Focused Review

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An integrated view of anti-inflammatory and antifibrotic targets for the treatment of NASH

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Summary

Successful development of treatments for non-alcoholic fatty liver disease and its progressive form, non-alcoholic steatohepatitis (NASH), has been challenging. Because NASH and fibrosis lead to progression towards cirrhosis and clinical outcomes, approaches have either sought to attenuate metabolic dysregulation and cell injury, or directly target the inflammation and fibrosis that ensue. Targets for reducing the activation of inflammatory cascades include nuclear receptor agonists (*e.g.* resmetirom, lanifibranor, obeticholic acid), modulators of lipotoxicity (*e.g.* aramchol, acetyl-CoA carboxylase inhibitors) or modification of genetic variants (*e.g. PNPLA3* gene silencing). Extrahepatic inflammatory signals from the circulation, adipose tissue or gut are targets of hormonal agonists (semaglutide, tirzepatide, FGF19/FGF21 analogues), microbiota or lifestyle interventions. Stress signals and hepatocyte death activate immune responses, engaging innate (macrophages, innate lymphocyte populations) and adaptive (auto-aggressive T cells) mechanisms. Therapies have also been developed to blunt immune cell activation, recruitment (chemokine receptor inhibitors), and responses (*e.g.* galectin-3 inhibitors, anti-platelet drugs). The disease-driving pathways of NASH converge to elicit fibrosis, which is reversible. The activation of hepatic stellate cells into matrix-producing myofibroblasts can be inhibited by antagonising soluble factors (*e.g.* integrins, cytokines), cellular crosstalk (*e.g.* with macrophages), and agonising nuclear receptor signalling. In advanced fibrosis, cell therapy with restorative macrophages or reprogrammed (CAR) T cells may accelerate repair through hepatic stellate cell deactivation or killing, or by enhancing matrix degradation. Heterogeneity of disease – either due to genetics or divergent disease drivers – is an obstacle to defining effective drugs for all patients with NASH that will be overcome incrementally.

Introduction

Non-alcoholic fatty liver disease (NAFLD) is the most common chronic liver disease in the world and its prevalence is increasing. While approximately 25% of the global population were affected by NAFLD in 2016, this number increased to about 30% in 2019, with the highest rates in Latin America and the Middle East.[1] The natural course of NAFLD is heterogeneous, with substantial inter-patient variability, which is influenced by several non-modifiable (age, sex, race/ethnicity, family history, genetics) and modifiable (lifestyle/diet/exercise, comorbidities, drugs, alcohol) risk factors.[2,3]

NAFLD can be broadly sub-classified into two categories, either non-alcoholic fatty liver (NAFL), the non-progressive form of NAFLD, or non-alcoholic steatohepatitis (NASH), the progressive form of NAFLD.[4] NAFL is characterised by the presence of hepatic steatosis typically in zone 3, with or without mild inflammation. Progression to NASH, is histologically characterised by the presence of three cardinal features, namely steatosis, lobular inflammation and hepatocyte ballooning, with or without fibrosis, which is typically perisinusoidal but can become panlobular as the disease advances. Resolution of NASH back to NAFL can be highly dynamic and may occur even within a short time frame.[5]

Among individuals with NASH, fibrosis progresses rapidly in some but relatively slowly in others.[4] For most at-risk patients, as well as for most patients with NAFLD, liver fat content is a prognostic marker of cardio- and cerebro-vascular risk, while inflammatory and cellular injury can drive NASH and fibrosis; the latter is critical because fibrosis stage is predictive of liver-specific morbidity and mortality. Ultimately, those with NASH and fibrosis may develop end-stage complications such as cirrhosis, portal hypertension or hepatocellular carcinoma (HCC), which portend a much worse prognosis.[6–10]

The development of a therapy for NASH has been surprisingly challenging, as no drug is currently approved for the treatment of NASH.[11] However, recent positive phase III clinical trial data for two agents, the farnesoid X receptor (FXR) agonist obeticholic acid and the thyroid hormone receptor (THR)-β agonist, resmetirom, offer hope of the first drug approvals for this serious illness. Two key regulatory agencies, the FDA and EMA, have agreed that a substantial histological improvement, defined as resolution of NASH and/or improvement in fibrosis

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Keypoints:

- Metabolic injury to the liver causes inflammatory processes that drive progression of NASH and liver fibrosis, supporting the concept of "anti-inflammatory" and "antifibrotic" therapeutic strategies.
- Metabolism, inflammation and fibrosis are closely intertwined, which can be leveraged by pharmacological approaches targeting several cellular components or molecular pathways of NASH at once.
- Fibrosis stage is the best predictor of liver-related and overall morbidity and mortality; the hepatic fat content serves as a prognostic marker of cardio- and cerebro-vascular risk.
- Pharmacological strategies targeting primarily metabolic processes aim at reducing metabolic injury and stress in hepatocytes to mitigate against activation of subsequent inflammatory signals and the cellular responses they elicit.
- Extrahepatic mediators, *e.g.* from the gut, adipose tissue or the endocrine system, are also being evaluated for the treatment of NASH, because they can impact inflammation and fibrosis as well.
- Anti-inflammatory strategies target the activation of immune sentinels, the subsequent recruitment of immune cells as well as the complex intercellular crosstalk of parenchymal and non-parenchymal cells.
- Liver macrophages – resident Kupffer cells and monocyte-derived macrophages – are key to the pathogenesis of NASH, as their context-dependent polarisation and enormous functional plasticity regulates inflammatory, fibrogenic and tissue repair responses.
- Pharmacological strategies targeting fibrogenesis focus on hepatic stellate cells as the main matrix-producing mesenchymal cells, by inhibiting the signals or intracellular processes culminating in their activation, or by reverting activated myofibroblasts to a quiescent state.
- Emerging therapeutic strategies include using cell therapy-based anti-inflammatory and antifibrotic approaches (*e.g.*, individualised/reprogrammed macrophages ['CAR-iMac'] or immune-regulatory mesenchymal stromal cells) to accelerate repair processes.
- Technological advances (artificial intelligence, spatial technologies, single-cell sequencing) may provide higher granularity on disease mechanisms, possibly guiding future personalised treatment approaches.

stage, in sequential liver biopsies will be sufficient as a surrogate marker reasonably likely to yield drug approval and long-term benefit (*i.e.*, reduced liver- or cardiovascular related events, mortality or liver transplantation).[12,13] This focus on inflammation and fibrosis, grounded on their association with disease progression and clinical outcomes, fuelled the concept of developing "anti-inflammatory" and/or "antifibrotic" targets to dissociate the metabolic disease from its detrimental tissue responses (*i.e.* inflammation, fibrosis) in the liver. However, inflammation and fibrosis represent evolutionarily conserved, endogenous defence mechanisms, so that targets for intervention must be selected carefully, striking a delicate balance between benefits and potential risks of dampening these defence mechanisms when the underlying drivers of the disease remain untargeted.

Because fibrosis is the most compelling predictor of outcomes, current and future therapies must improve fibrosis, by attenuating profibrotic inflammatory signalling and/or abrogating fibrosis directly. Accordingly, in this review article, we present an integrated view of anti-inflammatory and antifibrotic approaches to treat NASH.

From metabolic injury to inflammation: Targeting the activation of inflammatory cascades

Over the past decades, our understanding of mechanisms underlying NAFLD and NASH pathogenesis has advanced. Excessive energy substrates, especially carbohydrates, trigger hepatic *de novo* lipogenesis, while dietary fat and the metabolically overwhelmed and dysfunctional adipose tissue provide excessive free fatty acids. Although the initial liver triglyceride storage may act as a buffer, lipotoxicity – defined as the generation of injurious lipid metabolites – is a key feature of progressive NAFLD, involving production of reactive oxygen species, mitochondrial dysfunction and the development of endoplasmic reticulum stress.[3] Attenuating metabolic injury to hepatocytes should subsequently reduce inflammatory and fibrogenic responses (Fig. 1). Nonetheless, it is unclear whether targeting different pathways or enzymes to reduce fat will have differing impacts on the generation of lipotoxic species – addressing this issue will be important in helping to determine the key metabolic vulnerabilities that can be targeted to attenuate injury and inflammation in NASH. Current pharmacological strategies targeting primarily metabolic processes aim at reducing pathogenic mechanisms culminating in metabolic injury and stress to hepatocytes in order to reduce activation of subsequent inflammatory signals and the cellular responses they elicit. Since many of these signals also originate outside the liver in NAFLD, for example in the gut, adipose tissue or the endocrine system, targeting extrahepatic mediators is also being evaluated for the treatment of NASH.

