Steatotic Liver Disease: Pathophysiology and Emerging Pharmacotherapies

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**Abbreviations List**

- **AASLD** American Association for the Study of Liver Diseases
- **ALT** alanine aminotransferase
- **AMPK** adenosine monophosphate-activated protein kinase
- **APRI** aspartate aminotransferase to platelet ratio index
- **ASK1** apoptosis signal-regulating kinase 1
<table>
<thead>
<tr>
<th>Acronym</th>
<th>Definition</th>
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<tr>
<td>AST</td>
<td>aspartate aminotransferase</td>
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<tr>
<td>AT</td>
<td>adipose tissue</td>
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<tr>
<td>AUC</td>
<td>area under the receiver operating characteristic curve</td>
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<tr>
<td>BW</td>
<td>body weight</td>
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<td>CAP</td>
<td>controlled attenuation parameter</td>
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<tr>
<td>CI</td>
<td>confidence interval</td>
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<tr>
<td>CKD</td>
<td>chronic kidney disease</td>
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<tr>
<td>COVID-19</td>
<td>coronavirus disease 2019</td>
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<tr>
<td>CV</td>
<td>cardiovascular</td>
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<tr>
<td>CVD</td>
<td>cardiovascular diseases</td>
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<tr>
<td>DNL</td>
<td>de novo lipogenesis</td>
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<tr>
<td>EMA</td>
<td>European Medicines Agency</td>
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<tr>
<td>ENPP1</td>
<td>ectonucleotide pyrophosphatase/phosphodiesterase-1</td>
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<tr>
<td>FDA</td>
<td>Food and Drug Administration</td>
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<tr>
<td>FFA</td>
<td>free fatty acid</td>
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<tr>
<td>FGF</td>
<td>fibroblast growth factor</td>
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<tr>
<td>FIB-4</td>
<td>Fibrosis-4</td>
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<tr>
<td>FXR</td>
<td>farnesoid X receptor</td>
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<tr>
<td>GCKR</td>
<td>glucokinase regulator</td>
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<tr>
<td>GGT</td>
<td>glutamyl transferase</td>
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<tr>
<td>GIP</td>
<td>gastric inhibitory peptide</td>
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GLP-1  glucagon-like peptide-1
HbA1c  glycated hemoglobin
HCC  hepatocellular carcinoma
HOMA-IR  Homeostatic Model Assessment for Insulin Resistance
HR  hazard ratio
HSD17B13  Hydroxysteroid dehydrogenase type 13
HVPG  Hepatic Venous Pressure Gradient
ICER  Institute for Clinical and Economic Review
IL  interleukin
ION  index of NASH
IR  Insulin resistance
IRS  insulin receptor substrate
LASLD  lipodystrophy-associated steatotic liver disease
LDL-C  low-density lipoprotein cholesterol
LFC  liver fat content
LOXL2  Lysyl Oxidase-Like 2
LYPLAL1  lysophospholipase-like 1
MARC1  mitochondrial amidoxime-reducing component 1
MASH  metabolic dysfunction-associated steatohepatitis
MASLD  metabolic dysfunction-associated steatotic liver disease
MBOAT7  membrane bound-o-acyltransferase domain-containing 7
MELD  Mayo End-Stage Liver Disease
MERTK  MER proto-oncogene tyrosine kinase
miRNA  microribonucleic acid
MRI  Magnetic Resonance Imaging
MRI-PDFF  Magnetic Resonance Imaging-derived proton density fat fraction
MTTP  microsomal triglyceride transfer protein
NAFLD  non-alcoholic fatty liver disease
NAS  Non-alcoholic fatty liver disease Activity Score
NASH  non-alcoholic steatohepatitis
NFS  non-alcoholic fatty liver disease fibrosis score
O-MASLD  obesity-associated metabolic dysfunction-associated steatotic liver disease
OCA  obeticholic acid
OD  once daily
OR  odds ratio
OS-MASLD  obesity and sarcopenia-associated metabolic dysfunction-associated steatotic liver disease
OW  once weekly
PNPLA3  patatin-like phospholipase domain-containing 3
PPAR  peroxisome proliferator-activated receptor
RAs  receptor agonists
RCTs  randomized clinical trials
RR  relative risk

S-MASLD  sarcopenia-associated metabolic dysfunction-associated steatotic liver disease

SCD  stearoyl-CoA desaturase

SGLT-2is  sodium-glucose co-transporter 2 inhibitors

SLD  steatotic liver disease

T2DM  type 2 diabetes mellitus

THR  thyroid hormone receptor

TM6SF2  transmembrane 6 superfamily member 2

TNF  tumor necrosis factor

UCP2  uncoupling protein 2

UDCA  ursodeoxycholic acid

VLDL  very low-density lipoprotein

VLDL-C  very low-density lipoprotein cholesterol
Abstract

Steatotic liver disease (SLD) displays a dynamic and complex disease phenotype. Consequently, the metabolic dysfunction-associated steatotic liver disease (MASLD)/metabolic dysfunction-associated steatohepatitis (MASH) therapeutic pipeline is expanding rapidly and in multiple directions. In parallel, non-invasive tools for diagnosing and monitoring responses to therapeutic interventions are being studied, and clinically feasible findings are being explored as primary outcomes in interventional trials. The realization that distinct subgroups exist under the umbrella of SLD should guide more precise and personalized treatment recommendations and facilitate advancements in pharmacotherapeutics. This review summarizes recent updates of pathophysiology-based nomenclature and outlines both effective pharmacotherapeutics and those in the pipeline for MASLD/MASH, detailing their mode of action and the current status of phase 2 and 3 clinical trials. Of the extensive arsenal of pharmacotherapeutics in the MASLD/MASH pipeline, several have been rejected, whereas other, mainly monotherapy options, have shown only marginal benefits and are now being tested as part of combination therapies, yet others are still in development as monotherapies. Although the successful drug candidate (or combinations) remains elusive, such therapeutic approaches will ideally target MASH and fibrosis while improving cardiometabolic risk factors. Due to the urgent need for the development of novel therapeutic strategies and the potential availability of safety and tolerability data, repurposing of existing and approved drugs is an appealing option. Finally, it is essential to highlight that SLD and, by extension, MASLD should be recognized and approached as a systemic disease affecting multiple organs, with the vigorous implementation of interdisciplinary and coordinated action plans.
Significance Statement

SLD, including, among others, MASLD and MASH, is considered the most prevalent chronic liver condition affecting more than one-fourth of the global population. This review aims to provide the most recent information regarding SLD pathophysiology, diagnosis, and management options, in line with the most current advancements in the guidelines and clinical trials. Collectively, it is hoped that the information provided furthers the understanding of the current state of SLD with direct clinical implications and stimulates additional research initiatives.
Introduction

Closely linked with the obesity pandemic, non-alcoholic fatty liver disease (NAFLD) has become the most common cause of chronic liver disease, including its advanced forms, non-alcoholic steatohepatitis (NASH) and cirrhosis (Younossi et al., 2018; EASL-The Home of Hepatology, 2019; Riazi et al., 2022; Chong et al., 2023; Kouvari et al., 2023b). The prevalence of NAFLD is >90% in severely obese metabolic surgery candidates, >75% in patients with type 2 diabetes mellitus (T2DM), and approximately 70% in patients with cardiovascular (CV) diseases (CVDs), compared with over 30% for the global general adult population (Portillo-Sanchez et al., 2015; Younossi et al., 2019b; Riazi et al., 2022; Golabi et al., 2023). Notably, a substantial prevalence is also apparent in adolescents, almost reaching levels of disease observed in adults (Arshad et al., 2021; Muzurović et al., 2022c). As with adults, this may, in part, be related to increasing rates of obesity. By 2050, premature deaths associated with NAFLD in young adults are projected to increase 1.5-fold (Chong et al., 2023). Although obesity plays a significant role in the genesis of NAFLD, it is crucial to consider that even metabolically unhealthy nonobese adults with NAFLD demonstrate a 2.2-fold higher risk for all-cause mortality and a 3.2-fold higher risk for CV mortality (Xie et al., 2023). According to estimates, approximately 20% of patients with NAFLD have NASH (Younossi et al., 2016; Sanyal et al., 2019). Over subsequent decades, an additional 20% of these individuals are estimated to progress to cirrhosis, with a 2% yearly risk of developing hepatocellular carcinoma (HCC) (Singh et al., 2015; Huang et al., 2021). Of note, HCC can occur in non-cirrhotic forms of the NAFLD spectrum (Huang et al., 2021).

Although advanced forms of NAFLD, such as NASH and fibrosis, significantly and progressively increase overall mortality, its natural course is not linear but dynamic (Reddy et al., 2020; Looomba et al., 2021a) and varies according to a complex interplay of genetic determinants, behavioral risk factors, epigenetics and comorbidities (Kechagias et al., 2020; Meroni et al., 2020; Polyzos and Mantzoros, 2020; Muzurović et al., 2021, 2022; Angelidi et al., 2022). Recently, an independent committee of experts (Rinella et al., 2023) published "A multi-society Delphi consensus statement on new fatty liver disease nomenclature", and steatotic liver disease (SLD) was chosen as
an overarching term to encompass the various etiologies of steatosis while the term metabolic
dysfunction-associated SLD (MASLD) was chosen to replace NAFLD. Similarly, metabolic
dysfunction-associated steatohepatitis (MASH) was voted as the replacement term for NASH (Fig. 1).
Furthermore, there was consensus to change the definition to include the presence of at least one of
five cardiometabolic risk factors (Fig. 1). Those with no cardiometabolic parameters and no known
underlying cause were further deemed to have cryptogenic SLD (Fig. 1). A new category, termed
MetALD, was proposed to describe those with MASLD who consume greater amounts of alcohol
(140 g/week for females and 210 g/week for males) (Fig. 1). To highlight differences between the
new subgroups in terms of all-cause mortality, a recent study demonstrated that subjects with
MetALD and alcohol-associated (alcohol-related) liver disease with metabolic dysfunction show an
elevated risk of all-cause mortality, while “pure MASLD” was not associated with a significant
increase in all-cause mortality compared with individuals without hepatic steatosis (Li and Xie, 2023).
Due to the considerable overlap between the NAFLD and MASLD populations, in this review, we use
MASLD for studies on NAFLD to align with the latest updates in terminology updates. Further, we
exclusively refer to NASH as MASH as this is considered to minimize confusion in ongoing clinical
trials (Kim et al., 2023). Certainly, the recently introduced nomenclature is predominantly descriptive
and does not cover the broad pathophysiological mechanisms leading to SLD (Rinella et al., 2023).
Therefore, our group proposed a novel, pathophysiology-based, umbrella classification of SLD that
could help deliver personalized subcategory-oriented pharmacological interventions, ultimately
replacing the exclusive, nonspecific, and broad current MASLD-terminology (Valenzuela-Vallejo and
Mantzoros, 2022; Kokkorakis et al., 2023a) (Fig. 1). In Figure 1, we suggest a modified practical and
stepwise diagnostic approach for SLD and its subgroups, in line with the latest updates in terminology
and clinical practice.

While the scientific community, in general, acknowledges MASLD as a public health crisis,
tackling its various forms and identifying the disease (non-invasively) has been challenging,
demanding collaborative action across medical specialties, industry sectors, and governments
(Lazarus et al., 2020; Kanwal et al., 2021). Liver histology remains the gold standard for staging
MASLD severity and provides the basis for a robust scoring tool that exhibits reliability and
reproducibility. This assessment combines the unweighted sum of steatosis, lobular inflammation, and hepatic ballooning to specify The NAFLD Activity Score (NAS) (Kleiner et al., 2005) or Steatosis, Activity, and Fibrosis score (Rinella et al., 2019). However, in the absence of effective non-invasive, clinically available diagnostic tools and approved pharmacological treatments, MASLD remains an unmet clinical need and has been dubbed "The Silent Pandemic" (Lazarus et al., 2020). Since drug approval currently relies on liver biopsy, the field of MASLD/MASH urgently needs accurate non-invasive diagnostic options to replace this presently confirmatory but invasive method (Berzigotti et al., 2021; Valenzuela-Vallejo et al., 2022; Kouvari et al., 2023a; Kouvari et al., 2023b). Meeting this need would aid diagnostic and prognostic challenges, most notably disease monitoring during treatment and interpreting clinical trials.

In trials of individuals with MASH cirrhosis, the regulatory authorities recommend liver-related clinical outcomes endpoints (U.S. Food and Drug Administration, 2020; Aggarwal et al., 2022). Although CV and all-cause mortality should be considered as additional outcomes based on their interrelationship with advanced liver disease, these have not yet been the focus of the Food and Drug Administration (FDA) and other regulatory agencies (Kouvari et al., 2023a; Kouvari et al., 2023b, Polyzos and Mantzoros, 2020; Seo et al., 2023). The diversified and rich therapeutic pipeline in development for managing MASLD is driven by the complex disease pathophysiology, including CV outcomes, as well as the burdens of morbidity and mortality of this unmet clinical need, with annual direct medical costs in the United States as of 2016, of roughly $103 billion ($1,613 per patient) (Younossi et al., 2019d).

Patients with MASLD cirrhosis have predominantly liver-related events, whereas those with less advanced MASLD are at increased risk of extrahepatic malignancies and CV events, which represent the leading causes of death (Vilar-Gomez et al., 2018; Mantovani et al., 2020; E Muzzurović et al., 2022b; Simon et al., 2022). Specifically, in line with an increased burden of cancer attributed to high body mass index, individuals with MASLD, insulin resistance (IR), prediabetes, and T2DM are increasingly appreciated to be affected by, i.e., bladder cancer or colorectal tumors (Katsiki et al., 2018; Mantovani et al., 2018b; Tarantino et al., 2021; Liu et al., 2023; Tan et al., 2023). Additionally, a recent extensive meta-analysis has indicated that MASLD is linked to a moderately elevated long-
term risk, spanning a median duration of almost six years, for developing extrahepatic cancers, including gastrointestinal, breast, and gynecological cancers (Mantovani et al., 2022b). Therefore, treating MASLD/MASH must extend beyond liver disease alone (Przybyszewski et al., 2021).

Consistent with this, more than 140 pharmaceutical companies are currently developing therapies for MASLD/MASH (Clinical Trial Supply Services, 2023) targeting metabolic pathways, including carbohydrate and lipid modulation, inflammatory pathways, and fibrosis. In addition to monotherapies, combinations of pharmacological interventions are also being developed to target the above pathophysiological events (Przybyszewski et al., 2021). According to the recently published American Association for the Study of Liver Diseases (AASLD) Practice Guidelines on the Clinical Evaluation and Treatment of NAFLD, there are currently no FDA-approved drugs for the treatment of SLD, although the guidelines do suggest that drugs approved for the treatment of associated comorbidities, and potential benefit in SLD, may be considered (Rinella et al., 2023a). Previously, the European Association for the Study of the Liver, European Association for the Study of Diabetes, and European Association for the Study of Obesity Clinical Practice Guidelines for the management of MASLD stated that no specific therapy can be firmly recommended for the treatment of MASLD/MASH, and any drug treatment would be off-label (European Association for the Study of the Liver (EASL) et al., 2016). In this context, theoretically, combination-drug formulations with pleiotropic cardiometabolic effects would appear to be the most promising.

The present review provides insight into recent updates and prospects in the pipeline for the pharmacotherapy of MASLD/MASH, elucidating their mode of action and summarizing data relating to progress toward meeting phase 2 and 3 clinical trial endpoints. Further, SLD pathophysiology is briefly considered and mention is made of non-invasive testing approaches that are peripherally related to pharmacotherapies given that if novel pharmacotherapies are to reach the market they will likely need to be accompanied by accurate and accessible non-invasive tools.

**Literature search**

For this review, we identified references through searching the PubMed electronic database and the World Wide Web (press releases from early-phase clinical studies, ClinicalTrials.gov, Liver
Investigation: Testing Marker Utility in Steatohepatitis, Non-Invasive Biomarkers of Metabolic Liver Disease), with the latest access December 20, 2023. A search of the ClinicalTrials.gov site using the terms: NAFLD OR MASLD OR NASH OR MASH, interventional studies revealed 1,122 studies, of which 340 were identified as phase 2, 82 studies as phase 3, and 80 as phase 4 trials (most recent access December 20, 2023). While mostly encompassing pharmacological compounds, interventions included lifestyle and surgical procedures. In total, 320 studies had an active status (active, not recruiting), recruiting, not yet recruiting, or enrolling by invitation; 23 included a pediatric population. Currently, 92 phase 2 and 18 phase 3 trials for the pharmacotherapy of MASLD and MASH are ongoing (latest accessed December 20, 2023). Studies completed or terminated before 2016 were excluded to focus on recent updates. Studies with no results posted or with study designs identified as inferior to interventional (observational, cross-sectional) have also been excluded at the authors’ discretion. Exclusion criteria for phase 3 studies were: unknown status for ≥5 years, studies performed exclusively in children or special populations, e.g., patients with plaque psoriasis and coexisting MASLD or study results posted, but no FDA approval after ≥5 years of results being posted or last update provided. Medical Subject Headings and non-Medical Subject Heading terms were used, including non-alcoholic fatty liver disease, non-alcoholic steatohepatitis, fatty liver, liver fibrosis, steatotic liver disease, metabolic dysfunction-associated steatotic liver disease, metabolic dysfunction-associated steatohepatitis, liver biopsy, histology, histopathology, randomized controlled trial, interventional study, and clinical trial; combined with the names of substances or agents in the pipeline for the pharmacotherapy of MASLD/MASH. The authors aimed to include studies with outcomes that included liver histology, although less robust but frequently used non-invasive methods (e.g., Magnetic Resonance Imaging [MRI]-derived proton density fat fraction [MRI-PDFF], enhanced liver fibrosis, Fibrosis-4 [FIB-4] index, NAFLD fibrosis score [NFS] and parameters as measured by transient elastography) did not automatically relegate the study to non-inclusion. Reference lists of selected publications were also searched. Since our approach was to provide a narrative review, some articles not retrieved using this search strategy were added at the authors’ discretion if they were considered relevant. Finally, evidence from original articles, clinical trials, narrative reviews, systematic reviews, and meta-analyses were summarized.
Pathophysiology of steatotic liver disease – current understanding and impact of comorbidities

The pathogenesis of SLD involves an intricate and complex interorgan cross-talk (Keles et al., 2022), with the liver long being recognized as a barometer of metabolic health (Chitturi and Farrell, 2007). This interaction can be explained on many levels, from simple pathological explanations to in-depth metabolomics, which are beyond the scope of this article.

Genetic predisposition, IR, lipid and carbohydrate dysmetabolism, oxidative stress, inflammatory processes, and apoptosis are considered the main risk factors or ‘multiple hits’ that trigger hepatic steatosis and the progression to inflammation and fibrosis (Ferguson and Finck, 2021; Loomba et al., 2021a; Vuppalanchi et al., 2021) (Fig. 2). Since the above-mentioned mechanisms represent potential targets for pharmacotherapy, they serve as a focus for this review.

In light of the many parallel and distinct environmental, dysmetabolic, and genetic causes of SLD, the proposed “Mantzoros classification” offers a distinction of novel subgroups according to major contributing factors (Valenzuela-Vallejo and Mantzoros, 2022; Kokkorakis et al., 2023a). In line with other proposed changes in nomenclature, principally the multi-society Delphi consensus statement (Eslam et al., 2020a; Eslam et al., 2020b; Rinella et al., 2023), this classification acknowledges terms including, but not limited to MASLD, which could be further subdivided into, i.e., obesity-associated MASLD (O-MASLD), sarcopenia-associated MASLD (S-MASLD), or obesity and sarcopenia-associated MASLD (OS-MASLD) and lipodystrophy-associated SLD (LASLD) occurring in patients with abnormal adipose tissue (AT) depletion (possibly with ectopic lipid deposition) due to lipodystrophy syndromes that exhibit pronounced metabolic disturbances including IR (Kokkorakis et al., 2023a). The most distinctive component of the SLD umbrella classification, as an effect modifier, is the inclusion of a genetics-associated SLD; since it is mostly independent of lifestyle and environmental causes but associated with monogenic diseases (Kokkorakis et al., 2023a). In addition, endocrine-associated SLD refers mainly to thyroid dysfunction-associated SLD. SLD can have combined causes or other so far unclear etiologies, for which the term cryptogenic SLD was proposed (Valenzuela-Vallejo and Mantzoros, 2022; Kokkorakis et al., 2023a).
**Genetics-associated steatotic liver disease**

The importance of genetics and epigenetics in MASLD pathogenesis is underestimated as an effect modifier (Eslam and George, 2016) since a heritable component is estimated to be observed in between 35-61% of patients (Eslam and George, 2016; Loomba *et al.*, 2021a). The importance of genetic factors, especially in the absence of primary dysmetabolic abnormalities and obesity, and the specific characteristics of this sub-group of patients signifies the distinct nature of this population and should be appropriately addressed (Valenzuela-Vallejo and Mantzoros, 2022; Kokkorakis *et al.*, 2023a). All genes predisposing individuals to SLD appear to be involved in either glucose or fat metabolism (Fu *et al.*, 2022; Muzurović *et al.*, 2022c; Valenzuela-Vallejo *et al.*, 2023b).

MASLD has a hereditary component even beyond monogenic conditions (Valenzuela-Vallejo and Mantzoros, 2022). Specifically, hepatic steatosis may also occur in the absence of IR, for example, in carriers of a certain variant of the patatin-like phospholipase domain-containing 3 (*PNPLA3*) gene (Kantartzis *et al.*, 2009). Transmembrane 6 superfamily member 2 (*TM6SF2*) and membrane bound-acyltransferase domain-containing 7 (*MOAT7*) polymorphisms are considered the most robust genetic predictors of MASLD (Trépo and Valenti, 2020; Meroni *et al.*, 2021). Other gene mutations involve 17β-Hydroxysteroid dehydrogenase type 13 (*HSD17B13*) gene, lysophospholipase-like 1 (*LYPLAL1*), mitochondrial amidoxime-reducing component 1 (*MARC1*), mitochondrial uncoupling protein 2 (*UCP2*), ectonucleotide pyrophosphatase/phosphodiesterase-1 (*ENPP1*), insulin receptor substrate (*IRS*)-1, glucokinase regulator (*GCKR*), microsomal triglyceride transfer protein (*MTTP*), and MER proto-oncogene tyrosine kinase (*MERTK*) (Fig. 3). The metabolic phenotype associated with mutations in these genes is presented in Table 1.

Epigenetic mechanisms, e.g., deoxyribonucleic acid methylation or dysregulation of microribonucleic acid (miRNA) expression, appear to play an important role in the development and progression of MASLD. Hence miRNA-based therapeutic strategies, e.g., pharmacological analogs/inhibitors having a broad range of actions on hepatic metabolism, are being actively explored (Gjorgjieva *et al.*, 2019). Additionally, two highly promising and rapidly growing classes of nucleic acid therapeutics are antisense oligonucleotides and small interfering RNAs, which were recently comprehensively reviewed for the treatment of systemic diseases of liver origin and are expected to be
implicated in SLD as well (Gogate et al., 2023); therefore, we will be limiting discussions of such molecules to those necessary for the cohesiveness and comprehensiveness of this review.

**Endocrine-associated steatotic liver disease**

Thyroid dysfunction presents a pathogenic relationship with MASLD (Fig. 2). Key to endocrine-associated SLD are metabolic changes and oxidative stress, as well as a direct action of thyroid-stimulating hormone on hepatocytes (Hatziagelaki et al., 2022). Specifically, hypothyroidism-associated dyslipidemia and impaired hepatic β-oxidation favor the accumulation of triglycerides and lipotoxins, IR, and subsequently, DNL. Studies are currently investigating liver-specific thyroid hormone receptor (THR)-β agonists for treating MASLD in the hope of improved lipid homeostasis and mitochondrial respiration (Hatziagelaki et al., 2022).

