A roadmap for clinical trials in MASH-related compensated cirrhosis

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Abstract

Although metabolic dysfunction-associated steatohepatitis (MASH) is rapidly becoming a leading cause of cirrhosis worldwide, therapeutic options are limited, and the number of clinical trials in MASH-compensated cirrhosis is low as compared to those conducted in earlier disease stages. Moreover, designing clinical trials in MASH cirrhosis presents a series of challenges in relation to the understanding and conceptualisation of the natural history, regulatory considerations, inclusion criteria, recruitment, endpoints, and trial duration, among others. The first international workshop on the state of the art and future direction of clinical trials in MASH-compensated cirrhosis was held in April 2023 at Vall d'Hebron University Hospital in Barcelona (Spain) and was attended by a group of international experts on clinical trials from academia, regulatory agencies and industry, encompassing expertise in MASH, cirrhosis, portal hypertension, and regulatory affairs. The presented Roadmap summarizes important content of the workshop on current status, regulatory requirements and endpoints in MASH compensated cirrhosis clinical trials, exploring alternative study designs and highlighting the challenges that should be considered for upcoming studies on MASH cirrhosis.

[H1] Introduction

Metabolic dysfunction-associated steatotic liver disease (MASLD), formerly known as nonalcoholic fatty liver disease (NAFLD)[1], ranges from simple steatosis to metabolic dysfunction-associated steatohepatitis (MASH)[2,3]. Hepatic fibrosis often develops in patients with MASLD and can progress to cirrhosis, hepatocellular carcinoma and end-stage liver disease (Figure 1)[4]. According to modelling studies, global MASLD prevalence increased by 50.4% in the past three decades, from 25.26% (21.59–29.33) in 1990–2006 to 38.0% (33.71–42.49) in 2016–2019 (P<0.001), with an increased percentage of individuals experiencing advanced disease[5–9].

The progression of MASH and fibrosis is characterized by a highly variable natural history modified by endogenous (genetic, epigenetic) and exogenous disease modifiers (including diet, alcohol, and physical activity)[10]. From a pathophysiological perspective, and despite hepatic reparative mechanisms, MASLD is a complex disease affecting hepatocytes and numerous other cell types[11-14]. Metabolic drivers such as insulin resistance and adipose tissue dysfunction are essential in early disease evolution (Figure 2). The spectrum of compensated cirrhosis encompasses heterogeneous stages of severity associated with broadly diverse risks of cardiometabolic complications and liver decompensation, from early stage 4 fibrosis (F4) to severe portal hypertension and varying degrees of liver inflammation[15]. To adequately tailor treatments, future clinical trials should clearly define intervention, inclusion criteria and outcomes based on disease stage[16,17] (Figure 3).

Many pharmacological compounds have been and are currently tested in clinical trials[18]. Until now, there have been no Food and Drug Administration (FDA) or European Medicines Agency

(EMA) approved drugs for the treatment of MASH[19]. The publication of a positive trial in early February 2024[19] led to the first FDA approval of a drug to treat MASH[20], the agonist of the hepatic thyroid hormone beta-receptor resmetirom, which is now under consideration by EMA. The reasons for the prior multiple failures of apparently promising drugs and combinations, such as pioglitazone or obeticholic acid, are diverse, but among them, heterogeneity of the disease population and natural history, selection of the ideal target population, methods and criteria of diagnosis, and endpoints definition stand out[21]. Addressing the latter is difficult as disease evolution is slow, and surrogates for clinical outcomes have not been approved so far. These challenges are particularly problematic in MASLD cirrhosis owing to the disappearance of typical features of MASH in the advanced stages of the disease, uncertainties about the characteristics and accuracy of measurements of portal hypertension, the lack of knowledge on disease drivers and prevalence and nature of clinical events, and complexity of treatment of cardiometabolic co-morbidities of MASH in the cirrhotic stage[22]. Despite patients with MASH cirrhosis having an increased risk of liver decompensation and mortality, therefore having the highest need for efficacious treatment, clinical trials in this population have been relatively few, with very different designs in terms of inclusion and exclusion criteria, duration, definition of endpoints and assessments[23].

In April 2023, members of the Liver Unit organised a workshop on clinical trials in MASH cirrhosis at the Vall d'Hebron University Hospital in Barcelona (Spain). The event brought together international experts on clinical trials from academia, regulatory agencies and industry, encompassing expertise in MASH, cirrhosis, portal hypertension, and regulatory affairs. The meeting objectives were to review the current status and regulatory requirements

in MASH clinical trials, review study endpoints, and explore alternative options for designing clinical trials in patients with MASH-compensated cirrhosis. This Roadmap article was inspired by the presentations and discussions held during the workshop.

[H1] Current landscape and regulatory issues

[H2] Current scenario of clinical trials in MASH

Fibrosis is the primary predictor of morbidity and mortality in MASLD (liver-related complications and death, cardiovascular disease, and hepatic and extrahepatic malignancies), with prognosis correlating closely with the fibrosis stage, for example, from absence of fibrosis to cirrhosis in the F0 to F4 staging system[24]. Patients with compensated MASH cirrhosis are at high risk of liver-related events, which is even higher in those who have already exhibited hepatic decompensation. In a study published in 2022, Allen and colleagues found that the probability of liver-related events was 42% in 4 years in patients with compensated MASH cirrhosis, whereas it increased up to 65% in 2 years in patients with decompensated cirrhosis[25]. As the long-term goal is to prevent or reduce liver-related events (decompensation, transplantation, or cancer), surrogate endpoints are needed in clinical trials to assess the efficacy and safety of the interventions in the minimum possible number of individuals and time. Lifestyle changes such as diets low in refined sugars and unsaturated fats, physical exercise[26-28] and weight loss (through the former measures, bariatric surgery or the use of glucagon-like peptide 1 (GLP-1) receptor agonists and dual or triple glucagon agonists) are at the core of current MASLD clinical routine management for patients with MASH without cirrhosis but might not be as effective in MASH cirrhosis[29-33]. As for other

pharmacological options, obeticholic acid (discontinued) and resmetirom reached their phase III primary endpoint, but only in patients without cirrhosis[19,34–37] (Table 1). Potent anti-inflammatory and antifibrotic agents such as resmetirom and efruxifermin, respectively, or lanifibranor, which has both antifibrotic and anti-inflammatory activities, are warranted in the field of compensated MASH cirrhosis[38-40], alone[4-44] or in combination[45,46].

The REVERSE trial[47] (NCT03439254) included 919 participants to evaluate the efficacy of obeticholic acid, an agonist of FXR, leading to a reduction in bile acid synthesis in patients with compensated MASH cirrhosis. It adopted ≥ 1-stage histological improvement in fibrosis with no worsening of MASH following up to 18 months of therapy as the primary endpoint, which was not met after analysis. STELLAR-4 trial[48] (NCT03053063, 877 participants) evaluated selonsertib, a selective apoptosis signal-regulating kinase 1 inhibitor, and included ≥ 1-stage histological improvement in fibrosis with no worsening of MASH after 48 weeks of treatment as the primary endpoint, which was not met either. NAVIGATE[49] (NCT04365868, 357 participants) was a phase IIb–III trial testing belapectin, a galectin-3 inhibitor, the primary endpoint of which was the proportion of patients with MASH cirrhosis who developed new oesophageal varices at 78 weeks. NATiV3 (NCT04849728, 1000 participants) is a phase III clinical trial currently ongoing that evaluates lanifibranor, a pan-PPAR agonist for the treatment of MASH, fibrosis, and cirrhosis[50]. There is preclinical evidence on the efficacy of lanifibranor on cirrhosis and portal hypertension, so it is likely that trials will be set up in the coming years to test lanifibranor in MASH cirrhosis. Concerning phase II clinical trials, the one testing the FGF19 analogue aldafermin (ALPINE 4, NCT04210245, 160 participants)[52] used the reduction of enhanced fibrosis score (ELF) at week 48 as the primary endpoint, which was achieved in