Modulating hepatocytic cell metabolism and signalling to reduce inflammatory cascades

Several pharmacological compounds under late stage development (Fig. 1) target key metabolic pathways, including lipogenesis (*e.g.*, aramchol, inhibitors of acetyl-CoA carboxylase or fatty acid synthase), energy availability (*e.g.*, glucagon-like peptide 1 [GLP-1] receptor and/or glucagon agonists) or lipid handling (*e.g.*, fatty acid β-oxidation via nuclear receptors, such

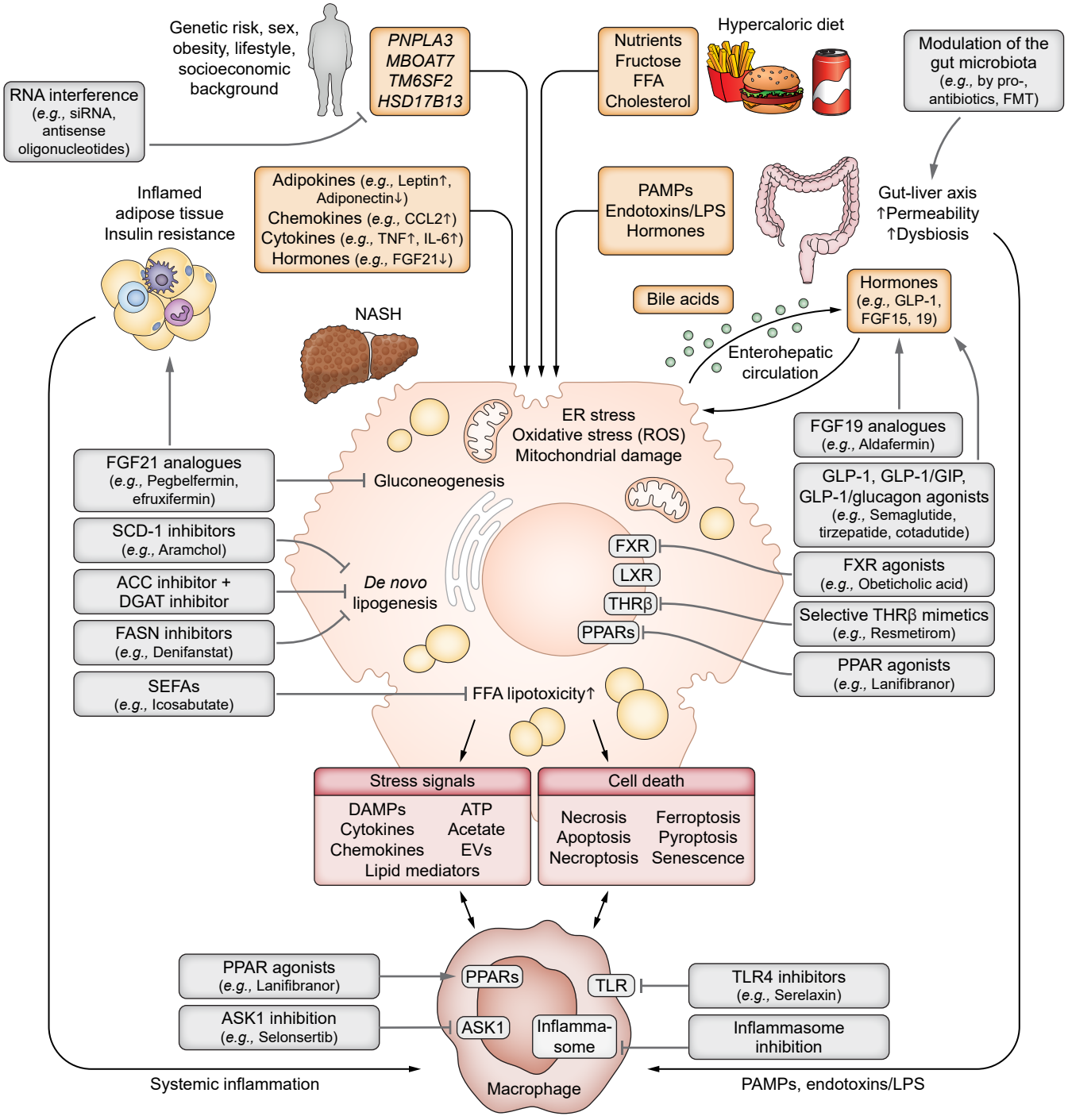

Fig. 1. From metabolic injury to inflammation: Targeting the activation of inflammatory cascades. Metabolic injury is influenced by various non-modifiable and modifiable, intra- and extrahepatic (risk) factors resulting in pathogenic cascades including ER stress, oxidative stress and mitochondrial dysfunction in hepatocytes. Lipotoxicity, as a consequence of FFA overload and increased *de novo* lipogenesis, leads to release of stress signals and induction of cell death mechanisms of the metabolically stressed hepatocyte, which in turn activate immune responses. Pharmacologic strategies target the metabolic dysregulation and injury of hepatocytes as well as extrahepatic inflammatory signals. Other approaches include modification of genetic risk factors or inflammatory activation of immune cells. ACC, acetyl-CoA carboxylase; ASK1, apoptosis signal-regulating kinase 1; ATP, adenosine triphosphate; CCL, chemokine (C–C motif) ligand; IL, interleukin; DAMPs, damage-associated molecular patterns; DGAT, diglyceride acyltransferase; ER, endoplasmic reticulum; EVs, extracellular vesicles; FASN, fatty acid synthase; FFA, free fatty acids; FGF, fibroblast growth factor; FMT, faecal microbiota transplantation; FXR, farnesoid X receptor; GIP, glucose-dependent insulinotropic polypeptide; GLP-1, glucagon-like peptide 1; LPS, lipopolysaccharide; LXR, liver X receptors; NASH, non-alcoholic steatohepatitis; PAMPs, pathogen-associated molecular patterns; PPAR, peroxisome proliferator-activated receptor; ROS, reactive oxygen species; SCD1, stearoyl-CoA desaturase 1; SEFAs, structurally engineered fatty acids; THR, thyroid hormone receptor; TNF, tumour necrosis factor.

as THR mimetics like resmetirom).[14] Long-chain omega-3 fatty acids are known to beneficially regulate inflammatory pathways in the course of NASH, but therapeutic efficacy is highly limited by peroxidation and subsequent degradation. Structurally engineered fatty acids, such as icosabutate, improve resistance to peroxidative processes, and thus represent a promising approach to maintaining the beneficial metabolic effects of long-chain omega-3 fatty acids.[15]

Key responses of hepatocytes to metabolic injury include the activation of inflammatory and pro-apoptotic signalling, *e.g.* via tumour necrosis factor (TNF)α and NF-κB,[3] favouring hepatocyte cell death pathways and/or senescence.[16,17] This is paralleled by upregulation of developmental pathways (*e.g.* Notch, Hedgehog, Hippo-YAP-TAZ) that are often expressed by bipotential progenitor cells or ductular cells, which can directly activate profibrotic cell-cell communication between hepatocytes and neighbouring cells.[18] Metabolic processes in both hepatocytes and non-parenchymal liver cells are modulated by nuclear receptors, a family of ligand-controlled transcription factors that regulate glucose, fat and cholesterol homeostasis.[19] Nuclear receptors are activated by a variety of ligands including hormones, lipids and bile acids.[20] Peroxisome proliferator-activated receptors (PPARs), liver X receptors (LXRs), FXR and THR (particularly THR-β) are the most extensively studied receptors that are targets for therapy.[20–22] Metabolic injury amplifies cell-cell-communication from hepatocytes to non-parenchymal and/or inflammatory cells.[23] Hepatocyte stress, and particularly cell death (including apoptosis, necrosis and necroptosis), stimulates the release of "signalling molecules" including cytokines/chemokines, lipids, extracellular vesicles and, ultimately, damage-associated molecular patterns.[24] By attenuating their impact on injury and inflammatory signalling, interventions targeting metabolic pathways may additionally have anti-inflammatory effects.

