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Design of the phase 3 MAESTRO clinical program to evaluate resmetirom for the treatment of nonalcoholic steatohepatitis

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Summary

Background: Non-alcoholic steatohepatitis (NASH) is a progressive form of non-alcoholic fatty liver disease (NAFLD) associated with steatosis, hepatocellular injury, inflammation and fibrosis. In a Phase 2 trial in adults with NASH (NCT02912260), resmetirom, an orally administered, liver-targeted thyroid hormone receptor-β selective agonist, significantly reduced hepatic fat (via imaging) and resolved NASH without worsening fibrosis (via liver biopsy) in a significant number of patients compared with placebo.

Aims: To present the design of the Phase 3 MAESTRO clinical programme evaluating resmetirom for treatment of NASH (MAESTRO-NAFLD-1 [NCT04197479], MAESTRO-NAFLD-OLE [NCT04951219], MAESTRO-NASH [NCT03900429], MAESTRO-NASH-OUTCOMES [NCT05500222]).

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Methods: MAESTRO-NASH is a pivotal serial biopsy trial in up to 2000 adults with biopsy-confirmed at-risk NASH. Patients are randomised to a once-daily oral placebo, 80 mg resmetirom, or 100 mg resmetirom. Liver biopsies are conducted at screening, week 52 and month 54. MAESTRO-NAFLD-1 is a 52-week safety trial in ~1400 adults with NAFLD/presumed NASH (based on non-invasive testing); ~700 patients from MAESTRO-NAFLD-1 are enrolled in MAESTRO-NAFLD-OLE, a 52-week active treatment extension to further evaluate safety. MAESTRO-NASH-OUTCOMES is enrolling 700 adults with well-compensated NASH cirrhosis to evaluate the potential for resmetirom to slow progression to hepatic decompensation events. Non-invasive tests (biomarkers, imaging) are assessed longitudinally throughout, in addition to validated patient-reported outcomes.

Conclusion: The MAESTRO clinical programme was designed in conjunction with regulatory authorities to support approval of resmetirom for treatment of NASH. The surrogate endpoints, based on week 52 liver biopsy, serum biomarkers and imaging, are confirmed by long-term clinical liver-related outcomes in MAESTRO-NASH (month 54) and MAESTRO-NASH-OUTCOMES (time to event).

1 | INTRODUCTION

Non-alcoholic steatohepatitis (NASH) is a progressive form of non-alcoholic fatty liver disease (NAFLD), defined as the presence of ≥5% hepatic steatosis with hepatocellular damage and inflammation, with or without fibrosis.[1,2] NAFLD/NASH is associated with a constellation of comorbid conditions, including obesity, type 2 diabetes, hypertension and dyslipidaemia.[1–3] In addition to cirrhosis and hepatocellular carcinoma, patients with more advanced NASH fibrosis have increased morbidity and mortality from cardiovascular disease (CVD).[3,4] Diagnosis of NASH is complicated by the requirement for a liver biopsy, and there remains a need for non-invasive tests (serum and imaging biomarkers) that can diagnose and stage NASH,[3,4] with or without fibrosis, as well as monitor response to potential treatments.

Currently, there is no approved treatment for NASH. In addition, the global burden of NASH is increasing with the rising prevalence of obesity and type 2 diabetes.[1,2] As such, NASH represents a high unmet medical need. Regulatory authorities have outlined approval pathways for potential NASH treatments, including the identification of possible endpoints and populations to be prioritised.[5,6] The Accelerated Approval of New Drugs for Serious or Life-Threatening Illnesses (21 CFR Part 314 Subpart H) pathway in the United States and conditional marketing authorisation in Europe are accelerated drug approval pathways based on achieving surrogate endpoints followed by confirmation of clinical benefit via reduction in quantitative clinical outcomes.[5,6]

Resmetirom (MGL-3196) is an orally administered, liver-targeted thyroid hormone receptor (THR)-β selective agonist in development for the treatment of NASH.[7] In patients with NASH, selectivity for THR-β may provide metabolic benefits of thyroid hormone that are mediated by the liver, including reduction of excess hepatic fat and atherogenic lipids/lipoproteins (low-density lipoprotein cholesterol [LDL-C], triglycerides, apolipoprotein B [apoB], lipoprotein (a) [Lp(a)],

apolipoprotein CIII [apoCIII]), while avoiding negative systemic effects of excess thyroid hormone in heart and bone.[8]

In a randomised, double-blind, placebo-controlled Phase 2 serial liver biopsy trial in adults with biopsy-confirmed NASH (NCT02912260), resmetirom-treated patients achieved a significantly greater reduction from baseline in hepatic fat (as measured by magnetic resonance imaging-proton density fat fraction [MRI-PDFF]) at week 12 and NASH resolution at week 36 (based on liver biopsy).[9] Furthermore, resmetirom treatment reduced liver enzymes as well as inflammatory and fibrosis biomarkers compared with placebo treatment.[9] In addition to improvements in NASH, resmetirom treatment resulted in clinically significant reductions in LDL-C, triglycerides, apoB, Lp(a) and apoCIII compared with placebo, potentially important beneficial effects of resmetirom in patients with NASH who more commonly die from CVD than progressive liver disease.[9]

These promising Phase 2 results led to the design and initiation of the Phase 3 MAESTRO clinical programme to further evaluate resmetirom for treatment of at-risk NASH, including a pivotal serial liver biopsy/outcomes trial (MAESTRO-NASH), the supporting safety and biomarker trials (MAESTRO-NAFLD-1, MAESTRO-NAFLD-OLE), and a second pivotal outcomes trial in adults with well-compensated NASH cirrhosis (MAESTRO-NASH-OUTCOMES).

2 | METHODS

2.1 | Rationale for the phase 3 MAESTRO clinical programme

Thyroid hormone, through activation of THR-β in hepatocytes, plays a central role in liver function, impacting a range of health

parameters, from levels of serum cholesterol and triglycerides to the pathological accumulation of lipotoxic fat in the liver.[10] Hepatic THR-β activity is key to normal hepatocellular function, including increasing lipophagy and β-oxidation, enhancing mitophagy and mitochondrial biogenesis, reducing reactive oxidative stress by limiting reactive oxygen species, and regulating cholesterol clearance.[8,10–12] However, patients with NASH have reduced levels of thyroid hormone activity in their liver (intrahepatic hypothyroidism), with resultant impaired hepatic function in part due to the inflamed state of the liver brought on by lipotoxicity, further resulting in reduced conversion of the prohormone thyroxine (T4) to the active hormone triiodothyronine (T3) within the liver.[13]

THR-β selective agonists have the potential to address this underlying pathophysiology of NASH. However, it is critical that any potential THR-β therapy avoid activity at THR-α, the predominant systemic THR responsible for activity in the heart and bone.[8] Resmetirom was selected for clinical development based on its enhanced THR-β selectivity in functional THR assays as well as its improved safety in preclinical animal models relative to other THR analogues.[7] Furthermore, resmetirom has shown targeted uptake into the liver, its site of action, avoiding virtually any uptake in tissues outside the liver.[7]

2.2 | Design of the phase 3 MAESTRO clinical programme

The MAESTRO clinical programme is comprised of four complementary Phase 3 trials designed to evaluate the safety and efficacy of resmetirom in patients with at-risk NASH (Figure 1). The three trials in patients with non-cirrhotic NASH (MAESTRO-NASH, MAESTRO-NAFLD-1, MAESTRO-NAFLD-OLE) and the overall Phase 1 and Phase 2 programme provide safety data in ≥1500 patients treated with the top resmetirom dose of 100 mg and >2000 patients treated with ≥80 mg, including many patients treated for 52 weeks and up to 54 months. Patient selection in these trials is designed around screening patients with ≥3 metabolic risk factors (obesity, type 2 diabetes, hypertension and dyslipidaemia) and using non-invasive testing. This programme depends on only one of the four trials (MAESTRO-NASH) requiring liver biopsy at screening and serial follow-up (Figure 2).