the 3mg arm but not in the 1 mg arm (the 0.3mg group was discontinued to limit exposure to suboptimal doses). Regarding fibrosis improvement, 15%, 21% and 23% of patients in the placebo (n=56), 1 mg (n=42) and 3 mg group (n=55), respectively, achieved fibrosis improvement ≥1-stage, and 13%, 16% and 20% achieved fibrosis improvement ≥1-stage without MASH worsening. Another trial with the GLP-1 receptor agonist semaglutide (NCT03987451, 71 participants) did not meet its primary endpoint: improvement in liver fibrosis of one stage or more without worsening MASH after 48 weeks[33]. One additional phase II trial with semaglutide and cilofexor–firsocostat (NCT04971785, 457 participants) has completed recruitment, but results have not been communicated ye[52]. Finally, MAESTRO-NAFLD1 was a phase III trial analyzing resmetirom 80 mg and 100 mg versus placebo in more than 1200 patients with MASLD, presumed MASH[53]. Interestingly, it included an open-label arm with 180 patients with compensated MASH cirrhosis. This trial provided the rationale for the ongoing MAESTRO-NASH-OUTCOMES trial[54] (NCT05500222), which has enrolled approximately 700 patients with compensated MASH cirrhosis in more than one hundred centres in North America and Europe.

In summary, the landscape of clinical trials in compensated MASH cirrhosis is characterized by an increasing number of ongoing trials and distinct pathophysiological features shaping the priority therapeutic targets. Rapid advances in basic science give us hope to identify new targets or even bring new insights into known targets.

[H2] Major challenges in MASH cirrhosis clinical trials

Amongst the current challenges of phase III trial endpoints and design in MASH-compensated cirrhosis, the baseline risk of clinical events and estimated effect size in MASH F4 phase III trial design stand out. Drug therapy approval in MASH-compensated cirrhosis requires a demonstration of its effect in preventing or delaying clinical outcomes[55]. Estimating baseline risk through observational cohorts[24,25,56,57] is reasonable, although intrinsic factors such as heterogeneity or liberal eligibility criteria, among others, might lead to potential overestimating of the risk of events. Moreover, the baseline risks from published randomized trials in MASH-compensated cirrhosis (for example, selonsertib, simtuzumab, emricasan) enable the estimation of a range of baseline risk of events of 3% (in early compensated cirrhosis) to 7% (in compensated cirrhosis with clinically significant portal hypertension (CSPH), that is, hepatic venous pressure gradient (HVPG) > 10 mmHg) per year, even with stringent selection criteria (Table 2). Establishing the effect size is critical when interpreting any intervention's benefit-to-risk ratio, yet this becomes difficult for complex clinical outcomes in non-communicable chronic metabolic diseases such as MASLD. Besides, there is a broad dynamic range of fibrosis within F4[17]. Thus, direct extrapolating effect size from histology (or other surrogates) to clinical outcomes in MASH F4 could lead to marked overestimation. Determining the effect size for clinical outcome trials, especially in the absence of standard of care, can be difficult to navigate. Thus, following current regulatory guidance and using realistic assumptions, designing and carrying out phase III pivotal trials in MASH F4 is challenging due to the large sample sizes required, well over a thousand patients (Table 2).

When analyzing the current challenges of phase IIb trial endpoints and design in MASH-compensated cirrhosis, histology has large and unmodifiable methodological limitations (see section 'Role of histology as an endpoint'). Using histologic features as primary endpoints for trials in cirrhosis is even more challenging than in non-cirrhotic MASH owing to the wide histological variation in cirrhotic stages, remarkably the lack of MASH-specific features such as severe steatosis or ballooning, in such advanced stages of liver disease. Moreover, biological limitations to histological fibrosis greatly affect the ability to power phase trials in MASH-compensated cirrhosis using 1-stage improvement in fibrosis (that is, cirrhosis regression) as the primary endpoint[33,55]. These two challenges with histology (methodological and biological) as the primary endpoint for a phase IIb trial seem largely unmodifiable and especially critical in cirrhosis, hindering the progress of potentially effective drugs to phase III trials.

Surrogate endpoint validation will require adequate phase III trials with sample sizes and study durations enough to enable liver (that is, cirrhosis decompensation and hepatocellular carcinoma) and non-liver (mostly cardiovascular, such as stroke or myocardial infarction) clinical outcomes to occur in both the treatment and placebo. Validated non-invasive tests (NITs) assessing liver fibrosis (for example, serum-based biomarkers or liver stiffness measured by elastography) represent the best alternative to cirrhosis regression by liver biopsy in phase IIb trials in MASH-compensated cirrhosis.

[H2] Regulatory authorities' position for MASH clinical development

EMA's draft reflection paper on regulatory requirements for developing medicinal products for chronic non-infectious liver diseases, including MASH, was published in November 2018 (EMA/CHMP/299976/2018)[58]. Intending to address and avoid potential pitfalls in drug development such high rates of trial failure either owing to discontinuation or negative results, the paper discusses vital considerations for developing drugs for MASH, including appropriate patient populations, using NITs to assess disease progression, and the need for well-designed clinical trials with relevant endpoints, tapping into aspects such as the use of liver biopsy and sample size calculation. An update on this reflection paper is currently being processed. Though not too many changes are anticipated, the inclusion of the cirrhotic population into clinical trials is not only desirable but necessary. This necessity is due to the high unmet medical need for therapies to treat MASH and its risk of disease progression, as well as to cover the full spectrum of the disease (treating and preventing cirrhosis) and, finally, to generate the necessary outcome data within an appropriate timeframe[11,25]. Including the cirrhotic population in the clinical trials design requires considering special features such as the current and past metabolic risk burden for the diagnosis to ensure MASLD aetiology and the presence of cirrhosis in case non-invasive inclusion criteria are intended[58]. Trial endpoints in compensated MASH cirrhosis depend on regulatory agencies' strategy (Table 3)[55,58]. Thus, cirrhosis regression remains a histological surrogate endpoint in the compensated cirrhosis population, whereas endpoints based on liver outcomes are like those previously postulated. The FDA guidance for trials in patients with NASH and compensated cirrhosis was published in June 2019[55]. It describes the FDA's current recommendations regarding the important components of a drug development program for compensated MASH cirrhosis, focusing on

the enrollment criteria, trial design, efficacy endpoints, and safety considerations for phase III trials. The FDA has suggested a complementary approach for MASH drug development, so MASH-compensated cirrhosis trials can support full-market approval for patients with MASH and fibrosis[59] (Table 3). Although the FDA seems open to accepting NITs for participants' identification and enrolment in clinical trials of compensated MASH cirrhosis, liver biopsy as a surrogate endpoint of clinical events remains current[55].

[H1] Implications of weight loss in clinical trials for MASH cirrhosis

[H2] Weight loss in MASH cirrhosis: classical approaches and novelties

Weight loss ameliorates adipose tissue dysfunction and improves diabetes, cardiovascular outcomes, and quality of life[27]. Additionally, weight loss can potentially resolve MASH and regress fibrosis—as demonstrated by histology and imaging (Figure 2). Yet, patients with cirrhosis are excluded from most studies evaluating weight-loss-inducing drugs for the treatment of MASH[60-63]. Moreover, changes in body weight and composition affect the risk of cirrhosis decompensation, with absolute losses of weight and adipose tissue preventing disease progression, whereas sarcopenia has been linked to an increased risk of decompensation. All these factors influence the natural history of MASH cirrhosis in patients before, during and after entering a clinical trial.

