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Review

Mechanisms and therapeutic insights into MASH-associated fibrosis

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Metabolic dysfunction-associated steatohepatitis (MASH) is a progressive liver disease posing a major global health concern, closely related to the rising prevalence of obesity. Liver fibrosis is the primary determinant of adverse outcomes in MASH. The increasing worldwide prevalence, economic impact, and adverse outcomes of MASH-associated fibrosis have spurred extensive research to elucidate its pathogenesis and to address its treatment. However, the intricate mechanism driving the transition from metabolic dysfunction to clinically significant fibrosis is not fully understood. Moreover, effective therapies, particularly direct antifibrotic agents, are still lacking, despite the recent approval of resmetirom and semaglutide for MASH-associated fibrosis. Here, we review current insights into the mechanism of MASH-associated fibrosis and provide a comprehensive overview of emerging therapeutic strategies.

Highlights

Metabolic dysfunction-associated steatotic liver disease (MASLD) is the hepatic manifestation of systemic metabolic disease and is fundamentally driven by dysregulated interorgan crosstalk among the liver, gut, adipose tissue, muscle, and brain.

The progression from MASLD to metabolic dysfunction-associated steatohepatitis (MASH)-associated fibrosis is orchestrated by complex intrahepatic cell–cell and cell–extracellular matrix (ECM) crosstalk.

Liver fibrosis is the key determinant of long-term outcomes including cirrhosis, hepatocellular carcinoma, and liver-related death in patients with MASH.

To date, the two FDA-approved drugs and most late-stage clinical trial candidates to reduce fibrosis in MASH primarily achieve their effects through weight loss and metabolic improvement. By contrast, direct antifibrotic therapies targeting fibrogenic cells and ECM turnover remain in early-phase trials or have been terminated.

The growing challenge of liver fibrosis in metabolic liver disease

Metabolic dysfunction-associated steatotic liver disease (MASLD) and its progressive form, MASH, represent the new nomenclature for what was previously termed non-alcoholic fatty liver disease (NAFLD) and non-alcoholic steatohepatitis (NASH) [1,2]. This terminological shift emphasizes the metabolic dysfunction that fundamentally underpins these conditions. Driven by the global epidemic of obesity, type 2 diabetes (T2D), and metabolic syndrome, the burden of MASLD has increased dramatically worldwide and it is now recognized as the leading cause of chronic liver disease [3,4]. The global prevalence of MASLD has risen steadily over the past three decades, now affecting approximately 30% of the adult population, with about a quarter progressing to MASH [5,6]. Alarmingly, MASH is projected to become the primary indication for liver transplantation within the next decade [7]. In the USA, it is already the most common indication for liver transplantation in patients with hepatocellular carcinoma (HCC) and the second most common indication in those without HCC [8,9].

MASH is histologically characterized by **hepatic steatosis** (see Glossary), **hepatocyte ballooning**, **lobular inflammation**, and varying degrees of fibrosis [10]. Among these features, liver fibrosis represents the primary determinant of long-term outcomes including cirrhosis, HCC, liver-related death, and cardiovascular outcomes in patients with MASH [11,12]. Consequently, fibrosis has become the central focus in both clinical diagnosis and therapeutic development for MASH. While the recent approval of Rezdiffra (resmetirom)[i,ii], an oral thyroid hormone receptor-β (THR-β) agonist, and Wegovy (semaglutide)[iii], an injectable GLP-1 receptor agonist, represent a meaningful advancement in MASH fibrosis treatment, effective therapies remain lacking, particularly those directly targeting fibrosis. Recent scientific and clinical advances have elucidated complex interactions involving multiple risk factors, interorgan crosstalk, intrahepatic cell–cell crosstalk, and cellular heterogeneity in the pathogenesis of liver fibrosis. These findings not only deepen our understanding of disease progression, but also reveal new therapeutic entry points. This review provides an integrated and up-to-date synthesis of these multidimensional

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advances, incorporating the latest mechanistic insights and emerging therapeutic strategies from recent clinical developments. By contextualizing these findings, it offers a timely and comprehensive overview of the current state of MASH-associated fibrosis.

Pathogenesis of MASH-associated fibrosis

Liver fibrosis is a dynamic process characterized by the net accumulation of **extracellular matrix (ECM)** resulting from chronic liver injury, ultimately distorting the liver architecture and increasing liver stiffness [13]. In the context of MASH, the pathogenic process of fibrosis is notably metabolic related, dynamic, and complex, with its progression and regression regulated by a web of metabolic, environmental, and genetic factors at the interorgan, cellular, and molecular levels (Figure 1).

Risk factors for MASH-associated fibrosis

The development of fibrosis in MASH is driven by an array of interconnected risk factors. At the forefront of MASH-associated fibrosis risk are metabolic factors. Epidemiological and clinical studies imply that MASLD is strongly associated with other metabolic disorders such as obesity,