MAESTRO-NASH (NCT03900429) is a 54-month randomised, double-blind, placebo-controlled trial in up to 2000 patients with at-risk NASH at ~200 sites worldwide (Figure 1A). Of the four Phase 3 trials, only MAESTRO-NASH requires a recent historic liver biopsy or a liver biopsy during screening to qualify for randomisation and allow assessment of the dual primary endpoints on serial liver biopsy at week 52. Screening of patients for MAESTRO-NASH requires the presence of three metabolic risk factors, a requirement that is consistent across all of the MAESTRO trials (Figure 2). The prescreening VCTE requirement was set at a higher liver stiffness measurement (VCTE ≥8.5 kPa) to identify patients likely to have significant non-cirrhotic NASH (fibrosis stage 2 or 3 [F2/F3]). To qualify

for randomisation, patients must meet screening non-invasive requirements, including ≥8% hepatic fat content (measured by MRI-PDFF), and have a liver biopsy with a minimum NAFLD activity score (NAS) (≥4) and fibrosis stage (F1B, F2 and F3) with a smaller percent F1 (A/C) [exploratory cohort]. MAESTRO-NASH is designed as a pivotal trial with 52-week liver biopsy data that will help support subpart H review with the US Food and Drug Administration (FDA) and review for conditional approval elsewhere. This trial continues blinded for 54 months to evaluate the number of composite clinical outcomes (all-cause mortality, liver transplant, liver-related events, histological progression to cirrhosis and confirmed increase in model for end-stage liver disease [MELD] score from <12 to ≥15) as well as long-term safety, as required by the FDA and European Medicines Agency (EMA).

MAESTRO-NAFLD-1 (NCT04197479) is a 52-week randomised, double-blind, placebo-controlled trial in 1400 patients at 80 sites in the United States. The primary objective is to evaluate the safety and tolerability of resmetirom 80 and 100 mg versus placebo (Figure 1B). Non-invasive identification of patients with NASH is a key focus of this trial. The presence of metabolic risk factors combined with non-invasive testing performed in sequential order (vibration-controlled transient elastography [VCTE] and controlled attenuation parameter [CAP]; magnetic resonance elastography [MRE], if available at the study site; and MRI-PDFF) identifies patients presumed to have NASH.[14–16] In addition to the three double-blind arms (resmetirom 80 mg, resmetirom 100 mg, placebo), MAESTRO-NAFLD-1 includes three open-label arms in patients with (1) non-cirrhotic NASH (100 mg), (2) well-compensated NASH cirrhosis (80 mg starting dose) and (3) moderate renal impairment. Approximately half of the patients in the open-label non-cirrhotic NASH arm are on background thyroxine treatment (for systemic hypothyroidism); this design allows comparison of the safety profile of resmetirom 100 mg in patients on thyroxine therapy versus patients not on thyroxine therapy in an open-label setting.

MAESTRO-NAFLD-OLE (NCT04951219) is a 52-week active treatment extension of MAESTRO NAFLD-1 and includes a 12-week double-blind run-in period in which patients are randomised to 80 or 100 mg of resmetirom. After week 12, all patients receive 100 mg of resmetirom for the duration of the trial (Figure 1B). This trial is designed to include 700 patients with presumed NASH to evaluate the safety and tolerability of resmetirom over an additional 52 weeks and thus provide up to 2 years of safety data for regulatory filing. Both the non-cirrhotic NASH and well-compensated NASH cirrhosis open-label arms from MAESTRO-NAFLD-1 are allowed to continue in MAESTRO-NAFLD-OLE.

MAESTRO-NASH-OUTCOMES (NCT05500222) is a randomised, double-blind, placebo-controlled trial in ~700 adults with well-compensated (Child-Pugh A 5–6) NASH cirrhosis (Figure 1C). This is an event-driven trial evaluating time to a composite clinical outcome (all-cause mortality, liver transplant and liver-related events including hepatic decompensation events [ascites, hepatic encephalopathy and gastroesophageal variceal haemorrhage], HCC
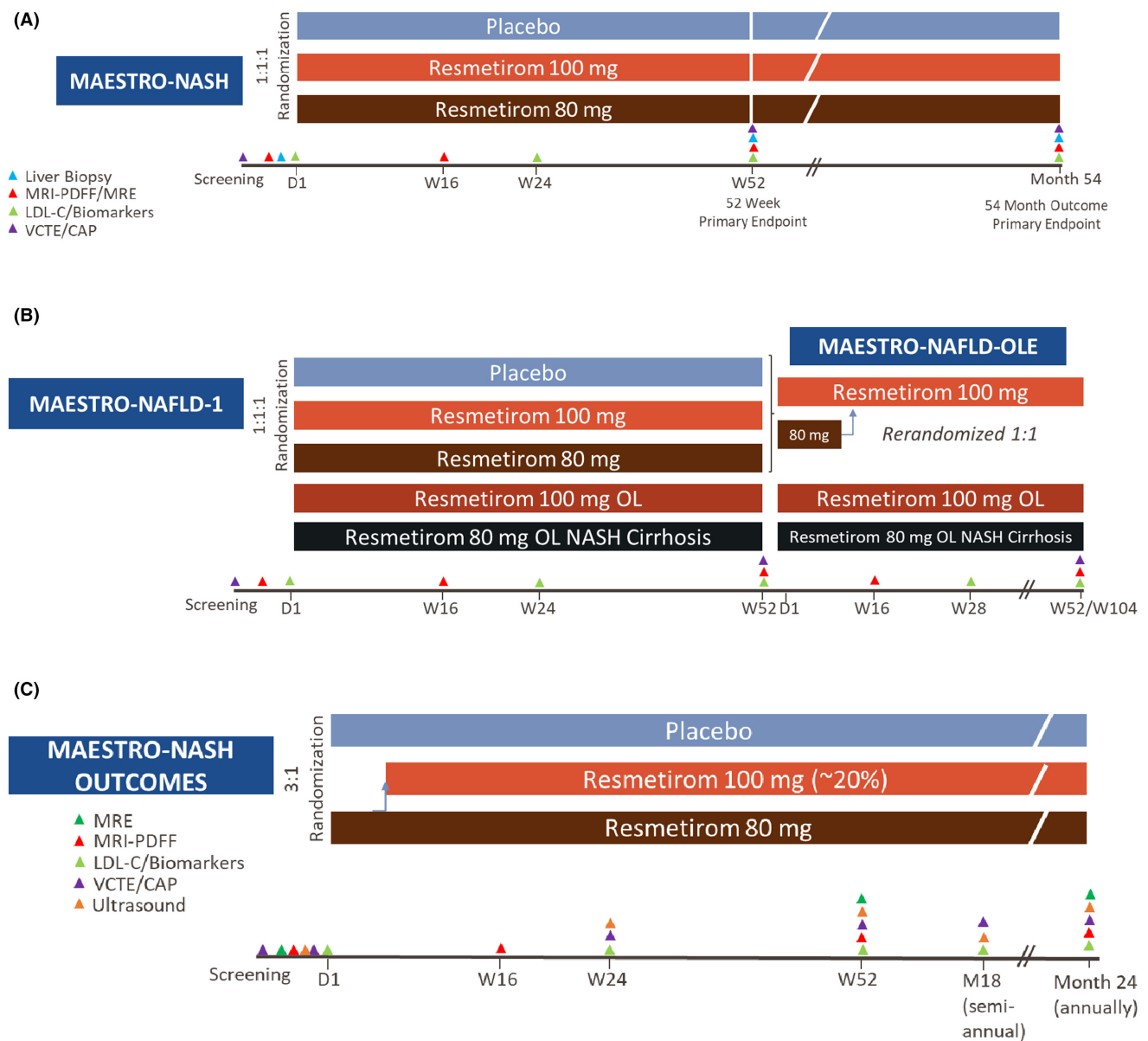
FIGURE 1 MAESTRO Study Design. Timeline for each of the four Phase 3 MAESTRO clinical trials with notation of endpoints and assessments. MAESTRO-NASH (A) is a pivotal study for subpart H approval at week 52 and continues for outcomes at month 54. The safety studies MAESTRO-NAFLD-1 and MAESTRO-NALFD-OLE (B) are sequential as shown. MAESTRO-NASH-OUTCOMES (C) is an event-driven study in adults with well-compensated NASH cirrhosis. CAP, controlled attenuation parameter; LDL-C, low-density lipoprotein cholesterol; MRE, magnetic resonance elastography; MRI-PDFF, magnetic resonance imaging-proton density fat fraction; NAFLD, non-alcoholic fatty liver disease; NASH, non-alcoholic steatohepatitis; OL, open-label; OLE, open-label extension; VCTE, vibration-controlled transient elastography.

and confirmed increase in MELD score from <12 to ≥15, as well as evaluating long-term safety in patients treated with resmetirom 80 mg versus placebo.