Metabolic pathways in hepatocytes are impacted by genetic factors, based on genome-wide association studies in large cohorts that have identified genetic susceptibility factors for NAFLD/NASH, such as polymorphisms in patatin-like phospholipase domain-containing protein 3 (*PNPLA3*), *MBOAT7* (membrane-bound O-acyltransferase domain 7) or *TM6SF2* (transmembrane 6 superfamily 2).[25] These genetic components critically determine how the liver, through regulation of hepatic mitochondrial function, handles the oversupply of substrates, such as sugars and lipids.[26] Consequently, gene modifying approaches such suppression of the risk variant *PNPLA3* rs738409 C>G (p.I148M) via RNA-interference (*e.g.* small-interfering RNA, antisense oligonucleotides), small molecules or targeting downstream pathways (*e.g.*, *HSD17B13*) are being explored in clinical trials using a personalised approach in patients with "at-risk" NASH.[27,28] Interestingly, *PNPLA3* and *MBOAT7* variants also directly impact fibrogenic signalling in stellate cells,[29,30] indicating that targeting the genetic determinants of lipid handling in hepatocytes may also have (additional) antifibrotic effects through their impact on non-parenchymal cells.[31] For example, the *PNPLA3* risk polymorphism also independently promotes fibrogenic activity in hepatic stellate cells (HSCs).[32] This may explain why genetic risk prediction by *PNPLA3* polymorphisms is not restricted to NAFLD, but also applies to other aetiologies of hepatic fibrosis such as alcohol-related liver diseases.[33]

Organ crosstalk in NAFLD: targeting extrahepatic inflammatory signals

The processes leading to metabolic injury are greatly influenced by extrahepatic stimuli. For instance, in cases of concurrent diabetes and insulin resistance, oxidative stress in other tissues due to hyperglycaemia may compound lipotoxicity in the liver.[34] As a multisystem disease, pathogenic pathways in the liver are fuelled by input from metabolic or inflammatory signals derived from the gut,[35] adipose tissue,[36] skeletal muscle[37] or bone marrow.[38] Together with specific extrahepatic signals, *e.g.* bacterial metabolites or pathogen-associated molecular patterns from the gut, these (systemic) mediators activate inflammatory responses.[39] For instance, bile acids are soluble mediators and key components of the gut-liver axis, connecting altered intestinal homeostasis (*e.g.* dysbiosis, increased permeability) to metabolic injury in NAFLD.[35] Their enterohepatic circulation allows them to act as hormones by stimulating nuclear receptors such as FXR in the gut and liver,[40] affecting functions of both organs via local and long-distance effects.

Interfering with gut-derived signals holds particular promise as a method to reduce inflammatory responses in the liver, since the gut and liver form an anatomically and functionally linked unit, the "gut-liver axis". Some of the obvious inflammatory mediators reaching the liver, particularly their resident macrophages (Kupffer cells), via the portal vein include nutrient components (fatty acids, carbohydrates, amino acids), bacterial metabolites (*e.g.*, trimethylamine, ethanol) and bacterial antigens (*e.g.*, lipopolysaccharide [LPS]).[35,41] On the contrary, the gut can also produce "anti-inflammatory" mediators with beneficial effects in the liver, including hormones (GLP-1, fibroblast growth factor [FGF]-19 or murine orthologue FGF-15), secondary bile acids or anti-inflammatory metabolites (*e.g.*, indole as a tryptophan metabolite).[35,42] Conceptually, reversing gut dysbiosis in NAFLD using pre-/pro-/antibiotics or faecal microbiota transplantation would reduce these inflammatory signals, reverse perturbations in gut barrier function (thereby further reducing endotoxin in the portal vein) and promote the endogenous production of beneficial mediators (FGFs, GLP-1, secondary bile acids). Ample preclinical evidence supports microbiota interventions to reduce NAFLD-related liver inflammation; however, evidence for long-term anti-inflammatory or antifibrotic effects in clinical trials is lacking.[43] Precision microbiome-centred therapies, including engineered bacteria, postbiotics and phages, may provide a promising avenue for the more individualised treatment of inflammation in NAFLD.[44]

Gut-derived hormones are already in clinical use for metabolic diseases (*e.g.* GLP-1 receptor agonists, such as semaglutide or liraglutide, GLP-1/GIP [glucose-dependent insulinotropic polypeptide] dual agonists, such as tirzepatide) or in advanced clinical development (e.g, GLP-1/glucagon dual agonists, such as cotadutide, or the FGF-19 mimetic aldafermin).[11,45] Their effects on inflammation and fibrosis in the liver are thought to be mainly indirect, *e.g*. via reducing energy supply and/or improving hepatocyte metabolism. The currently available data is inconclusive about GLP-1 receptor profiles on immune cells. While a recent analysis of human and mouse liver cell populations does not report GLP-1 receptor expression on

Kupffer cells,[46] some studies suggest that Kupffer cells express GLP-1 receptors and could thus also respond to GLP-1 receptor agonists.[47] As GLP-1 agonism has promoted NASH resolution in a phase II trial,[48] this mechanism is being intensively evaluated in larger studies.

Establishing inflammation in NAFLD: Targeting immune cell activation, recruitment and cellular crosstalk

Metabolic stress or injury to hepatocytes, that provokes tissue perturbation, and extrahepatic inflammatory signals (*e.g.* systemic circulating or gut-derived) are detected by immune sentinels in the liver that underlie a robust immune response.[39] This inflammatory response may further damage stressed hepatocytes, resulting in a vicious cycle and the full picture of necroinflammation (Fig. 2). However, inflammation in NAFLD is rarely linear in its progression, rather it fluctuates between flares and resolution. This may explain why the highly dynamic parameter of inflammation is a weaker prognostic feature than fibrosis, which is a more static parameter, when captured at a single time-point on liver histology.[7] However, we still have a somewhat limited understanding of the pathophysiology of inflammation in NAFLD, and we apply an oversimplified definition of inflammation (*i.e.* histological detection of leukocytes in the liver) that does not account for the distinct functions of immune cells that would allow us to differentiate between disease-promoting and resolving mechanisms of inflammation. Human studies and rodent models have provided compelling insights into the different immune cells and their presumed functions in NAFLD/NASH.[24,49] Still, it is likely that immune subsets are more complex and nuanced than we currently understand in their functions during NASH. Immune cell communication between hepatic parenchymal and non-parenchymal cells during NAFLD pathogenesis may be very disease stage- and localisation-dependent, and dynamic in nature, confounding our capacity to generate a complete

