confirming favourable tolerability of multiple monthly injections of ION224. Only one patient voluntarily withdrew from the study due to having moderate-grade injection-site reactions. The reduction in liver fat was not accompanied by hypertriglyceridemia or elevations in plasma glucose, changes in bodyweight, or gastrointestinal side effects. The paucity of adverse effects on the lipid profile, including no worsening of serum triglycerides with ION224, is notable and might be considered an added value. Hypertriglyceridemia has been observed with other investigational compounds that also work through modulating the de novo lipogenesis pathway such as acetyl-CoA carboxylase inhibitors[23,24] and fatty acid synthase inhibitors,[25] which might limit their clinical benefit, especially as dyslipidaemia is a common comorbidity in patients with MASH. Indeed, co-administration with a small molecule DGAT2 inhibitor has been used to counter the hypertriglyceridemia associated with acetyl-CoA carboxylase inhibitors.[26]

Liver transaminase levels were close to the ULN at baseline, making it challenging to detect further improvements in transaminase levels following treatment with ION224. However, a more prominent decrease in ALT and AST was observed in ION224-treated participants with elevated baseline levels (>1·5×ULN) versus placebo.

ION224 reduces de novo lipogenesis without adversely affecting the lipid profile. Furthermore, its potential long-term benefits downstream of DGAT2 inhibition—including improved glycaemic control, reduced fatty acid synthesis, and increased fatty acid oxidation, as demonstrated preclinically[13]—might provide additional therapeutic value for patients with MASH and comorbidities such as dyslipidaemia and diabetes. In addition to the unique mechanism of action, ION224's favourable safety profile, posology as a once-monthly injection, and absence of drug–drug interactions support its feasibility as a candidate for combination therapy with other investigational agents.

Key limitations of this trial include the small sample size that did not allow for adequate powering for the registrational endpoints (MASH resolution and fibrosis improvement), and although 37 (23%) of 160 participants were excluded from the pre-specified per-protocol set due to absence of final biopsy or other reasons, the effect size was large enough to achieve statistical significance. The adaptive design led to a smaller sample size for the 60-mg group. In addition, the duration of the study was limited to 1 year, and the assessment of histology with prolonged treatment or long-term outcomes was not possible. This study was not designed to assess the safety and efficacy of ION224 in patients with MASH-compensated cirrhosis. Multiplicity adjustment was not planned in this phase 2 trial. Furthermore, most of the participants were White, with approximately half being Hispanic or Latino, thereby restricting conclusions for broader populations. Infrequent alcohol assessments during the study (screening and at week 49) might introduce unrecognised non-compliance with protocol-mandated alcohol restrictions. There was a large placebo effect, particularly in about 11 (34%) of 32 patients in the placebo group with ≥30% MRI-PDFF reduction despite minimal weight loss. This placebo effect might be influenced by the Hawthorne effect, unreported dietary or lifestyle modifications, or changes in concomitant medications not captured in this study. However, when the threshold for liver fat reduction was raised to ≥50%, the only placebo responder was one patient with substantial weight loss (20% bodyweight lost compared with baseline) during the study.

In conclusion, this study demonstrated that antisense reduction of DGAT2 after 1-year treatment with ION224 leads to histological improvements, MASH resolution, and time-dependent reduction in hepatic steatosis without worsening of liver function, hyperlipidaemia, or glycaemic control. These results further support DGAT2 as a potential therapeutic target for MASH and support

further investigation of ION224 for the treatment of MASH in longer-term studies.

Contributors

SB, RL, and RG: conceptualised the study. EM, KY, SB, and DL: developed the methodology. DL and KY: performed the formal analysis, including directly accessing and verifying the data. EM and KY: project administration. RL and NA: clinical investigators. KY and DL: created visualisations. SB and EM: supervised the study. KY and EY: prepared the original draft. All authors reviewed the final manuscript, had full access to all the data in the study, and had final responsibility for the decision to submit the manuscript for publication.

Declaration of interests

EM, KY, DL, RG, and SB are employees of Ionis. RL reports grants from Arrowhead Pharmaceuticals, Astrazeneca, Boehringer-Ingelheim, Bristol-Myers Squibb, Eli Lilly, Galectin Therapeutics, Gilead, Intercept, Hanmi, Intercept, Inventiva, Ionis, Janssen, Madrigal Pharmaceuticals, Merck, Novo Nordisk, Pfizer, Sonic Incytes, and Terns Pharmaceuticals, and consulting fees from Aardvark Therapeutics, Altimmune, Arrowhead Pharmaceuticals, AstraZeneca, Cascade Pharmaceuticals, Eli Lilly, Gilead, Glympse bio, Inipharma, Intercept, Inventiva, Ionis, Janssen, Lipidio, Madrigal, Neurobo, Novo Nordisk, Merck, Pfizer, Sagimet, 89 bio, Takeda, Terns Pharmaceuticals, and Viking Therapeutics. NA reports grants, consulting fees, or speaker fees from 89bio, Akero Therapeutics, Arbutus Biopharma, AstraZeneca, BioAge, Boehringer Ingelheim, Bristol Myers Squibb, Corcept Therapeutics, Echosens, Fibronostics, Galectin Therapeutics, Genentech, Gilead Sciences, Healio, Hepagene Therapeutics, Intercept Pharmaceuticals, Inventiva Pharma, Ionis Pharmaceuticals, Ipsen, Lilly, Madrigal Pharmaceuticals, Merck, NGM Biopharmaceuticals, Noom, NorthSea Therapeutics, Novo Nordisk, Perspectum, Pfizer, PharmaIN, Poxel, Regeneron, Viking Therapeutics, and Zydus Pharmaceuticals.

Data sharing

Individual patient data will not be shared. ION224 is currently in development, and data are restricted to those directly involved in the trial.

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