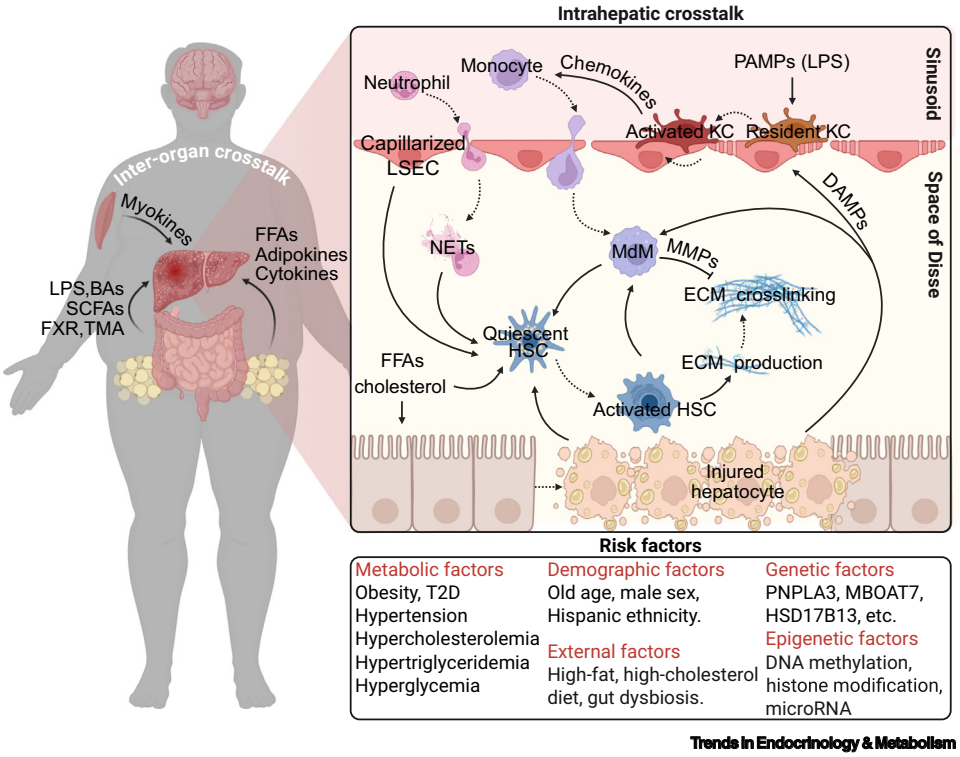

Figure 1. Pathogenic landscape of metabolic dysfunction-associated steatohepatitis (MASH)-associated fibrosis. The development of MASH-associated fibrosis is a complex and progressive process. Individual susceptibility to MASH is shaped by a combination of metabolic, demographic, environmental, genetic, and epigenetic factors. The fibrotic cascade is initiated by extrahepatic signals derived from the gut, adipose tissue, skeletal muscle, and brain. These inputs converge on the liver, triggering a cascade of intrahepatic cell–cell crosstalk and intracellular signaling events that drive fibrogenesis. Ultimately, the balance between extracellular matrix (ECM) synthesis and degradation is disrupted, leading to excessive ECM accumulation and the establishment of liver fibrosis. Abbreviations: BA, bile acid; FFA, free fatty acid; FXR, farnesoid X receptor; HSC, hepatic stellate cell; KC, Kupffer cell; LPS, lipopolysaccharide; LSEC, liver sinusoidal endothelial cell; PAMP, pathogen-associated molecular pattern; SCFA, short-chain fatty acid; T2D, type 2 diabetes. This figure was created using BioRender.

Glossary

Bariatric surgery: a surgical procedure designed to treat obesity and related health issues by modifying the stomach or intestine. Common types include gastric bypass, sleeve gastrectomy, and adjustable gastric banding. These surgeries restrict food intake, alter digestion, or both, resulting in significant weight loss and improved metabolic health.

Chemokines: small signaling proteins that direct the migration of immune cells to sites of inflammation, infection, or injury. They bind to specific receptors on target cells, guiding immune surveillance, tissue repair, and immune responses.

Chimeric antigen receptor (CAR) T cells: genetically engineered T cells that express synthetic receptors designed to recognize specific antigens on target cells, enabling targeted immune attack against cancers or diseased cells.

CpGs: regions in DNA where a cytosine nucleotide is followed by a guanine nucleotide linked by a phosphate bond. CpG sites are often found in clusters called CpG islands, commonly located near gene promoters, and are key sites for DNA methylation.

Damage-associated molecular patterns (DAMPs): a cascade of molecules such as high-mobility group box 1, ATP, mitochondrial DNA, and histones that are released by injured, dying, or dead cells. These endogenous danger signals activate the innate immune system by engaging pattern recognition receptors, promoting inflammation and tissue repair.

Extracellular matrix (ECM): a non-cellular 3D macromolecular network comprising collagens, proteoglycans, laminins, fibronectin, elastin, and other glycoproteins. In healthy liver, the ECM acts as a scaffold, providing structural integrity to the liver. During fibrosis, disrupted ECM turnover causes excessive deposition of fibrillar collagens, mainly types I and III, leading to increased tissue stiffness and disrupted liver architecture.

Hepatic crown-like structures: histological features in the liver where macrophages surround dying or dead lipid-laden hepatocytes, serving as a hallmark of localized inflammation and hepatocyte injury in MASH.

Hepatic steatosis: the pathological feature characterized by the excessive accumulation of fat within hepatocytes.

T2D, dyslipidemia, hyperglycemia, and hypertension [14,15]. Several demographic (male sex, older age, and Hispanic ethnicity) [16,17] and external factors (high-fat, high-cholesterol, high-glucose, high-fructose diet and gut microbiota dysbiosis) [18–20] have also been considered as risk factors for MASH-associated fibrosis.

Genetic predisposition plays a pivotal role in modulating individual susceptibility to MASH, particularly in lean patients. Large-scale genome-wide association studies have linked an increasing number of **single-nucleotide polymorphisms (SNPs)** to the risk of developing MASH and progressive fibrosis. The most well-established genetic variant is the patatin-like phospholipase domain-containing protein 3 (PNPLA3) rs738409 C>G (I148M) variant, which has been repeatedly confirmed in multiple cohorts [21–23]. PNPLA3^{I148M} hinders triglyceride and retinol ester turnover in hepatocytes and hepatic stellate cells (HSCs), respectively, leading to lipid accumulation and enhanced fibrogenesis [24–26]. The membrane bound *O*-acyltransferase domain containing 7-transmembrane channel-like 4 (MBOAT7–TMC4) rs641738 C>T variant has been linked to an increased risk of MASLD, MASH, fibrosis, and HCC [27,28]. Notably, hepatocyte-specific MBOAT7 deficiency in diet-induced models results in accentuated fibrosis without a concomitant rise in inflammatory markers, suggesting a more direct influence on fibrotic remodeling [29]. Other SNPs linked to MASH fibrosis include TM6SF2 rs58542926 [30], HSD17B13 rs72613567 [31,32], GCKR rs780094 [33], and MERTK rs4374383 [34], although most of them may not cause fibrosis directly.

Besides the genetic background and environment factors themselves, epigenetic modulations play a crucial role in gene–environment interactions, where environmental cues induce epigenetic changes that alter the expression and function of risk genes. Recent studies have shed light on the epigenetic changes, including DNA methylation, histone modification, and miRNA activities, in the development and progression of MASH. For instance, DNA methylation at specific **CpGs** of fibrosis-related genes [e.g., transforming growth factor-β (TGF-β), platelet-derived growth factor (PDGF), peroxisome proliferator-activated receptor (PPAR)α] differs between patients with mild and advanced MASH [35]. A recent study implicated THR-β-related DNA methylation and miR-34a-5p in reduced thyroid hormone signaling in MASH [36]. Combined inhibition of the epigenetic modulator histone deacetylase 2 and DNA methyltransferase 1 alleviated fibrosis in a minipig model of MASH [37]. Moreover, several circulating miRNAs, such as miR-21, miR-34a, miR-122, miR-192, and miR-193, are consistently associated with MASH [38]. Collectively, these findings underscore the pivotal role of epigenetic regulation in the development of MASH.

Interorgan crosstalk in MASH-associated fibrosis

MASH is the hepatic manifestation of systemic metabolic disease, fundamentally driven by dysregulated crosstalk among the liver, muscle, adipose tissue, gut, and brain (Figure 1). This interorgan dialog is mainly mediated by circulating factors, including organokines like adipokines and myokines, hormones, metabolites, and cytokines [39].