Regardless of the liver disease aetiology, overweight and obesity influence the progression towards decompensated cirrhosis. In a cohort of 173 patients with compensated cirrhosis (HVPG >5 mmHg but no varices on inclusion), overweight and obesity had a detrimental effect, increasing the risk of first clinical decompensation independent of portal pressure and

albumin[64]. The SportDiet study (NCT01409356, 60 participants)[65] determined that in patients with cirrhosis and overweight or obesity, overall weight loss obtained by an intensive lifestyle program (16 weeks) was safe and was associated with beneficial effects on portal pressure, cardio-metabolic health, and quality of life. Nonetheless, muscle mass reduction represents a potential risk in weight loss-inducing approaches, which needs particular attention in patients with cirrhosis, already prone to loss of muscle mass and sarcopenia[66]. The latter needs appropriate management and should be addressed through protein supplements and exercise[66]. In patients achieving weight loss through diet and exercise, several unanswered questions remain, such as the best type of diet and physical exercise and its efficacy [60].

Pharmacological and surgical approaches for inducing weight loss have largely affected the field of MASH drug development, including clinical trials on MASH cirrhosis with three main aspects to be highlighted: First, the use of both weight-loss inducing drugs and surgery are either relative or absolute exclusion criteria in most clinical trials[55,57]. Second, the bulk of evidence regarding weight loss-inducing drugs comes from clinical trials on obesity or type 2 diabetes, but compelling data from late-phase trials specifically designed for patients with MASH cirrhosis are limited (Table 1). However, such drugs as liraglutide or semaglutide are already used in routine clinical practice to treat patients who also have MASH cirrhosis, amongst other comorbidities[22]. Finally, no high-quality data from clinical trials on the efficacy and safety of bariatric surgery amongst patients with MASH has been available until currently[67]. The work carried out to answer these questions has shed light on important clinical management aspects for MASH in general, yet there remain several unsolved questions regarding patients with MASH and cirrhosis.

[H2] Weight-loss pharmacological treatments

GLP-1 receptor agonist therapy in MASLD with drugs such as semaglutide and liraglutide showed positive results in non-cirrhotic MASH[68,69]. However, a phase II trial (NCT03987451, 71 participants) on compensated MASH cirrhosis showed no benefit from using semaglutide 2.4 mg weekly in neither fibrosis improvement nor steatohepatitis resolution compared to placebo[33]. Tirzepatide is a dual glucose-dependent insulinotropic polypeptide and GLP-1 receptor agonist that promotes significant weight loss ($P<0.001$ compared to placebo in the pivotal trial including 2,539 participants, NCT04184622)[29]. Some evidence suggests that tirzepatide can benefit MASLD by reducing liver fat content, improving liver function tests, and potentially reducing liver inflammation[70]. However, prior studies did not assess histological changes, which data are expected to be provided by the phase II SYNERGY-NASH study (NCT 04166773, n=196)[71]. Research and development of GLP-1–glucagon co-agonists such as cotadutide[72,73] might represent another promising approach to weight loss-based MASH treatment and are under development. Other nutrient-stimulated peptides for obesity treatment currently in development are cagrilintide, pemvidutide, BI 456906 retatrutide, danuglipron and LY 3502970[74].

In brief, several drugs and bariatric and metabolic surgery techniques inducing weight loss are under current evaluation for treating MASH, but evidence amongst patients with compensated MASH cirrhosis is still preliminary and non-conclusive.

[H2] Bariatric or metabolic surgery and surgical risk

According to current publications, bariatric surgery and Roux-en-Y gastric bypass show comparable efficacy to lifestyle interventions in non-cirrhotic MASH in terms of liver improvement[29,34] despite conflicting data[75,76] and increased risk of alcohol use disorder[77,78]. Two systematic reviews concluded that bariatric surgery effectively improved MASH, specifically by reducing steatosis, histologic necroinflammatory activity, and liver fibrosis[79,80]. Indications for bariatric surgery currently align with general recommendations for metabolic syndrome[67]; however, endoscopic approaches might also be considered but need further evaluation, particularly in the setting of cirrhosis and portal hypertension. However, the role of bariatric surgery in the stage of MASH cirrhosis remains poorly defined.

After bariatric surgery, hepatic decompensation might occur in patients with or without previous cirrhosis[81], potentially caused by malnutrition, vitamin and/or nutrient deficiency or alcohol misuse[82]. Regarding the use of bariatric surgery in patients with cirrhosis, a systematic review from 2015[83] concluded that sleeve gastrectomy and adjustable gastric banding seem to be the safest bariatric surgical options for patients with Child-Pugh class A cirrhosis without portal hypertension. Another systematic review from 2018 highlighted that sleeve gastrectomy was the most frequent type of bariatric surgery in patients with cirrhosis and was even performed in patients with portal hypertension[84]. In a systematic review that included 467 patients with liver cirrhosis, Ahmed and colleagues concurred that sleeve gastrectomy seemed to be the safest technique, as compared to Roux-en-Y- gastrojejunostomy, gastric band, and biliopancreatic diversion[85]. However, the number of individuals with portal hypertension and Child-Pugh stage B cirrhosis was limited (that is, 11% and 3.2%, respectively)[85]. Notably, patients with obesity and with compensated cirrhosis are at low but not negligible risk for

perioperative mortality (<1%) following metabolic or bariatric surgery, but the expected benefits are substantial. Meanwhile, decompensation and mortality rates in decompensated cirrhosis seem to be substantially higher, yet good quality data is lacking in this regard[83-85]. For patients with decompensated MASH cirrhosis and a body mass index (BMI) >35 kg/m^2 who are candidates for liver transplantation, liver transplantation might be prioritized and performed prior to bariatric surgery[86].

Data on outcomes after bariatric surgery in patients with CSPH are scarce[67]. Thus, data from prospective studies are needed to define better the inclusion and exclusion criteria of bariatric surgery clinical trials in patients with MASH-compensated cirrhosis.

[H1] Endpoints in MASH-cirrhosis clinical trials

[H2] Role of histology as an endpoint

There is a clear divide between the trials designed from the MASH and the portal hypertension fields regarding various major criteria such as endpoints and treatment duration, with rare exceptions such as that of the MAESTRO-NASH OUTCOMES trial, which incorporated lessons from the cirrhosis and portal hypertension field for the design of the non-invasive and liver events-based substudy[54] (Table 1).

It is well established that there is an association between histologic disease activity and the progression of MASLD[87]. Conversely, cirrhosis regression has been proven to lead to improved clinical outcomestrials[88]. However, liver biopsy has several limitations, such as its invasive nature, sampling variability, and issues with both inter and intra-observer variability (for

example, the complexity of ballooned hepatocyte feature recognition) and placebo response[89-91]. Moreover, liver biopsy does not adequately capture the complicated relationship with the natural history of advanced chronic liver disease in MASH (for example, F3 and F4 sometimes overlap, no clear association with portal hypertension, massive variability within the F4 spectrum, different effects of inflammation)[91,92].

Other tools and histological features might be used to enhance the yield of liver biopsy for assessing liver tissue in samples from patients with cirrhosis. These have emerged mainly during the past five years and include digital pathology, zonal histology, spatial transcriptomics, organoids and artificial intelligence-based techniques such as neural networks, amongst others[92,93]. When MASH advances, the histological damage produced around the hepatic lobule and the portal space follows a metabolic gradient that is quite characteristic. These zonation-specific features have preliminarily been assessed as histologic endpoints in clinical trials and should be further exploited[94].