In addition to the well-established factors contributing to the pathogenesis of SLD mentioned above, there are several other contributing and/or associated disorders, including, but not limited to, metabolic and endocrine factors (e.g., polycystic ovarian syndrome, hypopituitarism, growth hormone deficiency and hypogonadism) (Arefhosseini et al., 2022).

Recently, a comprehensive review focused on the relationship between non-diabetic endocrinopathies and MASLD (Hutchison et al., 2023). Deficiency in growth hormone and panhypopituitarism pose significant risk factors for the rapid progression of MASLD, a condition that may show responsiveness to hormone replacement or agonist therapy (Hutchison et al., 2023). The thyroid axis is crucial in influencing hepatic energy metabolism, impacting processes such as hepatic lipogenesis and fatty acid oxidation (Hutchison et al., 2023). Notably, the liver is not only influenced by thyroid hormones but also possesses receptors for thyroid-stimulating hormone, and activation of these receptors can lead to developing hepatic steatosis (Hutchison et al., 2023). MASLD demonstrates marked sexual dimorphism, likely influenced by the pivotal role of sex hormones in regulating lipid, carbohydrate, and protein metabolism within and beyond the liver (Ji et al., 2023). For instance, elevated androgen levels characteristic of women with polycystic ovary syndrome considerably increase the risk of steatotic liver, IR, and obesity (Falzarano et al., 2022). Overall, alterations in the sex hormone axes are increasingly prevalent in individuals with MASLD (Hutchison
et al., 2023). Besides, exposure to excess glucocorticoids induces IR, a hallmark of metabolic syndrome, T2DM, and SLD (Chen et al., 2024); yet, the extent to which glucocorticoid-induced SLD is reversible and the timeline for such reversibility following the cessation of glucocorticoid use remain largely uncertain. Therefore, understanding the involvement of endocrine axes in the pathobiology of MASLD has been and will continue to be instrumental in paving the way for novel pharmacotherapies. Patients presenting with endocrine-associated SLD may warrant closer monitoring to assess the progression of fibrosis.

**Metabolic dysfunction-associated steatotic liver disease**

The development and progression of MASLD show strong bidirectional associations with IR and clinical manifestations of dysregulated metabolism. These relationships have fueled the proposed change in nomenclature from NAFLD to MASLD (Eslam et al., 2020b; Méndez-Sánchez et al., 2022; Valenzuela-Vallejo and Mantzoros, 2022; Kokkorakis et al., 2023a). Hyperinsulinemia is, directly and indirectly, involved in metabolic and inflammatory diseases, such as SLD (Muzurović et al., 2021; Hill et al., 2021), especially in O-MASLD (Fig. 3). More precisely, the consequences of IR in the liver and extrahepatic tissues are key factors in the pathogenesis of SLD (Vanni et al., 2010). An impaired response to insulin in AT is characterized by an increased rate of lipolysis, which contributes to ‘ectopic’ fat accumulation, further exacerbating IR and lipotoxicity (Katsiki and Mikhailidis, 2018; Byrne and Targher, 2020; Muzurović et al., 2021; Muzurović et al., 2022a). Hepatic IR results in an increase in hepatic gluconeogenesis, a decrease in insulin-mediated hepatic glycogen synthesis, and an increase in hepatic lipid synthesis (Samuel and Shulman, 2018). Consequently, agents that improve systemic insulin sensitivity are being evaluated for their efficacy in patients with SLD (Ferguson and Finck, 2021; Fu et al., 2022).

Continuous overnutrition and the resulting storage of excess calories leads to obesity (Romieu et al., 2017). A sedentary lifestyle and an unhealthy (high-fat and/or high-fructose) diet, rich in calories, refined sugars, and saturated fats and low in polyunsaturated fatty acids, micronutrients, and fiber, play a key role in the development and progression of O-MASLD (Romieu et al., 2017; Vancells Lujan et al., 2021) (Fig. 2, Fig. 3). Glucotoxicity and lipotoxicity are closely related negative
consequences of substrate overload. Both processes contribute to IR and impaired insulin secretion (Gastaldelli and Cusi, 2019). In addition to being important players in intra-hepatic lipid accumulation and possible progression to more advanced disease, excessive free fatty acid (FFA) availability, inflammation, and reactive oxygen species are driving forces of lipotoxicity (Delli Bovi et al., 2021) (Fig. 3). Glucotoxicity refers to the deleterious effects of chronic hyperglycemia that alter cellular glucose and lipid pathways (i.e., de novo lipid synthesis or insulin secretion from pancreatic beta-cells) and promotes cellular dysfunction (i.e., endoplasmic reticulum stress, oxidative stress, mitochondrial defects, accumulation of advanced glycation end-products and eventually cell death) (Samuel and Shulman, 2018). Short-, medium- and long-chain fatty acids can also indirectly impact insulin secretion (Keles et al., 2022).

In addition to the above stressors, there is a simultaneous increase in FFA influx into the liver from dietary sources, increased synthesis of triglycerides (de novo lipogenesis [DNL]), and a concomitant reduction in their outflow through very low-density lipoprotein (VLDL) particles (Ferguson and Finck, 2021) (Fig. 3). Targeting the enzymes involved in fructose metabolism (ketohexokinase) or DNL and the storage of FFA in triglycerides, including acetyl-CoA carboxylase, fatty acid synthase, stearoyl-CoA desaturase (SCD), and diacylglycerol acyltransferase, has been considered as a therapeutic approach in SLD (Samuel and Shulman, 2018; Ferguson and Finck, 2021). FFAs entering the portal circulation have three different fates: β-oxidation, re-esterification to triglycerides, and export as VLDL cholesterol (VLDL-C) or re-esterification and storage in the liver (Di Ciaula et al., 2020) (Fig. 3). Triglycerides are catabolized to FFAs through neutral lipolysis and/or lipophagy; however, in patients with SLD liver lipophagy appears to fail (Li and Peng, 2022). Therefore, restoring lipophagy impairment associated with the pathogenesis of MASLD through lipophagic molecules (including the mechanistic target of rapamycin complex 1 and adenosine monophosphate-activated protein kinase [AMPK]) may be an efficient therapeutic strategy (Li and Peng, 2022).

All the aforementioned mechanisms also contribute to the development of hepatic steatosis, which in a certain proportion of patients progresses to MASH (Younossi et al., 2016); mechanistically involving oxidative stress, mitochondrial dysfunction, and inflammatory cytokines (Hazlehurst et al.,
Lipotoxic liver injury from excessive availability of FFAs and a variety of their biologically active metabolites is an important cause of inflammation and increased oxidative stress in steatohepatitis (Delli Bovi et al., 2021). A healthy lifestyle has been promoted due to its antioxidant mechanisms; however, among several pharmacological antioxidants studied, the data on their efficacy for MASH remains contradictory (Delli Bovi et al., 2021). Oxidation of FFAs induces the generation of reactive oxygen species and facilitates the release of pro-inflammatory cytokines. This is further exacerbated by the obesity-induced release of interleukin (IL)-6 and tumor necrosis factor (TNF)-α (Huang et al., 2021) (Fig. 3). Certain FFAs (e.g., linoleic acid) disrupt mitochondrial function (Huang et al., 2021; Keles et al., 2022). However, it is still debated whether mitochondria have decreased or improved function in human MASLD (Koliaki et al., 2015). The above-mentioned enzyme, acetyl-CoA carboxylase, plays an important indirect role in inhibiting β-oxidation in mitochondria, therefore adding to the generation of reactive oxygen species (Loomba et al., 2018).

Additional risk factors involving disturbances in bile acids and intestinal microbiota have been suggested to be important in developing MASLD (Zarrinpar and Loomba, 2012). Probiotics, prebiotics, or fecal microbiota transplantation represent novel therapeutic approaches targeting gut microbiota dysbiosis and are being explored as therapeutic targets but also as predictive markers in the MASLD pipeline (Di Ciaula et al., 2020; Lang et al., 2020; Kanchanasurakit et al., 2022). Bile acids are reported to act as signaling molecules (Di Ciaula et al., 2020), and their homeostasis is tightly regulated by a complex interplay between the nuclear receptor farnesoid X receptor (FXR), the enterokine hormone fibroblast growth factor (FGF)-15, and FGF-19 (Di Ciaula et al., 2020). Targeting FXR activity thus represents a possible therapeutic approach for preventing and treating liver diseases (Badman et al., 2020; Patel et al., 2020; Loomba et al., 2021b). Bile acids indirectly affect the microbiota composition, which is involved in the balance of proinflammatory, anti-inflammatory, and metabolic pathways of glucose and FFAs (Arab et al., 2017). Another proposed mechanism by which bile acids may impact liver energy metabolism is via G protein-coupled receptor-mediated activation of Takeda G-protein-coupled receptor 5, which in turn increases deiodinase-2 expression, initiating thyroid hormone signaling by activating the pro-hormone thyroxine to the biologically active triiodothyronine molecule (Samuel and Shulman,
Triiodothyronine and glucagon exhibit treatment potential for metabolic diseases but also undesired effects. Coordinated triiodothyronine and glucagon effects synergize to correct obesity, glucose intolerance, hyperlipidemia, atherosclerosis, and steatohepatitis in metabolically compromised animal models (Finan et al., 2016). Consistent with a role in the underlying pathogenesis, a loss of protective gut bacteria and dysregulated FXR signaling in SLD increases intestinal permeability and hepatic inflammation (Huang et al., 2021). It has been further proposed that increased gut inflammation and dysbiosis are potential key steps in tumorigenesis, leading to MASLD-related HCC (Huang et al., 2021).

Excess activation of fibrogenesis in hepatic stellate cells leads to the progression of MASH and cirrhosis (Pellicoro et al., 2014). Intrahepatic inflammation and fibrosis are impacted by translocated gut bacteria, gut-derived lipopolysaccharides, and certain lipid metabolites (Pafili and Roden, 2021). The injured hepatocytes activate hepatic macrophage cells (Kupffer cells), which release proinflammatory cytokines IL-1β, IL-6, and TNF-α (Pafili and Roden, 2021). When Kupffer cells are activated, increase in size and accumulate excess fat, they experience an impaired lipid metabolism and attract other white blood cells as a result of their inflammatory phenotype (Leroux et al., 2012). Of note, this pathogenic mechanism involves comparable receptors and exhibits similarities to the development of atherosclerotic plaques driven by foam cells, emphasizing how common metabolic diseases affect the entire body (Kzhyshkowska et al., 2012). Recent paradigm shifts advocate liver fibrosis as a dynamic, bidirectional process with an inherent capacity for recovery and remodeling (Pellicoro et al., 2014). The switch in macrophage phenotype to a pro-resolution cell type facilitates such a remodeling (Pellicoro et al., 2014). Given the bidirectional nature of liver fibrosis, this suggests potential avenues for therapeutic approaches.

In individuals with SLD, hepatic IR can lead to overt T2DM if pancreatic beta-cell secretory capacity is impaired or is insufficient to compensate for these changes (Cusi et al., 2017). Evidently, there is a bidirectional relationship between MASLD/MASH and T2DM (Mantovani et al., 2018a; Corbin et al., 2023), which has led to the proposal to actively search for the other condition in an individual affected by any of the two diseases (European Association for the Study of the Liver (EASL) et al., 2016; Cusi et al., 2022; Rinella et al., 2023a) (Fig. 1, Fig. 2). Relevant to this, several
antidiabetic drugs have been evaluated for their direct and indirect efficacy in MASLD/MASH (Ghosal et al., 2021; Patikorn et al., 2021; Wei et al., 2021). Potential drug candidates include the glucagon-like peptide-1 (GLP-1) receptor agonists (RAs), which, in addition to established glucose lowering and anorexic effects, appear to decrease fat mass and its consequences, including organ-specific IR and hepatic lipid handling in MASH (Armstrong et al., 2016). Supporting this, the presence of T2DM, in addition to genetic risk factors, is a predictor of the rapid progression of hepatic fibrosis (Kechagias et al., 2020; Loomba et al., 2021a; Muzurović et al., 2021; Vuppalanchi et al., 2021; Castera et al., 2023). In individuals with T2DM and MASLD, several observational studies and meta-analyses have also reported the risk of chronic kidney disease (CKD) and additional microvascular complications such as neuropathy, but not retinopathy (Mantovani et al., 2022a).

Irrespective of the coexistence of cardiometabolic risk factors, such as T2DM or obesity, a growing body of epidemiological evidence suggests that SLD is an independent risk factor for CKD, possibly mediated by accelerated atherothrombosis (Byrne and Targher, 2020). Non-alcoholic fatty pancreas disease, a term fashioned to encompass functional fat accumulation in the pancreas associated with obesity and metabolic syndrome, has recently been gaining attention due to possible clinical consequences. Non-alcoholic fatty pancreas disease is strongly associated with MASLD, and its presence may aggravate it (Filippatos et al., 2022).

In regard to the involvement of skeletal muscle, in a seven-year study period, among patients without baseline SLD, 15% developed incident S-MASLD, with the highest incidence in the group with the lowest skeletal muscle mass (even after adjustment for several known SLD risk factors) (Li et al., 2020). Understanding skeletal muscle as an endocrine organ that secretes a variety of myokines (which contribute to FFA metabolism, and their reduced expression is associated with increased liver fat storage) may help us understand its role in the development of S-MASLD (Bhanji et al., 2017). Sarcopenia, a progressive and generalized loss of skeletal muscle mass, is observed in up to 60% of patients with end-stage SLD and is indicative of a poor prognosis (Bhanji et al., 2017; Aslam et al., 2023; Gielen et al., 2023). Specifically, a syndrome of “sarcopenic obesity” is now increasingly recognized and is applied to individuals who simultaneously show a triad of sarcopenia, IR, and obesity (Srikanthan et al., 2010; Axelrod et al., 2023). Furthermore, sarcopenia promotes IR
independent of obesity since skeletal muscle is the major tissue involved in insulin-mediated glucose utilization (Srikanthan et al., 2010). Treatment strategies that increase skeletal muscle mass by improving the secretory myokine profile may also improve S-MASLD-related outcomes (Cani et al., 2007; Barazzoni et al., 2023; Calvani et al., 2023; Hashimoto et al., 2023; Katsarou et al., 2023; Park and Choi, 2023; Polyzos and Mantzoros, 2023; Rolland et al., 2023; Yuan and Larsson, 2023). Activin type II receptor inhibition with bimagrumab, a monoclonal antibody, stimulates skeletal muscle growth, improves IR, and promotes excess AT loss (Heymsfield et al., 2021). Further, it is equally essential to recognize the role of myosteatosis, i.e., ectopic fat accumulation in skeletal muscle, which is observed to be increased in patients with obesity and MASH (Nachit et al., 2021). Myosteatosis may be an additional way of risk stratification within the S-MASLD group by introducing cut-offs for muscle fat and allowing the detection of adverse muscle composition (Linge et al., 2021).

Although obesity is an important risk factor for the development of MASLD, 20% of the global MASLD population have a lean phenotype (Ye et al., 2020), consistent with the pathophysiological complexity of SLD as well as indicating that prevention strategies may be directed at targeting modifiable risk factors. More specifically, rare cases of patients with SLD have LASLD, presenting with a lack of subcutaneous AT and, thus, with a degree of fat loss or fat redistribution (Fiorenza et al., 2011). Specifically, LASLD is an umbrella term used to determine a metabolic disorder characterized by either partial or complete lipoatrophy (loss of fat), ectopic AT accumulation (lipohypertrophy), and metabolic abnormalities, such as IR, acanthosis nigricans, hypertriglyceridemia, T2DM, and SLD, as a consequence of ectopic storage of FFAs in the liver (Fiorenza et al., 2011). Furthermore, the severity of such metabolic abnormalities typically correlates with the degree of fat loss (Fiorenza et al., 2011).

Lipodystrophy can be subclassified as congenital or acquired and generalized or partial. The details of congenital lipodystrophies are beyond the scope of this article and are discussed elsewhere (Patni and Garg, 2015). Abnormally low levels of leptin and adiponectin due to altered body fat mass are characteristic of LASLD (Polyzos et al., 2019b). Therefore, adiponectin and/or leptin replacement may represent specific therapeutic targets for LASLD (Polyzos et al., 2019b). A synthetic analog of...
Human leptin, metreleptin, is an effective treatment for congenital lipodystrophy (Rodríguez et al., 2015). Consequently, medications that increase the levels of adiponectin, such as peroxisome proliferator-activated receptor (PPAR)-γ, in combination with metreleptin appear to be the physiology-based treatments of choice for LASLD patients (Valenzuela-Vallejo and Mantzoros, 2022; Kokkorakis et al., 2023a).

**Combined causes-associated steatotic liver disease**

In some instances, cases of SLD arise from the overlap of all the above entities, involving various combinations of genetic predisposition, epigenetics, environmental risk factors, and IR with related complications, which lie in the background of obesity, sarcopenia, and lipodystrophy (Valenzuela-Vallejo and Mantzoros, 2022; Kokkorakis et al., 2023a).

**Cryptogenic steatotic liver disease**

In addition to the well-established factors contributing to the pathogenesis of SLD, it is very important to mention other, currently not distinctly recognized (cryptogenic), contributing disorders such as infections (e.g., Helicobacter pylori, human immunodeficiency virus infection, and coronavirus disease 2019 [COVID-19]) (Adenote et al., 2021; Iacob and Iacob, 2022; Makri et al., 2022). Thus, a multitude of signs and symptoms affecting different organ systems have been described in post-acute COVID-19 infection. The multisystemic nature of SLD and its association with immune-metabolic conditions and adverse outcomes pose research questions relating to the prevalence of SLD in patients with post-acute COVID-19 syndrome (Sanoudou et al., 2022). Certain COVID-19 treatments may also be hepatotoxic, cause drug-induced liver injury, or aggravate pre-existing liver lesions classically observed in SLD (Adenote et al., 2021). SLD was highly prevalent after hospital discharge and may represent a specific post-acute COVID state cluster phenotype with potential long-term cardiometabolic implications (Milic et al., 2022). This sub-category of cryptogenic SLD will be expanded in line with our growing understanding of the contributors to the pathophysiology of SLD.
Detection, risk stratification, and monitoring of steatotic liver disease progression

In the ideal case, individuals with MASLD should be identified before their condition progresses to MASH. Considering the high co-existence of MASLD/MASH with other cardiometabolic conditions, individuals with obesity, dyslipidemia, polycystic ovary syndrome, and T2DM should undergo annual liver screening, in line with the AASLD Practice Guidance published in 2023 (Fig. 1) (Rinella et al., 2023b). Due to the current absence of consensus regarding screening for SLD, in Figure 1, we propose a practical three-step risk stratification algorithm integrating important sub-populations of MASLD and SLD. However, the numerous drawbacks of invasive diagnostics present a distinct hurdle for the stratification of patients who should be prioritized for therapeutic intervention beyond lifestyle improvement (Sanyal et al., 2022). Currently, liver biopsy is the diagnostic gold standard for detecting MASLD/MASH and other diseases contributing to liver damage. However, if we aimed to perform a liver biopsy in 30% of the U.S. population (estimated MASLD prevalence), there would be approximately 9,310,000 minor complications, 4,410,000 cases of major bleeding, and 9,800 deaths (Thampanitchawong and Piratvisuth, 1999; Thomaides-Brears et al., 2022). In addition to these risks, the histopathological interpretation is subject to inter-reader and local variability (Neuberger et al., 2020; Mahjoubin-Tehran et al., 2021). The vast target population for such intervention is currently being managed according to sparse national and international guidelines, recommending sequential use of combinations of non-invasive biomarkers and risk stratification tools (European Association for the Study of the Liver (EASL) et al., 2016; Glen et al., 2016; Chalasani et al., 2018; Wong et al., 2018; Cusi et al., 2022; Valenzuela-Vallejo et al., 2022; Rinella et al., 2023a). The field of non-invasive tools and biomarkers (circulating, functional, and/or imaging) has evolved to offering point-of-care diagnostics using exhaled breath fingerprinting (Hydes et al., 2021; Sinha et al., 2022; Kouvari et al., 2023b); however, these technologies have neither been systematically compared nor validated against the current gold standard of liver biopsy (Sanyal et al., 2022). Non-invasive modalities to diagnose MASH and stages of liver fibrosis have promising diagnostic accuracy (with an area under the receiver operating characteristic curve [AUC] ranging between 0.76 and 0.90, predicting the risk of incorrect classification at <10-24%) (Younossi et al.,...
Similarly, machine learning algorithms increasingly emerge to use laboratory parameters and demographic data, but their overall performance fails to meet clinical relevance due to a lack of liver-biopsy-proven MASLD/MASH as the prediction outcome or the absence of external validation cohorts (Ji et al., 2022; Chang et al., 2023; Kouvari et al., 2023b; Peng et al., 2023). Recently, a large multi-center liver biopsy-based study (n=455 total serum samples) validated, confirmed, and compared the diagnostic performance of established and novel non-invasive MASLD indices (n=12 total non-invasive testing indices) (Kouvari et al., 2023b). Briefly, this study reported that the index of NASH (ION) demonstrated the highest differentiation ability among the other 11 non-invasive testing indices for the presence of MASLD vs controls (AUC=0.894), MASH vs MASL (AUC=0.747), presence of ballooning (AUC=0.839), and presence of inflammation (AUC=0.856) (Kouvari et al., 2023b). The aspartate aminotransferase to platelet ratio index (APRI), in turn, achieved the highest performance for differentiating between fibrosis stages F2-4 vs F0-1 (AUC=0.735) as well as between F3-4 vs F0-2 (AUC=0.845) (Kouvari et al., 2023b). Nonetheless, the aforementioned outcomes do not meet the required level of clinical performance, and continuous efforts are essential for enhancing the discriminatory abilities of current and novel non-invasive testing indices.

Based on the available evidence, MRI-PDFF, MRI-based corrected T1, and parameters as measured by transient elastography (FibroScan, Echosens, Paris, France), namely controlled attenuation parameter (CAP), are most frequently pursued as primary and/or secondary endpoints in randomized clinical trials (RCTs), usually to determine the degree of hepatic fibrosis. Furthermore, N-terminal type III collagen propeptide, enhanced liver fibrosis, and cytokeratin 18 seem to be the most promising biomarkers to assess physiological changes (Siddiqui et al., 2019; Hydes et al., 2021; Andersson et al., 2022; Jincheng Wang et al., 2022) in response to interventional treatment in a phase 2a trial design and may serve as primary and/or secondary endpoints (Ampuero and Romero-Gomez, 2020; Chen et al., 2023). Phase 2 trials additionally use other fibrosis biomarkers and blood-based biochemical laboratory findings as simple as alanine aminotransferase (ALT) and aspartate aminotransferase (AST) to report on MASH resolution. However, liver transaminases are less sensitive in detecting early steatotic liver disease, which, particularly in subjects with IR, might appear normal. As metabolic endpoint measures, glycated hemoglobin (HbA1c), Homeostatic Model
Assessment for IR (HOMA-IR), serum insulin, and lipid levels have been used in several trials (Rinella et al., 2019; Hydes et al., 2021). Recently, HbA1c levels were shown to be associated with the severity of liver steatosis in individuals without T2DM, subjects with prediabetes, and known T2DM (Hou et al., 2022; Xie et al., 2022).