Disruptions of the gut–liver axis, a bidirectional circuit linking the microbiota, intestinal barrier, and liver, have emerged as major drivers of MASH and its progression to fibrosis [40–42]. In particular, gut microbiota dysbiosis and increased intestinal permeability ('leaky gut') under harmful environmental conditions allow the translocation of detrimental microbial products, such as lipopolysaccharide (LPS), into the portal circulation [43]. On reaching the liver [44], LPS triggers inflammatory and profibrotic responses by activating toll-like receptor 4 (TLR4) on hepatic macrophages and HSCs [45,46]. Gut dysbiosis also alters bile acid (BA) metabolism, showing composition changes such as increased deoxycholic acid, which has been implicated in HCC pathogenesis in MASH [47,48]. Notably, intestinal farnesoid X receptor (FXR), a primary BA receptor, plays a potently

This results in a fatty liver, generally identified when the lipid content exceeds 5–10% of the liver weight.
Hepatocyte ballooning: swelling of hepatocytes with rarefied cytoplasm and cytoskeletal disruption, reflecting cell injury in steatohepatitis.
Lobular inflammation: infiltration of inflammatory cells, primarily lymphocytes, and macrophages, within the hepatic parenchyma, typically accompanied by hepatocyte injury.
Matrix metalloproteinases (MMPs): a family of zinc-dependent endopeptidases that degrade ECM components, playing key roles in tissue remodeling, wound healing, and fibrosis. In the liver, their activity is tightly controlled by TIMPs to maintain ECM homeostasis.
Single-nucleotide polymorphism (SNP): a genomic variant at a single base position in the DNA that can influence individual susceptibility to diseases.

antifibrotic role in MASH via the fibroblast growth factor (FGF)15 pathway in mouse and the FGF19 pathway in humans [49]. Other microbial metabolites like short-chain fatty acids, trimethylamine *N*-oxide, and even endogenous ethanol have also been linked to MASH [50].

Adipose tissue dysfunction is another extrahepatic driver of MASH, particularly in individuals with obesity. In dysfunctional white adipose tissue, impaired lipid storage capacity leads to the release of free fatty acids (FFAs) into the circulation. The subsequent uptake of these excessive FFAs by the liver disrupts lipid metabolism homeostasis and drives hepatic lipotoxicity. Notably, worsening liver fibrosis observed in some patients who undergo very rapid weight reduction following **bariatric surgery** may be attributed to the hepatic lipotoxicity caused by rapid fat mobilization [51]. Moreover, monocytes can be recruited into dysfunctional adipose tissue and differentiated into proinflammatory macrophages that secrete cytokines such as interleukin-1β (IL-1β). These cytokines not only act locally but also travel to the liver, where they exert proinflammatory and profibrotic effects. Dysfunctional adipose tissue also exhibits an altered secretion profile of adipokines, with reduced antifibrotic adiponectin and increased profibrotic leptin [52,53]. Specifically, adiponectin deficiency exacerbates CCl4-induced liver fibrosis [54], whereas administration of the adiponectin agonist ADP355 attenuates fibrosis in the same model [55]. Leptin treatment upregulates the mRNA expression of fibrogenesis genes in HSCs, whereas leptin deficiency attenuates liver fibrosis in methionine-choline-deficient (MCD)-fed ob/ob mice [56–58].

Other types of interorgan crosstalk, such as the skeletal muscle–liver and brain–liver axes, are also implicated in MASH fibrosis progression. The skeletal muscle–liver axis regulates MASH fibrosis primarily through myokines (e.g., myostatin, irisin) released from muscle tissue [39,59,60]. Insulin resistance in skeletal muscle also represents a crucial player in the skeletal muscle–liver axis in MASH pathogenesis. When muscle cells become less responsive to insulin, impaired glucose uptake leads to systemic hyperglycemia and hyperinsulinemia, thereby increasing hepatic lipotoxicity [59]. The brain–liver axis in MASH fibrosis involves intricate neural and hormonal signaling [39]. The centrally acting metabolic regulator FGF21 significantly influences MASH progression and represents a crucial drug target for MASH. FGF21 administration has been shown to reverse multiple diet-induced MASH fibrosis by directing its effects to both the central nervous system and hepatocytes [61]. Moreover, a recent retrospective cohort study in patients with MASLD found that the occurrence of cardiovascular disease correlated with a higher risk of liver fibrosis, and experimental models further demonstrated that myocardial infarction exacerbates liver injury in mice with pre-existing MASLD [62]. These findings highlight the novel concept that cardiovascular diseases may contribute to the progression of MASH [63]. However, further investigation is warranted to support this concept and to elucidate the heart–liver crosstalk involved in MASH progression.

Cellular landscape of MASH-associated fibrosis

Whereas the development of hepatic steatosis in early MASLD stages is driven by multisystemic metabolic disorders, the progression to and regression of MASH-associated fibrosis are primarily governed by intrahepatic crosstalk among resident and recruited cells, as well as the ECM. Key players in this intrahepatic crosstalk include hepatocytes, HSCs, liver sinusoidal endothelial cells (LSECs), macrophages, several other immune cells, and the ECM (Box 1 and Figures 1 and 2). The intrahepatic crosstalk is primarily mediated by paracrine loops involving metabolites, cytokines, **chemokines**, extracellular vesicles, **matrix metalloproteinases (MMPs)**, and direct contact (Figures 1 and 2) [64].

As MASH progresses, diverse cell populations in the liver undergo ‘reprogramming’, leading to distinct cellular subsets within different cell types, each exhibiting unique transcriptional signatures and

Box 1. Key cellular players in fibrosis progression and regression in MASH

Hepatocytes subjected to sustained metabolic stress and lipotoxicity undergo injury and cell death, releasing DAMPs, reactive oxygen species, and profibrotic molecules [10] that act as key initiators, shaping neighboring cells to drive fibrosis progression.

In MASH, quiescent HSCs turn into a proliferative, migratory, fibrogenic state due to profibrotic signals [10] and drive fibrosis by producing ECM, especially fibrillar collagens [58], which in turn interact with collagen receptors on HSCs like integrins, further activating them. During regression, activated HSCs either die or revert to a deactivated, quiescent-like state, re-expressing protective mediators to restore liver function.

KCs, which are the resident liver macrophages, are among the primary responders to DAMPs and gut-derived pathogen-associated molecular patterns. On activation, KCs release a cascade of cytokines and chemokines that amplify the local inflammatory milieu by recruiting other immune cells, such as monocyte-derived macrophages (MdMs) [10,70]. As MASH progresses, resident KCs gradually decline, which appears to be related to the injury severity, while MdMs increasingly populate the liver [71]. Hepatic macrophages play a dual role in MASH fibrosis. Profibrotic macrophages drive fibrosis primarily by providing profibrotic and survival signals to HSCs. By contrast, antifibrotic macrophages can resolve fibrosis by producing MMPs to break down the ECM.