Moreover, 'non-typical' MASH histologic features could be incorporated into histological endpoints. For instance, the seladelpar trial (NCT03551522, 181 participants) assessed portal inflammation, portal plasma cells, interface hepatitis and biliary injury[95]. Lessons drawn from MASH studies relying on mechanotransduction mechanisms[96] and liquid biopsy[97] could also be applied to generate useful and measurable endpoints. Machine-learning techniques are already used in MASH clinical trials as a supporting tool for pathologist readings of liver biopsy samples[92], though there is still limited data on cirrhosis. Second harmonic generation microscopy techniques[98], qFibrosis–qFIBS[99], and other machine learning tools[100,101] have

proved promising to assess fibrosis and steatohepatitis. They could be potentially used for predicting portal hypertension and/or liver-related events based on histological findings.

In summary, although histological endpoints have several limitations, which seem even more evident in MASH cirrhosis, liver biopsy continues to be the gold standard for diagnosis and staging of MASH, as well as the primary endpoint in the vast majority of MASH clinical trials. Although new, non-invasive tools and clinical endpoints are developed or validated to complement or replace biopsy, emerging techniques such as digital pathology seem promising for enhancing the performance of histological endpoints.

[H2] Hepatic venous portal gradient as a surrogate endpoint

HVPG is the best predictor of decompensation and outcome in patients with compensated cirrhosis[102]. However, there is an overall lack of studies supporting this evidence amongst patients with MASH, with most data originating from retrospective studies[103-108]. Notably, a distinctive correlation between HVPG thresholds and risk of decompensation seems to exist in MASH compared to other aetiologies, with MASH showing a lower HVPG threshold for decompensation[109,110].

Belapectin did not meet either the primary endpoint of reduction in HVPG or the clinically significant secondary endpoint of complications of cirrhosis[49]. In another clinical trial (NCT01672879, 258 participants) with simtuzumab, a monoclonal antibody against lysyl oxidase-like 2, CSPH at baseline was associated with a nearly 3-fold risk of liver-related complications compared with patients with HVPG <10 mmHg[111,112]. In line with other

aetiologies, the risk of clinical events increased with higher baseline HVPG, so each 1-mmHg increase in HVPG above 10 mmHg was associated with an 11% increase in the risk of hepatic decompensation[112]. After 24 months, estimated event-free survival was 92% (95% confidence interval (CI) 83–96) in patients with HVPG <10 mmHg versus 75% (95% CI 68–81) in those with CSPH (hazard ratio 2.83; 95% CI 1.33–6.02; *P*=0.007). Moreover, in a follow-up study involving 342 patients with strictly compensated MASH cirrhosis, a baseline HVPG ≥10 mmHg was linked to a 1-year and 2-year decompensation rate of 2.2% and 10.7%, respectively[113]. A baseline HVPG ≥16mmHg was associated with 1-year and 2-year decompensation rates of 8.7% and 13.5% in patients with MASH[113].

Decompensations at HVPG levels below the threshold defining CSPH led to the idea that MASH might behave hemodynamically differently than other aetiologies. Records of 138 patients with non-malignant ascites who underwent HVPG measurement showed that the HVPG gradient was lower in those with MASH (15 mmHg) compared to those with alcohol-related liver disease cirrhosis (18.2 mmHg, *P*=0.01) or hepatitis C virus (HCV) infection (17.2 mmHg, *P*=0.13)[114]. Patients with MASH can decompensate at HVPG <10 mmHg, and for any given value of HVPG, decompensation rates seem higher in MASH than in HCV infection[115].

When analyzing whether HVPG accurately reflects direct portal pressure, a higher individual variability was observed in MASH-related cirrhosis versus alcohol and viral-related cirrhosis [116]. Although the correlation between wedge hepatic vein pressure and portal vein pressure is almost 100% reliable in alcohol and viral-related cirrhosis, it is less accurate in MASH-related cirrhosis, with underestimation being the most common discrepancy[116], suggesting that

prognosis thresholds can vary slightly in MASH. A small case series including 38 patients showed that patients with MASH achieving an HVPG reduction ≥10% under non-selective beta-blocker treatment were protected from variceal bleeding[117].

Although HVPG fulfils the criteria for being considered a surrogate endpoint, optimal HVPG cut-offs predicting clinical decompensation in MASH cirrhosis remain to be determined. Additionally, the specific target for HVPG reduction to effectively decrease decompensation remains to be defined.

[H2] Endpoints based on non-invasive tests

When considering the use of biomarkers in drug development, it is essential to consider the specific context of use and population or setting that is being addressed using the biomarkers, endpoints, and other tools of FDA lexicon[118]. As mentioned, histological evaluation of MASLD is challenging, but several key considerations when proposing a new surrogate endpoint be adopted to support drug development, from their biological plausibility to the relationship between the proposed surrogate endpoint and the clinical outcome of interest, and the probability of such clinical outcome[119].

There is already ample data demonstrating that NITs have a good diagnostic performance to detect advanced fibrosis and cirrhosis, individually or when applied in combination or sequentially[120-122]. Thus, a range of tractable biomarkers are available to support optimized patient selection for clinical trials, although data specifically in cirrhosis are more limited. Indirect fibrosis biomarkers include ‘simple’ biomarker panels (for example, MASLD fibrosis

score, FIB4, aspartate aminotransferase (AST)-to-platelet ratio index, and markers of inflammation)[123-126]. In contrast, direct serum markers assess the dynamics of matrix turnover—fibrogenesis and fibrinolysis (for example, ELF, plasma Pro-C3 test, FIBC3 diagnostic panel, and ADAPT score)[127-129]. Therefore, these latter biomarkers have high biological plausibility and fulfil the first key requirement, making them good candidates as surrogate endpoints in the pharmacodynamic and response biomarker context of use[130]. Data also show that NITs that detect fibrosis have prognostic utility for long-term outcomes. These NITs include simple scores and direct collagen biomarkers like ELF, as well as elastography techniques such as vibration-controlled transient elastography or magnetic resonance elastography (MRE)[131-133]. Data showing that changes in NITs reflect changes in the probability of a clinical outcome are, however, more limited. This limitation is partly owing to a lack of consistency in biomarker measurement and reporting in clinical trials to date. Demonstrating sensitivity to change, serial FIB4 measurements in population cohorts have been shown to facilitate the monitoring of disease progression and regression[133,134]. Furthermore, post hoc analysis of the STELLAR trials (Table 1) showed that histological regression from cirrhosis is associated with improved outcomes correlated with biomarker changes, including ELF and VCTE[112,119]. Complementing these data, a single-centre study published in 2023 found that serial MRE measurements accurately detect disease progression[135].

There remains a need for more sensitive and specific, independently validated, and qualified biomarkers for use in MASH drug development, both in pre-cirrhotic and cirrhotic diseases. Although randomized clinical trials have generated important data to support biomarker

utility, there remains a pressing need for more research in the pharmacodynamic and response context of use.

[H2] Clinical endpoints

MASH natural course is slower than other aetiologies of liver diseases, such as alcohol-related liver disease and hepatitis C[24,25-136]. Although ascites, bleeding due to portal hypertension, encephalopathy, liver transplantation or Model for End-stage Liver Disease (MELD) >15, and all-cause mortality are appropriate clinical event definitions, serum levels of carnitine palmitoyl transferase increase by >2 is less clear[137]. New varices, their growth or progression to hepatocellular carcinoma might not constitute events suitable for a clinical endpoint [138]. In the case of alcohol-related liver disease, Rasmussen and colleagues proposed to use biomarker assessment at inclusion and retain only individuals fulfilling certain biomarker thresholds in such a way that with fewer participants and shorter trials, there were sufficient events[139]. Thus, predefined thresholds of various biomarkers can be used to select patients at higher risk of liver-related events and, therefore, enrich trial populations.