2.3 | Endpoints

The Phase 3 MAESTRO clinical programme is designed to evaluate a range of safety and efficacy endpoints with consistency across trials, allowing for analysis of the safety and efficacy of resmetirom across

the spectrum of presumed NASH, biopsy-confirmed NASH and well-compensated NASH cirrhosis.

MAESTRO-NAFLD-1 and MAESTRO-NAFLD-OLE are focused on safety-related endpoints, specifically monitoring for treatment-emergent adverse events (TEAEs) or serious adverse events (SAEs) in patients exposed to resmetirom 80 or 100 mg for up to 52 weeks (MAESTRO-NAFLD-1) and for up to 2 years for those who continue on treatment in the open-label extension (MAESTRO-NAFLD-OLE). These two trials are non-biopsy studies wherein NASH is diagnosed and the efficacy of resmetirom is evaluated via non-invasive testing (serum
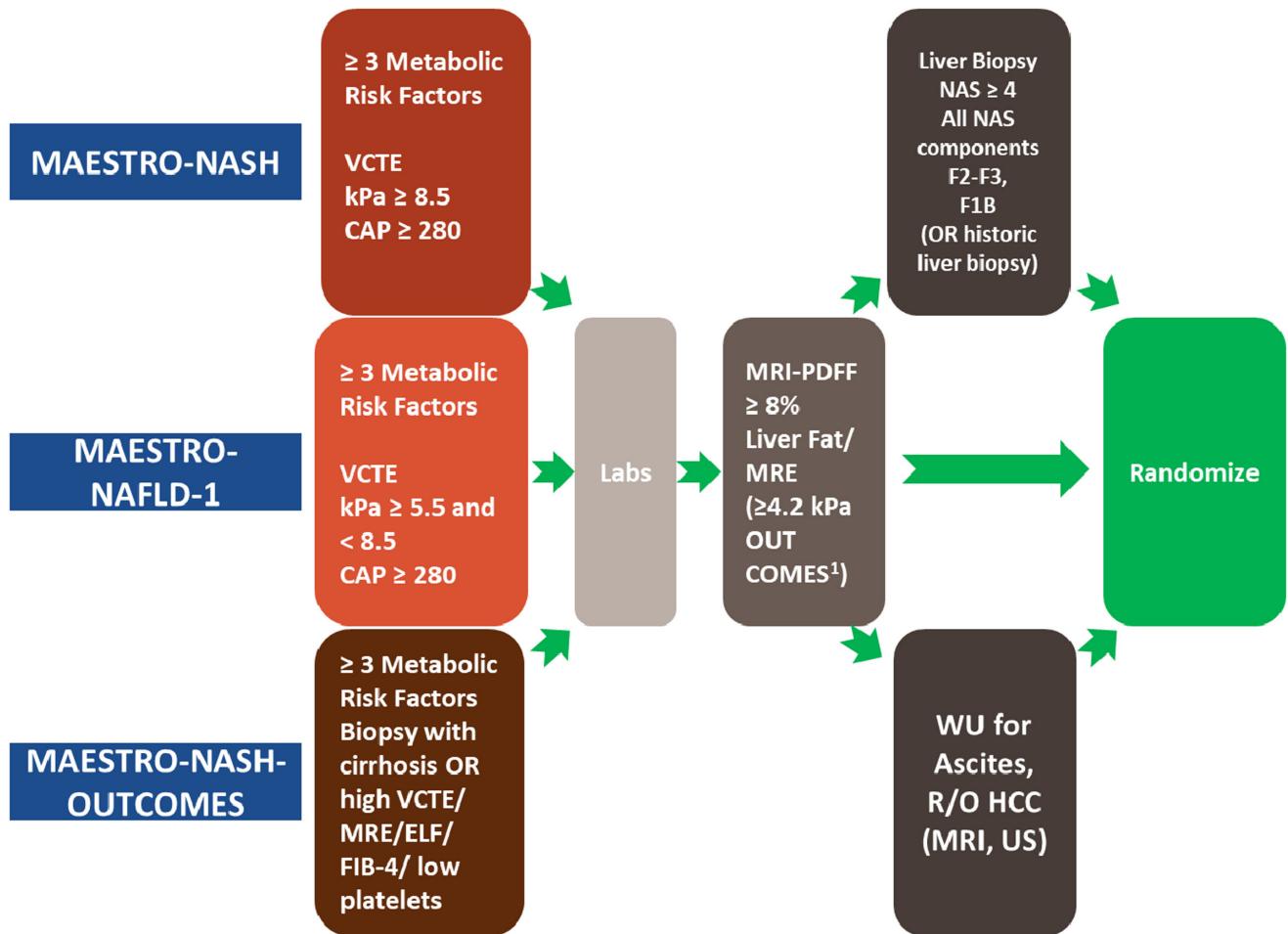
FIGURE 2 MAESTRO Screening Algorithm. MAESTRO-NASH required a liver biopsy to enter while MAESTRO-NALFD-1 utilised non-invasive testing only. MAESTRO-NASH-OUTCOMES could use a biopsy but was not required. [1]Patients without MRE or MRE ≥3.7 may qualify with platelets <140 K or ELF ≥10.25. CAP, controlled attenuation parameter; ELF, enhanced liver fibrosis; HCC, hepatocellular carcinoma; MRE, magnetic resonance elastography; MRI, magnetic resonance imaging; NAFLD, non-alcoholic fatty liver disease; NAS, NAFLD activity score; NASH, non-alcoholic steatohepatitis; US, ultrasound; VCTE, vibration-controlled transient elastography.

and imaging biomarkers). In addition to evaluating the safety profile of resmetirom, MAESTRO-NAFLD-1 and MAESTRO-NAFLD-OLE are representative of 'real-life' NASH, where liver biopsies are infrequently used to diagnose NASH, and thus will help inform clinical practice.

Because of the unpredictable rate of progression of NASH, it takes a long time to accrue enough outcomes to make an assessment of clinical outcomes.[17] For this reason and because of the significant unmet need, the FDA and EMA have expedited approval pathways using surrogate endpoints likely to predict clinical benefit.[5,6,18] For a surrogate endpoint to be clinically meaningful, it must measure how a patient feels, functions or survives.[18] Previous analyses have shown that NASH severity, as quantified by NAS and fibrosis stage, is strongly correlated with liver-related mortality and transplant-free survival and can therefore be used as histology-based surrogate endpoints in clinical trials.[3,4,19]

In MAESTRO-NASH, the dual primary endpoints at week 52 are:

- NASH resolution (achievement of a ballooning score of 0, inflammation score of 0/1) and ≥2-point NAS reduction with no worsening of fibrosis, or

- Fibrosis improvement by ≥1 stage with no worsening of NASH (measured by NAS).