Infiltrating neutrophils, which are traditionally linked to acute injury, promote fibrosis in experimental MASH models by releasing neutrophil extracellular traps, leading to HSC proliferation, migration, and activation [126].

Adaptive immune cells also contribute significantly to MASH pathogenesis [127]. $CD8^+$ T cells, activated by intestinal antigens and type I interferon (IFN) signals, aggravate MASH progression through the secretion of IFN-γ and TNF-α. $CD4^+$ T cells polarize toward IFN-γ-producing Th1 cells and IL-17-producing Th17 cells in MASH. An increased Th17:Treg ratio may be a biomarker of progression from MASLD to MASH. Natural killer T cells also infiltrate the MASH liver and activate HSCs by releasing proinflammatory and profibrotic cytokines. In parallel, B cells accumulate in the liver, where they secrete IL-6 and TNF-α, generate pathogenic antibodies, and modulate T cell responses [128]. Notably, B cell deficiency mitigates fibrosis through a MYD88-dependent but antibody-independent mechanism [128].

Last, chronic injury also leads to LSEC dysfunction, with loss of their fenestrae and formation of a basement membrane – namely, capillarization – which promotes fibrogenesis through increased expression of adhesion molecules and decreased nitric oxide production [129,130].

the adoption of varied and sometimes stage-dependent phenotypes that may contribute uniquely to disease progression. Recent single-cell RNA-seq (scRNA-seq) studies have expanded our understanding of the cellular plasticity and heterogeneity of MASH fibrosis. Understanding the specific roles of and interactions among these heterogeneous cell populations at the single-cell level helps to elucidate the mechanisms of MASH fibrosis and develop novel therapies.

HSCs, the primary fibrogenic cell type, exhibit remarkable plasticity in MASH by transitioning between distinct states that possess unique functions and gene expression profiles, ultimately dictating the balance between homeostasis and disease progression [65,66]. In the healthy liver, HSCs maintain a quiescent and non-proliferative phenotype and reside in the perisinusoidal space of Disse. They serve as the major reservoir for vitamin A through lecithin retinol acyltransferase and maintain liver homeostasis by secreting growth factors that regulate hepatocyte metabolism, zonation, and regeneration. On liver injury, quiescent HSCs transdifferentiate into activated HSCs, proliferative and contractile myofibroblasts that drive fibrogenesis [58]. This activation involves dramatic transcriptomic reprogramming, characterized by the upregulation of a core set of fibrogenic genes, thereby driving matrix deposition and fibrosis progression [58,66]. With prolonged injury, a subset of activated HSCs can undergo replicative senescence. Senescent HSCs adopt an inflammatory phenotype, despite limited ability to proliferate and produce ECM [67]. Thus, their selective clearance by **chimeric antigen receptor (CAR) T cells** is considered a promising direct antifibrotic therapy [68]. Conversely, on resolution of injury, activated HSCs can revert to a deactivated state that largely resembles the quiescent phenotype and helps to restore liver homeostasis, highlighting deactivation as another key therapeutic goal.

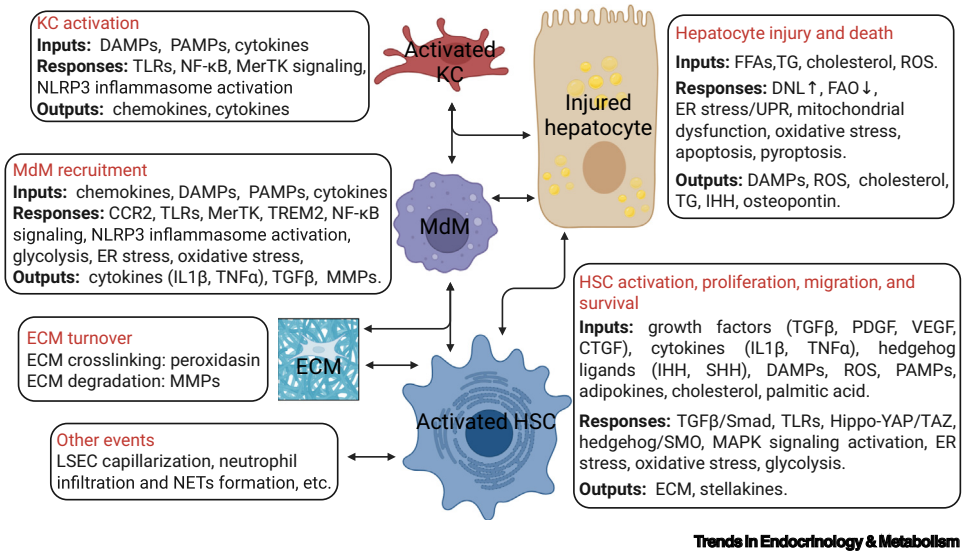

Figure 2. Key cellular and signaling events in metabolic dysfunction-associated steatohepatitis (MASH)-associated fibrosis: from intracellular responses to intercellular crosstalk. The development of fibrosis in MASH is orchestrated by a multilayered network of systemic, cellular, and molecular events. Initiated by systemic metabolic dysfunction, pathological inputs such as excess lipids, pathogen-associated molecular patterns (PAMPs), cytokines, and organokines converge on the liver and trigger cell-type-specific intracellular responses. Hepatocytes, for example, undergo lipotoxic stress [e.g., endoplasmic reticulum (ER) stress, mitochondrial dysfunction] and cell injury, resulting in the release of damage-associated molecular patterns (DAMPs), reactive oxygen species (ROS), and other pathological mediators. Kupffer cells (KCs) respond to systemic and hepatocyte-derived inputs via pattern recognition receptors [e.g., toll-like receptor (TLR) 4], activating innate immune pathways such as NF-κB, JNK, and the NLRP3 inflammasome, resulting in the release of proinflammatory and profibrotic cytokines and chemokines. Recruited via the CCL2–CCR2 axis, monocyte-derived macrophages (MdMs) activate innate immune and other cellular responses through receptors such as TLRs, MerTK, CD36, and triggering receptors expressed on myeloid cells 2 (TREM2), which shape their functional phenotype and effector output. Hepatic stellate cells (HSCs) integrate multiple fibrogenic cues through transforming growth factor-β (TGF-β)/Smad, TLRs, and Hippo–YAP/TAZ signaling, leading to their activation and excessive extracellular matrix (ECM) production. The secretory outputs of such cells can act as new inputs for neighboring cells, fueling complex and reciprocal intercellular crosstalk. This dynamic crosstalk establishes a fibrotic niche that ultimately culminates in progressive fibrosis in MASH. Abbreviations: FFA, free fatty acid; LSEC, liver sinusoidal endothelial cell; MMP, matrix metalloproteinase. This figure was created using BioRender.

The fate of HSCs (activation, senescence, deactivation, or death) is therefore a critical determinant of fibrosis progression and regression in MASH.