Hepatic outcomes have been monitored according to the stage of fibrosis as shown by enhanced liver fibrosis, FIB-4 index score, neo-epitope pro-peptide of type III collagen, NFS, and liver stiffness measurement by vibration-controlled transient elastography (Ampuero and Romero-Gomez, 2020; Mózes et al., 2022; Chen et al., 2023). The future promises that the use of omics (including metabolomics and lipidomics) will offer new non-invasive options (Perakakis et al., 2019; Masoodi et al., 2021; Keles et al., 2022), as well as measurements of serum circulating miRNAs, as novel biomarkers for MASH diagnosis in patients with MASLD (Kim et al., 2021). Integrated proteomics and ribonucleic acid sequencing approaches creating a proteo-transcriptomic map of MASLD can also be used to understand pathophysiological changes associated with MASLD and establish whether candidate circulating biomarkers originate from the liver (Govaere et al., 2023).

Future studies should further evaluate the non-invasive assessment of hepatic mitochondrial metabolism, reflecting the efficiency of mitochondrial β-oxidation, for monitoring pharmacodynamic activity (Loomba et al., 2018). Currently, liver histology remains the gold standard for staging MASLD severity, serving as a strong diagnostic tool with good reproducibility, the unweighted sum of steatosis, lobular inflammation, and hepatocellular ballooning scores such as NAS (Kleiner et al., 2005). Consequently, NAS remains the main endpoint of phase 3 trials (Rinella et al., 2019). NAS scores the level of steatosis (scores from 0-3), inflammation (scores from 0-3), and ballooning (scores from 0-2) (Kleiner et al., 2005, 2019). MASH resolution is defined as absent SLD or simple steatosis without steatohepatitis and a NAS score of 0-1 for inflammation, 0 for ballooning, and any value for steatosis with no worsening of liver fibrosis. Staging is based on the level of fibrosis according to the NASH Clinical Research Network methodology with a range from no fibrosis (F0) to cirrhosis (F4) (Brunt et al., 1999). Currently, the primary endpoints in clinical trials with MASH-related cirrhosis are usually clinical events, including cirrhosis decompensation, HCC, transplantation, and death (Polyzos and Mantzoros, 2020; U.S. Food and Drug Administration, 2020). More precisely, clinical
benefit can be verified by demonstrating superiority to placebo in delaying disease progression measured by a composite endpoint that includes the following: progression to cirrhosis on histopathology, reduction in hepatic decompensation events (e.g., hepatic encephalopathy, variceal bleeding, ascites), change in Mayo End-Stage Liver Disease (MELD) score from ≤12 to >15, Hepatic Venous Pressure Gradient (HVPG) <10 mmHg, liver transplantation, and all-cause mortality (Rinella et al., 2019; Ampuero and Romero-Gomez, 2020; Chen et al., 2023). Additionally, the resolution of MASH comprises another primary outcome in currently ongoing phase 2 and phase 3 clinical trials.

**Emerging pharmacotherapies for steatotic liver disease**

Clinical management of MASLD is crucially dependent on the stage of the disease. The first line management strategy for MASLD is lifestyle modification, and all other pharmacotherapies need to be accompanied by or developed based on this. Specifically, lifestyle modification with diet and regular physical activity targeting body weight (BW) loss of 7-10\% in addition to aggressive management of underlying metabolic comorbidities and cardiometabolic risk factors are the cornerstones of treatment for all patients (European Association for the Study of the Liver (EASL) et al., 2016; Chalasani et al., 2018; American Diabetes Association Professional Practice Committee, 2021; Cusi et al., 2022; He et al., 2023; Rinella et al., 2023a). Despite this, according to recent research, even for individuals who receive nutritional education and guidance, very few can sufficiently and consistently change their lifestyle (Stewart et al., 2015). Therefore, medical or surgical interventions should be considered if the desired weight loss cannot be achieved with lifestyle. Bariatric surgery, although typically reserved for patients with prevalent metabolic comorbidities, has proven a very effective treatment option for MASLD/MASH, associated with risk reduction for major adverse CV events (Fakhry et al., 2019; Aminian et al., 2021; Seeberg et al., 2022; Kokkorakis et al., 2023c; Verrastro et al., 2023) (Table 2). The need for specific pharmacological treatment for MASLD is supported by the fact that BW loss is a very elusive target, which is also difficult to sustain (Evert and Franz, 2017; Muzurović et al., 2020; Yan et al., 2022). The off-label use of pioglitazone is encouraged in biopsy-confirmed MASH with or without T2DM,
while vitamin E shows efficacy in biopsy-proven MASH without T2DM or cirrhosis (European Association for the Study of the Liver (EASL) et al., 2016; Chalasani et al., 2018; American Diabetes Association Professional Practice Committee, 2021; Cusi et al., 2022; Rinella et al., 2023a) (Table 2). The 2023 AASLD Practice Guidance acknowledges the use of liraglutide 1.8 mg subcutaneously daily (in T2DM), 0.6-3 mg subcutaneously daily (in patients with obesity), and semaglutide 0.4 mg subcutaneously daily, 0.25-2.4 mg subcutaneously once weekly (OW) in T2DM, obesity (Table 2). Furthermore, in carefully selected individuals with MASH and comorbid conditions such as T2DM, tirzepatide, and sodium-glucose co-transporter 2 inhibitors (SGLT-2is) should be considered, as the aforementioned guidance states (Rinella et al., 2023a) (Table 2).

The number of pharmaceuticals in the pipeline for MASH makes it necessary to categorize them into drug classes. The following have been proposed to broadly encompass the targeting of several of the most frequently considered of the 29 different mechanisms of action: FXR agonists, DNL inhibitors, THR-β modulators, PPAR agonists, GLP-1 RAs, antioxidants, vitamins, anti-apoptotic medications, anti-fibrotic, anti-inflammatory, antihypertensive, and other treatment options. According to a 2021 in-depth analysis of meta-analyses of both published and ongoing RCTs (Patikorn et al., 2021), only six interventions (12.2% of the included drug candidates) have demonstrated positive treatment effects against placebo based on the currently accepted histological endpoints for the FDA conditional approval. Compared with placebo, 16.3% of interventions were found to have significant positive treatment effects in any of the histological outcomes (improvement in inflammation, steatosis, ballooning, fibrosis, or reduction in NAS). Image-based outcomes, namely improvement of LFC assessed by MRI-PDFF, were reached in 22.4% of interventions, while 38.8% of interventions demonstrated improvements in ALT and/or AST (Patikorn et al., 2021). Of note, the clinical significance of therapeutic modalities remains to be elucidated since most studies evaluated surrogate endpoints, while the prevention of progression to cirrhosis and other important clinical liver outcomes for MASH were not investigated (Patikorn et al., 2021).
Targets related to metabolism

Anti-obesity medications and antidiabetic medications

MASH resolution with no worsening of fibrosis was shown in patients treated with the GLP-1 RAs (Pennisi et al., 2022; Muzurović et al., 2022b). Liraglutide and semaglutide are GLP-1RAs administered subcutaneously, bind reversibly to albumin, and lead to insulin release in pancreatic beta cells in the presence of hyperglycemia while also delaying gastric emptying. The benefits of MASLD/MASH treatment with GLP-1 RAs (or dual GLP-1/gastric inhibitory peptide [GIP] RAs) are predominantly weight-related, according to the data to date. The reduction in BW and the improved glycemic control resulting from the treatment with GLP-1 or dual GLP-1/GIP RAs are crucial factors in improving MASLD parameters. Other direct mechanisms are still the subject of debate (Wong et al., 2021; Nevola et al., 2023; Muzurović et al., 2022b). It is still controversial whether the liver expresses GLP-1 and GIP receptors, so their effects on the liver may be mediated indirectly (Muzurović et al., 2022b). Indeed GLP-1 RAs showed beneficial effects on the liver, including inhibition of hepatic gluconeogenesis indirectly via the gut-pancreas-liver axis and postprandial GLP-1-mediated insulin secretion (Jin and Weng, 2016). Furthermore, GLP-1 RAs increase glucose utilization by stimulating glycogen and lipid synthesis in the liver, independent of insulin (Kieffer and Habener, 1999). GLP-1 also shows some insulin-like effects by activating key signaling molecules downstream of IRS-2 (a key substrate for insulin signaling in the β-cells (Jin and Weng, 2016). GIP promotes the deposition of lipids in the liver (Keyhani-Nejad et al., 2015; Pfeiffer and Keyhani-Nejad, 2018). GIP release/signaling mediates the development of SLD in response to the consumption of high glycemic index foods (e.g., in response to high intake of carbohydrates, especially sucrose) (Keyhani-Nejad et al., 2015; Pfeiffer and Keyhani-Nejad, 2018).

In the first-ever RCT comparing two BW loss approaches for improving MASLD, liraglutide (3.0 mg once daily [OD] subcutaneously) has been shown to be effective in reducing (noninvasively assessed) hepatic steatosis and hepatocellular apoptosis in obese adults with MASLD (Khoo et al., 2017). In this phase 3 RCT, both lifestyle intervention and liraglutide groups had significant and similar reductions in BW (Khoo et al., 2017). However, the effects were not sustained after discontinuation since the liraglutide group significantly regained BW (1.8 ± 2.1 kg), as well as MRI-
measured liver fat and cytokeratin 18 levels in contrast with lifestyle modification (Khoo et al., 2019).

Liraglutide efficacy against placebo was demonstrated in the “Liraglutide efficacy and action in NASH – LEAN” phase 2 RCT involving 26 patients with MASH treated with liraglutide 1.8 mg daily subcutaneously for 48 weeks and who achieved biopsy-proven resolution of demonstrated MASH in 39%, compared with 9% in the placebo group (relative risk [RR] 4.3, 95% confidence interval [CI]: 1.0-17.7, p=0.019) (Matthew James Armstrong et al., 2016). The RCT, “Effect of Dulaglutide on Liver Fat in Patients With Type 2 Diabetes and Nonalcoholic Fatty Liver Disease - D-LIFT”, determined the effect of dulaglutide (add-on to usual care) in the treatment of 64 individuals with T2DM and MASH; dulaglutide, 0.75 mg OW subcutaneously for four weeks followed by 1.5 mg subcutaneously OW for 20 weeks, significantly reduced MRI-PDFF-measured liver fat content (LFC), corresponding to a 2.6-fold greater reduction, and improved serum γ-glutamyl transferase (GGT) activity (Kuchay et al., 2020).

A recent network meta-analysis of RCTs evaluated subcutaneously administered GLP-1 RA semaglutide compared with placebo, finding the GLP-1 RA to be superior for MASH resolution without worsening of fibrosis (odds ratio [OR] 6.89, 95% CI: 2.90-16.35). This places semaglutide among the most effective interventions, to date (Pennisi et al., 2022), based on its phase 2 trial performance (Newsome et al., 2021). In the “Investigation of Efficacy and Safety of Three Dose Levels of Subcutaneous Semaglutide Once Daily Versus Placebo in Subjects With Non-alcoholic Steatohepatitis”, the highest response rate documented so far in MASH trials was achieved; as the primary endpoint of the study (biopsy-proven resolution of MASH with no worsening of fibrosis) was achieved in 59% of patients with semaglutide (0.4 mg subcutaneously OD) compared with 17% in the placebo group (Newsome et al., 2021). The ongoing phase 3 research study on the effectiveness of semaglutide in individuals with MASH, “Research Study on Whether Semaglutide Works in People With Non-alcoholic Steatohepatitis - ESSENCE”, is evaluating semaglutide administered subcutaneously 2.4 mg OW in MASH with F2-3 fibrosis for 72 and 240 weeks; this trial is expected to be completed in 2028 (Chen et al., 2022) (Fig. 4, Table 4). Semaglutide 2.4 mg subcutaneously OW was evaluated for safety and efficacy in patients with MASH-related cirrhosis in a phase 2 RCT published in 2023; while no safety concerns were raised, semaglutide did not significantly improve
fibrosis versus placebo after 48 weeks, as assessed by biopsy (Loomba et al., 2023a). An additional semaglutide phase 2 RCT, “Study of Semaglutide for Non-Alcoholic Fatty Liver Disease, a Metabolic Syndrome With Insulin Resistance, Increased Hepatic Lipids, and Increased Cardiovascular Disease Risk - The SLIM LIVER Study”, was completed in September 2023 and investigated the effects of semaglutide on LFC in people living with human immunodeficiency virus, central adiposity, IR or pre-diabetes, and hepatic steatosis, without posted results yet (NCT04216589).

Tirzepatide is an OW subcutaneously injectable peptide (approved by the FDA for T2D in 2022; and in 2023 as an anti-obesity agent) engineered from the native GIP, leading to a substantial BW reduction (Jastreboff et al., 2022). Tirzepatide was approved by the FDA in 2022 for treating T2DM and in late 2023 for obesity. This novel dual GIP/GLP-1 RA has promising metabolic effects according to a sub-study of the randomized, phase-3 trial SURPASS-3 trial MRI-PDFF trial, which has assessed reductions in LFC in tirzepatide vs insulin degludec-treated individuals with T2DM (Gastaldelli et al., 2022). A phase 2 study of 5, 10, or 15 mg once weekly subcutaneously administered tirzepatide “A Randomized, Double-Blind, Placebo-Controlled Phase 2 Study Comparing the Efficacy and Safety of Tirzepatide Versus Placebo in Patients With Nonalcoholic Steatohepatitis - SYNERGY-NASH” included patients with and without T2DM and evaluated liver histology as the primary outcome, was recently completed, although results are yet to be posted (NCT04166773).

The dual GLP-1/glucagon RA cotadutide showed robust glucose-lowering and BW loss effects in patients with T2DM. Cotadutide administered subcutaneously OD is under development for treating MASH in T2DM with CKD (Parker et al., 2022). Its use was associated with improvement in hepatic parameters (AST, ALT activity, FIB-4, and NFS) after 54 weeks of treatment in participants with T2DM (Nahra et al., 2021), which supported the further evaluation of cotadutide in MASH in an ongoing phase 2 trial (NCT05364931) (Table 3). Another study investigating the effects of three different doses of a novel dual GLP-1/glucagon RA, survodutide (BI 456906), in patients with MASH and fibrosis (F1-F3), is currently at phase 2, was completed in December 2023, without posted results yet (NCT04771273). Notably, survodutide has received Fast Track designation from the FDA for adults with MASH (NCT04771273).
Additionally, according to a subgroup analysis of patients with MASLD presented at the 83rd American Diabetes Association Scientific Sessions in June 2023 from a landmark obesity trial (NCT04881760), among 98 patients with MASLD, those on the high dose (12 mg) of retatrutide (a novel triple GLP-1, GIP, and glucagon receptor agonist) experienced an 86% decrease in liver fat content over 48 weeks with most of the reduction occurring early on in treatment; moreover, resolution of MASLD was observed in 86% of patients receiving 12 mg at week 24 and 93% at week 48.

Indirectly activating GLP-1 and GIP, the oral dipeptidyl peptidase-4 inhibitor vildagliptin is currently being investigated in an ongoing phase 3 clinical trial as monotherapy or in conjunction with metformin for the pharmacotherapy of MASLD and T2DM and is expected to be completed in December 2028 (NCT03925701) (Fig. 4, Table 4).

Another dual agonist of glucagon and GLP-1 receptors, efinopegdutide (MK-6024), has completed a phase 2a RCT in which among 145 randomized participants (efinopegdutide 10 mg n = 72, semaglutide 1 mg n = 73, both administered subcutaneously once weekly for 24 weeks) there was shown that least squares mean relative reduction from baseline in LFC at week 24 was significantly (p <0.001) greater with efinopegdutide (72.7% [90% CI 66.8-78.7]) than with semaglutide (42.3% [90% CI 36.5-48.1]) (Romero-Gómez et al., 2023) (NCT04944992). Moreover, efinopegdutide is currently also under investigation in a phase 2 RCT of patients with precirrhotic MASH receiving subcutaneous injections in dose-escalation regimens once weekly, potentially including doses of 2 mg, 4 mg, 7 mg, and 10 mg for 52 weeks, expected to be completed in December 2025 (NCT05877547) (Table 3).

SGLT2is, widely prescribed in T2DM, comprise an orally-active drug class with established CV and renal protective properties (Wei et al., 2021). Several studies have reported that SGLT2is (empagliflozin, canagliflozin, dapagliflozin, licogliflozin, ipragliflozin, luseogliflozin, and tofogliflozin) exerted improvements in hepatic enzymes and other non-invasive tools for assessing hepatic steatosis or, in certain cases, also fibrosis in individuals with T2DM and MASLD (Kuchay et al., 2018; Shimizu et al., 2019; Wei et al., 2021; Yabiku, 2021; Bica et al., 2023; Borisov et al., 2023). Treatment with SGLT2is contributes to alleviating SLD by improving insulin sensitivity/IR, levels of triglycerides, and BW reduction due to caloric loss, mostly due to glycosuria (Androutsakos...
et al., 2022). In addition, SGLT2is show hepatoprotective effects through the reduction of DNL, hepatic inflammation, oxidative stress, endoplasmic reticulum stress, apoptosis, and increased $\beta$-oxidation in the liver (Androutsakos et al., 2022). Further, treatment with SGLT2is has been shown to ameliorate hepatic fibrosis and HCC development through reduced activation of hepatic satellite cells and p53/p21 pathways; however, the antifibrotic effects were not consistently shown across SGLT-2is class members (Androutsakos et al., 2022).

The effect of ertugliflozin on LFC, liver fibrosis, and glycemic control in individuals with T2DM and MASH/MASLD is similarly being investigated in the “Ertu-NASH Trial” (NCT05644717). While dapagliflozin 10 mg is continuing to be studied in T2DM with MASLD (NCT05459701; phase 4; no results posted), and the “Dapagliflozin Efficacy and Action in NASH - DEAN” (NCT03723252) ongoing phase 3 trial is assessing the efficacy of dapagliflozin on improving MASH as determined by liver biopsy, this SGLT2i has already been shown to reduce serum ALT activity in individuals with MASLD without T2DM (Tobita et al., 2021) (Fig. 4, Table 4). Another ongoing phase 3 clinical trial examining the safety of dapagliflozin in patients with MASLD, diagnosed by FibroScan CAP score $\geq$252 dB/m, is expected to be completed in April 2024 (NCT05308160) (Fig. 4, Table 4). Besides, an ongoing phase 3 RCT studies the impact of both dapagliflozin and empagliflozin on patients with MASLD and T2DM, estimated to be completed in October 2024 (NCT06117137) (Fig. 4, Table 4). Of note, in patients with T2DM and MASLD, dapagliflozin was shown to reduce biomarkers of hepatocyte injury and FGF-19, suggesting a disease-modifying effect in SLD (Eriksson et al., 2018; Sun et al., 2022). The effect of empagliflozin (10 mg) on liver steatosis was also under investigation in subjects without T2DM, in a phase 3 trial (IRCT20190122042450N1), in addition to improving liver steatosis, measures of liver fibrosis were improved, but non significantly, compared with placebo (Taheri et al., 2020). Interestingly, in individuals with T2DM, empagliflozin outperformed ursodeoxycholic acid (UDCA) and placebo in decreasing LFC (utilizing the MRI-PDFF) (Elhini et al., 2022).

In a 48-week randomized, open-label, parallel-group trial of 40 individuals with T2DM and biopsy-confirmed MASLD, daily 20 mg tofogliflozin or 0.5 mg glimepiride, a sulfonylurea drug, were compared head-to-head (Takeshita et al., 2022). Liver histological and metabolic improvement
in participants were not significantly different between the agents. Fibrosis scores improved in the tofogliflozin group (60%, $p=0.001$), whereas the change from baseline did not differ significantly between the treatment groups ($p=0.172$). Whereas only hepatocellular ballooning was improved in the glimepiride group (25%, $p=0.025$); steatosis (65%, $p=0.001$), hepatocellular ballooning (55%, $p=0.002$), and lobular inflammation (50%, $p=0.003$) were improved in the tofogliflozin group (Takeshita et al., 2022). Recently, a study with tofogliflozin, together with pemafibrate, a selective PPAR-α modulator, was initiated in a phase 2 clinical trial for the pharmacotherapy of MASH with liver fibrosis, expected to be completed in June 2025 (NCT05327127) (Fig. 3, Table 3).

Several systematic reviews and meta-analyses have shown that treatment with metformin results in an improvement, or a trend towards an improvement, in liver enzymes in patients with MASLD (Said and Akhter, 2017; Blazina and Selph, 2019; Jalali et al., 2020; Huang et al., 2022). Conversely, the evidence available to date on the effect of metformin on liver histology is heterogeneous and inconsistent, and is mostly derived from small trials using various scores and grading systems (Uygun et al., 2004; Bugianesi et al., 2005; Haukeland et al., 2009; Garinis et al., 2010; Torres et al., 2011; Kazemi et al., 2012; Sánchez-Muñoz et al., 2013; Handzlik et al., 2019).

Insulin sensitizers/peroxisome proliferator-activated receptor (PPAR) agonists

PPARs are nuclear receptor/transcription factors that regulate insulin action and lipid metabolism. Early RCTs in humans suggested that PPAR agonists are protective against MASH through various mechanisms, including decreasing inflammation and oxidative stress, inducing expression of genes involved in β-oxidation, and improving the secretion of beneficial adipokines (e.g., increasing adiponectin secretion) (Liss and Finck, 2017). The most studied PPAR (selective PPAR-γ) agonist, pioglitazone, is recommended for biopsy-proven MASH (European Association for the Study of the Liver (EASL) et al., 2016; Chalasani et al., 2018; American Diabetes Association Professional Practice Committee, 2021; Cusi et al., 2022; Rinella et al., 2023a) (Table 2).

The extensive body of evidence on pioglitazone argues that this PPAR-γ agonist is an effective option for MASH in patients with and without T2DM (Pennisi et al., 2022). However, pioglitazone reduced liver fibrosis to a significantly greater extent in individuals with T2DM (Bril et
al., 2018). In the landmark “Pioglitazone versus Vitamin E versus Placebo for the Treatment of Nondiabetic Patients with Nonalcoholic Steatohepatitis - PIVENS” phase 3 trial, 34% of pioglitazone (30 mg orally OD) treated patients versus vitamin E versus placebo reached biopsy-proven resolution of MASH, although the difference in the rate of improvement with pioglitazone compared with placebo was not significant (34% and 19%, respectively; p=0.04); given the two planned primary comparisons, p values <0.025 were considered to indicate statistical significance (Sanyal et al., 2010). A recent meta-analysis of 15 RCTs enrolling 4,499 patients (2,714 patients receiving interventions and 1,785 receiving placebo) estimated that pioglitazone 45 mg (OR 6.44, 95% CI: 2.59-16.06) and pioglitazone 30 mg (OR 2.63, 95% CI: 1.30-5.32) were significantly better than placebo for the resolution of MASH (Pennisi et al., 2022). Pioglitazone is also a viable option for combination therapy and is being investigated in several trials as an adjunct therapy to SGLT2is, metformin, and saroglitazar, another dual PPAR-γ agonist (Gawrieh et al., 2021). Pioglitazone 30 mg orally once daily is currently undergoing a phase 3 RCT vs empagliflozin 10 mg orally once daily for 24 weeks to study changes in non-invasive indices as well as liver enzymes as primary outcomes, expected to be completed in November 2024 (Fig. 4, Table 4) (NCT05605158). Compared with placebo, oral saroglitazar 4 mg OD improved LFC in a phase 2 trial (Gawrieh et al., 2021) and proceeded into a phase 3 trial as a combination therapy with vitamin E or monotherapy candidate (NCT04193982) (Fig. 4). While this trial was completed in October 2021 its current status is unknown without any posted results. Currently, there are three ongoing phase 2 RCTs administering saroglitazar magnesium 4 mg for the treatment of MASLD in combination with other diseases, as presented in Table 3 (NCT03617263, NCT05011305, NCT05211284). Another phase 2 RCT investigated the safety, tolerability, and efficacy of saroglitazar 4 mg in post-liver transplant recipients with MASLD and was expected to be completed in June 2023, without posted results yet (NCT03639623) (Table 3).