The European Union Innovative Medicines Initiative 2-funded LITMUS consortium had several interactions with regulatory agencies in the United States and the European Union regarding biomarker qualification in patients with MASLD and MASH[140]. When designing a clinical trial in MASH cirrhosis, the included population could consist of patients with MASH cirrhosis plus a feature of increased progression risks such as liver stiffness measurements by VCTE or ELF thresholds, varices, Child-Pugh 6 or HVPG ('enriched' population). An approximate annual

event rate of 8% is needed, but more liberal use of non-specific beta blockers following current Baveno consensus recommendations[141] might affect this, as more patients would potentially be receiving a drug that reduces the incidence of liver events, therefore requiring a sample size of around 700 patients for three years.

Investigating a drug's efficacy in only biomarker-positive patients accelerates drug development in personalized medicine. A large multinational trial with inclusion criteria that are not too restrictive is recommended. It should preferably be conducted in patients without decompensated cirrhosis yet in whom aetiological treatment might not arrive on time (Figure 3).

[H1] Alternatives in design for MASH cirrhosis clinical trials

[H2] Prognostic models for MASH cirrhosis

Prognostic models can be helpful in designing clinical trials, identifying the specific target population for the therapy under investigation, estimating the potential effect of the therapy, and helping in calculating the sample size for the clinical trial.

Agile 3+ was first designed to diagnose advanced fibrosis using liver stiffness measurements (LSM), platelet count, AST serum levels, alanine aminotransferase (ALT) serum levels, type 2 diabetes, biological sex and age. In a study conducted on 614 patients with biopsy-proven MASH (n=243 with F3-F4 fibrosis stage), the Agile 3+ score had a higher accuracy than LSM alone for predicting clinical events (being patients with Agile 3+ >0.68 those with the highest risk)[142]. On the contrary, data from randomized controlled trials on selonsertib and simtuzumab (Table 1) showed that LSM had similar performance for predicting liver-related

events in patients with F3-F4 MASLD fibrosis, compared to the model Agile 4[143]. ABIDE model includes the variables AST-to-ALT serum levels ratio, bilirubin serum levels, international normalized ratio, type 2 diabetes and the presence of oesophageal varices[144]. It showed good accuracy for predicting liver-related events in a cohort of 299 patients with biopsy-proven MASH with compensated cirrhosis; patients with a score $\geq$ 4.1 had the highest risk of decompensation. This model was first validated in an external cohort, including 244 patients with biopsy-proven MASH and cirrhosis[144]. As most of these models have been constructed on a subset of patients selected by liver biopsy, we need data on patients selected by NITs (patients with compensated advanced chronic liver disease- cACLD) covering the full spectrum of the disease[141]. Moreover, these models mainly focus on surrogate markers of fibrosis. Therefore, portal hypertension, a primary determinant of hepatic decompensation, is only partially addressed.

In 2022, the Baveno VII consensus incorporated the ANTICIPATE and ANTICIPATE-NASH models in their guidelines[141,145,146]. These two risk prediction models estimate the risk of presenting CSPH[141]. The former model was derived from a cohort of 518 patients with cACLD (mainly patients with HCV infection, 70%, whereas patients with MASLD accounted for 7%), and it was developed using LSM and platelet count[145]. In a later cohort validation study, it was found that BMI influenced the prediction of CSPH in patients with MASLD[146]. So, the model was reformulated for patients with MASLD adopting BMI in addition to platelet count and LSM (ANTICIPATE-NASH model). To test if the ANTICIPATE-NASH model could predict the risk of liver-related events, a multicentric retrospective cohort study with LSM including patients with MASLD (836 patients in total, 358 with hepatitis C, 248 with MASLD, 203 with alcohol-

related liver disease, and 27 with hepatitis B) was conducted[146]. After three years of follow-up, the number of liver-related events in patients with LSM <10 kilopascal (kPa) was very low, whereas the risk started to increase in those with LSM ≥10 kPa, and it was notable in patients with LSM >25 kPa, which was chosen as the best threshold to rule in CSPH, with a positive predicted value over 90% for all aetiologies except for patients with MASLD and with obesity[146]. The ANTICIPATE-NASH-LRE model was developed in a derivation cohort of 2638 patients with MASLD and validated using an external international cohort of 679 patients[147]. This model can easily select patients with MASLD at risk of LRE, showing good discrimination for predicting clinical events in the derivation and validation cohorts regardless of the presence of obesity.

In summary, accurate and validated prognostic models could help to select those patients at higher risk of presenting liver-related events, reducing sample size, time length and finally, the costs of clinical trials.

[H2] The role of ordinal outcomes

Fixed dichotomous analysis of categorical outcomes (for example, the modified Rankin Scale after stroke) is not a statistically efficient approach and usually requires a larger sample size to demonstrate efficacy[148]. Preferred approaches include ordinal outcomes (that is, classify patients following a pre-specified hierarchy) to avoid missing beneficial treatment effects[148]. The treatment effect should be modelled to enable testing a specific hypothesis, establishing the basis of predictions of the effects of the new treatment (that is, according to baseline risk), and enabling adjustment by baseline covariates (increasing efficiency).

In MASH, using an ordinal outcome to classify patients not reaching the decompensation event at the end of the trial could make phase III trials much more efficient, reducing their duration and sample size. Varices constitute the anatomical substrate for variceal bleeding, and their presence changes the management of patients with cirrhosis (beta-blocker therapy, banding, or scheduling follow-up endoscopies). It is also a biomarker of risk of decompensation that is, in addition, responsive to improvement in portal hypertension as varices might decrease in size or disappear with a decrease in portal pressure[149-151]. In fact, it has been considered a clinically relevant outcome for many years, as shown by the National Institute of Health-funded Timolol trial (NCT00006398, 213 participants), in which the primary endpoint was the development of varices in compensated cirrhosis[152]. However, it should be taken into account that the assessment of the presence and size of varices is limited by interobserver variability[151].

The use of varices in an ordinal outcome, rather than as part of a composite, does not interfere with assessing the effects of the new treatment on the hierarchically more relevant endpoint (decompensation). Designing a clinical trial with an ordinal scale that includes death, decompensation and, in those not reaching these endpoints, the assessment of varices presence and size could decrease the needed sample size by 3–4-fold (as compared to a dichotomous endpoint including only decompensation and death). Figure 4 exemplifies this using a hypothetical scenario with data from published studies.

Therefore, ordinal outcomes are a promising, although underutilized, approach to enhance the design of clinical trials in cirrhosis in general and MASH-compensated cirrhosis in particular.

[H1] Future directions in MASH-compensated cirrhosis clinical trials

The main challenges and needs that need tackling to move forward in the field of drug development in MASH-compensated cirrhosis are summarised in Table 4.

Relying on the lessons drawn from the cardiovascular disease field, we learned that clinical outcome trials are likely to remain important[153]. Thus, reliable clinical outcomes assessment might not be that different for patients with MASH cirrhosis to those analyzed in patients enrolled in large clinical trials with high-risk coronary heart disease[154]. Moreover, the way cardiometabolic outcomes are approached could be improved in future clinical trials in MASH cirrhosis, potentially leveraging design tools such as hierarchical or ordinal outcomes as well as adaptive designs, for example, platform and umbrella trials[155,156].

In trials enrolling patients with MASH and F3 fibrosis stage, there is likely to be a considerable number of participants with early F4 stage[157]. The selection of patients to enter clinical trials with MASH and compensated cirrhosis must recognize that liver biopsy is an imperfect tool and that sampling variability will inevitably misclassify patients who have cirrhosis as not having cirrhosis and vice versa. Trial designs should recognize this and use cACLD criteria for enrollment. In the field of MASH cirrhosis, the definition of cirrhosis regression as contemplated, for instance, by the EMA[58] (Table 3), is particularly problematic. The baseline NIT values were highly associated with the regression probability, suggesting that at least some apparent regression is simply sampling variability[153]. Moreover, the histological changes associated with apparent regression are predictable and could also result from sampling variability, whereas the longitudinal NIT changes were more modest as predictors[153]. Building

a consensus on NIT inclusion as study endpoints is paramount: a defined minimum set of biomarkers to be measured and reported in all future clinical trials. Accepting that a single ideal biomarker does not exist should form part of a global assessment of biomarker response to demonstrate consistent changes in multiple biomarkers across study arms and at the individual patient level.