Hepatic macrophages also exhibit considerable heterogeneity in MASH [69,70]. Advances in scRNA-seq and the growing efforts to identify novel cell populations have led to a diverse and not always standardized nomenclature for macrophage subsets in the MASH setting. Among the well-established subsets are resident Kupffer cells (KCs), despite their decline due to cell death and impaired self-renewal during MASH progression [71]. Monocyte-derived KCs (moKCs), which acquire phenotypic and transcriptional features similar to resident KCs, partially replenish this diminished pool [70]. Lipid-associated macrophages (LAMs) represent another subset receiving increasing attention. These cells are also identified in various studies under different names, such as scar-associated macrophages (SAMs), NASH-associated macrophages (NAMs), or triggering receptors expressed on myeloid cells 2 (TREM2)-expressing macrophages [69,70,72]. While all of them express markers like TREM2, CD9, and GPNMB, their specific phenotypes and functions exhibit differences based on the disease context, experimental model, and stage of progression. While some studies link LAMs/SAMs to increased fibrosis [72–74], others show that their key marker Trem2 plays an antifibrotic role in MASH [75,76], as its deletion

worsens fibrosis. Thus, the exact role of LAMs in MASH fibrosis is currently controversial and requires more research. Notably, the diverse subsets of hepatic macrophages in MASH livers occupy distinct spatial niches [77]. moKCs are typically found in liver sinusoids in healthier regions, occupying niches analogous to resident KCs, whereas LAMs accumulate preferentially in steatotic and fibrotic areas, sometimes clustering around dying or dead lipid-laden hepatocytes to form **hepatic crown-like structures** [74,78,79]. This spatial pattern implies that the local microenvironments (healthy, steatotic, inflamed, or fibrotic) encountered by recruited monocytes may influence their differentiation (toward moKCs or LAMs) and shape their functions (see Outstanding questions) [79].

Key signaling molecules, pathways, and events in MASH-associated fibrosis

Progressive liver fibrosis in MASH is a dynamic, multistep process driven by a cascade of cellular events. A series of interconnected signaling molecules and pathways orchestrates these events across multiple hepatic cell types (Figure 2).

The activation of HSCs together with their proliferation, migration, and survival collectively contribute to maintaining a profibrotic niche and are widely recognized as central drivers of fibrogenesis in the liver [58]. These events are mediated by numerous growth factors, notably TGF-β, as well as PDGF, and connective tissue growth factor (CTGF). Additional mediators include hedgehog ligands, cholesterol, **damage-associated molecular patterns (DAMPs)**, reactive oxygen species, IL-1β, tumor necrosis factor-α (TNF-α), osteopontin, LPS, leptin, and angiotensin [58]. These different sources of input converge on HSCs to generate a range of intracellular responses to exert profibrogenic effects (Figure 2).

TGF-β is recognized as a pivotal signaling molecule driving fibrogenesis in MASH. The established mechanism involves activated TGF-β binding to and activating its receptors TGF-β receptor 2 and TGF-β receptor 1 on the cell surface of HSCs, initiating a downstream signaling cascade that leads to the upregulation of profibrogenic genes (e.g., *TGFB1*, *COL1A1*, *ACTA2*). This occurs primarily through canonical Smad-dependent and noncanonical MAP kinase (MAPK) signaling [80]. Besides HSCs, liver macrophages and other cells also serve as crucial sources of TGF-β during MASH progression. For instance, MERTK has been shown to promote TGF-β secretion via the extracellular signal-regulated kinase (ERK) pathway in macrophages [81]. Other growth factors, including PDGF and CTGF, promote HSC proliferation, migration, and ECM production, primarily via the RAS/ERK, JAK/STAT, and PI3K/AKT pathways [58].

PDGF also stimulates HSC to produce sonic hedgehog ligand, whose hepatic levels correlate with the severity of ballooning, portal inflammation, and fibrosis stage in patients with MASH [82–84]. Its paralog, Indian hedgehog (IHH), has also emerged as a key profibrotic signal in this condition. Hepatocytes are the primary source of IHH, with TAZ shown to mediate its transcription and secretion in these cells [85]. Circulating IHH levels have been linked to fibrosis severity in patients with MASLD/MASH [86]. Furthermore, hepatocyte-specific IHH knockout reduces fibrosis in both HFD-induced MASH and HFD/diethyl-nitrosamine-induced HCC models [87]. Mechanistically, hedgehog ligands bind to patched 1 on the cell surface of HSCs, leading to activation of the smoothened homolog–GLI2 pathway and subsequent upregulation of fibrogenic gene expression in HSCs [88].

Cholesterol is a critical upstream trigger of the TAZ–IHH axis. Excess cholesterol stabilizes TAZ in hepatocytes, upregulating IHH transcription and secretion [89]. EHBP1 can blunt this process by restricting hepatocellular cholesterol uptake, thereby mitigating fibrosis in MASH [90]. Cholesterol also acts directly on HSCs. In mice fed a MCD diet, free cholesterol accumulated in HSCs, upregulates TLR4, and sensitizes the cells to TGF-β-induced activation [91]. Clinically, hepatic

cholesterol levels correlate with MASH and fibrosis severity, especially in PNPLA3 I148M carriers [92]. High-cholesterol diets are commonly used in many murine MASH models to induce significant fibrosis [93].

Glucose metabolism has emerged as a crucial regulator of liver fibrosis. Activated HSCs undergo metabolic reprogramming toward aerobic glycolysis, which provides energy and metabolic intermediates to support its proliferation and matrix synthesis. Inhibition of glycolysis has been shown to attenuate HSC activation and fibrogenesis. Mechanistically, glycolysis in HSCs is regulated by upstream signals such as CPEB4-mediated induction of PFKFB3 and hedgehog/HIF1α-driven upregulation of HK2 [94,95]. Moreover, glycolysis promotes the release of profibrotic extracellular vesicles and produces lactate, which epigenetically upregulates fibrogenic gene expression through histone lactylation [96,97].

Complementing the previously detailed mechanisms, additional intracellular pathways in HSCs that respond to extracellular cues include TLR signaling, Hippo–YAP/TAZ signaling, nuclear receptor signaling, ER stress responses, and oxidative stress pathways [98].

It is important to note that the pathogenesis of fibrosis in MASH involves much more than HSC activation. Additional contributory events encompass hepatocyte injury, KC activation, and immune cell infiltration, as well as ECM crosslinking and degradation. These processes, orchestrated by their respective signaling molecules and pathways, collectively shape the fibrotic landscape in MASH (Figure 2). The complex nature of these interactions underscores the necessity for multitargeted interventions to effectively address this pathology.