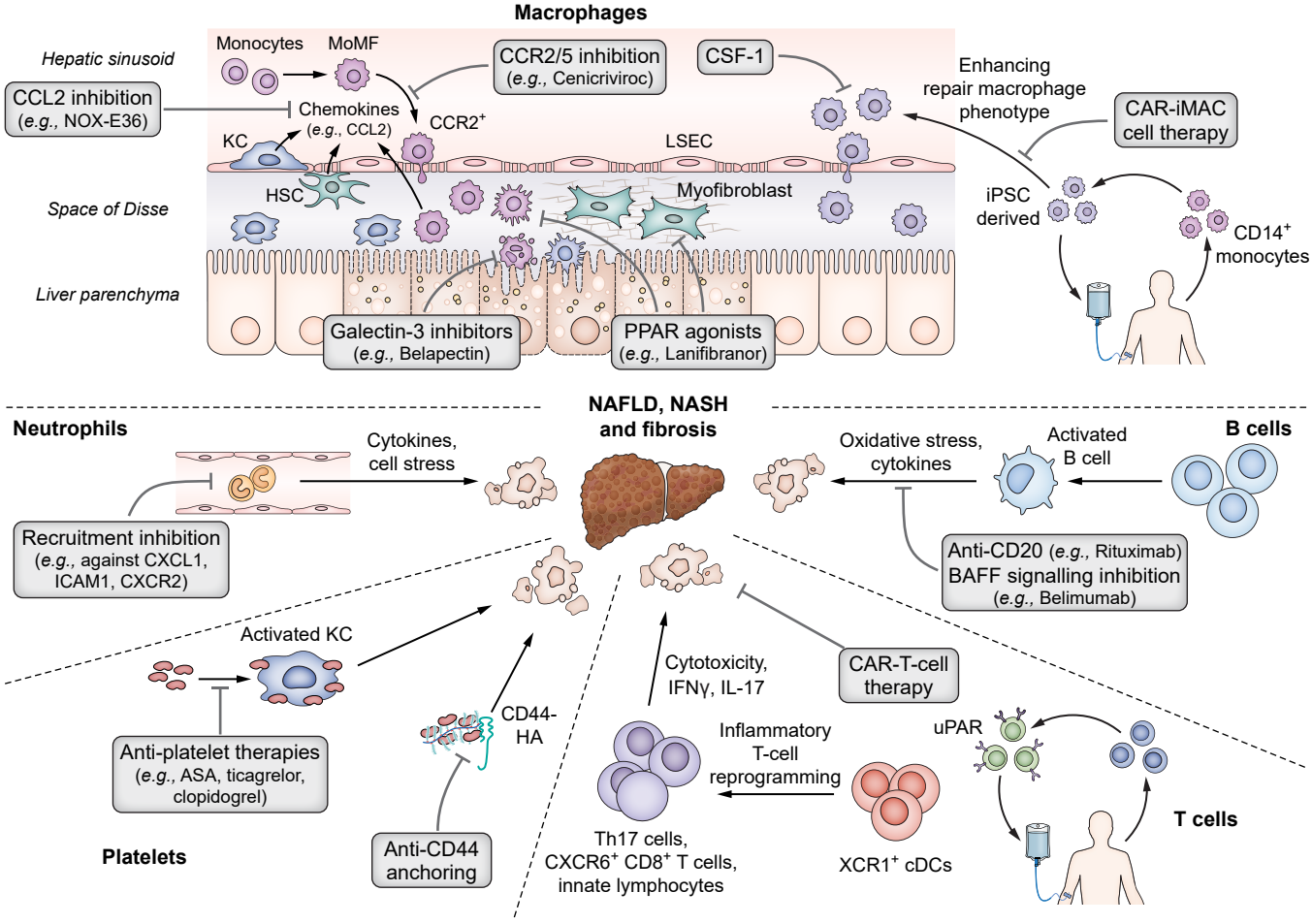

Fig. 2. Establishing inflammation in NAFLD: Targeting immune cell activation, recruitment and cellular crosstalk. Upon metabolic injury, parenchymal and non-parenchymal immune cells release inflammatory mediators such as chemokines, leading to the recruitment and hepatic accumulation of further immune cells. Pharmacologic strategies target the activation and polarisation of immune cells (macrophages, T cells), inhibit the hepatic infiltration of inflammatory immune cells (neutrophils, monocytes, macrophages) or modulate intercellular crosstalk. New therapeutic approaches include cell therapies with "reprogrammed" immune cells. ASA, acetylsalicylic acid; CAR-iMAC, CAR-expressing macrophages; CCL, chemokine (C–C motif) ligand; CCR, CC chemokine receptor; cDC, conventional (classical) DCs; CSF1, colony stimulating factor 1; CXCL1, C-X-C chemokine ligand 1; CXCR, C-X-C chemokine receptor; ECM, extracellular matrix; HSC, hepatic stellate cell; ICAM1, intercellular adhesion molecule-1; IFNγ, interferon gamma; IL, interleukin; iPSC, induced pluripotent stem cells; KC, Kupffer cell; LSEC, liver sinusoidal endothelial cells; MoMF, monocyte-derived macrophage; PPAR, peroxisome proliferator-activated receptors; uPAR, urokinase-type plasminogen activator receptor.

picture of these interactions.[23,24,50] The recent technological advances in biology and immunology, including single-cell multi-omics, have revolutionised our view on inflammation in NAFLD and promise to fill gaps in our knowledge. Current pharmacological strategies targeting the initiation and maintenance of hepatic inflammation either attempt to inhibit the primary recognition of injury and subsequent activation of inflammatory cascades, target the recruitment of inflammatory immune cells and/or modulate complex immune cell crosstalk.

Targeting the activation of inflammation: inhibition of TLRs, inflammasome or inflammatory signalling

Many cell types in the liver can act as immune sentinels, particularly non-parenchymal populations (sinusoidal endothelial cells, stellate cells) and liver-resident immune cells (innate lymphocyte populations and macrophages). Liver macrophages are key to the pathogenesis of NAFLD (Fig. 2), as they accumulate with disease progression in human biopsies.[50–52] Hepatic macrophages comprise ontogenetically and functionally distinct subsets, including tissue-resident Kupffer cells and infiltrating monocyte-derived macrophages, and have remarkable functional plasticity. When Kupffer cells sense hepatocyte stress and injury signals from other cells (or extrahepatic sources), they activate inflammatory signals, recruit monocytes (and other inflammatory cells) via chemokines and engulf cellular debris.[53,54] A series of elegant studies using single-cell RNA sequencing (scRNA-seq) has provided insights into hepatic immune cell heterogeneity that are unprecedented in their detail, revealing striking alterations, particularly in myeloid cells and macrophages in NAFLD,[55–60] and within related extrahepatic compartments including the bone marrow[61] and adipose tissue.[62] Kupffer cells comprise subsets/differentiation states, of which the CD206hi ESAM+ subtype (in mice) participates in fatty acid metabolism and may thereby directly drive NASH.[56]

Macrophages are equipped with receptors that recognise tissue injury or threats, including the LPS co-receptor CD14, immunoglobulin receptors including CD16 (FcγRIII), scavenger receptors like CD206 (mannose receptor) and pattern recognition receptors including toll-like receptors (TLRs, *e.g.* TLR4 or TLR9) that recognise pathogen- and/or damage-associated molecular patterns. For instance, activation of TLR4 via LPS leads to the release of pro-inflammatory cytokines (*e.g.*, TNF, interleukin [IL]-6), but similar effects are also observed if free fatty acids bind to TLR4.[63] Several strategies therefore aim to block danger recognition on macrophages and other cells, for example by inhibiting TLR4 via serelaxin or TAK-242, or through activation of the inflammasome, a multiprotein intracellular complex. In NAFLD and NASH, the NLR family pyrin domain containing 3 (NLRP3) inflammasome has been identified as a central driver of inflammation – via activation of caspase 1 and the release of inflammatory cytokines (*e.g.*, IL-1β) – and as an inducer of inflammatory ("pyroptotic") cell death.[64] Pharmacological inhibition of NLRP3 activation has been proposed for a wide range of inflammatory diseases,[65] but is currently still in early-stage development for NASH. Nonetheless, rodent models emphasise the importance of NLRP3 activation for inflammatory macrophage as well as fibrogenic stellate cell responses.[64] The key potential liability of this approach will be risk of infection, which will be monitored closely in clinical trials.