These histological endpoints are consistent with the FDA guidance document as reasonably likely to predict clinical benefit to support accelerated approval.[6] Additionally, these endpoints were initially explored in the Phase 2 trial.[9] As mentioned previously, composite clinical outcomes evaluated at month 54 are designed to support full approval and confirmation of clinical benefit. Safety of resmetirom in the MAESTRO-NASH trial is evaluated as described for the MAESTRO-NAFLD-1 and MAESTRO-NAFLD-OLE trials.

The design of MAESTRO-NASH was based on FDA/EMA guidelines and the Phase 2 trial.[5,6,9,18] Both the surrogate endpoints being evaluated at week 52 and the clinical outcomes at month 54 align with these recommendations. However, the NASH resolution endpoint in MAESTRO-NASH, the same as in the Phase 2 trial,[9] is more stringent than the agency-defined definition, as it requires achievement of a ≥2-point NAS reduction in addition to achievement of a ballooning score of 0 and an inflammation score of 0/1.

To extend the patient population to those with well-compensated NASH cirrhosis, MAESTRO-NASH-OUTCOMES was designed based upon FDA consultation and an FDA proposal for a parallel well-compensated NASH cirrhosis outcomes study to support full approval for non-cirrhotic NASH as well as a separate indication for well-compensated NASH cirrhosis.[20] MAESTRO-NASH-OUTCOMES evaluates composite clinical outcomes; however, unlike the 54-month outcomes portion of MAESTRO-NASH, this is an event-driven trial expected to last 2–3 years.[3,4,12,18]

2.4 | Resmetirom doses

Dosing was based on results from the Phase 2 trial, which demonstrated (1) the efficacy of a single daily dose of resmetirom 80 mg over placebo in significantly reducing hepatic fat, (2) that the magnitude of hepatic fat reduction predicts NASH resolution and fibrosis improvement and (3) that resmetirom 80 mg could achieve the level of hepatic fat reduction predictive of NASH resolution and fibrosis improvement.[9] Data from a multiple ascending dose Phase 1 study (NCT01519531) demonstrated that near maximal lipid-lowering effects were observed with 80 mg resmetirom.[12] However, in the 36-week open-label extension of the Phase 2 trial, patients were able to up-titrate their resmetirom dose to 100 mg, which led to an even greater reduction in hepatic fat without an increase in TEAEs.[21] Based on pharmacokinetics from Phase 1 studies, the 100-mg resmetirom dose results in an ~40–50% increase in drug exposure relative to the 80-mg dose. For these reasons, the Phase 3 MAESTRO clinical programme evaluates 80 and 100 mg resmetirom.

2.5 | Study objectives

In MAESTRO-NAFLD-1, the primary objective is to evaluate the safety and tolerability of resmetirom versus placebo for 52 weeks. Key secondary endpoints include percent change from baseline in LDL-C, apoB and triglycerides (in subgroup with baseline triglycerides ≥150 mg/dL) at week 24, percent change from baseline in hepatic fat (measured by MRI-PDFF) at week 16 and change from baseline in VCTE (in subgroup with baseline VCTE ≥7.2 kPa) and controlled attenuation parameter (CAP) at week 52 (Table 1). Other objectives include change in liver enzymes (alanine aminotransferase [ALT], aspartate aminotransferase [AST], gamma-glutamyl transferase [GGT]), liver stiffness by MRE and other non-invasive tests.

In MAESTRO-NAFLD-OLE, the primary objective is to evaluate the safety and tolerability of resmetirom for 52 weeks, and compare TEAEs at week 12 in patients randomised to 80 versus 100 mg resmetirom (Table 1). Secondary objectives include comparing the effect of 80 versus 100 mg resmetirom on percent change from baseline in LDL-C, apoB, Lp(a) (in subgroup with baseline Lp(a) >10 nmol/L), non-HLD-C, triglycerides (in subgroup with baseline triglycerides >150 mL) and apoCIII at week 12; percent change from baseline in LDL-C, apoB, Lp(a) (in subgroup with baseline Lp(a) >10 nmol/L), non-HLD-C, triglycerides (in subgroup with baseline triglycerides >150 mL) and apoCIII at weeks 28 and 52; comparing the effect of 80 versus 100 mg resmetirom on percent change from baseline in SHBG at week 12; percent change from baseline in sex hormone binding globulin at weeks 28 and 52; percent change from baseline in hepatic fat (measured by MRI-PDFF) according to original treatment at weeks 16 and 52. Two-year safety assessments are made in patients randomised to resmetirom in MAESTRO-NAFLD-1 who continue on resmetirom in MAESTRO-NAFLD-OLE.

In MAESTRO-NASH, the dual primary endpoints at week 52 are NASH resolution (achievement of a ballooning score of 0, inflammation score of 0/1) and ≥2-point NAS reduction with no worsening of fibrosis OR fibrosis improvement by ≥1 stage with no worsening of NASH (measured by NAS) (Table 1). The week 52 analysis is conducted in the first 900 patients with NASH with fibrosis stage 3 – at least half of randomised patients, fibrosis stage 2 (moderate fibrosis) or a small percentage with fibrosis stage 1B (moderate fibrosis) (termed the Primary *Week 52 population*). The key secondary endpoint is percent change from baseline in LDL-C at week 24. Other secondary endpoints include effects of resmetirom on other histological endpoints (fibrosis ≥2-stage responder, fibrosis resolution, composite of NASH resolution and fibrosis ≥1-stage improvement), liver enzymes (ALT, AST, GGT), cardiovascular and lipid parameters (triglycerides, apoB, apoCIII, Lp(a)), and relative and absolute change from baseline in hepatic fat (measured by MRI-PDFF). For the month 54 primary endpoint analysis, clinical benefit is confirmed by evaluating a composite endpoint of clinical outcomes that includes all-cause mortality, liver transplant, significant hepatic events including hepatic decompensation events (ascites, hepatic encephalopathy, gastroesophageal variceal haemorrhage), histological progression to cirrhosis and confirmed increase in MELD score from <12 to ≥15.

In MAESTRO-NASH-OUTCOMES, as an event-driven trial, the primary objective is to evaluate the potential for resmetirom to slow progression to hepatic decompensation events (ascites, hepatic encephalopathy, gastroesophageal variceal haemorrhage), increase in MELD score from <12 to ≥15, and other measures of liver failure (liver transplant) or all-cause mortality (Table 1).

2.6 | Sample size justification and statistical considerations

For MAESTRO-NAFLD-1, ~1400 patients are enrolled with the first ~30 patients receiving open-label resmetirom 100 mg. Thereafter, patients are randomised 1:1:1:1 to double-blind resmetirom 80 mg, double-blind resmetirom 100 mg, double-blind placebo or open-label resmetirom 100 mg. Randomisation switched to a 1:1:1 ratio between the three double-blind arms (resmetirom 80 mg, resmetirom 100 mg, placebo) after enrolling ~175 patients in the open-label arm. The open-label arm is analysed separately by descriptive analyses and compared to the other simultaneously randomised arms. For the hierarchically tested key secondary endpoints, ≥200 patients in each of the three double-blind arms

TABLE 1 Summary of the Phase 3 MAESTRO trials.