Therapeutic insights into MASH-associated fibrosis

The presence and stage of liver fibrosis are the most critical determinants of liver-related mortality and extrahepatic outcomes like cardiovascular disease in MASH [11]. Thus, the primary goals of MASH therapies are to attenuate fibrosis progression and regress fibrosis especially when cirrhosis has developed. Therapeutic strategies for MASH fibrosis are multifaceted (Figure 3), primarily focusing on improving metabolic homeostasis, which thereby indirectly regulates fibrosis progression. Simultaneously, direct antifibrotic therapies aim to disrupt the scarring process and reverse architectural distortion within the liver. Lipotoxicity-induced cell death, inflammation, and the gut–liver axis are also significant targets for MASH therapy.

Non-pharmacological interventions targeting weight loss and metabolic improvement

Weight loss remains the primary therapy for adults with MASH, particularly in overweight and obese individuals [99]. Accordingly, lifestyle intervention in the form of dietary changes and regular exercise is globally accepted as the first-line treatment for all patients with MASH [99]. It has repeatedly been demonstrated in clinical trials that weight reduction achieved by caloric restriction, either with or without increased exercise, leads to improvements in liver enzymes, steatosis, MASH, and fibrosis [100,101]. A reduction of 10% or more of body weight results in MASH resolution in approximately 90% of patients and fibrosis regression in about 45% of patients [102]. However, for most, it is difficult to achieve and maintain such weight loss through lifestyle modifications alone.

Bariatric surgery is an efficient alternative for patients with severe obesity [body mass index (BMI) ≥ 40 kg/m^2, or BMI ≥ 35 kg/m^2 with comorbidities, or BMI of 30–35 kg/m^2 with poorly controlled T2D and/or hypertension despite optimal medical therapy] [99,103]. Both Roux-en-Y gastric bypass and sleeve gastrectomy have been shown to induce substantial weight loss. A recent clinical trial showed that, at 1-year follow-up, approximately 60% of patients who received gastric

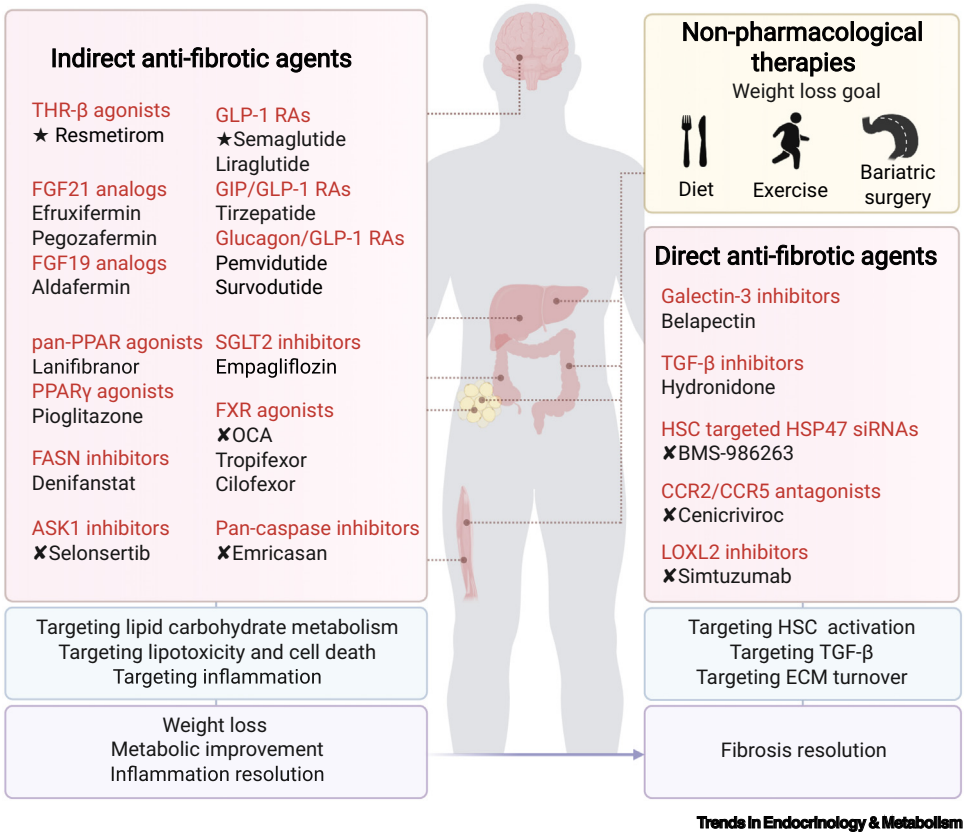

Figure 3. Therapeutic approaches for metabolic dysfunction-associated steatohepatitis (MASH)-associated fibrosis. Therapeutic strategies for MASH-associated fibrosis are multifaceted. Lifestyle modifications, including dietary changes and regular exercise aimed at achieving weight loss and improving metabolic homeostasis, are the fundamental intervention recommended for all individuals with MASH. Bariatric surgery offers a more efficient weight loss option for carefully selected individuals with severe obesity. Pharmacotherapy is presently dominated by metabolically targeted, indirect antifibrotic agents, led by the thyroid hormone receptor-β (THR-β) agonist resmetirom and the GLP-1 receptor agonist semaglutide (the two FDA-approved drugs for MASH fibrosis), in addition to numerous other agents in late-stage clinical trials, including fibroblast growth factor 21 (FGF21) analogs and peroxisome proliferator-activated receptor (PPAR) agonists. By correcting lipid carbohydrate dysregulation, reducing lipotoxicity-induced hepatocyte injury, and modulating immune activation, these agents achieve weight reduction, metabolic improvement, and inflammation resolution, thereby secondarily mitigating fibrosis. In parallel, direct antifibrotic candidates are under development, although most of them remain in early-phase trials or have been terminated. These therapies specifically target core fibrogenic processes and extracellular matrix (ECM) turnover to directly resolve fibrosis. By integrating these complementary therapeutic modalities, it may be possible to prevent, stabilize, and reverse fibrosis across the full clinical spectrum of MASH. ★, FDA-approved drugs for MASH-associated fibrosis; ×, drug development program has been discontinued. Abbreviations: ASK1, apoptosis signal-regulating kinase 1; FASN, fatty acid synthase; FXR, farnesoid X receptor; HSC, hepatic stellate cell; SGLT2, sodium-glucose cotransporter-2; TGF-β, transforming growth factor-β. Figure was created using BioRender.

bypass or sleeve gastrectomy achieved resolution of MASH without worsening of fibrosis, and about 40% achieved at least one-stage improvement in fibrosis without worsening of MASH [103]. However, these benefits following bariatric surgery are not guaranteed [51,104]. In a minority of patients, histological features such as hepatic fibrosis can actually deteriorate due to very rapid weight reduction following bariatric surgery [51]. Moreover, its application is restricted due to its invasive nature and risk of potential perioperative complications, as well as the requirement for continuous nutritional monitoring.