Following their recruitment, inflammatory intracellular signalling pathways in macrophages include NF-kB, apoptosis signal-regulating kinase 1 (ASK1), c-Jun N-terminal kinase or p38 mitogen-activated protein kinase.[51] The ASK1 inhibitor selonsertib did not lead to a histological benefit in terms of NASH resolution or fibrosis improvement in patients with NASH and advanced fibrosis or cirrhosis,[66] despite compelling anti-inflammatory and antifibrotic effects in preclinical models.[67] Newer, pharmacologically optimised ASK1 inhibitors are still being evaluated as potential therapies, given the compelling role of ASK1 in disease pathogenesis. Because inhibiting inflammatory signalling in macrophages is therapeutically challenging in NASH, augmenting anti-inflammatory signalling via targets that promote disease resolution may be the more realistic approach. In this regard, nuclear receptors modulate innate immune functions in NASH. Activation of PPARs, particularly PPARβ/δ stimulation, promote the anti-inflammatory polarisation of hepatic macrophages,[22,68] supporting the concept of pan-PPAR agonism in NASH and in line with promising clinical data on lanifibranor.[69] Similar observations on anti-inflammatory effects have been reported for FXR and LXR agonism in macrophages.[70]

Targeting immune cell recruitment: chemokine and chemokine receptor inhibition

In order to promote coordinated immune cell recruitment, macrophages, hepatocytes and other non-parenchymal cells release chemokines to attract immune cells to the site of injury.[53] ScRNA-seq has recently highlighted the importance of recruited monocyte-derived/bone marrow-derived macrophages in NASH.[24] These infiltrating monocyte-derived macrophages can promote both fibrogenesis[71] and fibrosis resolution.[72,73] Monocyte-derived macrophages can replace Kupffer cells (the resident phagocytes) and acquire a phenotype of "lipid-associated macrophages" (LAMs) or "scar-associated macrophages" (SAMs) that express TREM2, CD9 and osteopontin,[57–59] similar to adipose tissue LAMs.[62] While many of these discoveries have been based on mouse models, scRNA-seq analyses from human livers have also identified SAMs as a unique population located in the fibrotic niche of cirrhotic livers of different disease aetiologies.[74] Based on proteo-genomic data combined with spatial information, LAMs (SAMs) locate near intrahepatic bile ducts in homeostasis, but shift towards steatotic areas, fostered by increased C–C motif chemokine ligand 2 (CCL2) expression by HSCs.[75]

C–C motif chemokine receptor (CCR)2+ monocytes are important drivers of liver inflammation in NASH, and their therapeutic inhibition reduces NASH and fibrosis in rodent models.[71,76] The chemokine CCL2 and its cognate receptor CCR2 have therefore been tested as therapeutic targets in NASH (Fig. 2), for instance, by the dual CCR2/CCR5 inhibitor cenicriviroc or similar compounds (*e.g.* BMS-687681-02-020), the CCR2 inhibitor propagermanium, or an RNA-aptamer molecule inhibiting CCL2 (NOX-E36).[77] However, despite favourable tolerability as well as antifibrotic efficacy after 1 year of treatment in adults with NASH and liver fibrosis in a phase II trial,[78] cenicriviroc did not demonstrate sustained antifibrotic

efficacy after 1 year of treatment in a larger phase III clinical trial following interim analysis, leading to the termination of its development as a monotherapy in NASH.[79] At present, chemokine receptor inhibitors are only approved in highly specific indications, for example, the CCR5 inhibitor maraviroc for CCR5-tropic HIV strains, the C-X-C motif chemokine receptor (CXCR)4 antagonist plerixafor for haematopoietic stem cell mobilisation and the CCR4 antagonising antibody mogamulizumab for specific T-cell lymphomas. In NASH, the redundancy of the chemokine system, with many ligands and receptors that have overlapping targets, combined with the overlapping functions and adaptability to environmental cues of immune cell populations, likely explain the lack of efficacy of agents such as cenicriviroc in this complex and heterogeneous disease.

Modulating immune cell crosstalk: targeting platelets, neutrophils and lymphocytes

Macrophages act in concert with other cells to form the inflammatory infiltrate characteristic of NASH (Fig. 2). For instance, platelets bind to hepatic macrophages, and their activation further aggravates inflammation.[80] Observational studies indicate some benefits of anti-platelet compounds for patients with NASH and other indications, while pharmacologic inhibition of the hyaluronan-CD44 anchoring of platelets has been shown to be an effective anti-inflammatory strategy to attenuate NAFLD in mouse models.[80] Neutrophils are also abundant in NASH livers, particularly in early stages, and may contribute to inflammation by releasing toxic molecules including proteases, oxidants, cytokines and NETs (neutrophil extracellular traps).[81] With respect to fibrosis, traditional mouse models did not reveal a prime contribution of neutrophils to fibrogenesis.[82] Inhibitors of neutrophil infiltration are in early development (*e.g.*, targeting CXCR2, IL-8, or adhesion molecules), and may first be tested in alcohol-associated hepatitis[83] and/or hepatocellular carcinoma.[84]

Dendritic cells (DCs) that connect innate and adaptive immunity consist of different subsets, mainly conventional (classical) DCs (cDC1s and cDC2s) and plasmacytoid DCs. In NASH, XCR1 (X–C motif chemokine receptor 1)-expressing cDC1s are found abundantly in both patients with NASH and murine steatohepatitis, and correlate with NASH severity.[85] XCR1+ cDC1s promote inflammatory T-cell reprogramming, thereby aggravating NASH in mouse models.[85] Activated CD8+ T cells in NASH livers can, in cooperation with natural killer T (NKT) cells, induce hepatocyte injury.[86] More recently, a population of CXCR6+ Granzyme+ PD1+ CD8+ T cells was identified in NASH livers, which are directly activated by metabolic stimuli (including acetate and extracellular ATP) and collectively triggered auto-aggression against hepatocytes, in an antigen-independent fashion.[87] Other T-cell populations as well as B cells are further implicated in the progression of NASH.[88,89] Strategies to interrupt adaptive immunity, for example through broad immunosuppression (*e.g.*, glucocorticoids, azathioprine) or the specific targeting of B cells (with available compounds, such as rituximab or belimumab) or selected T-cell populations (*e.g.* with anti-IL-17 antibodies), are not suitable in NASH. Long-term risk of infection, specific side effects such as weight gain, and the lack of antigen-specific autoimmunity in NASH all argue against their use in the disease.

Innate lymphoid cells and unconventional T cells represent emerging but incompletely understood contributors to NAFLD pathobiology. Innate lymphoid cells (ILCs) consist, based on developmental and functional trajectories, of five subsets – natural killer (NK) cells, ILC1s, ILC2s, ILC3s and lymphoid tissue inducer cells.[90] Data on ILCs are emerging and still controversial. For instance, NK cells are considered anti-fibrogenic due to their capacity to eliminate HSCs,[91] but they may also support hepatic inflammation and alter metabolism.[92] Of note, ILC1, ILC2 and ILC3 populations have been implicated in metabolic disorders beyond NAFLD (*e.g.* obesity).[93,94] Unconventional T cells are a heterogeneous group of lymphocytes, which are abundant in the liver and represent the majority of intrahepatic T cells but only 10% of T cells in the blood. The most important subsets of unconventional T cells include NKT cells, $\gamma\delta$ T cells and mucosal-associated invariant T (MAIT) cells.[95] NKT cells are much more abundant in mouse livers than in humans, and they promote NASH, fibrosis and carcinogenesis in experimental models.[86,96,97] Given the opposing distribution of NKT cells and MAIT cells between human and mouse livers, findings about NKT cells from rodent models need to be interpreted with caution, and, from a functional perspective, MAIT cells may be the human counterpart of NKT cells.[39,98] Although the role of MAIT cells in the liver is evolving, experimental evidence clearly supports their profibrogenic activity in the chronically injured liver.[99,100] These innate and unconventional immune cell populations are affected by interventions on the gut-liver axis and could represent promising targets for future NASH therapies as well.

Targeting soluble mediators: inhibiting cytokines and other effector molecules

Many of the detrimental effects of inflammation in NASH – including death of hepatocytes and activation of fibrogenic myofibroblast populations – are mediated by soluble factors. Neutralising antibodies against cytokines including TNF, IL-1β, IL-6 or IL-12/IL-23 are approved for different inflammatory diseases. However, similar to the concern about using classical immunosuppressant drugs as highlighted above, their use in NAFLD is currently constrained by the lack of clinical data indicating beneficial effects on NASH and/or fibrosis, as well as their unfavourable safety profiles.[79] This may change once the heterogeneity of patients with NAFLD and their inflammatory status is better understood. Nonetheless, one important lesson from alcohol-associated hepatitis is that the broad application of steroids, anakinra (recombinant IL-1 receptor antagonist), pentoxifyllin (which inhibits TNF synthesis) or infliximab (anti-TNF antibody) does not provide benefit, and may aggravate this inflammatory condition.[101]

Similar to cytokines, galectin 3 is secreted by macrophages upon liver injury in NASH and has profibrotic activity.[102] The galectin 3 inhibitor belapectin (GR-MD-02) has been tested in a phase IIb clinical trial in 162 patients with NASH, cirrhosis and portal hypertension. While belapectin's safety and tolerability were good, belapectin did not improve clinically relevant endpoints including fibrosis regression. Nonetheless, a subgroup of patients showed improvement of hepatic venous pressure gradient,[103] prompting a trial to test its ability to prevent oesophageal varices.