Since first-generation insulin sensitizers are associated with side effects including bone fractures and edema, a second-generation oral insulin sensitizier MSDC-0602K was designed to target the mitochondrial pyruvate carrier preferentially, while minimizing direct binding to the transcriptional factor PPAR-γ (Harrison et al., 2020a). However, MSDC-0602K given orally OD did not significantly improve liver histology in T2DM or individuals without T2DM with MASH and F1-
F3 fibrosis (Harrison et al., 2020a). The phase 3 study “Study of MSDC-0602K, a Modulator of the Mitochondrial Pyruvate Carrier for Outcomes in Patients With Pre Type 2 Diabetes (T2D) or T2D and NAFLD/NASH, Assessed for ImpRoved Glycemic Control and Cardiovascular Outcomes-1 - MMONARCH-1”, MSDC-0602K, a modulator of the mitochondrial pyruvate carrier, is being given orally OD in subjects with evidence of prediabetes or T2DM and MASLD/MASH and is expected to enroll 1,800 participants with a completion date of September 2024 (NCT03970031) (Fig. 4, Table 4). More recently, a phase 3 RCT has been initiated with the aim of evaluating the efficacy and safety of diosmin, a selective PPAR-γ modulator, in individuals with MASH, without T2DM (NCT05942547) (Fig. 4, Table 4).

Elafibranor acts as an oral agonist of PPAR-α/δ. PPAR-α controls hepatic lipid flux by modulating β-oxidation and fatty acid transport and improves plasma lipids (by decreasing the triglyceride levels and increasing high-density lipoprotein cholesterol) (Pawlak et al., 2015). PPAR-δ agonists improve glucose homeostasis by enhancing insulin sensitivity and inhibiting hepatic glucose output, enhancing fatty acid transport/oxidation, and increasing the levels of high-density lipoprotein cholesterol (Bojic and Huff, 2013). Elafibranor showed modest preclinical and clinical effectiveness on the resolution of MASH but not on the histological resolution of fibrosis, thereby making elafibranor another candidate in the pursuit of effective drug combinations (Makri et al., 2022). Elafibranor, while demonstrating MASH resolution in a phase 2 RCT (Ratziu et al., 2016), failed to meet the primary endpoint of the “Phase 3 Study to Evaluate the Efficacy and Safety of Elafibranor Versus Placebo in Patients With Nonalcoholic Steatohepatitis - RESOLVE-IT” and was subsequently terminated (NCT02704403) (Fu et al., 2022) (Fig. 4).

Lanifibranor acts as an agonist of the three PPAR isoforms (PPAR-α, PPAR-δ, and PPAR-γ), therefore modulating fibrogenesis in addition to metabolic and inflammatory pathways (Francque et al., 2021) (Fig. 3). In the phase 2b trial “NASH Trial to Validate IVA337 Efficacy - NATIVE”, 1,200 and 800 mg orally OD doses of lanifibranor were favored over placebo for resolution of MASH without worsening of fibrosis (49% and 39%, respectively, vs 22%), improvement in fibrosis stage of at least one without worsening of MASH (48% and 34%, respectively, vs 29%), and resolution of MASH plus improvement in fibrosis stage of at least one (35% and 25%, respectively, vs 9%)
Thus, lanifibranor was the first agent to achieve both histological endpoints (Patikorn et al., 2021). Subsequently, lanifibranor was the first drug candidate to receive the “Breakthrough Therapy” designation in MASH since 2015 (Inventiva, 2020). According to a meta-analysis evaluating principal histological outcome measures for MASLD/MASH interventions, lanifibranor (OR 2.57, 95% CI: 1.12-5.88) was significantly better than placebo at ≥1 stage reduction of fibrosis without worsening of MASH (Pennisi et al., 2022). The phase 3 trial, NCT04849728, is ongoing (until September 2026) with no results posted as of yet, but as announced in January 2023, changes in the clinical development of lanifibranor are planned, including a new phase 3 trial in patients with MASH and compensated cirrhosis (Inventiva, 2020) (Fig. 4, Table 4). Besides, an ongoing phase 2 RCT of lanifibranor administration to individuals with T2DM and MASLD is expected to be completed in April 2024 (NCT03459079) (Table 3). Another ongoing phase 2 RCT of lanifibranor alone and combined with empagliflozin is expected to be completed in December 2023 (NCT05232071) (Table 3). In June 2022, a phase 2 RCT examining the effects of MET409, a PPAR-δ agonist, alone or in combination with empagliflozin, was expected to be completed; however, no results have been posted (NCT04702490).

**Pharmacological interventions targeting lipid metabolism**

Statins, known to inhibit the rate-limiting enzyme in cholesterol synthesis, 3-hydroxy-3-methylglutaryl-coenzyme A reductase, are under-prescribed in individuals with MASLD (Athyros et al., 2017), despite their well-known in vitro and in vivo anti-steatosis and anti-inflammatory potential (Horn et al., 2022; Ayada et al., 2023). A possible explanation for this may be the stance of national and international associations for the study of the liver that statins while safe but not beneficial for treating MASLD (Leoni et al., 2018). To this point, recent guideline updates for MASLD management in adults indicate that, in the presence of CV indications, statins are safe to be used in patients with MASH and compensated cirrhosis (Ando and Jou, 2021). A multi-center cohort study of 1,201 European individuals (n=107 subjects using statin) further concluded that statin use was, dose-dependently, associated with protection against liver damage (steatosis, MASH, and fibrosis stage F2-
F4) in individuals at risk of MASH; however, this benefit was limited in some genetic risk variants (Dongiovanni et al., 2015). Relevant histologic benefits were demonstrated in small-subject number biopsy studies (Tzanaki et al., 2022). It has been demonstrated that statins may also prevent MASLD among those with metabolic dysfunction (Lee et al., 2021; Ayada et al., 2023). The most recent meta-analysis found that statin use was significantly inversely associated with the prevalence of MASH (pooled-OR: 0.59, 95% CI: 0.44-0.79) and fibrosis (pooled-OR: 0.48, 95% CI: 0.33-0.70) in the general population (Ayada et al., 2023). Statins further lower the incidence of portal hypertension in cirrhotic MASH (Kim et al., 2017b).

Moreover, reductions in CVD events in comparison with subjects without liver disease have led to recommendations for statin use alone and/or preferably in combination with ezetimibe, the most commonly used nonstatin LDL-lowering drug (see below), or pioglitazone for the primary or secondary prevention of CVD, addressing the fact that CVD is the leading cause of death in individuals with MASLD/MASH (Glen et al., 2016; Athyros et al., 2017; Rinella et al., 2023a).

Notably, a large population-based study included 11,593,409 individuals from the National Health Information Database of the Republic of Korea (2010-2016) and demonstrated that statin use reduced the risk of new-onset MASLD and the risk of significant liver fibrosis once MASLD was developed (Lee et al., 2021). Previously, the prospective intention-to-treat “Greek Atorvastatin and Coronary Heart Disease Evaluation - GREACE” study showed that statin therapy is safe and has the potential to enhance liver function tests and lower the risk of CV problems in individuals with mild-to-moderately abnormal liver tests that may be due to MASLD (Athyros et al., 2010). Similarly, another study comparing atorvastatin with a more moderate regimen with simvastatin showed that the marked CV benefit stemming from intensive lipid-lowering treatment was more prominent in individuals with mildly-to-moderately elevated baseline ALT than those with normal baseline ALT; this study advocated the use of higher statins doses, in high-risk patients, even in cases of moderate liver enzyme elevations (Tikkanen et al., 2013).

Recently, an open-label phase 3 clinical trial set out to compare rosuvastatin 20 mg/day orally with coenzyme Q10 100 mg/day orally on patients with MASH and is expected to be completed in April 2024 (NCT05731596) (Fig. 4, Table 4). The primary outcomes will measure liver stiffness.
measurement and ultrasound score changes at 12 weeks. In the past, a prospective study including 20 biopsy-proven patients with MASH, suggested that rosuvastatin monotherapy improved MASH and resolved metabolic syndrome within one year (Kargiotis et al., 2015). An additional phase 2 RCT evaluating the efficacy of oral idebenone 200 mg, a synthetic analog of coenzyme Q10, in treating MASH, was expected to have been completed in May 2023, has, although results are yet to be posted (NCT04669158).

Ezetimibe, used in the management of hypercholesterolemia (10 mg OD), is an oral selective inhibitor of cholesterol absorption in the gut. Ezetimibe selectively blocks the Niemann-Pick C1-like 1 protein, in the jejunal brush border, which is integral to the uptake of intestinal lumen micelles into the enterocyte (Phan et al., 2012; Fras and Mikhailidis, 2021) and has been well studied in the context of MASLD/MASH with no meaningful histological benefit apparent with ezetimibe monotherapy (Loomba et al., 2015; Noto et al., 2022) (Loomba et al., 2015; Noto et al., 2022); however, combined with a statin, ezetimibe demonstrated reductions in MRI-PDFF-derived liver fat, especially in individuals with T2DM and severe liver fibrosis, as well as improved magnetic resonance elastography-derived measures for liver fibrosis (Cho et al., 2022). Sub-analyses of the effects of ezetimibe when added to statin therapy in the “Improved Reduction of Outcomes: Vytorin Efficacy International Trial - IMPROVE-IT” related to MASLD (as represented by NFS), T2DM and other patient subgroups are discussed elsewhere (Simon et al., 2018; Fras and Mikhailidis, 2021).

Omega-3 polyunsaturated fatty acids, prescribed as an oral supplementation, ameliorate hyperlipidemia through decreased triglyceride and VLDL-C levels. In addition, some studies have proposed that dietary omega-3 polyunsaturated fatty acids ameliorate IR by mediating anti-inflammatory effects and/or regulating mitochondrial function (Pahlavani et al., 2017). Dietary omega-3 polyunsaturated fatty acids also improve IR by mediating anti-inflammatory effects (Lee et al., 2020). Omega-3 has been investigated as monotherapy in two completed phase 3 clinical trials, yet no results have been posted since 2014 and 2015, respectively (NCT01277237, NCT00681408). However, the evidence for polyunsaturated fatty acid supplementation as monotherapy in MASLD management is currently not supported by robust evidence (Glen et al., 2016; Lee et al., 2020; Tzanaki et al., 2022).
Lastly, phosphatidylcholine, a naturally occurring compound, is thought to stimulate fatty acid β-oxidation and inhibit lipogenesis in hepatocytes, as well as contribute to the evacuation of fatty acids followed by their uptake and utilization in muscles (Buang et al., 2005; Gundermann et al., 2016; Maev et al., 2020). A phase 3 clinical trial of phosphatidylcholine supplementation was completed in 2019, with results yet to be posted (NCT04411862) (Fig. 4).

It is envisaged that novel pharmacotherapy targets implicated in MASH and dyslipidemia (for example, adenosine triphosphate citrate lyase), through actions on steatosis, dyslipidemia, and hyperinsulinemia, are expected to be further developed. Ultimately, transition from preclinical studies will eventually allow such approaches to add to the pharmacotherapeutic pipeline of MASLD/MASH (Momtazi-Borojeni et al., 2022; Morrow et al., 2022; Convertini et al., 2023).

Recently, a phase 3 RCT investigates the efficacy and safety of oral administration of two capsules of Orotic Acid Carnitine Complex Capsules (Godex®) (comprising Carnitine Orotate 150 mg, liver extract antitoxic fraction 12.5 mg, Adenine Hydrochloride 2.5 mg, Pyridoxine Hydrochloride 25 mg, Riboflavin 0.5 mg, Cyanocobalamin 0.125 mg, and Biphenyl Dimethyl Dicarboxylate 25 mg) in patients with MRI-PDFF ≥7% (suspected MASLD) expected to be completed in February 2027 (NCT06152991) (Fig. 4, Table 4).

Targeting de novo lipogenesis (DNL)

Firsocostat (GS-0976), a liver-targeted small molecular weight allosteric inhibitor of acetyl-CoA carboxylase, a rate-controlling enzyme in DNL that converts acetyl-CoA to malonyl CoA, has been evaluated in a phase 2 RCT that included 126 patients with MASH and fibrosis (Loomba et al., 2018). Median relative decreases in MRI-PDFF were significantly greater in patients given GS-0976 20 mg orally OD than those on placebo (Loomba et al., 2018). Despite convincing preclinical and preliminary clinical data of GS-0976 as a treatment for MASH, ACC-induced hypertriglyceridemia and subsequent long-term CV safety warrants further investigations (Alkhouri et al., 2020).

The ATLAS study tested firsocostat and cilofexor, an FXR agonist, alone and in combination with each other as well as with selonsertib, an orally bioavailable apoptosis signal-
regulating kinase 1 (ASK1) inhibitor, in participants with bridging fibrosis or compensated cirrhosis due to MASH. None of the treatments were successful (Loomba et al., 2021b).

Arachidyl amido cholanoic acid (aramchol) is an oral (up to 600 mg OD) SCD-1 targeting fatty acid-bile acid conjugate, inhibiting SCD-1, which converts saturated fatty acids into monounsaturated fatty acids (Leikin-Frenkel et al., 2010). SCD-1 downregulation enhances insulin sensitivity and reduces hepatic lipogenesis while promoting lipid oxidation (Ratziu et al., 2021). Recent data from the open-label component of the ARMOR trial demonstrated improvement in fibrosis improvement after at least 48 weeks of aramchol given orally OD in individuals with MASH (Royikoroha, 2022). This reinforced the anti-fibrotic activity of aramchol observed in a phase 3 trial of individuals with MASH, as assessed by NAS, where fibrosis improvement by ≥1 stage without worsening MASH in 29.5% vs 17.5% in the placebo arm and MASH resolution without worsening fibrosis was achieved in 16.7% of aramchol 600 mg OD vs 5%, respectively (Ratziu et al., 2021). The current state of the ARMOR phase 3 clinical trial is suspended as the interim analysis of data from the open-label part has indicated successful achievement of its objectives, and its expected completion date is June 2027; however, the commencement of the double-blind part is facing delays owing to the formulation process of Aramchol Meglumine (NCT04104321) (Fig. 4).

Oltipraz, a synthetic dithiolethione, functions as an antisteatotic agent by inhibiting liver X receptor α activity (Brooks et al., 2009). Oltipraz inhibits fatty acid synthesis through the AMPK-dependent p70 ribosomal S6 kinase-1 pathway and by indirectly mediating increases in the sterol regulatory element-binding protein-1c gene (Kim et al., 2017c). AMPK is a serine/threonine kinase that plays a vital role in regulating fat and carbohydrate metabolism, and it may modulate liver X receptor α activity and decrease expression of the sterol regulatory element-binding protein-1c gene, a key regulator of lipid production (Winder and Hardie, 1999). In a phase 2 trial, 24-week treatment with oltipraz (30 or 60 mg orally twice daily) significantly reduced LFC, demonstrating its antisteatotic efficacy in a dose-response fashion (Kim et al., 2017c). Oltipraz has already completed a phase 3 clinical trial in 2016; however, no results have been posted since then (NCT02068339) (Fig. 4).
Thyroid hormone receptor-β (THR-β) modulators

Resmetirom (MGL-3196) is a liver-directed, orally active, selective THR-β agonist designed to improve MASH by increasing hepatic fat metabolism and reducing lipotoxicity (Harrison et al., 2019; Karim and Bansal, 2023). THR-β is highly expressed in the liver and is essential in reducing cholesterol and triglycerides, promoting liver regeneration, reducing apoptosis, and improving insulin sensitivity (Sinha et al., 2019). Resmetirom is 28-fold selective for THR-β over THR-α, and consequently, this relative selectivity avoids unwanted systemic actions (in bone and heart) of thyroid hormone excess that are largely mediated through THR-α (Kelly et al., 2014; Karim and Bansal, 2023). MASH resolution with no worsening of fibrosis was shown in phase 2 RCTs of once daily, oral administration of MGL-3196 (resmetirom) 80 or 100 mg versus matching placebo (OR 4.5, 95% CI; 1.0-21.9) (Harrison et al., 2019; Patikorn et al., 2021). Also, initiated in June 2023, a phase 2 RCT has studied TERN-501, a TRH-β agonist, as monotherapy or in combination with TERN-101, an FXR agonist, in patients with MASH, without posted results as of yet (NCT05415722). Preliminary data from the MAESTRO-NASH 52-week serial liver biopsy phase 3 study in >950 patients indicates that several non-invasive imaging endpoints (MRI-PDFF and markers of fibrosis) were met when administering resmetirom to individuals with MASH (Harrison et al., 2022). Furthermore, as announced in December 2022, resmetirom achieved both FDA-proposed liver histological improvement endpoints, which could lead to accelerated approval for treating MASH with liver fibrosis (Madrigal Pharmaceuticals, 2023). Currently, there are three ongoing phase 3 clinical trials with resmetirom, i.e., MAESTRO-NASH (NCT03900429), MAESTRO-NAFLD-OLE (NCT04951219), and MAESTRO-NASH-OUTCOMES (NCT05500222) (Harrison et al., 2023a), as presented in more detail in Table 4. Recently, the results of the “A Phase 3 Study to Evaluate Safety and Biomarkers of Resmetirom (MGL-3196) in Non Alcoholic Fatty Liver Disease Patients (MAESTRO-NAFLD1)” phase 3 RCT were published, demonstrating no difference between treatment arms (80 or 100 mg) regarding treatment-emergent adverse events (primary outcome, up to 52 weeks of treatment and four weeks of follow-up), while randomizing 1,143 patients in total (NCT04197479, Harrison et al., 2023b)). Treatment-emergent adverse events observed included diarrhea and nausea at the initiation of treatment in excess of placebo (NCT04197479, Harrison et
al., 2023b)). The main secondary outcomes (included least square means difference from placebo at 80 mg, 100 mg resmetirom) were low-density lipoprotein cholesterol (LDL-C) (−11.1%, −12.6%), apolipoprotein B (−15.6%, −18.0%), triglycerides (−15.4%, −20.4%), 16-week hepatic fat (−34.9%, −38.6%), (p<0.0001) and liver stiffness (−1.02, −1.70) and 52-week hepatic fat (−28.8,−33.9) (NCT04197479, (Harrison et al., 2023b)).

Considering the above, the Institute for Clinical and Economic Review (ICER) has issued a conclusive Evidence Report, stating that the existing evidence for resmetirom sufficiently proves its overall health benefits compared to lifestyle management for MASH (The Institute for Clinical and Economic Review, 2023). According to analyses from the ICER, resmetirom is projected to meet commonly accepted cost-effectiveness thresholds if it is priced within the range of $39,600 to $50,100 per year (The Institute for Clinical and Economic Review, 2023) This pricing assumption is based on the belief that short-term improvements in liver fibrosis will lead to long-term reductions in cirrhosis.

VK2809, another liver-targeted thyromimetic, is a selective small molecular weight activator of the THR-β receptor (Fig. 3). VK2809, a prodrug, undergoes first-pass liver extraction and requires cytochrome P450 cleavage to generate the negatively charged THR-β agonist VK2809A (formerly known as MB07344) (Erion et al., 2007). In a recent phase 2 RCT, VK2809 taken orally in ascending doses of 1.0, 2.5, 5.0, and 10.0 mg significantly reduced LFC (Lian et al., 2020). Currently, VK2809 is undergoing another phase 2 RCT, with the same doses as above, for 52 weeks to study LFC changes in individuals with biopsy-proven MASH (NCT04173065) (Table 3).

Another ongoing phase 2 RCT is studying the THR-β oral agonist, ASC41, among noncirrhotic subjects with MASH, and is evaluating histological changes from baseline to week 52 (NCT05462353) (Table 3). Other emerging THR-β agonists, such as ALG-055009, are currently under development for MASH and are soon expected to proceed to phase 2 RCTs (NCT05090111).

**Targets related to inflammation and immune activation**

Given the fact that TNF-α contributes to IR and liver damage in patients with MASH, the use of pentoxifylline, a competitive non-specific phosphodiesterase inhibitor and blocker of TNF-α...
production, has been considered expedient (Adams et al., 2004). The anti-inflammatory properties of pentoxifylline (orally three times a day)/400 mg/day were thus evaluated for efficacy in MASH as a monotherapy (Mahjoubin-Tehran et al., 2021), and most notably, in an earlier phase 2/3 trial, where no significant changes in ALT and liver histology were determined compared with placebo (Van Wagner et al., 2011). Fibrosis improvement was observed in a greater proportion (35%) of patients in the pentoxifylline-treated group compared with placebo (15%); however, it did not reach statistical significance (Zein et al., 2011). More recently, a phase 3 clinical trial of 50 participants with MASH investigating pentoxifylline as monotherapy was completed in October 2022, but results are not yet available (NCT05284448) (Fig. 4).

Ongoing phase 2 clinical trials are currently targeting a wide spectrum of inflammatory pathways (Table 3). Notably, studies of patients with biopsy-proven MASLD/MASH and fibrosis are investigating the properties of JKB-12, a long-acting toll-like receptor 4 antagonist, rencofilstat, a cyclophilin inhibitor, and norursodeoxycholic acid, a sidechain shortened derivative of UDCA, all having MASH resolution as their primary outcome (NCT04255069, NCT05402371, NCT05083390).

Previously, the anti-inflammatory, antioxidant, and anti-fibrotic properties of UDCA have been tested in individuals with MASH; however, UDCA therapy, while effectively reducing serum ALT and GGT activities, does not necessarily lead to significant effects on liver histology (Traussnigg et al., 2019; Yoon et al., 2021; Lin et al., 2022). Furthermore, UDCA was shown to improve lipid profile and hepatic steatosis independent of BW loss, in addition to a positive effect on surrogate markers of atherosclerosis (Nadinskaia et al., 2021). Interestingly, the anti-inflammatory effects of 81 mg of aspirin have been studied for changes in intrahepatic lipid content, as quantified by 1H magnetic resonance spectroscopy, in a completed phase 2 trial of biopsy-proven early-stage liver fibrosis among individuals with MASLD; however, no results have been posted yet (NCT04031729).

ASK1 is involved in mitogen-activated p38 kinase and c-Jun N-terminal kinase signaling pathways, which play important roles in inflammation and fibrosis, promotion of lipotoxicity affecting, especially myofibroblasts, macrophages, and hepatocytes (Hayakawa et al., 2012). However, selonsertib, a selective inhibitor of ASK1, was ineffective in preventing cirrhosis (Harrison et al., 2020c; Patikorn et al., 2021).
Targets related to cell death (apoptosis and necrosis)

The A3 adenosine receptor is overexpressed in pathological liver cells (Fishman et al., 2019). Namodenoson orally 25 mg every 12 h, a selective agonist of the A3 adenosine receptor, and FM101, a highly selective A3 adenosine receptor modulator, are currently undergoing phase 2 clinical trials, for the pharmacotherapy of MASH. These trials have the number of adverse events as their primary outcomes (NCT04697810 to be completed in 2025, NCT04710524 was expected to be completed in August 2023) (Table 3). Namodenoson induces a robust anti-inflammatory effect in the liver, mediated via the de-regulation of the Wnt/β-catenin pathway, while FM101 is a biased ligand with functional activities both as a G protein agonist and a β-arrestin antagonist (Park et al., 2020). Further, a recently initiated phase 2 clinical trial is investigating the role of dasatinib, a European Medicines Agency (EMA)-approved (for leukemia) inhibitor of several targets, including BCR-ABL, SRC family (SRC, LCK, YES, FYN), c-KIT, EPHA2, and PDGFRβ, plus quercetin, a quinone reductase 2 inhibitor, in senescence in MASLD-related fibrosis (NCT05506488) (Table 3).