Metabolically targeted and other indirect antifibrotic therapies

Many pharmacological therapies for MASH fibrosis were initially antidyslipidemic, antiobesity, or antidiabetes drugs, and repurposed due to their metabolic benefits. Several promising agents are advancing from preclinical investigations to late-stage clinical trials (Figure 3). A notable milestone was the 2024 FDA approval of resmetirom (Rezdiffra™, MGL-3196), an oral, liver-directed, selective THR-β agonist for the treatment of patients with MASH and moderate to advanced liver fibrosis[i,ii]. Resmetirom met both primary endpoints at 52 weeks in the MAESTRO-NASH Phase 3 trial [105]. Mechanistically, resmetirom reduces lipotoxicity and improves mitochondrial function in hepatocytes while also improving serum lipid profiles, providing a dual hepatic and cardiovascular benefit that distinguishes it from many earlier therapeutic candidates. Notably, further Phase 3 MAESTRO-NAFLD-1 trial data (announced in May 2025) showed that treatment for up to 2 years with resmetirom reduced liver stiffness, fibrosis biomarkers, and the risk of clinically significant portal hypertension in 122 patients with compensated MASH cirrhosis (F4c)[iv]. However, its ultimate efficacy remains to be determined in the ongoing Phase 3 MAESTRO-NASH OUTCOMES trial.

The therapeutic landscape further expanded in August 2025 with the approval of Wegovy (semaglutide), an injectable GLP-1 receptor agonist also for the treatment of patients with MASH with moderate to advanced fibrosis[iii]. Semaglutide met both primary endpoints at 72 weeks in 36.8% of subjects compared with 22.4% with placebo in the Phase 3 ESSENCE trial [106]. Other powerful incretin-based therapies are also advancing, including newer dual incretin agonists, such as tirzepatide [a glucose-dependent insulinotropic polypeptide (GIP)/GLP-1 RA] and survodutide (a glucagon/GLP-1 RA). Both tirzepatide and survodutide demonstrated potent metabolic benefits, MASH resolution, and reduced liver fibrosis in Phase 2 trials, and two large Phase 3 trials of survodutide (LIVERAGE and LIVERAGE-Cirrhosis) are currently enrolling patients. Mechanistically, these incretin-based therapies drive significant weight loss (by increasing satiety through actions on hypothalamic centers, slowing gastric emptying, and inhibiting caloric intake), improve glycemic control (by stimulating glucose-dependent insulin secretion and inhibiting glucagon secretion), and reduce hepatic fat and inflammation, thereby indirectly inhibiting fibrosis progression.

PPAR agonists, such as the pan-PPAR agonist lanifibranor and the PPARγ agonist pioglitazone, primarily improve lipid metabolism, glucose homeostasis, and insulin sensitivity. Lanifibranor demonstrated MASH resolution and fibrosis improvement in the Phase 2b NATIVE trial [107]. Enrollment for the Phase 3 NATiV3 trial of lanifibranor was completed in April 2025. Pioglitazone, an oral drug approved for T2D, is controversial as to whether it is recommended for treating MASH due to a lack of large Phase 3 trial data demonstrating significant histological improvement in both steatohepatitis and liver fibrosis [99,108].

Sodium-glucose cotransporter-2 (SGLT2) inhibitors (e.g., empagliflozin) improve glycemic control and promote weight loss by inhibiting glucose reabsorption in the proximal renal tubules. Clinical trials have shown that SGLT2 inhibitors reduce body weight and improve dyslipidemia, hepatic steatosis, and liver injury. While some studies reported a reduction in noninvasive fibrosis markers, these findings were not consistent across trials [109–111]. More importantly, histological assessment of liver fibrosis was not performed in these trials. Consequently, although the EASL-EASD-EASO guidelines support the use of SGLT2 inhibitors for MASLD treatment, there is currently insufficient clinical evidence to recommend them specifically for the treatment of MASH [99].

FGF21 analogs (e.g., pegozafermin, efruxifermin) are emerging as promising therapeutic agents for MASH fibrosis by modulating glucose and lipid metabolism. FGF21 is a pleiotropic hormone-like protein that acts on multiple organs, including the liver, adipose tissue, and central

nervous system, to exert its therapeutic effects in MASH fibrosis [61]. The Phase 2b ENLIVEN trial in patients with MASH and F2F3 fibrosis showed that pegozafermin ameliorated fibrosis in 22–27% of subjects versus 7% for placebo at 24 weeks [112]. Two Phase 3 ENLIGHTEN programs for pegozafermin are currently enrolling patients globally. In the Phase 2b HARMONY trial in patients with MASH and F2–F3 fibrosis, efruxifermin achieved a ≥1-stage improvement in liver fibrosis without worsening of MASH in 39–41% of subjects versus 20% for placebo [113]. More recently, efruxifermin achieved a ≥1-stage fibrosis improvement without MASH worsening at week 96 in 21–29% of patients with compensated MASH cirrhosis versus 11% in the placebo group in the Phase 2b SYMMETRY trial [114], with global Phase 3 SYNCHRONY programs currently underway.

Fatty acid synthase (FASN) inhibitors, such as denifanstat, reduce lipogenesis in hepatocytes and HSCs, thereby decreasing lipid accumulation and fibrogenesis. In the Phase 2b FASCINATE-2 trial, denifanstat met both primary endpoints at 52 weeks in patients with biopsy-confirmed MASH and F2–F3 fibrosis [115]. Based on these findings, Phase 3 trials (FASCINATE-3 and FASCINIT) have been initiated to further evaluate its efficacy.

By targeting BA homeostasis, FXR agonists such as obeticholic acid have shown antifibrotic effects in Phase 3 clinical trials [116], yet the FDA has denied its approval due to pruritus and dyslipidemia that could increase the risk of cardiovascular disease. The new-generation FXR agonists (e.g., tropifexor, cilofexor, TERN-101) with improved tolerability are now being investigated in earlier stages.

While numerous drugs targeting inflammation and cell injury have shown promise for MASH treatment in preclinical studies, clinical translation has proved challenging. For instance, the dual chemokine receptor (CCR)2 and CCR5 antagonist cenicriviroc failed to meet fibrosis endpoints in the Phase 3 AURORA trial [117], despite promising early-stage data. Apoptosis signal-regulating kinase 1 (ASK1) inhibitor selonsertib also failed in Phase 3 STELLAR trials for MASH fibrosis [118]. Similarly, the pan-caspase inhibitor emricasan failed in the Phase 2b ENCORE-NF trial for MASH fibrosis [119]. Other emerging strategies such as genetically focused drugs, specialized pro-resolving mediators, and gut–liver axis modulators such as probiotics and synbiotics have shown therapeutic benefits in preclinical MASH studies and are awaiting further clinical evidence [120,121].