From inflammation to fibrosis: Targeting fibrogenesis

The disease-driving pathways of NASH converge in fibrosis (Fig. 3), an aberrant wound-healing process resulting not only in scarring (extracellular matrix deposition), but also in the loss of functional hepatocytes and chronic inflammation.[104] Wound healing in the liver is dynamic and reversible,[105] with ample evidence of fibrosis regression after effective treatment of viral hepatitis, and even following bariatric surgery in patients with NASH.[106] Because fibrosis severity is the main predictor of

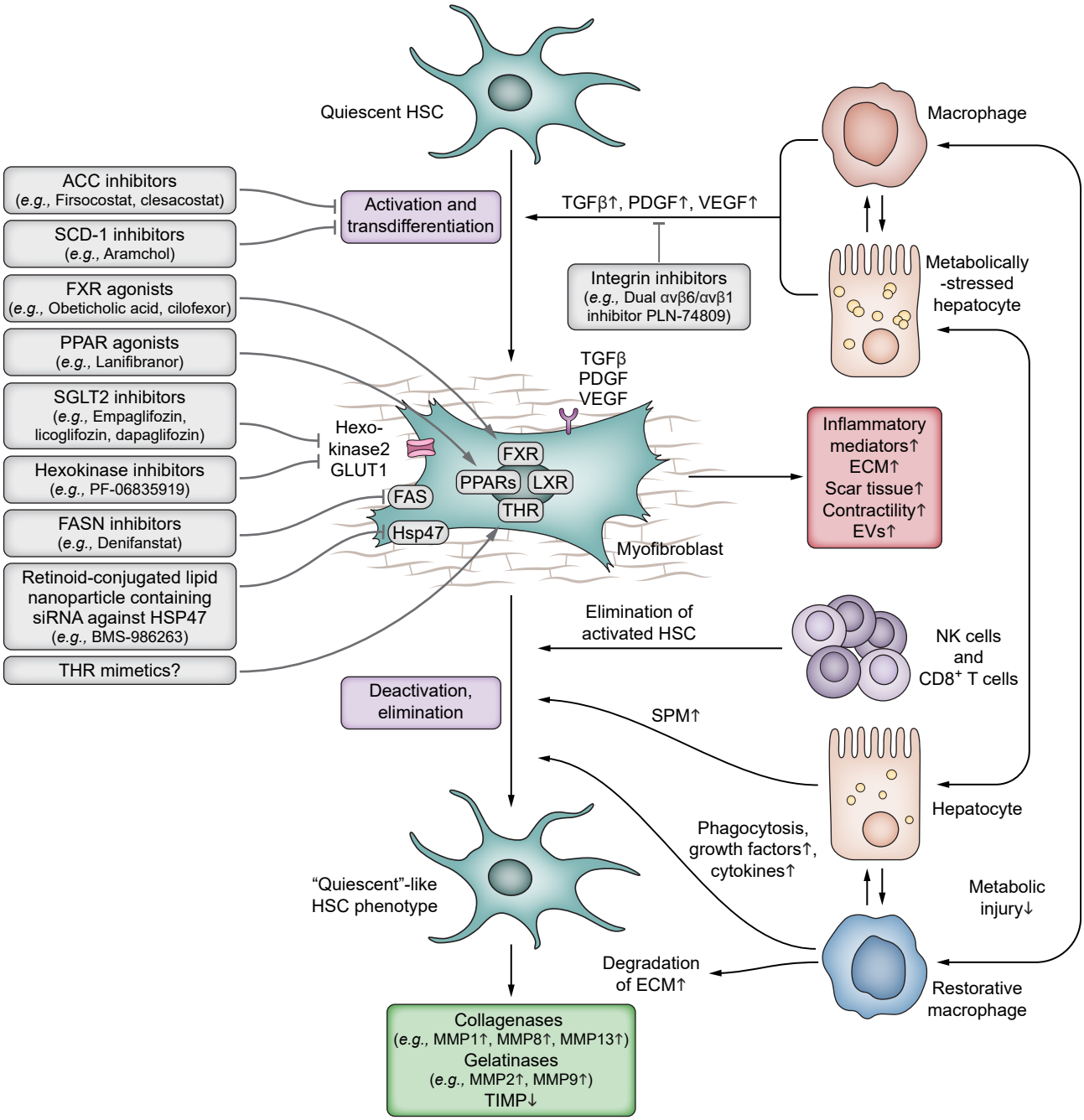

Fig. 3. From inflammation to fibrosis: Targeting fibrogenesis. During progression of chronic liver injury, pro-fibrogenic mediators and cell-cell interactions lead to activation and transdifferentiation of quiescent HSCs to extracellular matrix-producing myofibroblasts. Upon cessation of metabolic injury, pro-resolving factors and anti-inflammatory reprogrammed immune cells revert myofibroblasts to a "quiescent"-like HSC phenotype or support elimination of activated HSCs, initiating tissue repair and regeneration. Pharmacologic strategies target the interaction of HSCs with inflammatory cells and HSC-activating mediators, metabolic dysregulation in HSCs and energy dependent signalling pathways needed for transdifferentiation to myofibroblasts. Drug delivery systems enable HSC-specific gene silencing approaches. ACC, acetyl-CoA carboxylase; ECM, extracellular matrix; EVs, extracellular vesicles; FASN, fatty acid synthase; FXR, farnesoid X receptor; HSC, hepatic stellate cell; LXR, liver X receptors; MMP, matrix metalloproteinases; NK cell, natural killer cells; PDGF, platelet-derived growth factor; PPAR, peroxisome proliferator-activated receptor; SCD1, stearoyl-CoA desaturase 1; SGLT2, sodium glucose linked transporter 2; SPM, specialised pro-resolving mediators; TGFβ, transforming growth factor beta; THR, thyroid hormone receptor; TIMP, tissue inhibitor of matrix metalloproteinases; VEGF, vascular endothelial growth factor.

liver-related, as well as overall, morbidity and mortality, efforts to directly attenuate fibrosis remain a high priority for emerging NASH therapies.[6,7] Nonetheless, simply because fibrosis is the most important predictor of death does not establish that reversing fibrosis will improve all outcomes. While cirrhosis regression may be associated with improved clinical outcomes in patients with NASH,[107] extrahepatic comorbidities, especially cardiovascular events, may not be attenuated upon antifibrotic therapy. The impact of therapies on extrahepatic components of the metabolic syndrome will depend on whether the drug's targets are expressed in extrahepatic tissues and whether they contribute to the pathogenesis of these conditions. Current pharmacological strategies targeting fibrogenesis focus on HSCs as the main matrix-producing mesenchymal cells, with current efforts aiming to inhibit the signals or intracellular processes culminating in HSC activation, or revert activated myofibroblasts to a quiescent state.