The oral pancaspase inhibitor emricasan (inhibits caspase 1, which mediates the generation of the inflammasome and initiates the inflammatory cascade, and inhibits caspase 8, which induces hepatocyte apoptosis) failed to decrease clinical events in patients with decompensated MASH-cirrhosis and affect MELD scores. In a separate study in MASH fibrosis stage F1-F3, emricasan 5 or 25 mg administered orally twice a day did not improve liver histology and may have worsened fibrosis and ballooning (Harrison et al., 2020b; Frenette et al., 2021).

Targets related to fibrogenesis and collagen turnover

Inhibition of galectin-3, a previously investigated novel liver anti-fibrosis target, is appealing in individuals with MASH as galectin-3 appears to be crucial in the process of liver fibrosis (Kram, 2023). Galectin-3 is required for transforming growth factor-β mediated myofibroblast activation and extracellular matrix production, and its expression is up-regulated in established fibrotic liver disease (Henderson et al., 2006). Currently, the infusible galectin-3 inhibitor, belapectin, is undergoing a phase 3 clinical trial to determine the proportion of patients with MASH developing new esophageal...
varices as the primary outcome, whereas cirrhosis-related events and changes in liver stiffness are also measured (NCT04365868) (Fig. 4, Table 4). In this study, belapectin, 2 mg/kg lean body mass, is administered intravenously every other week (NCT04365868). Previously, belapectin evaluated in patients with cirrhosis was not associated with a significant reduction in HVPG or fibrosis when compared with placebo in a phase 2b study of 162 patients with MASH (Chalasani et al., 2020). However, in a subgroup analysis of patients without esophageal varices, belapectin infusion reduced HVPG and the development of varices (Chalasani et al., 2020).

FGF-21, an essential regulator of energy metabolism in the liver, is implicated in bile acid pathways (Tillman and Rolph, 2020) (Fig. 3). FXR activation and PPAR-α induce hepatic expression and secretion of FGF-21 (Cyphert et al., 2012). There is a close relationship between circulating FGF-21 levels and LFC, and consequently, aberrant FGF-21 signaling is an important pathophysiological step in the development and progression of SLD (Tucker et al., 2019). Also, in a recent genome-wide association and Mendelian randomization study, FGF-21 demonstrated a strong causal effect on enhanced lipid profile, decreased alcohol consumption and C-reactive protein concentrations, and liver function biomarkers, including fibrosis (Larsson et al., 2022). Pegbelfermin (PGBF/BMS-986036), a subcutaneously administered PEGylated FGF-21 analog, regulates energy balance, insulin sensitivity, and lipid metabolism (Sanyal et al., 2018) when given in daily or weekly regimens. In a phase 2a study in patients with MASH and stage 1-3 fibrosis (NCT02413372), treatment with PGBF was associated with significant reductions in hepatic steatosis measured by MRI-PDFF and improvements in biomarkers of fibrosis (Sanyal et al., 2018). The “Pegbelfermin in Patients With Nonalcoholic Steatohepatitis and Compensated Cirrhosis - FALCON 2” trials were designed to assess 48-week PGBF treatment in MASH and stage 3 fibrosis or cirrhosis (Abdelmalek et al., 2021). In the phase 2 RCT “Pegbelfermin in Patients With Nonalcoholic Steatohepatitis and Stage 3 Fibrosis - FALCON 1”, MASH improvement without worsening of fibrosis was achieved in 24-31% of subjects across the 10, 20, and 40 mg dose arms compared with placebo (14%); however, the trial did not meet the primary endpoint because of a lack of dose-dependent response rate differentiation (Abdelmalek et al., 2021; Brown et al., 2023). Four other genetically engineered variants of FGF-21 are currently undergoing phase 2 clinical trials for the pharmacotherapy of MASH (NCT04880031, NCT04929483,
NCT05039450, NCT04767529) (Table 3). Another ongoing phase 2 study, initially expected to be completed in 2023, was investigating the properties of MK-3655 that can activate the FGF-21 receptor complex through selective activation of the β-Klotho/FGFR1c receptor complex (NCT04583423). This trial has, however, been terminated for business reasons (NCT04583423). Besides, a phase 2 clinical trial, completed in January 2022, investigated the safety and effects of pegazafermin (BIO89-100), a long-acting engineered glycopegylated recombinant FGF-21 agonist, in subjects with biopsy-confirmed MASH or MASLD, and those at high-risk of MASH; however, no results have been posted yet (NCT04048135). Recently, the results of the phase 2b RCT of subcutaneous pegazafermin administration of 15 mg or 30 mg weekly or 44 mg every two weeks in individuals with biopsy-confirmed MASH and a fibrosis stage F2 or F3, “Study Evaluating the Safety, Efficacy and Tolerability of BIO89-100 in Subjects With Biopsy-confirmed Nonalcoholic Steatohepatitis (NASH) (ENLIVEN)” were published, demonstrating significant improvement in fibrosis in all treatment groups compared to placebo, without worsening of MASH (NCT04929483) (Loomba et al., 2023b). These results also support a benefit regarding MASH resolution without worsening of fibrosis (Loomba et al., 2023b). Even though other nonclinical studies with FGF-21 analogs and early clinical studies with two FGF-21 analogs have reported effects on bone turnover, this RCT did not identify any evidence for reduced bone mass density or fractures (Talukdar et al., 2016; Kim et al., 2017a; Rader et al., 2022; Loomba et al., 2023b). Currently, efrouxifermin (50 mg, subcutaneous injection) is the only FGF-21 analog undergoing a phase 3 RCT estimated to enroll 600 individuals with suspected or confirmed diagnosis of NASH/MASH or NAFLD/MASLD or non-invasively diagnosed NASH/MASH or NAFLD/MASLD and the expected completion date is October 2026 (Fig. 4, Table 4) (NCT06161571).

FGF-19 has a role in the de novo synthesis and export of triglycerides from the liver, fatty acid oxidation, inflammation, steatotic endoplasmic reticulum stress, and cytotoxicity (Sciarrillo et al., 2021) (Fig. 3). Patients with SLD tend to have lower levels of circulating FGF-19 (Alisi et al., 2013). Furthermore, patients with IR in the context of MASLD display hepatic resistance to FGF-19, which could exacerbate SLD progression (Alisi et al., 2013). Aldafermin, an FGF-19 analog administered as a daily subcutaneous injection, failed to meet the primary endpoint of improved liver
fibrosis of at least one stage with no worsening of MASH in the evaluation of efficacy, safety, and tolerability of aldafermin in a phase 2b, randomized, double-blind, placebo-controlled, multicenter study in subjects with stage 2/3 fibrosis (“Aldafermin in patients with non-alcoholic steatohepatitis - ALPINE 2/3”; (Harrison et al., 2022a). Aldafermin also underwent a phase 2 clinical trial, “Study of Aldafermin (NGM282) in Subjects With Compensated Cirrhosis - ALPINE 4”, that was completed in February 2023 but is currently without posted results (NCT04210245).

The enzyme Lysyl Oxidase-Like 2 (LOXL2) mediates collagen crosslinking and fibrotic matrix stabilization during hepatic fibrosis (Ikenaga et al., 2017). Inhibition of LOXL2 can decrease cell numbers, colony formation, cell growth, and proliferation, as well as increasing apoptosis (Puente et al., 2019). Simtuzumab, a monoclonal antibody against LOXL2, was studied in subjects with compensated cirrhosis secondary to MASH. Subcutaneous injections of simtuzumab (75 or 125 mg) given weekly were, like all other pharmacological options assessing this clinical endpoint, ineffective in decreasing hepatic venous pressure gradient (Harrison et al., 2018).

**Other targets**

**Farnesoid X receptor (FXR)**

FXR exhibits crucial effects in the regulation of fat homeostasis and has been extensively studied for the pharmacotherapy of MASH (Adorini and Trauner, 2023). Activation of FXR leads to lower lipid levels as it suppresses DNL and oxidation, increases triglyceride hydrolysis and clearance, and decreases VLDL secretion from the liver (Stofan and Guo, 2020). The bile acid derivative 6-ethylchenodeoxycholic acid (obeticholic acid [OCA]) is a potent activator of the nuclear FXR that has been reported to benefit histological features in MASH (Li and Chiang, 2014; Neuschwander-Tetri et al., 2015). The histological endpoint for FDA approval of ≥1-point improvement in fibrosis with no worsening of MASH was demonstrated in patients treated with OCA (RR 1.9, 95% CI 1.4-2.8) (Patikorn et al., 2021), leading to the clinical trial “Randomized Global Phase 3 Study to Evaluate the Impact on NASH With Fibrosis of Obeticholic Acid Treatment - REGENERATE” the first and, so far, the only phase 3 RCT to reach this point (Younossi et al., 2019c). Improvement in fibrosis as an endpoint was achieved in 37 (12%) patients in the placebo group, 55 (18%) in the OCA 10 mg group...
(p=0.045), and 71 (23%) in the OCA 25 mg group (p=0.0002). However, the resolution of MASH as the endpoint was not achieved (Younossi et al., 2019c). According to a meta-analysis of 15 RCTs, OCA had the highest probability of achieving the desired ≥1 stage of fibrosis improvement without worsening of MASH (Pennisi et al., 2022). Based on an interim analysis of REGENERATE at 18 months, dynamic changes in selected non-invasive testing separated histologic responders from non-responders, suggesting that non-invasive testing may be useful in assessing histologic response to OCA therapy (Rinella et al., 2022). Another phase 3 clinical trial, “Study Evaluating the Efficacy and Safety of Obeticholic Acid in Subjects With Compensated Cirrhosis Due to Nonalcoholic Steatohepatitis - REVERSE”, evaluated the efficacy and safety of OCA in subjects with compensated cirrhosis due to MASH and was completed in September 2022, although the results have been submitted to ClinicalTrials.gov and await Quality Control Review (NCT03439254) (Fig. 4). Currently, there is phase 3 clinical trial of OCA therapy for patients with biopsy-proven MASH which was initially expected to be completed in 2025 but has already been completed in September 2023; however, without posted results yet (NCT02548351) (Fig. 4).

The ICER concluded that, based on the assumption that short-term improvements in liver fibrosis will lead to long-term reductions in cirrhosis after OCA therapy, OCA would be considered cost-effective if it was priced in the range of $32,600 to $40,400 per year (The Institute for Clinical and Economic Review, 2023).

Tropifexor is a highly potent synthetic non-bile acid FXR agonist. Treatment with tropifexor (10-90, 140 or 200 µg, orally) led to a significant dose-dependent decline in serum ALT activity from baseline and liver fat content (measured by MRI-PDFF) compared with placebo, which was sustained for up to 48 weeks of treatment (Sanyal et al., 2023). However, tropifexor monotherapy failed to achieve improvement in rates of resolution of MASH and is now being investigated in a combination therapy approach with a CCR 2/5 inhibitor, cenicriviroc, for potential antifibrotic activity (NCT03517540) (Pedrosa et al., 2020). A similar combination therapy approach with cilofexor, a nonsteroidal FXR agonist, was evaluated for its effects in patients with noncirrhotic MASH in a phase 2 RCT, where it provided significant declines compared with placebo in MRI-PDFF-measured hepatic steatosis, liver biochemistry, and serum bile acids (Patel et al., 2020).
The novel oral FXR agonist, EDP-305, appears to be providing the rationale for a longer-term trial assessing histological endpoints in patients with MASH based on its ability to reduce ALT levels and LFC (Ratziu et al., 2022) (NCT03421431) (Table 3). In addition, two new FXR agonists, HPG1860 and CS0159, have completed phase 2 clinical trials for the pharmacotherapy of MASH without posted results yet; the first was completed in March 2023 and the latter in November 2023, respectively (NCT05338034, NCT05591079). Similarly, other FXR agonists, including MET642 and HEC96719, have completed phase 2 trials without posting results as of yet (NCT04773964, NCT05397379, respectively). The study's sponsor terminated a previously ongoing phase 2 RCT, “A Randomized, Double-blind Study to Assess the Safety and Efficacy of EDP-305 in Subjects With Liver-biopsy Proven NASH - ARGON-2” of EDP-305, an FXR agonist, to prioritize combination treatment approaches (NCT04378010). The sponsor mentioned that this decision was not based on safety concerns (NCT04378010).

Targeting chemokine receptors

Antagonists of chemokine receptor CCR2/5 can improve fibrosis by altering hepatic macrophage subsets and reducing monocyte infiltration (Puengel et al., 2022). Cenicriviroc, an oral CCR 2/5 inhibitor targeting the steatotic, inflammatory, and/or fibrotic pathways, was evaluated in the CENTAUR RCT. In this phase 2b clinical trial, histological improvement in fibrosis of >1 point with no worsening of MASH was shown in patients treated with cenicriviroc (OR 2.2, 95% CI: 1.1-4.3) (Ratziu et al., 2020; Patikorn et al., 2021). The majority of patients treated with cenicriviroc who achieved the set fibrosis response at year one maintained the improvement at year two, with greater effects observed in advanced fibrosis (Ratziu et al., 2020). A phase 3 evaluation for the treatment of subjects with MASH F2/F3 fibrosis (“A Study for the Efficacy and Safety of Cenicriviroc (CVC) for the Treatment of Liver Fibrosis in Adults With Nonalcoholic Steatohepatitis - AURORA”; NCT03028740) was terminated early due to lack of efficacy based on the results of the planned interim analysis.
Antioxidants, vitamins, and supplements

Generally, antioxidants are molecules that neutralize free radicals. Febuxostat, a non-purine-selective inhibitor of xanthine oxidase, is undergoing a phase 2 clinical trial for the pharmacotherapy of MASLD/MASH (NCT05574036) (Table 3).

Vitamin E as a treatment option for MASH or MASLD has been explored in two large RCTs (Sanyal et al., 2010; Lavine et al., 2011). A high vitamin E dosage (533.6 mg/day [800 IU] for 96 weeks) was found to be superior to placebo for the treatment of MASH in adults without diabetes (Sanyal et al., 2010) in the “Pioglitazone versus Vitamin E versus Placebo for the Treatment of Nondiabetic Patients with Nonalcoholic Steatohepatitis - PIVENS” trial, more precisely, the rate of improvement in MASH was 43% vs 19%, respectively; (p=0.001). According to a meta-analysis of high-quality RCTs, including a subset of individuals with T2DM, vitamin E therapy significantly improved steatosis, hepatocyte ballooning, and lobular inflammation while not significantly improving fibrosis (Said and Akhter, 2017). Previously, vitamin E was investigated as a combination therapy with low-dose spironolactone (mineralocorticoid-receptor antagonist), to ameliorate its dose-dependent side effects (Polyzos et al., 2017). In a recent network meta-analysis, a combination regimen of pioglitazone and vitamin E had the highest probability of being the most effective therapeutic regimen for achieving the FDA requirement of MASH resolution without worsening fibrosis (Pennisi et al., 2022).

Currently, there are four ongoing phase 2 clinical trials investigating the efficacy of vitamin E, with one study including tocotrienol, which belongs to the vitamin E family, in the pharmacotherapy of MASLD/MASH (NCT05573204, NCT05574036, NCT04801849, NCT02581085) (Table 3). Additionally, a phase 2 clinical trial examining vitamin D3 in patients with a steatotic liver diagnosis determined by ultrasound and T2DM was completed in April 2023 without posted results yet (NCT05578404). Recently, a phase 3 clinical trial of patients with ultrasound-diagnosed MASLD taking vitamin D3 as a single oral dose of cholecalciferol 300,000 IU followed by oral cholecalciferol 800 IU daily for four months was completed without posted results to date (NCT05613192) (Fig. 4, Table 4).
An additional phase 3 clinical trial has been initiated to investigate the effects of the supplement N-acetylcysteine, typically used to treat paracetamol overdose and to loosen thick mucus in individuals with chronic bronchopulmonary disorders, on leptin as an IR marker in individuals with MASLD (NCT05589584) (Fig. 4). This trial is expected to be completed in December 2023 and assesses the effect of N-acetylcysteine on leptin as the primary outcome.

**Antihypertensive agents**

The role of the α-2 adrenergic receptor agonist, Guanabenz (WY-8678), in the pharmacotherapy of MASLD/MASH has been investigated in a phase 2 clinical trial completed in June 2023 (NCT05084404); results are not yet available.

Lisinopril, an oral angiotensin-converting enzyme inhibitor, is currently undergoing a phase 2 clinical trial of patients with MASH determined by imaging, having a Fibroscan liver stiffness of ≥12 kPa, with multiple MASLD/MASH-related outcomes (NCT04550481), were completed in October 2023, without posted results yet.

The mineralocorticoid receptor antagonist spironolactone was previously examined in a combination regimen with vitamin E, showing a significantly decreased MASLD liver fat score (Polyzos et al., 2017). A phase 2 clinical trial of patients with biopsy-proven MASH was completed in July 2023 and investigated the effects of 100 mg orally OD spironolactone on changes in liver stiffness as determined by magnetic resonance elastography, without posted results yet (NCT03576755).

**Plant-based agents, prebiotics, probiotics, and synbiotics**

Several plant-based agents and food derivatives (e.g., caffeine) have been examined in preclinical and clinical studies for their potential hepatoprotective effects; however, reliable clinical studies in the context of MASLD are limited (Salvoza et al., 2022; M et al., 2023) and robust trials are required to appropriately address their safety (Rizzo et al., 2023). The hepatoprotective properties of silymarin, an effective antioxidant with supposedly antifibrotic actions, have historically been
attributed to this milk thistle field extract (Salvoza et al., 2022). However, the effect of silymarin on patients with MASH remains inconclusive (Wah Kheong et al., 2017; Navarro et al., 2019).

Curcumin, a plant-based polyphenol compound with reported anti-inflammatory and wide-ranging actions, has been shown to exhibit favorable effects on liver ultrasonographic findings and liver enzymes (Ngu et al., 2022).

Prebiotics, probiotics, and synbiotics are likely to be effective treatments for adult patients with MASLD while improving microbiota composition. Although AST and ALT cannot exactly define the severity of MASLD, unlike the results from biopsy or imaging tests, they are important indicators that can monitor the status of the disease and provide benefits for clinical management (Kanchanasurakit et al., 2022). Xue et al. designed a phase 4 study to determine the effect of consecutive fecal microbiota transplantation on liver fat accumulation as measured by MRI. In this 12-week study, 20 patients will be randomized for infusion of allogenic (lean donor) or autologous feces (NCT04465032). It is hoped that the long-term efficacy of fecal microbiota transplantation in treating MASLD will be determined by the quality of research (Jieyi Wang et al., 2022). Currently, two phase 2 clinical trials, expected to be completed in 2024 and 2025, are investigating the role of fecal microbiota transplantation in MASLD and MASH (NCT04371653, NCT05622526) (Table 3).

**Growth Hormone**

Growth hormone exerts effects on white AT lipolysis that result in impairment of hepatic and peripheral insulin sensitivity (Doycheva et al., 2022). The non-peptide agonist of the ghrelin receptor and a growth hormone secretagogue, ibutamoren 25 mg OD orally, is currently undergoing a phase 2 clinical trial of obese or overweight patients with MASLD/MASH determined by liver biopsy, measuring changes in intrahepatic lipid content by 1H magnetic resonance spectroscopy as the primary outcome (NCT05364684) (Table 3). Similarly, a phase 2 clinical trial of patients with MASH determined by biopsy or 1H magnetic resonance spectroscopy is studying the efficacy of tesamorelin (2 mg OD subcutaneously), a synthetic analog of human growth hormone-releasing hormone, with the
same primary outcome as the above-mentioned study and both are expected to be completed in December 2024 (NCT03375788) (Table 3).

**RNA interference**

The recognition of the genetic basis of MASLD provides the stepping-stone for initiating new therapeutic approaches (Fig. 3). Herein, we provide only limited coverage of this topic due to a recent comprehensive review summarizing liver targeting with nucleic acid therapeutics in the same journal (Gogate *et al.*, 2023). For instance, gene silencing techniques are enabled by degrading targeted mRNAs using interfering RNA (Valenzuela-Vallejo *et al.*, 2023b; Sachan *et al.*, 2024). Notably, HSD17B13 expression appears to be an appropriate target for the development of drugs to treat MASLD since it facilitates the progression of MASLD by directly stabilizing the intracellular lipid droplets and by indirectly activating hepatic stellate cells (Wang *et al.*, 2022). Heat shock proteins contribute significantly to fibrosis-based disorders, including liver fibrosis, by boosting collagen synthesis and deposition (Abd El-Fattah and Zakaria, 2022). Two novel RNAi drugs, GSK4532990, inhibiting the expression of 17 hydroxysteroid dehydrogenase, and BMS-986263, degrading HSP47 mRNA, are currently undergoing two phase 2 clinical trials in patients with MASH and NAS ≥4 and are expected to be completed in December 2025 and December 2026, respectively (NCT05583344, NCT04267393) (Table 3). These nucleic and molecular techniques appear to be promising and exhibit enormous potential for targeted pharmacotherapies.

**Other ungrouped emerging agents**

Blockade of leaked bacterial endotoxin and improved intestinal permeability via the laxative drug lubiprostone, which selectively stimulates type 2 chloride channels in epithelial cells thereby causing an efflux of chloride into the intestinal lumen, has been presented as a therapeutic strategy for MASLD (Kessoku *et al.*, 2020). A phase 3 clinical trial of patients with MASLD, as determined by ultrasound or computed tomography, has recently been completed and utilized lubiprostone 24 μg
orally, investigating its effects on ectopic fat as quantified by changes measured with MRI-PDFF and FibroScan with CAP in one year (NCT05768334) (Fig. 4).

Another phase 3 clinical trial of postmenopausal 45-70-year-old women with MASH determined by biopsy or MASLD by imaging, expected to be completed in May 2027, is currently investigating the properties of a cutaneous patch providing 100 μg of estradiol daily (NCT04833140) (Fig. 4, Table 4). Specifically, estradiol, which binds to estrogen receptors alpha and beta, is expected to reduce the degree of liver fibrosis (Klair et al., 2016; Kim et al., 2023) (NCT04833140).

Leptin is a targeted pharmacotherapy for situations of lipoatrophy with leptin deficiency and requiring leptin replacement therapy (Kokkorakis et al., 2023c). Specifically, leptin therapy in the form of recombinant-human-methionyl-leptin metreleptin, administered by subcutaneous injections for 12 months, was examined in common forms of MASLD (NCT01679197), on the basis that recombinant leptin therapy reverses MASH in leptin-deficient lipodystrophy (Akinci et al., 2021). MASH scores from paired liver biopsies in patients with MASH associated with both relative leptin deficiency and partial lipodystrophy have reductions in hepatic steatosis and injury in response to exogenous leptin therapy, in addition to promoting fat deposition (Akinci et al., 2021).

Additional ongoing phase 2 clinical trials of unknown or other pathways for the pharmacotherapy of MASLD/MASH are presented in Table 3.

Safety and tolerability issues

Relatively high rates of adverse events have been experienced by patients receiving several of the MASLD/MASH drug candidates, as well as in individuals with MASLD receiving a placebo (Said and Akhter, 2017; Kim et al., 2017c; Loomba et al., 2018; Chen et al., 2022). Since the SLD spectrum encompasses many generations of patients, age considerations should be taken into account, and most notably, with young women of reproductive age, fetal safety must be considered (El Jamaly et al., 2022). The prevalence of MASLD in pregnancy has nearly tripled in the last decade, and due to maternal complications MASLD should be considered a high-risk obstetric condition (Sarkar et al., 2020). At the other end of the age spectrum – in the elderly, age-related changes in body composition, such as sarcopenia, accompanied by aggravated IR and metabolically unfavorable ectopic fat...
deposition, (Eslam, et al., 2020a) represent additional important factors that are also associated with MASLD (Lee et al., 2015).