The selection of appropriate clinical endpoints for phase III trials is critical. Appropriate major clinical outcomes are variceal bleeding, ascites or hepatic encephalopathy requiring treatment, liver transplant, or all-cause death. The incorporation of the development of oesophageal varices into this endpoint is debatable. MELD score >15 should be used as a major surrogate endpoint. There are caveats to the use of hepatocellular carcinoma as it is not traditionally considered a decompensating liver event, might occur in patients with MASLD but without cirrhosis, and requires long-duration trials.

As clinical trials on MASH-decompensated cirrhosis start to be set up, there are issues regarding participants and lessons learned from both MASH in general and compensated cirrhosis in particular. For instance, whether participants in clinical trials on MASH-compensated cirrhosis might be eligible for trials on decompensated disease after a washout period. Another area that requires further elucidation is to what extent an aetiological treatment might change disease trajectory in decompensated disease where, in contrast to viral hepatitis and alcohol, not all disease mechanisms will be potentially targeted, even with combination therapy.

To accelerate drug development in cirrhosis, enrichment strategies must be developed using prognostic models to identify patients who stand to gain the most from treatment. Recognizing that there is continued development of models to predict decompensation identifies one approach to enriching the population to be enrolled in trials. Importantly, however, it is unclear whether therapies primarily targeting liver injury and fibrosis will be efficacious at the very late stages of compensated cirrhosis, in which the absolute risk of decompensation is greatest.

Additionally, prospectively validating proposed surrogate outcomes in phase III randomised trials is critical to establish those within the regulatory framework. In phase II liver disease clinical trials, we have many unvalidated surrogate endpoints, including histology, imaging or blood-based biomarkers, making decisions regarding which drug candidates to progress to phase III challenging[158]. The optimal selection of surrogate endpoints remains to be defined but seems likely to include both blood-based and imaging NITs (such as MRE)[159].

Future clinical trials in MASH, in general, and in cirrhosis, in particular, should be able to tackle the underreporting of alcohol consumption. Mallet and colleagues found that alcohol intake is a major attributable risk factor for liver disease progression in patients with type 2 diabetes[160]. Using ethylglucuronide analysis in hair and urine, Staufer and colleagues detected repeated moderate to excessive alcohol consumption in 28.6% of 184 patients with presumed MASLD[161]. Systematic screening of excessive alcohol consumption should be, therefore, a priority prior to recruitment and once patients are randomly assigned, as the effect of alcohol

consumption in the risk of decompensation makes patients with compensated cirrhosis a particularly susceptible population, therefore, largely affecting trial outcomes.

Finally, delivering large clinical trials, including patients with MASH cirrhosis, is challenging. Considering efficient trial designs, underpinned by non-invasive and risk-based enrollment criteria, is likely to yield a step change in recruitment. Leveraging electronic healthcare records to define clinical progression in MASH cirrhosis from clinical data is a further mechanism through which large clinical effectiveness trials and cost-effectiveness analyses can be facilitated[162].

[H1] Conclusions

The landscape of MASH clinical trials is complex and might be overwhelming, and the progressive approval of drugs with partial efficacy with add more variables to the equation. In the particular case of clinical trials for patients with MASH cirrhosis, even more difficulties will be faced. Some drugs might not be adequate in the cirrhotic phase, and new optimized designs for clinical endpoints will have to be developed to deliver results in reasonable timeframes and with contained costs.

[H1] Data availability

The calculations for the illustrative scenarios shown in Table 2 and Figure 4 are based on published data (references 24,25,111,113,171 and 172, 173, respectively). The excel sheets with the data used, and the calculations will be provided upon request.

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Author contributions

All authors researched data for the article. J.M.P., Q.M.A, S.A., R.B., A.B., S.M.F., J.G.A., V.H-G., M.P., T.R., I.A.R., F.T., and J.G. contributed substantially to discussion of the content. J.M.P., Q.M.A., S.A., R.B., A.B., S.M.F., J.G.A., V.H-G., A.A.R., F.T., and J.G. wrote the article. All authors reviewed and/or edited the manuscript before submission.

Competing interests

JMP reports having received consulting fees from Boehringer Ingelheim and Novo Nordisk. He has received speaking fees from Gilead, and travel expenses from Gilead, Rubió, Pfizer, Astellas, MSD, CUBICIN, and Novo Nordisk. He has received educational and research support from Gilead, Pfizer, Astellas, Accelerate, Novartis, Abbvie, ViiV, and MSD. QMA is coordinator of the EU IMI-2 LITMUS consortium, which is funded by the EU Horizon 2020 programme and EFPIA. This multistakeholder consortium includes industry partners. He has received research grant funding from AstraZeneca, Boehringer Ingelheim, and Intercept Pharmaceuticals, Inc.; has served as a consultant on behalf of Newcastle University for Alimentiv, Akero, AstraZeneca, Axcella, 89bio, Boehringer Ingelheim, Bristol Myers Squibb, Galmed, Genfit, Genentech, Gilead, GSK, Hanmi, HistoIndex, Intercept Pharmaceuticals, Inc., Inventiva, Ionis, IQVIA, Janssen, Madrigal, Medpace, Merck, NGM Bio, Novartis, Novo Nordisk, PathAI, Pfizer, Poxel, Resolution Therapeutics, Roche, Ridgeline Therapeutics, RTI, Shionogi, and Terns. He has served as a speaker for Fishawack, Integritas Communications, Kenes, Novo Nordisk, Madrigal, Medscape, and Springer Healthcare; and receives royalties from Elsevier Ltd. SA works for Boehringer-Ingelheim. SF has acted as consultant for Abbvie, Actelion, Aelin Therapeutics, AgomAb, Aligos Therapeutics, Allergan, Alnylam, Astellas, Astra Zeneca, Bayer, Boehringer Ingelheim, Bristol-Meyers Squibb, CSL Behring, Coherus, Echosens, Dr. Falk Pharma, Eisai, Enyo, Galapagos, Galmed, Genetech, Genfit, Genflow Biosciences, Gilead Sciences, Intercept, Inventiva, Janssens Pharmaceutica, PROMED. Praha, Julius Clinical, Madrigal, Medimmune, Merck Sharp & Dome, Mursla Bio, NGM Bio, Novartis, Novo Nordisk, Promethera, Roche, Siemens Healthineers. He has been a lecturer for Abbvie, Allergan, Bayer, Eisai, Genfit, Gilead Sciences, Janssens Cilag, Intercept, Inventiva, Merck Sharp & Dome, Novo Nordisk, Promethera and Siemens. His institution has received grants from Astellas, Falk Pharma, Genfit, Gilead Sciences, GlympsBio, Janssens Pharmaceutica, Inventiva, Merck Sharp & Dome, Pfizer, Roche. PR works for Madrigal Pharmaceuticals. ES contributions do not represent the official position of the BfArM or EMA/CHMP. IR has received honoraria for consulting from Boehringer Ingelheim and Roche, fees for educational material from Norgine, and fees for adjudication committees for AstraZeneca and Novo Nordisk. FT's lab has received research funding from Allergan, Bristol-Myers Squibb, Gilead and Inventiva. He has received honoraria for consulting or lectures from Astra Zeneca, Gilead, AbbVie, Boehringer, Madrigal, Intercept, Falk, Inventiva, Merz, Pfizer, Alnylam, NGM, CSL Behring, Novo Nordisk, Novartis. JG has received consulting fees from Boehringer Ingelheim, speaking fees from Echosens and travel expenses from Gilead and Abbie. JMP received funds from European Commission/EFPIA IMI2 853966-2, IMI2 777377, H2020 847989, HLTH-101136299, ISCIII PI19/01898 and PI22/01770, Barcelona City Council-"La Caixa" Foundation 22S07286-001 and SR20-00386, and Next Generation EU-IBEC Q6922. QMA is an NIHR Senior Investigator supported by the Newcastle NIHR Biomedical Research Center and the Liver Investigation: Testing Marker Utility in Steatohepatitis (LITMUS) project, which received funding from the Innovative Medicines Initiative 2 Joint Undertaking, under grant agreement No. 777377. This Joint Undertaking receives support from the European Union's Horizon 2020 research and innovation program and EFPIA. SF holds a senior clinical investigator fellowship from the Research Foundation Flanders (FWO) (1802154N). FT is supported by the German Research Foundation (DFG Ta434/8-1, SFB/TRR 296 and CRC1382, Project-ID 403224013). JG was partially funded by grant PI21/00691 from Instituto de Salud Carlos III and co-funded by the European Union (ERDF/ESF, "Investing in your future"). CIBERehd is supported by Instituto de Salud Carlos III.