Trial	Patient population[a]	Estimated size	Primary endpoint	Key secondary endpoint(s)	Duration
MAESTRO-NAFLD-1	NAFLD/presumed NASH and cohort of patients with well-compensated NASH cirrhosis (Child Pugh A 5–6)	~1400 patients	Week 52: safety and tolerability as measured by the incidence of TEAEs	Week 24: percent CFB in LDL-C, apoB, triglycerides (in subgroup with baseline triglycerides >150 mg/dL) Week 16: percent CFB in hepatic fat (measured by MRI-PDFF) Week 52: CFB in VCTE (in subgroup with baseline VCTE ≥7.2 kPa) and CAP	52 weeks
MAESTRO-NAFLD-OLE	Patients who complete MAESTRO-NAFLD-1 or screen fail MAESTRO-NASH or MAESTRO-NASH-OUTCOMES	~1000 patients	Week 52: safety and tolerability as measured by the incidence of TEAEs	NA	52 weeks
MAESTRO-NASH	Biopsy-confirmed NASH, based on liver biopsy obtained ≤24 weeks before randomisation with F1A/1C, F1B, F2, or F3 fibrosis and NAS ≥4 with a score of ≥1 in steatosis, ballooning and inflammation Patients with F1A/1C fibrosis must have elevated PRO-C3 (>14 ng/mL) at screening	Up to 2000 patients to be enrolled for 54 months ~900 patients are needed for 52-week dual primary endpoints	Week 52: dual primary endpoints: • NASH resolution (ballooning of 0, inflammation of 0/1) and ≥2-point NAS reduction with no worsening of fibrosis • Fibrosis improvement by ≥1 stage with no worsening of NASH (measured by NAS) Month 54: Composite clinical outcomes (all-cause mortality, liver transplant and significant hepatic events including hepatic decompensation events (ascites, hepatic encephalopathy, gastroesophageal variceal haemorrhage), historical progression to cirrhosis and confirmed increase in MELD score from <12 to ≥15	Week 24: percent CFB in LDL-C	54 months
MAESTRO-NASH-OUTCOMES	(a) Histologic evidence of compensated NASH cirrhosis, OR (b) historic liver biopsy showing non-cirrhotic NASH, or (c) no liver biopsy. For (b) or (c), then also 1 of the following: MRE ≥4.2 kPa, ELF ≥10.5, platelets <140K, or FIB-4 ≥ 3	~700 patients	Time to experiencing a composite clinical outcome (all-cause mortality, liver transplant and significant hepatic events including hepatic decompensation events (ascites, hepatic encephalopathy, gastroesophageal variceal haemorrhage) and confirmed increase in MELD score from <12 to ≥15	Week 28: percent CFB in LDL-C Week 52: percent CFB in hepatic fat (measured by MRI-PDFF) (in subgroup with baseline hepatic fat ≥8%)	Event-driven trial; presumed to take 2–3 years

Abbreviations: apoB, apolipoprotein B; CAP, controlled attenuation parameter; CFB, change from baseline; ELF, enhanced liver fibrosis; FIB-4, fibrosis-4 score; LDL-C, low-density lipoprotein cholesterol; MELD, model for end-stage liver disease; MRE, magnetic resonance elastography; MRI-PDFF, magnetic resonance imaging-proton density fat fraction; NA, not applicable; NAFL, non-alcoholic fatty liver; NAFLD, non-alcoholic fatty liver disease; NASH, non-alcoholic steatohepatitis; OLE, open-label extension; TEAE, treatment-emergent adverse event; VCTE, vibration-controlled transient elastography.

[a]All patients had ≥3 metabolic risk factors to be included in screening and ≥ 18 years of age. Metabolic Risk factors include: Large waist or Body mass index (BMI) ≥30, Dyslipidaemia (raised TGs >150 or receiving treatment for elevated lipids), Dyslipidaemia (reduced HDL cholesterol); hypertension (BP >140/90 on two occasions or receiving BP lowering medications), Type 2 diabetes or evidence of insulin resistance derived by HOMA-IR.

provide >90% power to demonstrate a statistically significant difference between each resmetirom dose and placebo at the two-sided 0.025 significance level in percent change in LDL-C, assuming a ≥13.5% difference in percent change from baseline at week 24 between the resmetirom and placebo arms with a within-treatment standard deviation of 16%. Other key lipid secondary endpoints and percent change in hepatic fat (week 16) between the resmetirom and placebo arms have ≥90% power. The number of patients in each arm facilitates subgroup analyses and more precisely determines the magnitude of the treatment effect.

For MAESTRO-NASH, the week 52 primary endpoint analysis is conducted in the first 900 patients who have NASH with fibrosis stage 3 – at least half of randomised patients, fibrosis stage 2 (moderate fibrosis) or a small percentage with fibrosis stage 1B (moderate peri-sinusoidal fibrosis). The sample size was estimated based on response rates in the Phase 2 trial.[9] For the month 54 primary endpoint analysis, a sample size of 1500 patients (500 per treatment arm) is required for the endpoint here is progression to cirrhosis or clinical outcome. The alpha of 0.05 is split between the week 52 and month 54 analyses for a total overall study alpha of 0.05. Key secondary endpoints are hierarchically tested.

For MAESTRO-NASH-OUTCOMES, ~700 patients are planned for enrolent. Patients are randomised 3:1 to resmetirom 80 mg or placebo. This sample size provides 90% power to compare time to composite clinical outcome with resmetirom versus placebo, assuming an annual decompensation rate of 5% for resmetirom and 10% for placebo and a 12-month uniform enrolment. Based on exponential survival, this equates to a hazard ratio of 0.4868. Overall, ~92 composite clinical outcomes are required.

3 | STUDY PROCEDURES

3.1 | Eligibility

In MAESTRO-NAFLD-1, patients aged ≥18 years with ≥3 metabolic risk factors, suspected or confirmed NAFLD or NASH, ≥8% hepatic fat (measured by MRI-PDFF), and liver stiffness by VCTE/MRE consistent with fibrosis stage ≥1 and <4, are eligible (Figure 1A). Patients completing MAESTRO-NAFLD-1 have the opportunity to roll over into MAESTRO-NAFLD-OLE. In addition, patients who screen fail from MAESTRO-NASH with a liver biopsy result of F2 or F3 fibrosis with NAS 3, steatosis 1, ballooning 1 and inflammation 1, OR with F2 or F3 fibrosis with NAS 3 and ballooning 0, OR with F1A or F1C fibrosis with NAS ≥4 (≥1 in all components) and PRO-C3 ≤14 ng/mL are eligible for inclusion in MAESTRO-NAFLD-OLE.

In MAESTRO-NASH, patients aged ≥18 years with ≥3 metabolic risk factors, definite steatohepatitis, NAS ≥4 with a score of ≥1 in all components (steatosis, ballooning and inflammation), and F1A/1C, F1B, F2 or F3 fibrosis confirmed by central reading of a liver biopsy obtained within 6 months of randomisation are eligible.[6] Patients with F1A/1C fibrosis must also have elevated PRO-C3 (>14 ng/mL)

at screening to be eligible and are included for exploratory analysis only. Patients with F1B, F2 or F3 fibrosis are included in the primary analysis at week 52 and month 54.

In MAESTRO-NASH-OUTCOMES, patients aged ≥18 years with ≥3 metabolic risk factors and well-compensated NASH cirrhosis are eligible. Approximately 70% of enrolled patients have liver biopsy evidence of NASH cirrhosis, preferably confirmed by a central liver biopsy review. If no central review of a historic biopsy is possible or the patient has not had a liver biopsy confirming cirrhosis, clinical evidence of NASH cirrhosis based on a combination of non-invasive testing criteria (elevated VCTE, MRE, enhanced liver fibrosis [ELF] score and/or FIB-4 and low platelet count [≥2 tests achieving a value consistent with cirrhosis]) are used to enable screening and confirm eligibility. Confirmation of diagnosis and establishment of well-compensated NASH cirrhosis requires additional testing during the screening period (MRE and other biomarker thresholds, rule out of HCC and ascites).

In all MAESTRO trials, patients are not included if they currently consume or have a history of consumption of significant alcohol for a period of >3 consecutive months within 1 year prior to screening or any other documented causes of chronic liver disease.

3.2 | Blinding

During the conduct of MAESTRO-NAFLD-1, MAESTRO-NASH and MAESTRO-NASH-OUTCOMES, patients, investigators and the sponsor are blinded to individual treatment assignments, except for the open-label arms of MAESTRO-NAFLD-1. Results of several laboratory tests (e.g. SHBG, FT4 and lipids) will be blinded to study personnel and investigators throughout the study to preserve the blind. If necessary to ensure safety throughout the trials, a Data Monitoring Committee has access to unblinded individual patient data. To maintain the integrity of the month 54 analysis in MAESTRO-NASH, only a minimal number of personnel is unblinded to the patient-level data at the time of the week 52 analysis to facilitate regulatory filings and required public disclosures.