Liver and renal function considerations

Current data support a favorable safety profile of elafibranor; however, reversible serum creatinine elevations occur with its use (Ratziu et al., 2016). Renal function will most certainly be an important consideration with MASLD treatment since comorbidities (T2DM, obesity) impact CKD risk in individuals with MASLD/MASH. Liver drug metabolism is subject to alterations in MASLD, potentially increasing adverse reactions due to significant alterations in the hepatic enzyme activity (Merrell and Cherrington, 2011).

Cardiovascular safety

The increased prevalence of CVD in patients with MASH dictates the need for pharmacological treatment of MASH should not further increase CVD risk. In general, pharmacological interventions used to treat MASH should confer a neutral or preferably beneficial cardiometabolic effect. Assessment of the impact of therapy on cardiometabolic risk factors should be included as secondary endpoints in phase 2 and phase 3 trials (Rinella et al., 2019). Of note, it has been suggested that decreased NAS is associated with an improved CVD risk (Rinella et al., 2019).

Bile acid-derived FXR agonist OCA and acetyl-CoA carboxylase inhibitors are associated with increases in total and LDL-C and triglyceride levels, respectively (Neuschwander-Tetri et al., 2015; Loomba et al., 2018). Therefore, a priority will be to develop non-bile acid FXR agonists (e.g., tropifexor, also known as LJN452) with improved pharmacokinetics and tolerability (Badman et al., 2020). Since FXR activation influences plasma lipoprotein concentrations, the CONTROL study demonstrated that atorvastatin 10 mg/day added to OCA decreased the LDL-C to below baseline as early as four weeks after initiation (Pockros et al., 2019). Statins reduce CVD risk in individuals with MASLD with dyslipidemia, and their use is safe and recommended (Athyros et al., 2017; Eslam et al., 2020c). Notably, the novel proprotein convertase subtilisin/kexin type 9 inhibitors represent an
alternative pharmacological approach targeting plasma LDL-C concentrations, with reassuring recent data regarding hepatic safety in MASLD (Zhou et al., 2022) (Rimbert et al., 2021).

Currently, in addition to FXR agonists and acetyl-CoA carboxylase inhibitors, saroglitazar and lanifibranor offer benefits in MASLD, but vigilance against CVD risk is warranted (Zhou et al., 2022). With vitamin E use, a potential increase in all-cause mortality and hemorrhagic stroke was reported, in addition to increased prostate cancer risk (Hadi et al., 2018).

Hepatocellular carcinoma (HCC)

MASLD-associated HCC has shown a steep increase in prevalence and is becoming the leading form of HCC in the developed world (Younossi et al., 2019a), while its prognosis is generally poor since it presents at an advanced stage in older patients with co-morbidities (Geh et al., 2021). Consequently, any novel MASLD/MASH therapy that could potentially interfere with the progression to HCC would offer added benefits to the anticipated MASH/fibrosis regression. As with all stages of MASLD, in HCC prevention, dietary restriction and exercise are recommended (Gutiérrez-Cuevas et al., 2022; Jeong, 2023). One of the agents frequently reported to have anti-tumor effects is metformin, an insulin sensitizer with multiple molecular mechanisms, that has been studied in the context of MASLD but has not been associated with MASH-histologic improvement (Said and Akhter, 2017). However, in a large case-control study, metformin use resulted in a 7% reduction in the risk of HCC for each incremental year in individuals with T2DM (Chen et al., 2013). Statin use in patients with MASH cirrhosis was associated with a lower risk of developing HCC (hazard ratio [HR] 0.40, 95% CI: 0.24-0.67, p=0.001) in a dose-dependent manner (Pinyopornpanish et al., 2021).

The therapeutic use of FGF-19 analogs could be limited by the potential for ectopically produced FGF-19 to induce HCC; furthermore, OCA could increase serum FGF-19 concentrations (Samuel and Shulman, 2018). However, the FXR agonists were found to suppress HCC in cell culture (Orabi et al., 2021).

Pruritus
Mild pruritus occurs early after bile acid-derived FXR agonist OCA therapy is initiated, while not worsening over time (Neuschwander-Tetri et al., 2015). However, pruritus was also noted in placebo-treated patients with MASH (Neuschwander-Tetri et al., 2015). The mechanism of pruritus induction by FXR agonists supposedly reflects direct or indirect activation of the G-protein coupled bile acid receptor, Takeda G-protein-coupled receptor 5, or alternatively could involve the production of an endogenous pruritogenic metabolite or bile acid (Badman et al., 2020).

**Future perspective and lessons learned from challenges of the trials in the steatotic liver disease pipeline**

The diseases associated with the SLD spectrum represent a global healthcare challenge and demand attention from the public to diverse scientific communities and policymakers (Boutari et al., 2023). A generalized acknowledgment will likely aid in promoting the role of MASLD in national action plans (Lazarus et al., 2022b). Improved recognition and dialogue between experts could be facilitated by an appropriate and more specific disease classification (Valenzuela-Vallejo and Mantzoros, 2022). Therefore, multiple groups have proposed the replacement of NAFLD’s (currently MASLD) nomenclature (Eslam et al., 2020a; Hill et al., 2021; Méndez-Sánchez et al., 2022; Valenzuela-Vallejo and Mantzoros, 2022), which led to the recently voted and approved introduction of SLD. Extending this definition, our group proposed a pathophysiology-based umbrella definition of SLD and its phenotypic subclassification for a more personalized and precise pharmacological approach (Fig. 1) (Kokkorakis et al., 2023a).

Identifying and developing effective pharmacotherapeutics for MASLD/MASH has been hindered by many factors, including the complex biology underlying SLD, which needs to be further unraveled to deliver more treatment options. It is possible that a reason for discontinued clinical trials is that targeting inflammation and fibrosis focuses on processes distal in the pathophysiological cascade (Vuppalanchi et al., 2021), instead of the initial lipotoxic injury to hepatocytes. This is in line with the growing popular opinion that targeting only one of the parallel mechanistic pathways is not sufficient, which is further evidenced by the initially promising agents (e.g., elafibranor, cenicriviroc, selonsertib, belapectin), that successfully performed in early phase studies only to subsequently fail in
phase 3 clinical trials (Rinella and Noureddin, 2020). Consequently, combination therapies or monotherapy with multimodal targeting and pleiotropic effects (analogous to metabolic surgery success (Truong and Noureddin, 2022) or GLP-1 RAs) are most promising. Furthermore, an individualized approach will be needed to evaluate person-specific pathogenesis and risk factors, including an individual’s genetic signature – approaching precision medicine (Ramos et al., 2022).

Importantly, combination therapies offer another advantage, i.e., a lower dose of an individual agent and consequently less chance of adverse events (Makri et al., 2022).

Despite the advantages of oral drug delivery, the various biological barriers in the gastrointestinal tract reduce the bioavailability of several drugs used for the SLD disease spectrum. Therefore, injectables are frequently required. Furthermore, nanoparticles are being actively explored as viable options since they avoid their premature release before reaching the liver by incorporating small molecules and nucleic acid therapeutics (Moosavian et al., 2021; Salunkhe et al., 2021).

Currently, unsuccessful phase 3 studies should be carefully studied and used to shepherd the development of established requirements for the progression of MASLD/MASH drug candidacy to advanced trial stages (Fig. 4). In addition to the in-depth and realistic interpretation of preliminary data, the chances of failure can be minimized by following the design of a successful placebo-controlled phase 2b study and studying a similar population in phase 2 as in phase 3 (Rinella and Noureddin, 2020). The so far ubiquitous failure of phase 3 agents has been suggested to be irrespective of the endpoint, whether clinical, histological, or HVPG outcome-driven (Chalasani et al., 2020; Garcia-Tsao et al., 2020; Harrison et al., 2020b; Harrison et al., 2022a). We speculate that adding cardiometabolic outcomes and pronounced BW loss (>10%) to the spectrum of requirements for MASLD/MASH pharmacological interventions could provide extrahepatic benefits and improve overall clinical outcomes (Polyzos et al., 2020; Vuppalanchi et al., 2021; Kokkorakis et al., 2023c).

Drug repositioning, i.e., assessment of existing available drugs, is a time-efficient and appealing option in the MASLD/MASH pipeline since the pharmacological options already approved for the treatment of comorbidities are known for their pleiotropic effects, and at the same time, their long-term safety and efficacy have already been studied. This has already been recognized and explored in the combination therapies regiments, for example, with GLP-1 RAs, PPAR agonists, SGLT2is,
Another challenge to be overcome is the development of an animal model that reliably replicates the MASLD pathobiology and disease progression, ensuring the transfer of preclinical results that may offer a more solid foundation for clinical trials that are currently plagued by failing to meet required endpoints (Drenth and Schattenberg, 2020). Another complicating factor is that the development pathway for MASH treatment appears to have been further delayed by a lack of harmony between the FDA and the EMA (EMA, 2018; U.S. Food and Drug Administration, 2020). While the EMA requires both the resolution of steatohepatitis and the improvement of fibrosis by ≥1 stage as co-primary endpoints (EMA, 2018), the FDA only requires the achievement of one MASH endpoint (improvement of ≥1 stage in fibrosis with no worsening of MASH or improvement in MASH resolution with no worsening of fibrosis) (U.S. Food and Drug Administration, 2020). While liver histology is subject to inter-reader and sampling variability, limitations of non-biopsy methods are the next vital obstacle to overcome in the drug development (Mahjoubin-Tehran et al., 2021). The ubiquitous need for specific non-invasive markers to serve as both the basis for drug introduction and intervention monitoring has not been met (Polyzos et al., 2020). Several trials have been designed to address the critical issues plaguing the MASH therapeutic landscape and are in progress to directly link non-invasive evaluation to outcomes independent of histologic change (Rinella and Noureddin, 2020; Rinella et al., 2022). This could decrease the need for liver biopsy and a much-desired increase in the utilization of non-invasive tools and clinical outcomes as primary endpoints in clinical trials (Aggarwal et al., 2022). Furthermore, non-invasive tools for diagnosing liver fibrosis may be used to identify patients at higher risk of CVD (Tamaki et al., 2021; Seo et al., 2023). A further consideration is that the MASH trials are known for a robust placebo effect (Han et al., 2019; Rinella and Noureddin, 2020; Vuppalanchi et al., 2021), a finding that should be used to inform sample size estimations for future trials. The placebo changes in histologic measures and magnetic resonance spectroscopy are significant in more than a third of the included subjects, according to a meta-analysis of 39 clinical trials (Han et al., 2019).
The low response rates, with <20-30% of participants of trials demonstrating MASH resolution and fibrosis regression, can be attributed to disease heterogeneity in included subjects, which are not stratified according to the individual underlying pathological mechanisms (e.g., genetic predisposition, the presence or absence of comorbidities) (Eslam et al., 2020a). Notably, the misclassification of subjects suffering from this heterogenous disease results from the SLD umbrella term’s exclusive (instead of preferred, inclusive) nomenclature. Describing these distinct types of SLD with this uniform name reflects the limited understanding of its pathophysiology (Kokkorakis et al., 2023a). Using the highly unspecific and overarching umbrella diagnosis of MASLD and consequently including all subjects (with discrete underlying pathophysiology) hinders potential advances in developing effective pharmacotherapies (Kokkorakis et al., 2023a). This calls for innovative, adaptive trial designs, as suggested by Eslam et al. (Eslam et al., 2020a), such sub-phenotype stratification of patients into trial arms could also distinguish relevant biomarkers or non-invasive tools for each subgroup of individuals with MASLD (Chen et al., 2023).

Since the hepatoprotective effects of statins, including chemoprevention of HCC (Pinyopornpanish et al., 2021), have been demonstrated in experimental studies and meta-analyses, it is crucial to promote their use. Despite clinicians remaining reluctant to prescribe these agents, they appear to have the potential to limit the disease burden of MASLD (Ayada et al., 2023). Furthermore, since MASLD is associated with a substantial CVD burden, new MASLD/MASH drugs may need to be compatible with statin use and potential drug-drug interactions considered. Furthermore, there is a consensus that MASH treatment regimens should not increase CV risk and should either be neutral or, preferably, confer a beneficial effect on the metabolic comorbidities (Rinella et al., 2019; Zhou et al., 2023). Given that GLP-1 RAs, SGLT2is, and glucagon receptor agonists are prominent in the MASLD/MASH pipeline of phase 3 RCTs, we can anticipate a simultaneous enhancement of the CV profile in patients receiving these agents.

Last but not least, MASLD/MASH brings about substantial non-clinical burdens such as economic strain (Younossi et al., 2019d) and a significant patient-reported deterioration in the quality of life (McSweeney et al., 2020). Amelioration of MASH corresponds with improvement in several health-related quality of life and patient-reported outcome domains (Younossi et al., 2022).
Nevertheless, it remains uncertain whether achieving histological improvement of fibrosis and resolution of MASH will lead to a reduced risk of cirrhosis and its associated complications and fewer concurrent health issues. Of particular significance is the ambiguity surrounding the optimal duration of treatment after the trial period. Specifically, determining whether the treatment should be administered for a finite period until MASH resolution is attained or if it should be maintained lifelong, comparable to the management of cardiometabolic diseases, carries significant implications for overall lifetime costs. Moreover, metabolic drugs that positively impact cardiometabolic risk by reducing IR and weight may exert a more substantial influence on mortality and morbidity compared to those with antifibrotic or anti-inflammatory mechanisms of action. Over a mean life expectancy of approximately 21 years in a simulated cohort, a pharmaceutical intervention for MASLD was estimated to be cost-effective in diminishing fibrosis and mortality only if the yearly drug cost does not surpass $12,000 (Rustgi et al., 2022). For instance, with an estimated annual cost of $36,000 (which lies within the commonly accepted cost-effectiveness thresholds according to the ICER – regarding the emerging pharmacotherapies), the incremental cost-effectiveness ratio would be $2,517,676 per quality-adjusted life year gained, surpassing a price that can be economically justified (Rustgi et al., 2022).

These burdens significantly add to the need for urgent action. Public health policies should focus on preventing obesity, T2DM, and related cardiometabolic abnormalities even after approved pharmacotherapy is available (Byrne and Targher, 2020; Kokkorakis et al., 2023b). The key challenge will be raising nationwide and global awareness, including World Health Organization action plans (World Health Organization, 2013) that are currently disappointingly sparse (Lazarus et al., 2022b). There is a current paradoxical lack of national MASLD clinical guidelines and a pronounced lack of inclusion of MASLD in the treatment strategies for related cardiometabolic conditions such as T2DM (Lazarus et al., 2022b). Documenting all burdens associated with SLD could raise public awareness regarding the link between obesity and MASLD (Polyzos et al., 2019a; Kokkorakis et al., 2023c). Consequently, policymakers must carefully assess the trade-offs they are prepared to make to attain fair and inclusive access to such innovative pharmaceutical advancements. Additionally, targeting childhood obesity, which is fueling the MASLD burden of future generations, comprises an essential
healthcare topic and a growing population challenge (Lazarus et al., 2022a). The high prevalence of MASLD in the young, including women of childbearing age, is becoming a relevant concern since MASLD is associated with a substantial increase in maternal complications and adverse fetal outcomes (El Jamaly et al., 2022; Chen et al., 2024). The impact of peripartum maternal MASLD in developing neonatal/pediatric MASLD, beyond the association with maternal obesity and gestational diabetes, requires further studies isolating these effects (Hershman et al., 2019).

Conclusions

Research into the basis of SLD and the subsequently developed therapeutic pipeline is vast and an expedition with many destinations. This could be driven by discriminating between different pathophysiological mechanisms, introducing non-invasive prognostic and diagnostic tools (comprised of a set of validated biomarkers) as well as therapeutic intervention response monitoring, and promoting clinical outcomes as primary outcomes in interventional trials. Although the perfect drug candidate (or combination) for MASH is currently elusive, as summarized in the present review, it will likely target both steatohepatitis and fibrosis in addition to cardiometabolic risk factors. SLD must be recognized and approached as a systemic disease affecting multiple organs. Acknowledging that distinct groups exist under the umbrella of SLD would guide more precise, coordinated, and personalized therapeutic recommendations, ultimately shepherding the development of novel pharmacotherapeutic interventions.

Lastly, it is highly essential to critically summarize clinical research progress, gain insight from failures and successes, and eventually guide future research efforts. In the meantime, before the approval of licensed drugs, promoting a healthy lifestyle as a prevention option in different populations, especially the young, and enhancing the use of favorable cardiometabolic treatments is mandatory.

Author Contributions

Michail Kokkorakis contributed to the conception and research design, research data discussion, design, drafting, figure and table creation, and critical review of the manuscript. Emir Muzurović
contributed to the conception and research design, wrote the manuscript, contributed to the research data discussion, and reviewed the data and manuscript. Špela Volčanšek contributed to the conception and research design, wrote the manuscript, contributed to the research data discussion, drafting of figures and table creation, and reviewed the data and manuscript. Marlene Chakhtoura and Michael A. Hill contributed to the critical review of the manuscript. Dimitri P. Mikhailidis contributed to the conception and research design, drafting, and critical review of the manuscript. Christos S. Mantzoros envisioned this review and its structure, was responsible for overseeing the team, contributed to the conception, research design, and critical review of the manuscript. All authors reviewed and approved the final version of the manuscript.

Declaration of interests

MK has no conflicts of interest to declare. EM has given talks or attended conferences sponsored by Novo Nordisk, Boehringer Ingelheim, AstraZeneca, Medtronic, Merck Sharp & Dohme, Novartis, Sanofi and Servier. ŠV has given talks or attended conferences sponsored by Eli Lilly, Novo Nordisk, Boehringer Ingelheim, Sanofi, Medtronic, and Abbott. DPM has given talks, acted as a consultant, or attended conferences sponsored by Amgen and Novo Nordisk. MC and MAH report no relevant conflicts of interest. CSM reports grants through his institution from Merck and Boehringer Ingelheim, has been a shareholder of and has received grants through his Institution and personal consulting fees from Coherus Inc. and AltrixBio, he reports personal consulting fees from Novo Nordisk, reports personal consulting fees and support with research reagents from Ansh Inc., collaborative research support from LabCorp Inc., reports personal consulting fees from Genfit, Lumos, Amgen, Corcept, Intercept, and Regeneron, reports support (educational activity meals through his institution or national conferences) from Amarin, Novo Nordisk, Astra Zeneca, Boehringer Ingelheim and travel support and fees from TMIOA, Elsevier, the California Walnut Commission, Collège International de Recherche Servier and the Cardio Metabolic Health Conference. None is directly related to the work presented herein.
Data Availability Statement

The authors declare that all the data supporting the findings of this study are contained within the paper.

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An international multidisciplinary consensus statement on MAFLD and the risk of CVD.

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**Figure and Table legends**

Figure 1. Modified diagnostic criteria for Steatotic Liver Disease proposed by the authors. FIB-4 has yet to be fully validated as the most appropriate first-line NIT and, therefore, might be replaced by more accurate diagnostic or prognostic options. Abbreviations: FIB-4, Fibrosis 4 Index; NIT, non-invasive tool; ELF, Enhanced Liver Fibrosis; VCTE, Vibration-controlled Transient Elastography; MRE, Magnetic Resonance Elastography; HTN, hypertension; HCC, hepatocellular carcinoma; HBsAg, Hepatitis B Surface Antigen; HBsAb, Hepatitis B Surface Antibody; HBcAb, Hepatitis B Core Antibody; ANA, antinuclear antibodies; AMA, antimitochondrial antibodies; ASMA, Anti-Smooth Muscle Antibody; A1At, alpha-1 antitrypsin; BMI, body mass index; BP, blood pressure; HDL, high-density lipoprotein; WC, waist circumference; HbA1c, hemoglobin A1c; M, male; F, female.

Figure 2. Steatotic liver disease is a multifactorial disease, and its close interplay with comorbidities from the metabolic spectrum are displayed.

Figure 3. Pathophysiology and target pathways of novel pharmacotherapies for steatotic liver disease. The key pathophysiology of steatotic liver disease and the main etiological causes of some subgroups are depicted. Emerging therapies and therapeutic targets in the pathophysiological continuum of steatotic liver disease, as presented herein, are indicated in the respective pathophysiological pathways, numbered in Latin. The dotted arrows refer to interactions/procedures taking place outside the hepatocytes. Abbreviations: ApoB, apolipoprotein B; VLDL, very low-density lipoprotein; TNF-a, Tumor necrosis factor-alpha; DNL, de novo lipogenesis; MASLD, metabolic dysfunction-
associated steatotic liver disease; LASLD, lipodystrophy-associated SLD; FFA, free fatty acids; O-MASLD, obesity-associated MASLD; S-MASLD, sarcopenia-associated MASLD; OS-MASLD, obesity and sarcopenia-associated MASLD; PNPLA3, patatin-like phospholipase domain-containing protein 3; UCP2, mitochondrial uncoupling protein 2; MERTK, proto-oncogene tyrosine-protein kinase MER; LYLAL1, lysophospholipase like 1; TM6SF2, transmembrane 6 superfamily 2 human gene; MTTP, microsomal triglyceride transfer protein; HSD17B13, 17β-hydroxysteroid dehydrogenase type 13; MBOAT7, lysophospholipid acyltransferase 7; GCKR, glucokinase regulatory protein; MARC1, mitochondrial amidoxime-reducing component 1; IRS-1, insulin receptor substrate 1; ENPP1, ectonucleotide pyrophosphatase/phosphodiesterase 1; MASH, metabolic dysfunction-associated steatohepatitis; GLP-1 RAS, glucagon-like peptide-1 receptor agonist; SGLT-2i, sodium-glucose transport protein 2 inhibitors; PPAR, peroxisome proliferator-activated receptor; PUFAs, polyunsaturated fatty acids; THR-β, thyrotropin-releasing hormone β; FGF, fibroblast growth factor; LOXL2, lysyl oxidase homolog 2; ASK1, apoptosis signal-regulating kinase 1; RNAi, ribonucleic acid interference.

Figure 4. MASH/MASLD phase 3 pharmacotherapeutics with the main modes of activity. Several agents might have more than one mode of activity; herein, the main mechanism of action is conceptualized for each agent. This figure depicts with pink some of the agents that have been discontinued in their respective phase 3 trials; however, more are not presented herein. Abbreviations: MASLD, metabolic dysfunction-associated steatotic liver disease; MASH, Metabolic dysfunction-associated steatohepatitis.

Table 1. Prominent genes implicated in Steatotic Liver Disease along with their main metabolic phenotypes. Abbreviations: MASLD, metabolic dysfunction-associated steatotic liver disease; MASH, Metabolic dysfunction-associated steatohepatitis; PNPLA3, patatin-like phospholipase domain-containing 3; TM6SF2, transmembrane 6 superfamily member 2; MBOAT7, membrane bound-o-acyltransferase domain-containing 7; HSD17B13, 17β-hydroxysteroid dehydrogenase type 13; LYLAL1, lysophospholipase-like 1; MARC1, mitochondrial amidoxime-reducing component 1;
UCP2, mitochondrial uncoupling protein 2; ENPP1, ectonucleotide pyrophosphatase/phosphodiesterase-1; IRS-1, insulin receptor substrate; GCKR, glucokinase regulator; MTTP, microsomal triglyceride transfer protein; MERTK, MER proto-oncogene tyrosine kinase; WAT, White Adipose Tissue; DNL, De novo lipogenesis; VLDL, Very low-density lipoprotein; FFA, Free fatty acids.