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Key points

- MASH-compensated cirrhosis is a broad entity encompassing persons with differing drivers of disease and differing risks of clinical outcomes.
- Identification of persons at risk of clinical outcomes using existing non-invasive tests (NITs) and prognostic models will enrich clinical trial samples.
- A combination of NITs evaluating fibrosis and measures of portal pressure might be used to guide drug development in phase II clinical trials.
- A core outcome set of NITs and clinical outcomes needs to be developed for implementation and reporting in all clinical trials.

Table 1. Phase IIb and III clinical trials in patients with MASH compensated cirrhosis.

Trial	Drug(s)	Population	Duration	Primary endpoint(s)	Secondary endpoint(s)
Completed					
ALPINE 4 Phase IIb NCT04210245 [52]	Aldafermin	F4 (CRN NASH Score) N=160	48w	Change in ELF at week 48	Fibrosis, improvement in ALT, AST, Pro-C3 and liver stiffness
GS-6624 Phase IIb NCT01672879 [111]	Simtuzumab	Ishak fibrosis stage ≥ 5 (MASH[a] or cryptogenic cirrhosis) N=258	96w	Change in HVPG at week 96	LRE
STELLAR-4 Phase III NCT03053063 [48,163]	Selonsertib	F4 (CRN NASH Score) N=877	48w	≥1-stage improvement in fibrosis without worsening of MASH[a]	LRE; NASH resolution without worsening of fibrosis
IDN-6556-14 Phase IIb NCT02960204 [164,165]	Emricasan	Compensated and decompensated (1 event, 25%) cirrhosis, HVPG≥12mm Hg N=263	48w	Change in HVPG at week 24	Biomarkers (aminotransferases, caspases, cytokeratins) and LRE
REVERSE Phase III NCT03439254 [47]	Obethicolic acid	F4 (CRN NASH score) N=919	72w	≥1-stage improvement in fibrosis without worsening of MASH[a]	Fibrosis (Ishak), MASH resolution
NN9931-4492 Phase IIb NCT03987451 [33]	Semaglutide	F4 (CRN NASH score) N=71	48w	≥1-stage improvement in fibrosis without	MRI-PDFF, MRE, MASH resolution, etc

				worsening of MASH[a]	
Atlas Phase IIb NCT03449446 [166]	Selonsertib + cilofexor + firsocorstat	F3 & F4 (CRN NASH score) N=172 (F3) & 220 (F4)	48w	≥1-stage improvement in fibrosis without worsening of MASH[a]	MASH resolution and biomarkers
Falcon-2 Phase IIb *NCT03486912* [167]	Pegbelfermin	F4 (CRN NASH score) N=155	48w	≥1-stage improvement in fibrosis without worsening of MASH[a]	MRI-PDFF
Ongoing					
MAESTRO-NASH-OUTCOMES Phase III NCT05500222 [54]	Resmetirom	Liver Forum consensus for CT within 5 years and current cirrhosis clinical–imaging N=700	144w	Any event of all-cause mortality, liver transplant, ascites, hepatic encephalopathy, gastroesophageal variceal haemorrhage, and increase of MELD from <12 to ≥15 due to liver disease	Lowering of LDL-C, apolipoprotein B, and triglyceride lowering and reduction of liver fat as determined by MRI-PDFF
BMS-986263 Phase IIb NCT04267393 [168]	BMS-986263	F4 (CRN NASH score) N=270	12w	≥1-stage improvement in fibrosis without worsening of MASH[a]	5 histologic (CRN, Ishak, CPA), AEs
GS-US-454-6075	Firsocostat–	F4 (CRN NASH score)	72w	≥1-stage improvemen	MASH resolution

Phase IIb NCT04971785 [52]	Cilofexor + Semaglutide	N=457		t in fibrosis without worsening of MASH[a]	
BI 1366-0029 Phase IIb NCT05282121 [169]	BI 685509 +/- empaglifozin	CSPH (oesophageal varices + HVPG≥10mm Hg) N=80	8w	Change in HVPG	LRE, AEs
Symmetry Phase IIb NCT05039450 [170]	Efruxifermin	F4 (CRN NASH score) N=200	36w	Fibrosis improvement without worsening of MASH[a]	3 histologic + 1 NITs + anthropometric measures+ tolerability
NAVIGATE Phase IIb–III NCT04365868 [49]	Belapectin	MASH[a] CSPH N=357	78w	New oesophageal varices	New oesophageal varices requiring treatment, bleeding varices requiring hospitalization

[a]NASH in the original study

AE, adverse event; ALT, alanine aminotransferase; AST, aspartate aminotransferase; CPA, Collagen proportionate area; CNR NASH score, Clinical Research Network NASH score; CSPH, clinically significant portal hypertension; ELF, Enhanced liver fibrosis score; HVPG, Hepatic venous pressure gradient; LDL-C, low-density lipoprotein cholesterol; LRE, liver-related event; MELD, model for end-stage liver disease; MRE, magnetic resonance elastography; MRI-PDFF, magnetic resonance imaging-proton density fat fraction; NASH, nonalcoholic steatohepatitis; NIT, non-invasive test.

Table 2. Different approaches in pivotal clinical trials in MASH cirrhosis (F4) under current regulatory guidance and using realistic assumptions.

Baseline risk of liver events	HR	N	Follow-up (years)
3%	0.4	700-800	3
	0.7	3750-5250	4-5
	0.8	5000-5500	8
5%	0.5	700-800	3
	0.7	2250-2750	4-5
	0.8	5700	5
7%	0.55	700-800	3
	0.7	1600-2000	4-5
	0.8	4000-5000	4-5

The calculations are based on arbitrary decision rules to show the large variability of clinical trial design scenarios and required sample sizes, in particular, depending on the estimated risk of liver events. Sample sizes were calculated assuming alpha error 0.05, power 90% and an arbitrary range of feasibility scenarios (recruitment rates 0.15-0.35 patients per month per site, 300-600 sites and various scenarios of risk for liver events) based on refs 24,25,111,113,171.

HR: hazard ratio

Table 3. Current Food and Drugs Administration and European Medicine Agency guidance on endpoints for MASH in patients with cirrhosis.