Targeting signals promoting HSC activation: TGF-β and integrin inhibitors

During fibrogenesis, quiescent HSCs, which display some adipocyte-like features (*e.g.* lipogenic gene expression) and store vitamin A (retinol), become activated and transdifferentiate into extracellular matrix-producing myofibroblasts.[104] HSC activation in NASH is mainly driven by: i) soluble signals such as platelet-derived growth factor (PDGF), vascular endothelial growth factor, and transforming growth factor (TGF)-β; ii) by paracrine interactions with macrophages and other immune cells, as well as hepatocytes and endothelial cells; it is also accompanied by metabolic reprogramming.[70,108] ScRNA-seq allows for high-resolution transcriptomic profiling of mesenchymal subpopulations during liver fibrosis, revealing that chemokine/cytokine release and extracellular matrix production are diversified among HSC/myofibroblast subpopulations owing to a higher degree of cellular heterogeneity than previously appreciated.[109–111] In advanced (human or rodent) hepatic fibrosis, HSC directly interacts with surrounding HSCs, driven by autocrine pathways that amplify fibrogenesis.[112] Moreover, the evolution of a dense autocrine network indicates that as fibrosis advances, the repertoire of therapeutic targets evolves, so that treatments for early disease may be less effective in advanced fibrosis. In the human liver, scar-associated mesenchymal cells expressing high levels of fibrogenic genes and the myofibroblast marker PDGF-RA are topographically restricted to fibrotic septae and linked to ligand-expressing SAMs and endothelia.[74] In addition, quiescent and activated HSCs are transcriptomically distinct in human livers, with activated HSCs downregulating vitamin A storage-related genes and upregulating profibrogenic genes such as collagen I.[113]

While the fundamental role of HSCs in liver fibrosis has been well known for decades, therapeutic targeting of these cells has remained challenging. Whereas targeting inflammatory cells and blocking their fibrogenic interactions with HSCs seems plausible, targeting the key pro-fibrogenic cytokines – particularly secreted TGFβ – has not yet been successful.[114] Targeting TGF-β directly with antibodies (*e.g.*, fresolimumab or metelimumab) has been largely terminated due to dose-limiting adverse events, since systemic inhibition of the cytokine is immunosuppressive and may promote cancer.[114] However, inhibiting the activation of TGF-β by targeting the αv-containing subset of integrins at the cell surface (*e.g.*, integrins αvβ1, αvβ3, αvβ5, αvβ6 and αvβ8) is promising. Preclinical models support this approach in NASH fibrosis.[115] Several integrin inhibitors are currently in clinical trials (mostly for pulmonary fibrosis), including PLN-74809, a dual αvβ6/αvβ1 integrin inhibitor, in patients with liver fibrosis.[116]

Targeting HSC activation: lipid metabolism, autophagy and nuclear receptors

Upon injury, HSCs become activated, increasing their contractility, secreting inflammatory mediators, and synthesising extracellular matrix components, which mechanically stabilise injured tissue and enable migration of immune, mesenchymal and endothelial cells into the repairing tissue.[104] HSC activation into myofibroblasts requires energy, so that HSC activation necessitates an intense metabolic reprogramming through activation of glycolysis, glutaminolysis (using glutamine as an additional source of ATP), and lipogenesis.[70] Although metabolic regulation is far better characterised in hepatocytes than in HSCs, there is convincing evidence that some of the metabolic drugs targeting *de novo* lipogenesis, particularly acetyl-CoA carboxylase and fatty acid synthase inhibitors, can also suppress fibrogenesis directly. The acetyl-CoA carboxylase inhibitors firsocostat, as well as clesacostat (PF-05221304), both reduced liver fibrosis in animal models, independently of their anti-steatotic effects, and HSC activation *in vitro*.[117,118] Very similar observations were obtained with aramchol, an inhibitor of the lipogenic enzyme stearoyl-CoA desaturase 1, on HSCs *in vitro*.[119] The selective inhibition of stearoyl-CoA desaturase 1 in HSCs reduced their activation and inhibited fibrosis in rodent models.[120] Of note, a phase III trial evaluating aramchol in individuals with NASH is currently suspended while a new formulation of this compound with higher bioavailability ('aramchol meglumine') is being developed. Most recently, the fatty acid synthase inhibitor denifanstat (TVB-2640) also demonstrated antifibrotic activity in HSCs while improving NASH.[121] Intracellular lipids released during autophagy act as an additional energy source for HSC activation, revealing another therapeutic vulnerability that could be targeted by antifibrotic strategies.[122] These encouraging findings from preclinical systems emphasise the relevance of lipid metabolism as an energy source for HSC activation, indicating that some of the metabolic compounds in phase II/III clinical development in NASH may actually have additional direct antifibrotic effects by reducing HSC activation.[123–125]

Inhibiting glucose metabolism represents another example of targeting the intrinsic metabolic pathways of HSCs to block their activation. During HSC transdifferentiation to myofibroblasts *in vitro*, glucose transporters (*e.g.*, glucose transporter 1) and glycolytic enzymes (*e.g.*, hexokinase 2) are induced, and gluconeogenesis is downregulated.[70] Thus, hexokinase inhibitors (*e.g.*, PF-06835919) or SGLT2 (sodium glucose linked transporter 2) inhibitors like empaglifozin, licoglifozin or dapaglifozin may attenuate HSC activation and fibrogenesis. However, clinical evidence on SGLT2 inhibitors as a treatment specifically for NASH is currently limited.[126] As noted above, autophagy (*i.e.* the lysosome-dependent orderly degradation and recycling of cellular components) may generate energy during HSC activation,[127] such that inhibiting autophagy would be antifibrotic. On the contrary, autophagy itself can also be

antifibrotic by inhibiting the release of fibrogenic extracellular vesicles from HSCs,[128] making it difficult to predict whether autophagy inhibitors or inducers (*e.g.*, rapamycin or carbamazepine, respectively) would be the preferred drug strategy in liver fibrosis.

The picture is even more complex for compounds targeting nuclear receptors. HSCs express several nuclear receptors that are targets of investigational drugs for NASH, including low levels of FXR, high levels of LXR, robust levels of THR-α (but low levels of THR-β) and different isoforms of PPARs.[129] FXR activation, as promoted by agonists like obeticholic acid or cilofexor, is reportedly antifibrotic in HSCs. While rodent models have demonstrated a striking reduction in portal hypertension with FXR agonism,[130] its effect on HSC activation may possibly be more preventive than therapeutic *in vitro*.[131] Consistently, clinical data with obeticholic acid reported an improvement of liver fibrosis in non-cirrhotic NASH,[132] but not in patients with compensated cirrhosis. For THR agonists and thyromimetics, THR-α stimulation has an antifibrotic effect on HSCs, suggesting that the fibrosis improvement in patients treated with the THR-β agonist resmetirom is more likely a consequence of reduced metabolic injury.[21] This interpretation is different for PPAR agonists. The pan-PPAR agonist lanifibranor has antifibrotic activity in patients with NASH.[69] Rodent models suggest that its PPAR-γ agonistic function reduces HSC activation, favouring a less activated and more quiescent phenotype.[68]

Nanoparticle-mediated drug delivery to HSCs: gene silencing or activation

Several features of HSCs can be exploited for targeted drug delivery via nanomedicine formulations; these functions include vitamin A storage, or expression of the PDGF receptor or integrins.[133] Activating or inhibiting molecules could be delivered to interfere with collagen synthesis either directly or via micro-RNA-controlled transcriptional inhibition.[134] Currently, a retinoid-conjugated lipid nanoparticle containing small-interfering RNA against heat-shock protein 47 (HSP47, BMS-986263) is being tested in a phase II clinical trial for patients with compensated NASH cirrhosis, since it demonstrated favourable safety and some efficacy in patients regressing from hepatitis C virus-associated fibrosis.[135] If successful HSC targeting can be achieved in clinical trials, the in-depth understanding of HSC/myofibroblast subpopulations, their activation pathways and their differential effector functions may create multiple opportunities to deliver antifibrotic therapies.[108] However, HSC specificity is critical as some "HSC-specific nanoparticles" target macrophages, possibly through their phagocytic capacity.[136]

Augmenting scar-free tissue repair and regeneration: Targeting fibrosis regression