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<th>Gene</th>
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<th>DNL</th>
<th>VLDL</th>
<th>FFA</th>
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<th>Insulin</th>
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= indicates no change; ↑ indicates an increase; ↓ indicates a decrease. Abbreviations: MASLD, metabolic dysfunction-associated steatotic liver disease; MASH, Metabolic dysfunction-associated steatohepatitis; PNPLA3, patatin-like phospholipase domain-containing 3; TM6SF2, transmembrane 6 superfamily member 2; MBOAT7, membrane bound-o-acyltransferase domain-containing 7; HSD17B13, 17β-Hydroxysteroid dehydrogenase type 13; LYPAL1, lysophospholipase-like 1; MARC1, mitochondrial amidoxime-reducing component 1; UCP2, mitochondrial uncoupling protein 2; ENPP1, ectonucleotide pyrophosphatase/phosphodiesterase-1; IRS-1, insulin receptor substrate; GCKR, glucokinase regulator; MTTP, microsomal triglyceride transfer protein; MERTK, MER proto-oncogene tyrosine kinase; WAT, White Adipose Tissue; DNL, De novo lipogenesis; VLDL, Very low-density lipoprotein; FFA, Free fatty acids.

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Genetics of non-alcoholic fatty liver disease and associated metabolic disorders; Hernaez Avances en Diabetología 2011

Genetic variants regulating insulin receptor signalling are associated with the severity of liver damage in patients with non-alcoholic fatty liver disease; Dongiovanni et al, Gut 2009

Table 2. Steatotic liver disease modifying modalities – current management. Abbreviations: FDA, Food and Drug Administration; MASLD, metabolic dysfunction-associated steatotic liver disease; T2DM, type 2 diabetes mellitus; MASH, Metabolic dysfunction-associated steatohepatitis; PPARγ, peroxisome proliferator-activated receptor-gamma; IU, international units; S.c., subcutaneous administration; GLP-1 RA, glucagon-like peptide-1 receptor agonist; BMI, body mass index; GIP, gastric inhibitory polypeptide; SGLT-2is, sodium-glucose co-transporter 2 inhibitors; CVD, cardiovascular disease.

<table>
<thead>
<tr>
<th>Treatment modality</th>
<th>Mode of action</th>
<th>Patient population</th>
<th>FDA-Approval for</th>
<th>Considerations and level of evidence</th>
<th>Cardiac benefit</th>
<th>Improvement of MASH</th>
<th>Fibrosis improvement</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lifestyle Modification</td>
<td>Using diet and exercise to achieve 7-10% weight loss</td>
<td>Adult/ Pediatric MASLD Obesity</td>
<td>Both overweight/obese and nonobese MASLD can benefit from weight loss</td>
<td>+</td>
<td>+</td>
<td>+</td>
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<td></td>
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<td></td>
<td>Mediterranean-type diet is advisable (Islam et al., 2020); (Cusi et al., 2022)</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td>Patients with MASLD should not consume heavy amounts of alcohol (Chalasani et al., 2018)</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td>Patients with clinically significant hepatic fibrosis (≥F2) should abstain from alcohol use completely (Rinella et al., 2023)</td>
<td></td>
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</tr>
</tbody>
</table>
**Grade B-C**

(American Diabetes Association Professional Practice Committee, 2021, p. 4)

**Grade B; Intermediate/High Strength of Evidence** (Cusi et al., 2022)

**Grade B1**

(Chalasani et al., 2018)

<table>
<thead>
<tr>
<th>Pioglitazone</th>
<th>PPARγ modulator</th>
<th>Biopsy-proven MASH and fibrosis with and without T2DM</th>
<th>T2DM</th>
<th>Not studied in MASH cirrhosis</th>
</tr>
</thead>
<tbody>
<tr>
<td>30-45 mg orally daily</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td></td>
</tr>
</tbody>
</table>

**Grade A**

(American Diabetes Association Professional Practice Committee, 2021, p. 4)

**Grade A; High Strength of Evidence**

(Cusi et al., 2022)

<table>
<thead>
<tr>
<th>Vitamin E</th>
<th>Biopsy-proven MASH and fibrosis with and without T2DM</th>
<th>N/A</th>
<th>Not recommended to treat MASH in patients with T2DM, MASLD without liver biopsy, MASH cirrhosis, or cryptogenic cirrhosis</th>
</tr>
</thead>
<tbody>
<tr>
<td>&gt;800 IU/day</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Liraglutide</th>
<th>GLP-1 RA</th>
<th>Biopsy-proven MASH and fibrosis</th>
<th>Obesity</th>
<th>T2DM</th>
<th>for chronic weight management in individuals with a BMI of ≥27 kg/m² and MASLD or MASH, clinicians should give preference to semaglutide 2.4 mg/week (best evidence) or liraglutide 3 mg/day (Cusi et al., 2022).</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.8 mg s.c. daily (T2DM)</td>
<td>+</td>
<td>+</td>
<td>-</td>
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</tr>
<tr>
<td>0.6–3 mg s.c. daily (obesity)</td>
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</tr>
</tbody>
</table>

Not studied in MASH cirrhosis

<table>
<thead>
<tr>
<th>Semaglutide</th>
<th>Biopsy-proven MASH and fibrosis</th>
<th>Obesity</th>
<th>T2DM</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.4 mg s.c. daily</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>0.25–2.4 mg s.c. weekly</td>
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</tr>
</tbody>
</table>

**Grade A**

(American Diabetes Association Professional Practice Committee, 2021, p. 4)

**Grade A; High Strength of Evidence**

(Cusi et al., 2022)

<table>
<thead>
<tr>
<th>Tirzepatide</th>
<th>GIP/GLP-1 RA</th>
<th>T2DM or obesity with MASLD</th>
<th>Obesity</th>
<th>T2DM</th>
</tr>
</thead>
<tbody>
<tr>
<td>GLP-1 RA</td>
<td>+</td>
<td>N/A</td>
<td>N/A</td>
<td></td>
</tr>
<tr>
<td>Bariatric Surgery</td>
<td>Obesity</td>
<td>Biopsy-proven MASH and patients with fibrosis who meet criteria for metabolic weight loss surgery</td>
<td>In otherwise eligible patients with compensated MASH or cryptogenic cirrhosis, foregut bariatric surgery may be considered (Chalasani et al., 2018)</td>
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<tr>
<td></td>
<td></td>
<td>Should be considered as a therapeutic option in patients who meet criteria for metabolic weight loss surgery (Rinella et al., 2023)</td>
<td></td>
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</tr>
<tr>
<td></td>
<td></td>
<td>Contraindicated in decompensated MASH cirrhosis (Rinella et al., 2023)</td>
<td></td>
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<tr>
<td></td>
<td></td>
<td>Clinicians should consider bariatric surgery as an option to treat MASLD (Grade B; Intermediate Strength of Evidence) and improve cardiometabolic health (Grade A; High/Intermediate Strength of Evidence) in persons with MASLD and a BMI of ≥35 kg/m² (≥32.5 kg/m² in Asian populations), particularly if T2DM is present</td>
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<tr>
<td></td>
<td></td>
<td>It should also be considered an option in those with a BMI of ≥30 to 34.9 kg/m² (≥27.5 to 32.4 kg/m² in Asian populations) (Grade B; Intermediate/Weak Strength of Evidence; BEL 2) (Cusi et al., 2022)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>SGLT-2is</td>
<td>T2DM with MASLD</td>
<td>T2DM</td>
<td>To offer cardiometabolic benefit in persons with T2D and MASLD, clinicians must consider treatment with GLP-1 RAs, pioglitazone, or SGLT-2 inhibitors; however, there is no evidence of benefit for treatment of steatohepatitis with SGLT-2 inhibitors (Cusi et al., 2022)</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Grade A; High Strength of Evidence</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>(Cusi et al., 2022)</td>
<td></td>
</tr>
<tr>
<td>Statins</td>
<td>HMG-CoA reductase inhibitors</td>
<td>Cardiovascular indications and MASH with compensated cirrhosis</td>
<td>Hyperlipidemia</td>
<td>In patients with cardiovascular indications, statins can be safely used in patients with NASH and compensated cirrhosis (Ando et al., 2021)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Grade B1</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>(Ando et al., 2021)</td>
</tr>
</tbody>
</table>

American Diabetes Association Professional Practice Committee (2021) 4. Comprehensive Medical Evaluation and Assessment of Comorbidities: Standards of Medical Care in Diabetes—2022. *Diabetes Care* 45:S46–S59. Abbreviations: FDA, Food and Drug Administration; MASLD, metabolic dysfunction-associated steatotic liver disease; T2DM, type 2 diabetes mellitus; MASH, Metabolic dysfunction-associated steatohepatitis; PPARγ, peroxisome proliferator-activated receptor-gamma; IU, international units; S.c., subcutaneous administration; GLP-1 RA, glucagon-like peptide-1 receptor agonist; BMI, body mass index; GIP, gastric inhibitory
polypeptide; SGLT-2is, sodium-glucose co-transporter 2 inhibitors; CVD, cardiovascular disease.


European Association for the Study of the Liver (EASL), European Association for the Study of Diabetes (EASD), and European Association for the Study of Obesity (EASO) (2016) EASL-EASD-EASO Clinical Practice Guidelines for the management of non-alcoholic fatty liver disease. *Diabetologia* **59**:1121–1140.


Table 3. MASLD and MASH medications in ongoing Phase 2 identified from Clinicaltrials.gov.
<table>
<thead>
<tr>
<th>Drug</th>
<th>Mechanism of action</th>
<th>Dose</th>
<th>Indication</th>
<th>Primary Outcome(s)</th>
<th>Sample size</th>
<th>Expected completion</th>
<th>Registration number</th>
</tr>
</thead>
<tbody>
<tr>
<td>APHD-012</td>
<td>Distal jejunal-release dextrose</td>
<td>12 g daily</td>
<td>Patients with or without MASLD/MAS H and obesity</td>
<td>Changes from baseline in % weight change</td>
<td>150</td>
<td>Mar 2024</td>
<td>NCT05385978</td>
</tr>
<tr>
<td>Cotadutide</td>
<td>GLP-1 RA and GCGR agonist</td>
<td>300 and 600 μg</td>
<td>Patients with non-cirrhotic MASH with fibrosis</td>
<td>Resolution of MASH without worsening of liver fibrosis in participants with non-cirrhotic MASH with fibrosis; resolution of MASH without worsening of liver fibrosis; improvement of liver fibrosis by at least one stage without worsening of MASH based on biopsy</td>
<td>54</td>
<td>Apr 2024</td>
<td>NCT05364931</td>
</tr>
<tr>
<td>Semaglutide</td>
<td>GLP-1 RA</td>
<td>Subcutaneously once weekly</td>
<td>MASH determined by liver biopsy or MASLD documented by imaging</td>
<td>Point decrease in MASLD activity score</td>
<td>84</td>
<td>Aug 2024</td>
<td>NCT03884075</td>
</tr>
<tr>
<td>Semaglutide  (SEMA) with the fixed-dose combination (FDC) of Cilofexor/ Firsocostat (CILO/FIR)</td>
<td></td>
<td></td>
<td></td>
<td>Worsening of MASH is defined as a ≥ 1-point increase in hepatocellular ballooning or lobular inflammation; MASH resolution is defined as lobular inflammation of 0 or 1 and hepatocellular ballooning of 0</td>
<td>457</td>
<td>Dec 2024</td>
<td>NCT04971785</td>
</tr>
<tr>
<td>Tirzepatide</td>
<td>Gastric Inhibitory Polypeptide/Glucagon-Like Peptide-1 Analogue</td>
<td>5 mg, 10 mg, or 15 mg subcutaneously once weekly</td>
<td>Participants with a histologic diagnosis of MASH with stage 2 or 3 fibrosis by liver biopsy with overweight</td>
<td>Percentage of participants with absence of MASH with no worsening of fibrosis on liver histology</td>
<td>196</td>
<td>Feb 2024</td>
<td>NCT04166773</td>
</tr>
<tr>
<td>Tirzepatide</td>
<td></td>
<td>0.25 mg subcutaneously once weekly</td>
<td>Patients with MASLD advanced fibrosis (F3 and F4; defined by NFS of &gt; 0.676) and T2DM</td>
<td>Change in liver stiffness in terms of kPa; Change in liver fat quantification</td>
<td>30</td>
<td>Feb 2025</td>
<td>NCT05751720</td>
</tr>
<tr>
<td>HM15211</td>
<td>Long-acting triple agonist that targets glucagon receptors, gastric inhibitory peptide, and glucagon-like peptide 1</td>
<td>n/a</td>
<td>Patients with noncirrhotic MASH with liver fibrosis stage F1-F3 determined by liver biopsy and MRI-PDF with ≥8% steatosis</td>
<td>Proportion of subjects who achieve resolution of steatohepatitis on overall histopathological reading and no worsening of liver fibrosis</td>
<td>217</td>
<td>Nov 2025</td>
<td>NCT04505436</td>
</tr>
<tr>
<td>Efinopegdutide</td>
<td>GLP-1 RA and GCGR agonist</td>
<td>Subcutaneous injection in dose-</td>
<td>Histological confirmation of MASH, defined as non-alcoholic</td>
<td>Percentage of participants with MASH resolution without worsening of fibrosis at week 52; Percentage of participants who</td>
<td>300</td>
<td>Dec 2024</td>
<td>NCT05877547</td>
</tr>
<tr>
<td>Drug</td>
<td>Mechanism of action</td>
<td>Dose</td>
<td>Indication</td>
<td>Primary Outcome(s)</td>
<td>Sample size</td>
<td>Expected completion</td>
<td>Registration number</td>
</tr>
<tr>
<td>-----------------------------</td>
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</tr>
<tr>
<td>Saroglitazar Magnesium</td>
<td>Dual PPAR agonist at the subtypes α and γ</td>
<td>4 mg</td>
<td>Women with MASLD and Polycystic ovary syndrome (PCOS)</td>
<td>Change in hepatic fat content from baseline following 24 weeks of treatment as measured by MRI-PDFF</td>
<td>90</td>
<td>Jul 2024</td>
<td>NCT03617263</td>
</tr>
<tr>
<td>Lanifibranor</td>
<td>Pan-PPAR agonist</td>
<td>400 or 800 mg/day</td>
<td>Patients with hepatic steatosis determined by Magnetic Resonance and Spectroscopy (1H-MRS) and uncontrolled T2DM</td>
<td>Change in IHTG quantified by proton 1H-MRS</td>
<td>54</td>
<td>Apr 2024</td>
<td>NCT03459079</td>
</tr>
<tr>
<td>Lanifibranor alone and in combination with Empagliflozin</td>
<td>Lanifibranor 800 mg or Lanifibranor 800 mg + Empagliflozin 10 mg</td>
<td>Patients with MASH based on histology or cT1&gt;875msecs by LiverMuliScan and T2DM</td>
<td>Absolute change in HbA1c from baseline (week 0) to week 24</td>
<td>63</td>
<td>Dec 2023</td>
<td>NCT05232071</td>
<td></td>
</tr>
<tr>
<td>Saroglitazar Magnesium</td>
<td>Dual PPAR agonist at the subtypes α and γ</td>
<td>2 or 4 mg</td>
<td>Patients with MASH and liver fibrosis determined by liver biopsy</td>
<td>Resolution of steatohepatitis with no worsening of fibrosis</td>
<td>240</td>
<td>Dec 2023</td>
<td>NCT05011305</td>
</tr>
<tr>
<td>Saroglitazar Magnesium</td>
<td>Dual PPAR agonist at the subtypes α and γ</td>
<td>4 mg</td>
<td>Patients with MASH determined by liver biopsy and human immunodeficiency virus</td>
<td>Improving MASH, defined as an improvement of at least 2 points (without worsening of fibrosis) in the NAS</td>
<td>160</td>
<td>Mar 2025</td>
<td>NCT05211284</td>
</tr>
<tr>
<td>Pemafibrate (K-877-ER) and Tofogliflozin (CSG452)</td>
<td>K-877 is a selective PPARα modulator (SPPARMα)</td>
<td>n/a</td>
<td>Patients with MASH and liver fibrosis determined by liver biopsy</td>
<td>Improvement in disease activity and no worsening of liver fibrosis</td>
<td>300</td>
<td>Jun 2025</td>
<td>NCT05327127</td>
</tr>
</tbody>
</table>

*Insulin sensitizers/peroxisome proliferator-activated receptors (PPAR) agonists*

Saroglitazar: A dual PPAR agonist at the subtypes α and γ, available as a 4 mg tablet or 2 mg, 4 mg, 7 mg, and 10 mg doses. It is indicated for women with MASLD and Polycystic ovary syndrome (PCOS) to change in hepatic fat content from baseline following 24 weeks of treatment as measured by MRI-PDFF. The expected completion is July 2024, and the registration number is NCT03617263.

Lanifibranor: A pan-PPAR agonist available in 400 or 800 mg/day doses. It is indicated for patients with hepatic steatosis determined by Magnetic Resonance and Spectroscopy (1H-MRS) and uncontrolled T2DM to change in IHTG quantified by proton 1H-MRS. The expected completion is April 2024, and the registration number is NCT03459079.

Lanifibranor alone and in combination with Empagliflozin: Lanifibranor 800 mg or 800 mg + Empagliflozin 10 mg. It is indicated for patients with MASH based on histology or cT1>875msecs by LiverMuliScan and T2DM to achieve an absolute change in HbA1c from baseline (week 0) to week 24. The expected completion is December 2023, and the registration number is NCT05232071.

Saroglitazar Magnesium: A dual PPAR agonist at the subtypes α and γ available as a 2 mg or 4 mg tablet orally administered daily. It is indicated for patients with MASH and liver fibrosis determined by liver biopsy to resolve steatohepatitis with no worsening of fibrosis. The expected completion is December 2023, and the registration number is NCT05011305.

Saroglitazar Magnesium: A dual PPAR agonist at the subtypes α and γ available as a 4 mg orally daily. It is indicated for patients with MASH determined by liver biopsy and human immunodeficiency virus to improve MASH, defined as an improvement of at least 2 points (without worsening of fibrosis) in the NAS. The expected completion is March 2025, and the registration number is NCT05211284.