<table>
<tr><th>Regulatory authority</th><th colspan="2">EMA</th><th>FDA</th></tr>
<tr><td>Guidance</td><td colspan="2">Draft Reflection paper on chronic non-infection liver disease
EMA/CHMP/299976/2018</td><td>Guidance for Trials in Patients with NASH and Compensated Cirrhosis</td></tr>
<tr><td rowspan="2"></td><td>Compensated MASH[a] cirrhosis</td><td>Trial endpoints according to CMA development strategy</td><td rowspan="2">Randomized, placebo-controlled, double-blind clinical trials</td></tr>
<tr><td>Interim analysis</td><td>Primarily ‘full approval’</td></tr>
<tr><td>Endpoints</td><td>Regression of cirrhosis (histology)</td><td>Composite endpoint</td><td>Composite endpoint</td></tr>
<tr><td>Description</td><td>≥ 1 stage improvement in liver fibrosis, no decompensation event, no increase in MELD, no deterioration or re-occurrence of features of MASH[a] activity</td><td>Decompensation events (variceal bleeding, hepatic encephalopathy, ascites), MELD score ≥ 15, liver transplantation, all-cause death</td><td>Complications of ascites, variceal haemorrhage, hepatic encephalopathy, MELD score ≥ 15, Liver transplantation, all-cause death</td></tr>
<tr><td>Challenges</td><td colspan="2">MELD-score: upper limit for inclusion needed when included in composite; needs justification and careful evaluation since influenced by diseases and medication; consider MELD-Na, MELD-3.

HCC: might not be sensitive to change, not recommended as part of the composite.

Oesophageal varices: without signs of high risk for bleeding not adequate surrogate; presence, evaluation of diameter and stigma features not recommended as part of the primary composite.

HVPG: not recommended due to invasiveness; potential use in early development.</td><td>Histological improvements in fibrosis can be proposed and justified; however, as a surrogate endpoint is insufficient to support accelerated approval so drugs are evaluated under the traditional approval pathway.</td></tr>
</table>

[a]NASH in the original study

CMA, conditional marketing authorisation; EMA, European Medicines Agency; FDA, Food and Drug Administration; HCC, hepatocellular carcinoma*;* HVPG, hepatic venous pressure gradient; MELD, model for end-stage liver disease; NASH, nonalcoholic steatohepatitis

Table 4. Main challenges and needs in compensated MASH cirrhosis clinical trials

Trial design challenges	Clinical challenges	Needs
Defining the patient population	Compensated cirrhosis is a histological diagnosis Contemporary clinical practice uses NITs to define the presence of cACLD rather than cirrhosis cACLD encompasses a range of disease phenotypes with differing degrees of risks of clinical outcomes	Acceptance of NITs for identification of cACLD Understanding that NITs predict risk of clinical outcomes Validated NIT-based prediction models to select persons for trial entry
Participant numbers	MASH cACLD is increasingly common Large trials, whereas commonly delivered in cardiovascular disease, are considered not to be feasible in MASH	Efficient trial designs incorporating participant selection using NITs and electronic data capture
Identifying appropriate therapies	Persons at different stages within cACLD might respond differently to different therapies Those with MASH cirrhosis and who are at high risk of decompensation might not benefit from aetiological treatment	Understanding of disease drivers across the stages of MASH cACLD Development of therapies that meaningfully affect disease drivers in all stages
Characterising treatment response – phase II	Measures that predict clinical outcomes are recognised, e.g. liver fibrosis, HVPG Liver histology is imperfect	Definition of a core outcome set of NITs to be captured and reported in all trials Availability of NITs in all areas

	NITs have good performance in cross-sectional analysis Little data in MASH cirrhosis to confirm dynamic changes in NITs predict clinical outcomes	Validation of response biomarkers
Characterising treatment response – phase III	Clinical outcomes – decompensation and the development of HCC – are increased in persons with MASH cACLD Other clinical outcomes, such as cardiovascular events and extrahepatic cancers, are also common Surrogate outcomes of decompensation are not validated	Definition of a core liver clinical outcome set Methods to understand the effect of non-liver outcomes in persons with MASH cACLD Efficacious therapies to enable validation of surrogate outcomes

cACLD, compensated advanced chronic liver disease; HCC, hepatocellular carcinoma*;* HVPG, hepatic venous pressure gradient; MASH, metabolic dysfunction-associated steatohepatitis; NITs, non-invasive tests.

Fig. 1. Main stages and pathophysiological mechanisms in the natural history of MASLD. Although the prevalence of simple steatosis in the general population is high, only a proportion of individuals will progress to more advanced stages of the disease. Liver fibrosis is the main driver of disease progression and prognosis, but to reach advanced stages of fibrosis, steatohepatitis must be present. However, steatosis and steatohepatitis might be absent or largely reduced in patients with MASH cirrhosis owing to the replacement of fat and inflammation by scarring. Note that regression is also possible at each stage of the disease, although less so at advanced stages.

The estimated numbers of people affected by at any stage of MASLD were calculated using as denominator the approximated current European Union population (448 million) and the estimated prevalence of MASLD and MASH, cirrhosis and HCC were taken from references 7-

9. HCC, hepatocellular carcinoma; M, millions; MASLD, metabolic dysfunction-associated steatotic liver disease; MASH, metabolic dysfunction-associated steatohepatitis

Fig. 2. Effect of metabolic drivers on different levels of MASLD natural history. a. MASH features evolution. Metabolic drivers and MASH histological features are less relevant as the disease progresses from low fibrosis (F0-1-2) to severe fibrosis (F3-4). **b.** Imbalance between damaging and repairing liver mechanisms in MASH.

cACLD, compensated advanced chronic liver disease; CSPH, clinically significant portal hypertension; dACLD, decompensated advanced chronic liver disease; MASLD, metabolic dysfunction-associated steatotic liver disease; MASH, metabolic dysfunction-associated steatohepatitis; PH, portal hypertension

Fig. 3. Summary of the pathophysiology, natural history and treatment modalities of MASH cirrhosis. As the disease progresses, mainly through an increase in liver fibrosis, there is also an increase in portal hypertension, which in the case of MASH does not only correlate with fibrosis but with other mechanisms (for example, specific mechanotransduction and steatosis-related pathways). The more advanced the disease, the less efficacious the measures aimed to control or remove the aetiology of liver disease (the components of metabolic syndrome in the case of MASH) and the more relevant those measures aimed at either preventing or mitigating the complications, particularly once the cirrhosis decompensates

CSPH, clinically significant portal hypertension; F4, liver fibrosis grade 4; HVPG, hepatic venous pressure gradient; MASLD, metabolic dysfunction-associated steatotic liver disease; MASH, metabolic dysfunction-associated steatohepatitis

Fig. 4. Oesophageal varices as ordinal outcomes in patients with cirrhosis. Example of the use of oesophageal varices in an ordinal outcome to assess the efficacy of an intervention for compensated cirrhosis. If only decompensation is considered an event, any patient not reaching decompensation is considered a ‘success’. Owing to the low number of events, trials would need a large sample size. However, these patients could be further classified by an endoscopy at the end of the trial as having large varices, small varices and no varices. Assuming

that the effect on the most severe outcome (decompensation) would be consistent with an effect on the less severe outcome (varices), the use of this ordinal outcome could markedly increase the power of the randomized trial[172]. The provided frequencies and sample sizes are illustrative. The percentages in each category in the placebo group are based on the expected distribution of patients with decompensation, large, small and no varices in the cohort study by Pennisi and colleagues[173], by factoring in the rates of progression at two years of patients with no varices, small varices and large varices. The expected distribution in the treatment group for a pooled OR of 0.65 and the approximated sample sizes using an ordinal outcome for the OR: 0.65 and OR: 0.80 effect size were calculated with the R hmisc package[174]. HR, hazard ratio; OR, odds ratio

ToC blurb

Metabolic dysfunction-associated steatohepatitis (MASH), a primary cause of chronic liver disease (CLD), often leads to advanced CLD stages such as cirrhosis. This Roadmap summarizes the current landscape and challenges of MASH-cirrhosis clinical trials and explores a way forward for future studies.