In MAESTRO-NAFLD-OLE, investigators and patients are blinded to treatment during the 12-week lead-in period (80 or 100 mg resmetirom). From weeks 12 through 52, all patients receive open-label resmetirom 100 mg.

3.3 | Screening procedures

Both MAESTRO-NAFLD-1 and MAESTRO-NASH utilise a sequential non-invasive test screening strategy, including the requirement for ≥3 metabolic risk factors, and key inclusion criteria using a modified version of the International Diabetes Foundation criteria.[22] FIB-4 is not included as a screening test because many patients with at-risk NASH have near normal liver enzymes and/or ALT predominant elevations and normal platelets. Instead, in the MAESTRO clinical programme, AST (≥20 mg/dL [men]/≥17 mg/dL [women])

and VCTE (MAESTRO-NAFLD-1: 5.5–8.5 kPa; MAESTRO-NASH: ≥8.5 kPa) are used to enrich for patients with NAFLD/NASH. Eligible patients based on medical history, concomitant medications and screening labs are then evaluated for hepatic fat (measured by MRI-PDFF) with ≥8% required before the liver biopsy is performed (in MASTRO-NASH). See Figure 2 for the MAESTRO-NAFLD-1, MAESTRO-NASH and MAESTRO-NASH OUTCOMES screening processes. The screening algorithm, including the metabolic risk factors, VCTE and MRI-PDFF, is unique among Phase 3 NASH trials and improved the MAESTRO-NASH biopsy screen failure to an acceptable rate.[23]

3.4 | Histological grading

For MAESTRO-NASH, two highly qualified central pathologists follow a standardised criterion for the NASH Clinical Research Network (CRN) scoring system to ensure consistency between histology readings. Fibrosis and the key features of NASH (steatosis, ballooning, inflammation) are graded according to the NASH-CRN criteria.[24]

All liver biopsy specimens are read centrally using glass slides as the primary evaluation and digitised images as a secondary assessment. At week 52, month 54, or early termination, biopsies are centrally read for eligibility and separately read in a primary analysis by two central pathologists. Groups of slides, defined by time of biopsy, are read by both central pathologists. Secondary review includes reading of paired biopsy digitised images. Intra-reader and inter-reader consistency are determined. In addition to grading by two central pathologists, digitised biopsy images are evaluated using two exploratory artificial intelligence algorithms, developed by PathAI and HistoIndex.[25,26]

3.5 | Patient-reported outcomes

Health-related quality-of-life assessments are performed throughout all MAESTRO trials. Three patient-reported outcome measures assess change in health outcomes from baseline: (1) The NAFLD/NASH Chronic Liver Disease Questionnaire (CLDQ) comprised 29 questions in 6 domains,[27] (2) The Short Form-Liver Disease Quality of Life (SF-LDQOL) comprised 36 liver-specific questions split into 9 scales and 36 non-liver-specific questions for a total of 72 questions,[28] and (3) The Work Productivity and Activity Index (WPAI)-NASH: The WPAI-NASH is a validated system designed consisting of 4 domains to measure impairment in work and activities.[29]

Patients complete the questionnaires at the time of study visits using a handheld device to capture their responses. The investigator and/or research staff review the instructions to complete the questionnaires with patients. Patients complete the questionnaires with limited assistance from the Investigator and/or research staff.

3.6 | Safety

In all MAESTRO trials, patients are closely monitored for signs/symptoms consistent with a clinically significant cardiovascular event (e.g. MACE) and/or cardiac toxicity. Monitoring includes physical examination, vital signs, 12-lead electrocardiogram with rhythm strip, clinical laboratory tests and adverse event assessment. These assessments are performed on a regular, ongoing basis at predefined study visits throughout each study. Adjudication of possible MACE is performed by the Cardiovascular Event Adjudication Committee and potential hepatic events by a Hepatic Adjudication Committee.

3.7 | Human studies and patients

All studies are conducted in full compliance with the International Council for Harmonisation Guidance on General Considerations for Clinical Trials and approved by the institutional review board and independent ethics committee at each study sites. Prior to participation, all patients provide written informed consent in accordance with the Declaration of Helsinki, the United States Code of Federal Regulations and Good Clinical Practice guidelines.

4 | DISCUSSION

4.1 | Non-invasive screening and prediction of at-risk NASH

To enrich the population in the MAESTRO clinical programme and reduce screen failure rates at liver biopsy in MAESTRO-NASH, the unique requirement for the presence of ≥3 metabolic risk factors and protocol-specified ≥8% hepatic fat resulted in 70.6% of screened patients having qualifying liver biopsies.[23] In addition to patients enrolled in the open-label arm of MAESTRO-NAFLD-1 who are eligible to continue open-label treatment in MAESTRO-NAFLD-OLE, new patients, who screen fail from MAESTRO-NASH with a liver biopsy result of F2 or F3 fibrosis with NAS 3, steatosis 1, ballooning 1 and inflammation 1, OR with F2 or F3 fibrosis with NAS 3 and ballooning 0, OR with F1A or F1C fibrosis with NAS ≥4 (≥1 in all components) and PRO-C3 ≤14 ng/mL, are eligible for open-label resmetirom 100 mg treatment in MAESTRO-NAFLD-OLE. This design maximises opportunities for patients to participate in the MAESTRO clinical programme, an efficient proactive approach in a disease state with high unmet need and unpredictable progression.

4.2 | Diagnosis of NASH

Globally, the estimated prevalence of NASH is ~1.5% to 6.45% of the general population and much higher in those with type 2 diabetes.[30–33] Based on 2004–2016 data from the United Network for Organ Sharing/Organ Procurement and Transplantation

Network database, NASH was the second leading cause of liver transplant overall (and leading cause in women).[34] As such, the FDA views NASH with liver fibrosis as a serious and life-threatening condition and NASH is an important area of drug development, especially in at-risk NASH of F2-F4.[2,6]

NASH is typically diagnosed non-invasively using a combination of approaches including patient assessment for metabolic risk factors, imaging (ultrasound, VCTE) and simple laboratory assessments, coupled with ruling out other causes of liver disease (e.g. alcohol, viral hepatitis, autoimmune hepatitis), leading to low sensitivity and specificity for diagnosis of at-risk NASH.[1,2,6] As shown in recent studies and in the MAESTRO-NASH screening paradigm,[23,35–37] NASH may be more accurately diagnosed using more advanced imaging technologies (MRI-PDFF, MRE, VCTE) and fibrosis biomarkers such as enhanced liver fibrosis (ELF) and PRO-C3. This strategy led to accurate diagnosis of NASH with significant fibrosis (F2/F3) about 70% of the time.

4.3 | Biopsy review and endpoints

Regulatory agencies continue to require liver biopsy for diagnosis and serial evaluation in clinical trials of drugs for treatment of NASH while recognising that liver biopsies may have high variability and poor reader concordance, particularly in scoring of inflammation and ballooning.[38,39] To address the poor intra-reader and inter-reader evaluation of liver biopsies, two central readers review the MAESTRO-NASH data in a blinded fashion. The central readers are trained to score similarly using shared baseline digitised images. Primary, secondary and artificial intelligence reviews of the biopsies are conducted in an attempt to achieve high concordance.