Pemafibrate (K-877-ER) and Tofogliflozin (CSG452): K-877 is a selective PPARα modulator (SPPARMα). It is indicated for patients with MASH and liver fibrosis determined by liver biopsy to improve disease activity and no worsening of liver fibrosis. The expected completion is June 2025, and the registration number is NCT05327127.
<table>
<thead>
<tr>
<th>Drug</th>
<th>Mechanism of action</th>
<th>Dose</th>
<th>Indication</th>
<th>Primary Outcome(s)</th>
<th>Sample size</th>
<th>Expected completion</th>
<th>Registration number</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pioglitazone</td>
<td>Selective agonist at peroxisome proliferator-activated receptor-gamma (PPARγ)</td>
<td>15 mg/day</td>
<td>Patients with MASH determined by liver biopsy and T2DM</td>
<td>The proportion of patients with liver histological improvement of ≥2 points in NAS with a ≥1-point reduction in either ballooning or lobular inflammation and no increase in fibrosis stage by MASH Clinical Research Network scoring criteria</td>
<td>166</td>
<td>Aug 2027</td>
<td>NCT04501406</td>
</tr>
<tr>
<td>(SGLT2) inhibitor</td>
<td></td>
<td></td>
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<tr>
<td>Drugs targeting lipid metabolism</td>
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<tr>
<td>Ketohexokinase Inhibition (KHKi)</td>
<td>Enzyme catalyzing the first committed step in fructose metabolism is thought to reduce intrahepatic lipid (IHL) content</td>
<td>300 mg daily</td>
<td>Patients with MASLD with hepatic steatosis (i.e. IHL ≥ 5.56%)</td>
<td>Insulin-mediated suppression of endogenous glucose production (EGP) in µmol/kg/min measured during the 2-step hyperinsulinemic-euglycemic clamp</td>
<td>17</td>
<td>Dec 2023</td>
<td>NCT05463575</td>
</tr>
<tr>
<td>PF-06865571</td>
<td>PF-06865571 is a diacetylgluceral acyltransferase 2 inhibitor (DGAT2i)</td>
<td>PF-06865571 1 25, 75, 150, 300 mg twice daily</td>
<td>Patients with MASLD determined by liver biopsy with either F2 or F3 fibrosis</td>
<td>Resolution of MASH without worsening of fibrosis or improvement in fibrosis by 1/&gt;= or both</td>
<td>258</td>
<td>Feb 2024</td>
<td>NCT04321031</td>
</tr>
<tr>
<td>PF-06865571 with PF-05221304</td>
<td>PF-05221304 is an Acetyl-CoA carboxylase (ACC) inhibitor</td>
<td>PF-06865571 1 150, 300 mg twice daily + PF-0522130 4 5, 10 mg twice daily</td>
<td></td>
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</tr>
<tr>
<td>ION224</td>
<td>A ligand-conjugated antisense (LICA) medicine designed to reduce the production of DGAT2</td>
<td>n/a</td>
<td>Patients with MASH determined by liver biopsy, liver fat ≥ 10% as assessed by MRI-PDFF</td>
<td>At least 2-point reduction in NAS with at least 1-point Improvement in hepatocellular ballooning or lobular inflammation, and without worsening in fibrosis stage at end of treatment</td>
<td>160</td>
<td>Mar 2024</td>
<td>NCT04932512</td>
</tr>
<tr>
<td>Atorvastatin</td>
<td>Competitively inhibits the enzyme 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) reductase</td>
<td>40 mg orally daily</td>
<td>Patients with MASH and liver fibrosis stage ≥2 determined by liver biopsy</td>
<td>One overall score of MASH improvement will be derived from improvement in NAS score or no worsening in fibrosis</td>
<td>70</td>
<td>Dec 2024</td>
<td>NCT04679376</td>
</tr>
<tr>
<td>HTD1801</td>
<td>Contains two active moieties, berberine and ursodeoxycholic acid (UDCA)</td>
<td>1250 mg twice daily</td>
<td>Patients with MASH and liver fibrosis stage 2 or 3 determined by liver biopsy with overweight or obesity</td>
<td>A decrease of ≥2-points in NAS with ≥1-point decrease of either lobular inflammation or ballooning and no worsening of fibrosis; or resolution of MASH (defined as the overall histopathologic interpretation of 1) no MASLD or 2) MASLD (simple or isolated steatosis) without steatohepatitis AND a NAS of 0 for ballooning and 0-1 for inflammation and no worsening of fibrosis.</td>
<td>210</td>
<td>Dec 2024</td>
<td>NCT05623189</td>
</tr>
<tr>
<td>ALN-HSD</td>
<td>Targets hydroxysteroid dehydrogenase</td>
<td>n/a</td>
<td>Patients with biopsy-proven MASH with Change in the continuous quantitative liver fibrosis score measured by second harmonic</td>
<td></td>
<td>300</td>
<td>Dec 2026</td>
<td>NCT05519475</td>
</tr>
<tr>
<td>Drug</td>
<td>Mechanism of action</td>
<td>Dose</td>
<td>Indication</td>
<td>Primary Outcome(s)</td>
<td>Sample size</td>
<td>Expected completion</td>
<td>Registration number</td>
</tr>
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<td>----------------------------------------------------------------------------</td>
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</tr>
<tr>
<td>Thyroid hormone receptor (TRH)-β modulators</td>
<td>expression using RNA interference</td>
<td></td>
<td>fibrosis with genetic risk factors</td>
<td>generation/two-photon excitation microscopy</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>VK2809</td>
<td>Selective THR-β Agonist</td>
<td>1.0, 2.5, 5.0, 10.0 mg</td>
<td>Patients with MASH determined by liver biopsy and MRI-PDFF with ≥ 8% liver fat fraction</td>
<td>Relative change in liver fat content (assessed by MRI-PDFF)</td>
<td>248</td>
<td>Jun 2024</td>
<td>NCT04173065</td>
</tr>
<tr>
<td>ASC41</td>
<td>THR-β agonist</td>
<td>2 or 4 mg orally daily</td>
<td>Patients with ≥7.5% steatosis on screening MRI-PDFF</td>
<td>To evaluate the effect of ASC41 compared with placebo in noncirrhotic subjects with MASH by a histological change</td>
<td>180</td>
<td>Jun 2024</td>
<td>NCT05462353</td>
</tr>
<tr>
<td>Targets related to inflammation and immune activation</td>
<td></td>
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</tr>
<tr>
<td>JKB-122</td>
<td>Long-acting toll-like receptor 4 (TLR4) antagonist</td>
<td>n/a</td>
<td>Patients with MASH and NAS &gt;4.0 determined by liver biopsy</td>
<td>MASH resolution on liver histology in non-cirrhotic MASH patients with stage 2 or 3 fibrosis; ≥ 1 stage fibrosis improvement on liver histology in non-cirrhotic MASH patients with stage 2 or 3 fibrosis</td>
<td>300</td>
<td>Dec 2023</td>
<td>NCT04255069</td>
</tr>
<tr>
<td>Norursodeoxycholic Acid</td>
<td>Side-chain shortened derivative of UDCA</td>
<td>500 mg thrice daily</td>
<td>Patients with MASH and liver fibrosis determined by liver biopsy</td>
<td>Number of participants with resolution of MASH, assessed by centrally scored liver histology; and no worsening of fibrosis AND/OR improvement of fibrosis, and no worsening of NAS</td>
<td>363</td>
<td>Apr 2025</td>
<td>NCT05083390</td>
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<tr>
<td>Rencofilstat</td>
<td>Cyclophilin inhibitor that binds to Cyclophilin A, blocking its binding to specific receptors on inflammatory cells</td>
<td>75, 150, or 225 mg daily</td>
<td>Patients with MASH determined by liver biopsy</td>
<td>Proportion of subjects with improvement in fibrosis by at least 1 stage (MASH CRN system) OR MASH resolution without worsening of fibrosis</td>
<td>336</td>
<td>Sep 2025</td>
<td>NCT05402571</td>
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<tr>
<td>Targets related to cell death (apoptosis and necrosis)</td>
<td></td>
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<tr>
<td>Namodenoson</td>
<td>A3 adenosine receptor agonist</td>
<td>25 mg every 12 hours</td>
<td>Patients with MASH determined by liver biopsy, NAS ≥4 and concomitant biopsy-proven stage 1-3 hepatic fibrosis</td>
<td>MASLD activity score (NAS); Adverse events (AEs)</td>
<td>114</td>
<td>Oct 2025</td>
<td>NCT04697810</td>
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<tr>
<td>Dasatinib and Quercetin</td>
<td>Dasatinib inhibits several targets, including BCR-ABL, SRC family (SRC, LCK, YES, FYN), c-KIT, EPHA2, and PDGFRβ; Quercetin is a quinone reductase 2 (QR2) inhibitor</td>
<td>Dasatinib 100 mg + Quercetin 1000 mg</td>
<td>Patients with MASLD with fibrosis score ≥2, but no cirrhosis</td>
<td>Improvement of fibrosis with at least 1-point without worsening of fibrosis and MASLD score based on histology</td>
<td>30</td>
<td>Oct 2025</td>
<td>NCT05506488</td>
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<tr>
<td>Targets related to fibrogenesis and collagen turnover</td>
<td></td>
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<tr>
<td>Drug</td>
<td>Mechanism of action</td>
<td>Dose</td>
<td>Indication</td>
<td>Primary Outcome(s)</td>
<td>Sample size</td>
<td>Expected completion</td>
<td>Registration number</td>
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<tr>
<td>BOS-580</td>
<td>genetically engineered variant of human fibroblast growth factor 21 (FGF21)</td>
<td>n/a</td>
<td>Patients with hepatic fat fraction (HFF) measured by MRI-PDFF ≥10</td>
<td>The effects of BOS-580 on safety and tolerability will be assessed</td>
<td>180</td>
<td>Dec 2024</td>
<td>NCT04880031</td>
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<tr>
<td>BIO89-100</td>
<td>Long-acting engineered glycopegylated recombinant FGF21 agonist</td>
<td>15, 30, or 44 mg once a week</td>
<td>Patients with MASH determined by liver biopsy and F2 or F3 per MASH CRN System and NAS ≥4</td>
<td>Proportion of participants with histological resolution of MASH without worsening of fibrosis; Proportion of participants with ≥1 stage decrease in fibrosis stage with no worsening of MASH</td>
<td>222</td>
<td>Sep 2024</td>
<td>NCT04929483</td>
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<tr>
<td>Efruxifermin (EFX) Fusion protein of human IgG1 Fc domain linked to a modified human FGF21 (Fc-FGF21)</td>
<td>EFX 28 or 50 mg</td>
<td>Patients with MASH determined by liver biopsy and compensated MASH-caused cirrhosis</td>
<td>Proportion of subjects who achieve ≥1 stage improvement in fibrosis (based on MASH CRN fibrosis score) and no worsening of steatohepatitis</td>
<td>200</td>
<td>Apr 2024</td>
<td>NCT05039450</td>
<td></td>
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<tr>
<td>Efruxifermin (EFX) Fc-FGF21</td>
<td>EFX 28 or 50 mg</td>
<td>Patients with MASH determined by liver biopsy and FibroScan measurement &gt; 8.5 kPa</td>
<td>Change from baseline in liver fibrosis with no worsening steatohepatitis assessed by MASH CRN system</td>
<td>128</td>
<td>May 2024</td>
<td>NCT04767529</td>
<td></td>
</tr>
</tbody>
</table>

**Other targets**

**Farnesoid X receptor**

**Obeticholic Acid vs Vitamin E**

| Obeticholic Acid | Obeticholic acid 10 mg once daily | Patients with MASH | Change from baseline fibrosis stage at 6 months; fibrosis improvement (≥ 1 stage), with no worsening of MASH, detected by fibroscan device; Change from baseline MASH condition at 6 months; MASH resolution, with no worsening of fibrosis, with the study considered successful if either 1ry end point is met; Change from baseline steatosis stage at 6 months detected by Fibroscan device | 59         | Sep 2024            | NCT05573204         |

**Antioxidants and Vitamins**

**Febuxostat vs Vitamin E**

| Febuxostat | Febuxostat at 80 mg orally daily | Patients with MASH determined by non-invasive tests | Change in fibrosis stage; fibrosis improvement (≥ 1 stage), with no worsening of MASH; Change from baseline MASH condition at 6 months; MASH resolution, with no worsening of fibrosis; Change from baseline steatosis stage | 70         | Feb 2024            | NCT05574036         |

| Vitamin E  | Vitamin E 400 mg twice daily     | Patients with MASLD determined by FibroScan CAP>280 dB/m | Relative change in alanine aminotransferase (ALT) from baseline to 24 weeks | 200        | Dec 2024            | NCT04801849         |

**Tocotrienol (TCT)**

<p>| Tocotrienol (TCT) | Tocotrienol (TCT) capsules following AM | Patients with End Stage Liver Disease with clinically-diagnosed MASLD or | Effect of oral TCT on model for End-Stage Liver Disease (MELD) score | 70         | Dec 2028            | NCT02581085         |</p>
<table>
<thead>
<tr>
<th>Drug</th>
<th>Mechanism of action</th>
<th>Dose</th>
<th>Indication</th>
<th>Primary Outcome(s)</th>
<th>Sample size</th>
<th>Expected completion</th>
<th>Registration number</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Phosphodiesterase inhibitors</strong></td>
<td></td>
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<tr>
<td>MN-001</td>
<td>Leukotriene receptor antagonism, inhibition of phosphodiesterase, 5-lipoxygenase, phospholipase C, and thromboxane A2</td>
<td>n/a</td>
<td>Patients with FibroScan CAP score ≥ 248 dB/m and T2DM</td>
<td>Mean change in controlled attenuation parameter (CAP) score by sound-based elastography; Mean change from baseline in fasting serum triglyceride levels</td>
<td>40</td>
<td>Dec 2024</td>
<td>NCT05464784</td>
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<tr>
<td>Cilostazol</td>
<td>Cilostazol is a selective inhibitor of phosphodiesterase type 3 (PDE3)</td>
<td>50 mg tablet twice daily</td>
<td>Patients with MASLD confirmed by imaging exams</td>
<td>ALT and Aspartate Aminotransferease (AST) will be evaluated in U/L</td>
<td>120</td>
<td>Dec 2024</td>
<td>NCT04761848</td>
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<tr>
<td>ZSP1601</td>
<td>Pan-phosphodiesterase inhibitor</td>
<td>50 or 100 mg twice daily</td>
<td>Patients with MASH determined by liver biopsy, NAS≥4, and fibrosis score F2 or F3</td>
<td>Percent of patients with improvement of steatohepatitis and no worsening of liver fibrosis or improvement in liver fibrosis greater than or equal to one stage and no worsening of the steatohepatitis</td>
<td>180</td>
<td>Dec 2026</td>
<td>NCT05692492</td>
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<td><strong>Fecal Microbiota Transplantation</strong></td>
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<tr>
<td>PRIM-DJ2727</td>
<td></td>
<td>n/a</td>
<td>Patients with MASLD without cirrhosis based on imaging or clinical judgment and T2DM</td>
<td>Microbiome diversity in fecal samples as indicated by the Shannon Diversity Index; Microbiome richness in fecal samples as indicated by the number of taxonomies per participant; Number of participants with an increase in flora diversity in fecal samples</td>
<td>12</td>
<td>Dec 2024</td>
<td>NCT04371653</td>
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<tr>
<td><strong>Growth Hormone</strong></td>
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<tr>
<td>Ibutamoren</td>
<td>Non-peptide agonist of the ghrelin receptor and a growth hormone secretagogue</td>
<td>25 mg daily</td>
<td>Patients with MASLD/MASH determined by liver biopsy and liver fibrosis stage ≥1</td>
<td>Improvement of fat fraction by proton density by MRI and no worsening of activity or fibrosis; resolution of MASH without worsening of fibrosis; no worsening of fibrosis or activity; number of AEs, serious AEs, AEs resulting in discontinuation of study treatment, AEs of special interest, and changes in vital signs and laboratory values</td>
<td>96</td>
<td>Mar 2025</td>
<td>NCT05622526</td>
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<tr>
<td>Tesamorelin</td>
<td>Synthetic analogue of human growth hormone-releasing hormone (GRF)</td>
<td>1.4 or 2 mg daily subcutaneously</td>
<td>Patients with hepatic steatosis determined by liver biopsy or 1H-MRS</td>
<td>Liver Fat Content as measured by hydrogen-magnetic resonance spectroscopy</td>
<td>76</td>
<td>Dec 2024</td>
<td>NCT03375788</td>
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<tr>
<td><strong>RNA interference</strong></td>
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<tr>
<td>GSK4532990</td>
<td>GalNAc-conjugated RNA interference</td>
<td>n/a</td>
<td>Patients with MASLD NAS ≥ 1 Stage Improvement in histological fibrosis with no</td>
<td></td>
<td>246</td>
<td>Dec 2025</td>
<td>NCT05583344</td>
</tr>
</tbody>
</table>
### Table 4. MASLD and MASH medications in ongoing Phase 3 identified from Clinicaltrials.gov.

<table>
<thead>
<tr>
<th>Drug</th>
<th>Mechanism of action</th>
<th>Dose</th>
<th>Indication</th>
<th>Primary Outcome(s)</th>
<th>Sample size</th>
<th>Expected completion</th>
<th>Registration number</th>
</tr>
</thead>
<tbody>
<tr>
<td>SNP-610</td>
<td>SNP-610 has multiple mechanisms of action, including inhibition of</td>
<td>2 tablets once a day orally</td>
<td>Patients with MASH as evidenced by imaging or other diagnostic assessments</td>
<td>Absolute change from baseline in serum alanine aminotransferase (ALT/GPT)</td>
<td>80</td>
<td>Dec 2024</td>
<td>NCT03468556</td>
</tr>
<tr>
<td>AZD4831</td>
<td>Myeloperoxidase inhibitor</td>
<td>n/a</td>
<td>Patients with MASH determined by liver biopsy and there is fibrosis F2-F3</td>
<td>ALT change from baseline and over placebo to week 12</td>
<td>90</td>
<td>Jul 2024</td>
<td>NCT05638737</td>
</tr>
<tr>
<td>Synthroid</td>
<td>Synthetic form of thyroxine (T4)</td>
<td>25, 50, or 75 μg daily</td>
<td>Patients with MASH determined by liver biopsy, overweight or obesity, T2DM</td>
<td>Improvement in NAS by 2 points</td>
<td>128</td>
<td>Mar 2029</td>
<td>NCT05526144</td>
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<tr>
<td>Semaglutide</td>
<td>Glucagon-like peptide-1 receptor (GLP-1R) agonist</td>
<td>Subcutaneously once weekly</td>
<td>Patients with MASH and liver fibrosis stage 2 or stage 3 determined by</td>
<td>Resolution of steatohepatitis and no worsening of liver fibrosis; Improvement in liver fibrosis and no</td>
<td>1200</td>
<td>Jul 2029</td>
<td>NCT04822181</td>
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</tbody>
</table>

**Abbreviations:** MASLD, metabolic dysfunction-associated steatotic liver disease; MASH, Metabolic dysfunction-associated steatohepatitis; IU, international units.
<table>
<thead>
<tr>
<th>Drug</th>
<th>Mechanism of action</th>
<th>Dose</th>
<th>Indication</th>
<th>Primary Outcome(s)</th>
<th>Sample size</th>
<th>Expected completion</th>
<th>Registration number</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dapagliflozin</td>
<td>Sodium-glucose co-transporter-2 (SGLT-2) inhibitor</td>
<td>10 mg orally</td>
<td>Patients with MASH determined by liver biopsy</td>
<td>Improvement in scored liver histological improvement over 12 months</td>
<td>100</td>
<td>Jul 2024</td>
<td>NCT03723252</td>
</tr>
<tr>
<td>Dapagliflozin</td>
<td>SGLT-2 inhibitor</td>
<td>10 mg orally</td>
<td>Patients with MASLD and FibroScan shows CAP score ≥252 dB/m</td>
<td>Safety outcome measure</td>
<td>75</td>
<td>Apr 2024</td>
<td>NCT05308160</td>
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<tr>
<td>Dapagliflozin Empagliflozin</td>
<td>SGLT-2 inhibitors</td>
<td>n/a</td>
<td>Patients with MASLD</td>
<td>Number of participants with treatment-related adverse events as assessed by CTCAE v4.0; Number of participants with tight controlled diabetes mellitus</td>
<td>150</td>
<td>Oct 2024</td>
<td>NCT06117137</td>
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<tr>
<td>Vildagliptin vs Vildagliptin/Metformin</td>
<td>Dipeptidyl peptidase-4 inhibitor</td>
<td>Vildagliptin 50 mg twice daily</td>
<td>Patients with MASLD and type 2 diabetes mellitus (T2DM)</td>
<td>Number of patients with improved MASLD and insulin resistance</td>
<td>120</td>
<td>Dec 2028</td>
<td>NCT03925701</td>
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<tr>
<td>MS-DC-0602K</td>
<td>Insulin sensitizer designed to preferentially target the mitochondrial pyruvate carrier while minimizing direct binding to the transcriptional factor PPARγ</td>
<td>Orally once daily</td>
<td>Patients with pre-T2DM or T2DM and evidence of MASLD/MASH</td>
<td>Change in glycosylated hemoglobin (HbA1c); Change in the weighted average of standardized AST, cytokeratin 18 (CK-18), and HbA1c values (standard deviations)</td>
<td>1800</td>
<td>Sep 2024</td>
<td>NCT03970031</td>
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<tr>
<td>Empagliflozin vs Pioglitazone</td>
<td>Empagliflozin is a SGLT-2 inhibitor</td>
<td>Empagliflozin 10 mg</td>
<td>Patients with MASH based on liver ultrasonography</td>
<td>Change in fibrosis index based on the 4 factors (FIB-4); Change in aspartate transaminase-to-platelet ratio index (APRI); Change in liver enzymes</td>
<td>56</td>
<td>Nov 2024</td>
<td>NCT05605158</td>
</tr>
<tr>
<td>Diosmin</td>
<td>Selective PPARγ modulator</td>
<td>600 mg twice daily</td>
<td>Patients with established diagnosis of MASH based on liver ultrasonography, without T2DM, with/without hypertension</td>
<td>Change in ultrasound including MASLD fibrosis (risk) score</td>
<td>48</td>
<td>Jun 2025</td>
<td>NCT05942547</td>
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<tr>
<td>Lanifibranor</td>
<td>Pan-PPAR agonist</td>
<td>800 or 1200 mg/day</td>
<td>Patients with MASH and liver fibrosis stage 2 or 3</td>
<td>Resolution of MASH and improvement of fibrosis</td>
<td>1000</td>
<td>Sep 2026</td>
<td>NCT04849728</td>
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</table>
## Drugs targeting lipid metabolism

<table>
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<th>Drug</th>
<th>Mechanism of action</th>
<th>Dose</th>
<th>Indication</th>
<th>Primary Outcome(s)</th>
<th>Sample size</th>
<th>Expected completion</th>
<th>Registration number</th>
</tr>
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<tbody>
<tr>
<td>Rosuvastatin vs Coenzyme Q10 (CoQ10)</td>
<td>Rosuvastatin inhibits 3-hydroxy-3-methyl-glutaryl coenzyme A (HMG-CoA) reductase, which is the rate-limiting step in cholesterol synthesis</td>
<td>Rosuvastatin 20 mg oral Tablet Coenzyme Q10 100 mg oral capsule</td>
<td>Patients with MASH based on liver ultrasonography</td>
<td>Change in liver stiffness measurement (LSM); Change in ultrasound score</td>
<td>46</td>
<td>Apr 2024</td>
<td>NCT05731596</td>
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<tr>
<td>CoQ10 improves lipid metabolism and ameliorates obesity by regulating CaMKII-mediated PDE4 inhibition</td>
<td></td>
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<td></td>
</tr>
<tr>
<td>Orotic Acid Carnitine Complex Capsules (Godex®)</td>
<td>Modulator of mitochondrial free fatty acid transport and oxidation</td>
<td>2 capsules of Godex® three times a day orally</td>
<td>Patients with an magnetic resonance imaging-derived proton density fat fraction (MRI-PDFF) ≥ 7% indicating evidence of intrahepatic fat deposition, suspected of having MASLD</td>
<td>Change in intrahepatic fat content measured by MRI-PDFF</td>
<td>196</td>
<td>Feb 2027</td>
<td>NCT06152991</td>
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## Thyroid hormone receptor (TRH)-β modulators

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<th>Drug</th>
<th>Mechanism of action</th>
<th>Dose</th>
<th>Indication</th>
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<th>Sample size</th>
<th>Expected completion</th>
<th>Registration number</th>
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<tbody>
<tr>
<td>Resmetirom (MGL-3196)</td>
<td>Selective thyroid hormone receptor-β agonist</td>
<td>80 or 100 mg</td>
<td>Patients with MASLD determined by liver biopsy</td>
<td>Effect of once daily, oral administration of 80 or 100 mg resmetirom versus placebo on the incidence of adverse events.</td>
<td>1000</td>
<td>Apr 2026</td>
<td>NCT04951219</td>
</tr>
<tr>
<td>Resmetirom (MGL-3196)</td>
<td>Selective thyroid hormone receptor-β agonist</td>
<td>80 or 100 mg</td>
<td>Patients with MASLD determined by liver biopsy</td>
<td>Any event of all-cause mortality, liver transplant, ascites, hepatic encephalopathy, gastroesophageal variceal hemorrhage, and confirmed increase of MELD score from &lt;12 to ≥15 due to liver disease</td>
<td>700</td>
<td>Nov 2025</td>
<td>NCT05500222</td>
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<tr>
<td>Resmetirom (MGL-3196)</td>
<td>Selective thyroid hormone receptor-β agonist</td>
<td>80 or 100 mg</td>
<td>Patients with MASH and fibrosis</td>
<td>Effect of 80 or 100 mg MGL-3196 vs matching placebo on liver biopsy (MASH Clinical Research Network score) at week 52 compared with baseline</td>
<td>2000</td>
<td>Jan 2028</td>
<td>NCT03900429</td>
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## Targets related to fibrogenesis and collagen turnover

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<th>Drug</th>
<th>Mechanism of action</th>
<th>Dose</th>
<th>Indication</th>
<th>Primary Outcome(s)</th>
<th>Sample size</th>
<th>Expected completion</th>
<th>Registration number</th>
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<tbody>
<tr>
<td>Belapectin</td>
<td>Inhibition of galectin-3</td>
<td>Optimal dose of belapectin</td>
<td>Patients with MASH determined by liver biopsy</td>
<td>Development of new esophageal varices at 18 months of treatment</td>
<td>357</td>
<td>Dec 2024</td>
<td>NCT04365868</td>
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<tr>
<td>Efruxifermin</td>
<td>Fibroblast growth factor 21 (FGF21) analog</td>
<td>50 mg</td>
<td>Patients with suspected or confirmed diagnosis of MASH</td>
<td>Safety and tolerability will be assessed through the reporting of extent of exposure (weeks)</td>
<td>600</td>
<td>Oct 2026</td>
<td>NCT06161571</td>
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### Drug Therapy Table

<table>
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<th>Indication</th>
<th>Primary Outcome(s)</th>
<th>Sample size</th>
<th>Expected completion</th>
<th>Registration number</th>
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</thead>
<tbody>
<tr>
<td>N-acetyl Cysteine</td>
<td></td>
<td>High dose 2400 mg/day</td>
<td>Patients with MASLD</td>
<td>Assessment of the effect of NAC on leptin</td>
<td>60</td>
<td>Dec 2023</td>
<td>NCT05589584</td>
</tr>
<tr>
<td>Estradiol</td>
<td>Binds to subtypes of the estrogen receptor: estrogen receptor alpha and estrogen receptor beta</td>
<td>100 μg of estradiol daily</td>
<td>Patients with MASH determined by biopsy or MASLD by imaging</td>
<td>Reduction in degree of liver fibrosis</td>
<td>60</td>
<td>May 2027</td>
<td>NCT04833140</td>
</tr>
</tbody>
</table>

**Other targets**

- **NASH/MASH or NAFLD/MASLD**
- **or non-invasively diagnosed**
- **NASH/MASH or NAFLD/MASLD**

- **adverse events**
  - (frequency & severity of events), clinical laboratory tests, ECGs, ultrasounds, vital sign assessments, and concomitant medication usage

**Abbreviations:** MASLD, metabolic dysfunction-associated steatotic liver disease; MASH, Metabolic dysfunction-associated steatohepatitis.
Fig. 3

MASLDMASH emerging treatment options

Ⅰ Targeting metabolism: GLP-1 RAs, SGLT-2i, insulin, PPAR agonists
Ⅱ Targeting lipid metabolism: statins, ezetimibe, n-3 PUFAs
Ⅲ Targeting DNL: firsocostat, aramchol, oltipraz
Ⅳ TRH-β modulators: resmetirom, VK2809

Ⅴ Targets related to cell death (apoptosis and necrosis): namodenoson, dasatinib, quercetin
Ⅵ Targets related to fibrogenesis & collagen turnover: belaplatin, EGF-19/21, LOXL2, ASK1
Ⅶ Gene silencing biological therapies in development: RNAi
Ⅷ Future agents that may specifically affect mitochondrial function
Ⅸ Different therapeutic agents may have several targets