Direct antifibrotic therapies

Although no direct antifibrotic therapies have been clinically approved for MASH, accumulating preclinical evidence highlights their potential value, especially if combined with therapies targeting upstream metabolic dysregulation. Direct antifibrotic strategies aim to reduce scar formation and promote fibrosis regression. Targeting HSC activation is a central strategy, as they are the primary ECM producers in MASH [121]. HSC-directed oligonucleotide therapies have been tested. BMS-986263, a vitamin A-coupled lipid nanoparticle that delivers siRNA to collagen chaperone heat shock protein 47, was designed to disrupt collagen maturation. A Phase 2 trial in patients with HCV and advanced fibrosis showed histological improvements, while the Phase 2 trial in patients with compensated MASH cirrhosis was terminated due to lack of efficacy [122]. Targeting of specific HSC populations represents a novel cell therapy concept. CAR T cells are being engineered to target and clear specific HSC populations, such as senescent HSCs or fibroblast activation protein-expressing HSCs [68,121]. However, careful targeting is crucial to avoid depleting 'good' HSCs.

TGF-β is also a primary target due to its potent profibrogenic activity. However, systemic TGF-β antagonism poses safety concerns due to its pleiotropic roles. Alternative strategies focus on

inhibiting integrin-mediated TGF-β activation or selectively reducing the release of latent TGF-β binding protein-bound TGF-β [121]. However, clinical evidence for their efficacy in MASH is still lacking. Another agent targeting TGF-β is hydronidone, a pirfenidone analog that inhibits TGF-β/p38-driven collagen production in HSCs. A Phase 3 clinical trial of hydronidone for chronic hepatitis B-associated fibrosis is ongoing in China [123] and a Phase 2 trial for MASH fibrosis is planned to initiate in the USA in 2025.

Galectin-3 inhibitors (e.g., belapectin) are considered promising direct antifibrotic therapies due to their implication in inflammation and HSC activation [121]. Belapectin is being evaluated in the Phase 2b/3 NAVIGATE trial in patients with MASH cirrhosis and portal hypertension. Topline results from the 18-month treatment period showed that treatment with belapectin resulted in fewer patients experiencing worsening of liver stiffness in the treatment arms[v].

Direct targeting of ECM remodeling offers another therapeutic strategy. Previous efforts, such as lysyl oxidase-like 2 inhibitors aimed at reducing ECM stiffness, experienced setbacks with the failure of simtuzumab in two Phase 2 MASH trials of patients with MASH and bridging fibrosis or compensated cirrhosis [124]. A more recent development, RTX001, is an engineered autologous macrophage therapy that overexpresses IL-10 and MMP9 to enhance both anti-inflammatory and ECM degrading activities. The EMERALD Phase 1/2 trial of RTX001 is currently recruiting patients with end-stage liver disease (including MASH cirrhosis).

Concluding remarks and future perspectives

Growing evidence over the past decade indicates that MASH-associated fibrosis arises from complex, multipathogenic processes sustained by multicellular and multiorgan networks. Despite remarkable advances in our understanding of the underlying mechanisms and a parallel expansion in therapeutic development in MASH, the progression from therapeutic target discovery to clinical approval remains slow, with frequent trial setbacks. To date, only Rezdiffra (resmetirom) and Wegovy (semaglutide) have been approved by the FDA for treatment of stage F2–F3 fibrosis in MASH, and notably, no pharmacological therapies are available for patients with compensated cirrhosis (F4). Moreover, most clinical candidates alleviate fibrosis indirectly through metabolic improvements and weight loss. This raises the question of whether upstream metabolic interventions alone are sufficient for patients with established cirrhosis, or whether a shift toward direct antifibrotic or combination strategies is warranted (see Outstanding questions). In practice, however, the development of direct antifibrotic agents targeting fibrogenic cells and ECM turnover has proved challenging, with most candidates either discontinued or still in early-phase trials. Accordingly, identifying safe and tractable direct antifibrotic targets remains a key priority of future research.

These challenges also highlight a crucial reality: the same drugs do not work in all patients, reflecting the complexity and heterogeneity of MASH fibrosis. A 'one-size-fits-all' approach is unlikely to be universally effective. The future of treatment is evolving toward multifaceted and personalized strategies. Combination therapies that target distinct yet complementary pathways are emerging as the most promising approach, particularly for patients with more advanced fibrosis or who do not respond adequately to monotherapy. Future preclinical and clinical research efforts should thus focus on the safe and rational integration of metabolic modulators with direct antifibrotic strategies. In parallel, precision medicine approaches incorporating genetic, epigenetic, and biomarker profiling are expected to further individualize therapy and improve clinical outcomes [125].

From a translational perspective, overcoming the slow pace and high attrition rate in MASH therapeutic development is critical. Harnessing cutting-edge technologies will be essential to improve

Outstanding questions

The fate of recruited monocytes (differentiate into moKCs, LAMs, or other subsets) in MASH is primarily determined by the location to which they are recruited and the local microenvironment at that site. So, what signaling cues drive their recruitment from distinct sites and how do signals differ between injured regions and relatively healthier regions?

In the context of MASH fibrosis, are LAMs/SAMs observed in steatotic/fibrotic liver regions only derived from fresh recruitment, or do local proliferation and migration from other regions after recruitment also contribute to the presence of those cells?

Can therapies targeting upstream metabolic dysfunction provide meaningful clinical benefits in MASH patients with compensated cirrhosis (F4), or should therapeutic strategies in this population shift toward direct antifibrotic approaches?

What are the most feasible and safe targets for direct antifibrotic therapies, and how can these be effectively combined with metabolically focused agents to enhance fibrosis reversal in MASH?

When a MASH therapy with significant hepatic benefits also poses potential cardiovascular risks (e.g., hypertriglyceridemia with ACC inhibitors, elevated LDL-C with certain FXR agonists), could combination strategies with agents that mitigate these risks without compromising hepatic efficacy represent a promising solution?

the efficiency and predictability of translating scientific advances into approved therapies. Human liver organoids, for instance, are a powerful tool that closely mimics human liver architecture and disease-specific pathways. Their enhanced physiological relevance enables patient-specific modeling and drug testing, providing advantages over conventional animal models and 2D cultures. Moreover, machine learning-based multiomics integration holds promise for identifying precise therapeutic targets and stratifying patients. Future progress will require the development of new technologies alongside the optimization and integration of existing ones; for example, to address critical limitations of liver organoid systems such as incomplete maturation and limited physiological fidelity.

Author contributions

Y.Z. drafted the manuscript and generated figures. B.C. edited the manuscript.

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Declaration of interests

The authors declare no competing interests.

Resources

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[ii]https://ir.madrigalpharma.com/news-releases/news-release-details/madrigal-pharmaceuticals-announces-fda-approval-rezdiffratm

[iii]www.fda.gov/drugs/news-events-human-drugs/fda-approves-treatment-serious-liver-disease-known-mash

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