In addition, MAESTRO-NASH employs a more stringent definition of NASH resolution, including the requirement for a ≥2-point NAS reduction plus absence of ballooning, inflammation 0/1 and no worsening of fibrosis to help avoid variability that leads to a high rate of 'apparent' response in the NASH resolution endpoint. Pathologists commonly disagree in the assessment of ballooning.[39] The NASH resolution endpoint defined by regulatory agencies allows a 1-point reduction in the ballooning score as the only change to be called 'NASH resolution' in baseline biopsy with NAS of 4 with ballooning 1 and inflammation 1 that occurs in ~25% of baseline biopsies. This NASH resolution endpoint results in high apparent response rate in the placebo arm (or treatment arm) that is not accompanied by improvement in any other parameter and may simply result from disagreement between pathologists as to whether a 'few ballooned cells' are present.[38]

4.4 | Totality of non-invasive data

The MAESTRO clinical programme provides a large database of non-invasive testing data over several years across a full spectrum of NASH from relatively early NASH with mild fibrosis through patients progressing to decompensated NASH cirrhosis. Further, the programme is expected to provide data across various non-invasive tests correlated to liver biopsy at baseline, with treatment and with clinical outcomes. The biomarkers – liver enzymes, simple tests such as FIB-4 and a variety of proprietary tests (e.g. ELF, PRO-C3), imaging tests (e.g. VCTE, CAP, MRI-PDFF, MRE, cT1) and combination tests (e.g. MAST, FAST) will provide insight into patient identification, risk stratification and monitoring. This is anticipated to afford a strong bridge from the regulatory endpoints (based on liver biopsy) to the various methods used in clinical practice throughout the world to diagnose and monitor patients (based on access to specific non-invasive tests).

4.5 | Size of safety database

Drug development for potential NASH treatments can be challenging due to the slow progression of liver fibrosis over several years. The magnitude of the benefit that a patient receives with lifelong treatment of NASH must be balanced against the safety profile of the drug. Patients with NASH often are pre-disposed to other diseases,[1,2] and the investigational drug should not worsen comorbidities, including type 2 diabetes, dyslipidaemia and CVD, or cause liver injury.

The FDA stated that NASH is a common disease and trials that provide a sufficiently large pre-approval safety database will facilitate the assessment of risk and benefit. In accordance with the International Conference on Harmonisation E1A guidance,[40] which recommends a minimum number of patients for enrolment in a trial for drugs intended for chronic administration, the size of the pre-approval safety database should ensure that low-frequency adverse event(s) can be detected and appropriately described for an assessment of risk and benefit. Additionally, the regulators stated that the size of a single placebo-controlled trial, adequately powered for efficacy, might not be sufficient to support the drug's safety and allow for the overall risk–benefit assessment necessary for drug approval; this is a particular concern in NASH, in which millions of patients might be exposed to the drug once approved. To meet the requirements for a large safety database, MAESTRO-NAFLD-1 and MAESTRO-NAFLD-OLE were added to further evaluate and characterise the safety profile of resmetirom in additional patients at the same doses being tested in MAESTRO-NASH.

5 | CONCLUSIONS

The MAESTRO clinical programme was designed in conjunction with regulatory authorities to support an approval of resmetirom for treatment of NASH. The surrogate assessments of efficacy (liver biopsy, biomarkers, imaging) are supported by long-term clinical outcomes that assess mortality, progression to cirrhosis and hepatic

decompensation events. The full programme will provide a broad and long-term assessment of resmetirom in patients spanning the breadth of at-risk NASH, providing insight into patient identification and risk stratification as well as monitoring of treatment response in the real world.

AUTHOR CONTRIBUTIONS

Stephen A. Harrison: Conceptualization (equal); investigation (equal); methodology (equal); supervision (lead); writing – original draft (equal). **Vlad Ratziu:** Conceptualization (equal); investigation (equal); methodology (equal); supervision (lead); writing – review and editing (equal). **Quentin M. Anstee:** Conceptualization (equal); methodology (equal); writing – review and editing (equal). **Mazen Noureddin:** Conceptualization (equal); investigation (equal); methodology (equal); supervision (equal); writing – review and editing (equal). **Arun J. Sanyal:** Conceptualization (equal); investigation (equal); methodology (equal); supervision (equal); writing – review and editing (equal). **Jörn M. Schattenberg:** Conceptualization (equal); investigation (equal); methodology (equal); supervision (equal); writing – review and editing (equal). **Pierre Bedossa:** Conceptualization (equal); investigation (equal); methodology (equal); writing – review and editing (equal). **Mustafa R. Bashir:** Conceptualization (equal); investigation (equal); methodology (lead); supervision (equal); writing – review and editing (equal). **David Schneider:** Conceptualization (equal); investigation (supporting); methodology (supporting); supervision (supporting); writing – review and editing (equal). **Rebecca Taub:** Conceptualization (lead); investigation (equal); methodology (lead); supervision (lead); writing – original draft (lead). **Meena Bansal:** Conceptualization (equal); investigation (equal); methodology (equal); supervision (equal); writing – review and editing (equal). **Kris V. Kowdley:** Conceptualization (equal); investigation (equal); methodology (equal); supervision (equal); writing – review and editing (equal). **Zobair M. Younossi:** Conceptualization (equal); investigation (equal); methodology (equal); supervision (equal); writing – review and editing (equal). **Rohit Loomba:** Conceptualization (lead); investigation (equal); methodology (lead); supervision (equal); writing – review and editing (equal).

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REFERENCES

1. Harrison SA, Loomba R, Dubourg J, Ratziu V, Noureddin M. Clinical trial landscape in NASH. Clin Gastroenterol Hepatol. 2023;21(8):2001–14.
2. Rinella ME, Neuschwander-Tetri BA, Siddiqui MS, Abdelmalek MF, Caldwell S, Barb D, et al. AASLD practice guidance on the clinical assessment and management of nonalcoholic fatty liver disease. Hepatology. 2023;77:1797–835.
3. Younossi ZM, Stepanova M, Rafiq N, Makhlouf H, Younoszai Z, Agrawal R, et al. Pathologic criteria for nonalcoholic steatohepatitis: interprotocol agreement and ability to predict liver-related mortality. Hepatology. 2011;53:1874–82.
4. Angulo P, Kleiner DE, Dam-Larsen S, Adams LA, Bjornsson ES, Charatcharoenwitthaya P, et al. Liver fibrosis, but No other histologic features, is associated with long-term outcomes of patients with nonalcoholic fatty liver disease. Gastroenterology. 2015;149:389–397.e10.
5. European Medicines Agency. Reflection Paper on Regulatory Requirements for the Development of Medicinal Products for Chronic Non-infectious Liver Diseases (PBC, PSC, NASH). 2018 Available from: https://www.ema.europa.eu/en/documents/scientific-guideline/reflection-paper-regulatory-requirements-development-medicinal-products-chronic-non-infectious-liver_en.pdf
6. US Department of Health and Human Services; Food and Drug Administration; Center for Drug Evaluation and Research (CDER). Noncirrhotic Nonalcoholic Steatohepatitis With Liver Fibrosis: Developing Drugs for Treatment Guidance for Industry. 2018 Available from: https://www.fda.gov/media/119044/download
7. Kelly MJ, Pietranico-Cole S, Larigan JD, Haynes NE, Reynolds CH, Scott N, et al. Discovery of 2-[3,5-dichloro-4-(5-isopropyl-6-oxo-1,6-dihydropyridazin-3-yloxy)phenyl]-3,5-dio xo-2,3,4,5-tetrahydro[1,2,4]triazine-6-carbonitrile (MGL-3196), a highly selective thyroid hormone receptor beta agonist in clinical trials for the treatment of dyslipidemia. J Med Chem. 2014;57:3912–23.
8. Saponaro F, Sestito S, Runfola M, Rapposelli S, Chiellini G. Selective thyroid hormone receptor-beta (TRbeta) agonists: new perspectives for the treatment of metabolic and neurodegenerative disorders. Front Med (Lausanne). 2020;7:331.
9. Harrison SA, Bashir MR, Guy CD, Zhou R, Moylan CA, Frias JP, et al. Resmetirom (MGL-3196) for the treatment of non-alcoholic steatohepatitis: a multicentre, randomised, double-blind, placebo-controlled, phase 2 trial. Lancet. 2019;394:2012–24.
10. Sinha RA, Bruinstroop E, Singh BK, Yen PM. Nonalcoholic fatty liver disease and hypercholesterolemia: roles of thyroid hormones, metabolites, and agonists. Thyroid. 2019;29:1173–91.
11. Ritter MJ, Amano I, Hollenberg AN. Thyroid hormone signaling and the liver. Hepatology. 2020;72:742–52.
12. Taub R, Chiang E, Chabot-Blanchet M, Kelly MJ, Reeves RA, Guertin MC, et al. Lipid lowering in healthy volunteers treated with multiple doses of MGL-3196, a liver-targeted thyroid hormone receptor-beta agonist. Atherosclerosis. 2013;230:373–80.
13. Bohinc BN, Michelotti G, Xie G, Pang H, Suzuki A, Guy CD, et al. Repair-related activation of hedgehog signaling in stromal cells promotes intrahepatic hypothyroidism. Endocrinology. 2014;155:4591–601.
14. Noureddin M, Lam J, Peterson MR, Middleton M, Hamilton G, le TA, et al. Utility of magnetic resonance imaging versus histology for quantifying changes in liver fat in nonalcoholic fatty liver disease trials. Hepatology. 2013;58:1930–40.
15. Younossi Z, Alkhouri N, Cusi K, Isaacs S, Kanwal F, Noureddin M, et al. A practical use of noninvasive tests in clinical practice to identify high-risk patients with nonalcoholic steatohepatitis. Aliment Pharmacol Ther. 2023;57(3):304–12.
16. Kugelmas M. The use of current knowledge and non-invasive testing modalities for predicting at-risk non-alcoholic steatohepatitis and assessing fibrosis. Liver Int. 2023;43:964–74.
17. Harrison SA. Challenges and opportunities in NASH drug development. Nat Med. 2023;29:562–73.
18. FDA Regulatory Considerations for NASH Clinical Trial Endpoints. 2018; Available from: http://www.globalengage.co.uk/nash/Omokaro.pdf.
19. Kleiner DE, Brunt EM, Wilson LA, Behling C, Guy C, Contos M, et al. Association of histologic disease activity with progression of nonalcoholic fatty liver disease. JAMA Netw Open. 2019;2:e1912565.
20. US Department of Health and Human Services; Food and Drug Administration; Center for Drug Evaluation and Research (CDER). Nonalcoholic Steatohepatitis with Compensated Cirrhosis: Developing Drugs for Treatment Guidance for Industry. 2019 Available from: https://www.fda.gov/media/127738/download
21. Harrison SA, Bashir M, Moussa SE, McCarty K, Pablo Frias J, Taub R, et al. Effects of resmetirom on noninvasive endpoints in a 36-week phase 2 active treatment extension study in patients with NASH. Hepatol Comm. 2021;5:573–88.
22. International Diabetes Federation. The IDF Consensus Worldwide Definition of the Metabolic Syndrome. 2006 Available from: https://www.idf.org/e-library/consensus-statements/60-idfconsensus-worldwide-definitionof-the-metabolic-syndrome
23. Harrison SATR, Karsdal MA, Franc J, Bashir M, Barbone JM, Neff G, et al. Algorithm for predicting advanced NASH fibrosis on

screening biopsy in resmetirom phase 3 MAESTRO-NASH clinical trial. Hepatology. 2020;72(S1):1008A.

24. Kleiner DE, Brunt EM, van Natta M, Behling C, Contos MJ, Cummings OW, et al. Design and validation of a histological scoring system for nonalcoholic fatty liver disease. Hepatology. 2005;41(6):1313–21.

25. Taylor-Weiner A, Pokkalla H, Han L, Jia C, Huss R, Chung C, et al. A machine learning approach enables quantitative measurement of liver histology and disease monitoring in NASH. Hepatology. 2021;74:133–47.

26. Liu F, Goh GB, Tiniakos D, Wee A, Leow WQ, Zhao JM, et al. qFIBS: an automated technique for quantitative evaluation of fibrosis, inflammation, ballooning, and steatosis in patients with nonalcoholic steatohepatitis. Hepatology. 2020;71:1953–66.

27. Younossi ZM, Stepanova M, Younossi I, Racila A. Validation of chronic liver disease questionnaire for nonalcoholic steatohepatitis in patients with biopsy-proven nonalcoholic steatohepatitis. Clin Gastroenterol Hepatol. 2019;17:2093–2100.e3.

28. Kanwal F, Spiegel BM, Hays RD, Durazo F, Han SB, Saab S, et al. Prospective validation of the short form liver disease quality of life instrument. Aliment Pharmacol Ther. 2008;28(9):1088–101.

29. Reilly MC, Zbrozek AS, Dukes EM. The validity and reproducibility of a work productivity and activity impairment instrument. Pharmacoeconomics. 1993;4(5):353–65.

30. Younossi ZM, Koenig AB, Abdelatif D, Fazel Y, Henry L, Wymer M. Global epidemiology of nonalcoholic fatty liver disease-meta-analytic assessment of prevalence, incidence, and outcomes. Hepatology. 2016;64(1):73–84.

31. Younossi ZM, Golabi P, de Avila L, Paik JM, Srishord M, Fukui N, et al. The global epidemiology of NAFLD and NASH in patients with type 2 diabetes: a systematic review and meta-analysis. J Hepatol. 2019;71(4):793–801.

32. Williams CD, Stengel J, Asike MI, Torres DM, Shaw J, Contreras M, et al. Prevalence of nonalcoholic fatty liver disease and nonalcoholic steatohepatitis among a largely middle-aged population utilizing ultrasound and liver biopsy: a prospective study. Gastroenterology. 2011;140(1):124–31.

33. Harrison SA, Gawrieh A, Roberts K, Lisarti CJ, Schwope RB, Cebe KM, et al. Prospective evaluation of the prevalence of non-alcoholic fatty liver disease and steatohepatitis in a large middle-aged US cohort JHEP.

34. Noureddin M, Vipani A, Bresee C, Todo T, Kim IK, Alkhouri N, et al. NASH leading cause of liver transplant in women: updated analysis of indications for liver transplant and ethnic and gender variances. Am J Gastroenterol. 2018;113(11):1649–59.

35. Boyle M, Tiniakos D, Schattenberg JM, Ratziu V, Bugianessi E, Petta S, et al. Performance of the PRO-C3 collagen neo-epitope biomarker in non-alcoholic fatty liver disease. JHEP Rep. 2019;1(3):188–98.

36. Newsome PN, Sasso M, Deeks JJ, Paredes A, Boursier J, Chan WK, et al. FibroScan-AST (FAST) score for the non-invasive identification of patients with non-alcoholic steatohepatitis with significant activity and fibrosis: a prospective derivation and global validation study. Lancet Gastroenterol Hepatol. 2020;5(4):362–73.

37. Vali Y et al. Biomarkers for staging fibrosis and non-alcoholic steatohepatitis in non-alcoholic fatty liver disease (the LITMUS project): a comparative diagnostic accuracy study. Lancet Gastroenterol Hepatol. 2023;714–25.

38. Davison BA, Harrison SA, Cotter G, Alkhouri N, Sanyal A, Edwards C, et al. Suboptimal reliability of liver biopsy evaluation has implications for randomized clinical trials. J Hepatol. 2020;73(6):1322–32.

39. Brunt EM, Clouston AD, Goodman Z, Guy C, Kleiner DE, Lackner C, et al. Complexity of ballooned hepatocyte feature recognition: defining a training atlas for artificial intelligence-based imaging in NAFLD. J Hepatol. 2022;76:1030–41.

40. ICH-E1A. The Extent of Population Exposure to Assess Clinical Safety: For Drugs Intended for Longterm Treatment of Non-Life-Threatening Conditions. 1995 Available from: https://www.fda.gov/media/71180/download