Hepatic fibrosis is dynamic and reversible prior to the development of cirrhosis, and even cirrhosis may be reversible if not too advanced; however, specific features of advanced liver disease such as capillarisation of the liver sinusoids might be less reversible.[105] The mechanisms underlying disease regression are less well understood, but several molecular and cellular principles have been identified based on mouse models[24] as well as samples from patients with NASH following disease regression, for example after bariatric surgery or lifestyle modifications.[137] These mechanisms include [138]: i) cessation of metabolic injury, which is accompanied by the release of specialised pro-resolving mediators that act as "stop signals" for inflammation [139]; ii) shifting the intrahepatic balance from inflammation to restoration of normal architecture – associated events include polarisation of macrophages towards a restorative phenotype with efferocytosis of apoptotic cells, secretion of regenerative growth factors and anti-inflammatory cytokines,[52] and recruitment of neutrophils to resolve inflammation through release of specialised pro-resolving mediators [140]; iii) deactivation or elimination of myofibroblasts by reverting them to a quiescent HSC-like phenotype, via HSC senescence or by elimination of activated HSCs via NK cells or T cells [91,105,141]; iv) degradation of extracellular matrix, which is executed primarily by matrix metalloproteinases (MMPs) including the collagenases MMP1, MMP8, MMP13, the gelatinases MMP2, MMP9, and the metalloelastase MMP13.[142] Full matrix degradation also requires reduced levels of MMP-inhibitory proteins (*e.g.* TIMP [tissue inhibitor of MMP]) and the presence of phagocytic macrophages.[142] However, the matrix protein composition in advanced fibrosis may be relatively resistant to matrix degradation, particularly in the case of collagen cross-linking and elastin deposition. The antibody simtuzumab was administered to block the collagen cross-linking activity of LOXL2 (lysyl oxidase-like 2), but it did not demonstrate antifibrotic efficacy in clinical trials involving patients with advanced fibrosis or cirrhosis.[143] Some have argued that there was insufficient proof that the antibody reached its target, prompting more recent efforts to antagonise LOXL2 using small molecules. So far, targeting matrix proteins or fibrolysis (*e.g.* MMP, TIMP inhibitors) have not progressed to advanced clinical development in NASH. Understanding the matrix composition, or matrisome, during fibrosis resolution is an ongoing area of research, and new molecular targets are likely to emerge.[144]

Cell therapy-based anti-inflammatory and antifibrotic approaches

Since HSCs/myofibroblasts, matrix proteins and fibrolytic pathways are challenging therapeutic targets, cell-based therapies are being explored to augment anti-inflammatory and antifibrotic activity. Because of the technical and logistical requirements, antifibrotic cell therapies are most often tested in selected patients with advanced fibrosis and cirrhosis.[145]

In selected haematological malignancies, cytotherapy with reprogrammed T cells has entered clinical practice, wherein patient's T cells are isolated and then equipped *ex vivo* with chimeric antigen receptors (CAR) for highly cell-specific targeting. Such CAR T cells could be principally programmed to reduce liver fibrosis. In mouse models, CAR T cells directed against uPAR (urokinase-type plasminogen activator receptor), which is abundantly present on senescent cells, ameliorates experimental liver fibrosis.[146] Nonetheless, the clinical translation of this approach is challenging because uPAR is not only expressed on senescent cells, but also on several immune cell populations including neutrophils and macrophages, and cellular senescence has cell type-dependent functions in fibrosis that may undermine efforts to deplete senescent cells as a therapy. On the other hand, other efforts to target fibrogenic cells in the heart by targeting cell surface FAP-1 (fibroblast activating protein) are very encouraging, and may be adaptable to liver disease.[147,148]

Macrophages can adopt a repair phenotype that promotes fibrosis regression and proper tissue regeneration, supporting the concept of programmed macrophage transplantation as a therapeutic strategy (Fig. 2). However, proper macrophage differentiation is probably required, because mouse models of fibrosis have demonstrated that the adoptive transfer of "non-programmed" immature bone marrow monocytes aggravates liver fibrosis, while the intravenous administration of properly (*ex vivo*) differentiated macrophages ameliorates hepatic fibrosis.[149,150] The feasibility of such an approach was shown in a phase I clinical trial including nine patients with compensated cirrhosis.[151] In this proof-of-concept trial, apheresis-derived autologous CD14+ monocytes were differentiated *ex vivo* into a restorative phenotype (CD14+, high 25F9, CD206, CD163 and CD169). The single injection of different doses of these macrophages appeared safe and was associated with a (slight) reduction in the model of end-stage liver disease score.[151] Nonetheless, the clinical efficacy of this approach is unknown, and many questions remain unanswered, including the efficiency of engraftment, cell viability, phenotype stability, and whether macrophages are the optimal cell for this purpose. Possibly, the immune response following cell therapy rather than the cells themselves account for the benefit.[152] Also, for repeated injections, a more reliable cell source than autologous cells would be desirable. In this respect, induced pluripotent stem cells could be used to generate individualised and reprogrammed macrophages (termed 'CAR-expressing macrophage cells', or 'CAR-iMac'.[153] Regardless, efficient cytotherapy will require long-term stability of a "repair macrophage" phenotype. New protocols are developing to ensure that transplanted macrophages retain their desired epigenetic and transcriptional profiles.[154]

Mesenchymal stromal cells (MSCs) are another potential option for cell therapy, since this population of fibroblast-like cells from the bone marrow exerts immune-regulatory functions and can even express MMPs. While MSC transfer led to promising antifibrotic effects in mouse models, clinical trials on MSCs in humans have yielded mixed results.[145] Since MSCs appear to exert many of their beneficial actions via the release of extracellular vesicles, the use of MSC-derived extracellular vesicles instead of cells could be an interesting cell-free, less immunogenic, and less toxic alternative antifibrotic strategy.[155]

Challenges in translation from anti-inflammatory/antifibrotic target identification to drug approval

As outlined above, the in-depth understanding of the pathogenic mechanisms initiating, propagating and resolving inflammation and fibrosis in NAFLD provides a complex picture of potential anti-inflammatory and antifibrotic targets (Figs 1-3). However, despite the relevance of inflammation and fibrosis to NAFLD-related outcomes, no "pure" anti-inflammatory or antifibrotic compound has been approved for the treatment of NASH. This is likely related to the complexity of the processes driving inflammation and fibrosis, but also to redundancy in inflammatory and fibrogenic signalling. In order to ensure tissue homeostasis or – in the case of injury – damage control, the body has evolved many overlapping mechanisms to preserve inflammation and fibrogenesis. In fact, many cellular components of liver fibrosis show a tremendous capacity to adapt their phenotypes towards the hepatic microenvironment, making it challenging to treat the disease if the tissue injury is ongoing. This would, however, support the concept of combinatorial treatment for NASH, as combining anti-inflammatory strategies could amplify the efficacy of metabolic drugs and overcome signalling redundancies.[156] However, clinical trials using combinations have been disappointing despite promising preclinical data in mouse models.[125,157] Another strategy to overcome mitigation by redundant pathways could be to deliberately target processes at the initiation step of the activation cascade, thus reducing pathogenic processes before highly conserved inflammatory/fibrogenic pathways are being activated.

Nonetheless, we often describe these processes in a hierarchical order; for example, metabolic injury leads to inflammation, which leads to fibrosis. However, this construct is oversimplified and incorrect as these responses are non-hierarchical, but rather intertwined. For instance, lipid metabolism is not restricted to hepatocytes, but affects macrophage inflammatory responses as well as HSC activation and fibrogenesis. Gut-derived mediators stimulating nuclear receptors not only target macrophages as classical scavengers, but also influence hepatocyte metabolism and HSC activation. Moreover, targeting critical downstream pathways of inflammation and fibrosis, for example by depleting immune cells or antagonising cytokines, carries a high risk of undermining important immune surveillance functions,[158] making such approaches unacceptably risky for a chronic condition such as NASH.

Technology advances at a fast pace, and it will be important to integrate the rapidly emerging data from patient samples, advanced *in vitro* models and preclinical rodent models into novel actionable therapeutic targets. In doing so, detailed insights will surely emerge to clarify the complex communication circuits between metabolism, inflammation and fibrosis in NAFLD and NASH, culminating in better, stage-specific and personalised treatments. Detailed features of inflammation and fibrosis should be captured in greater depth and granularity from liver tissues, potentially supported by novel AI (artificial intelligence) approaches,[159] rather than using current descriptive, semi-quantitative scoring systems that are limited by being highly subjective and one-dimensional. Combined with a growing arsenal of targets and greater clarity about cellular and genetic heterogeneity, a more integrated and tailored approach to combat inflammation and fibrosis during NAFLD progression will most likely emerge.

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Abbreviations

ASK1, apoptosis signal-regulating kinase 1; CAR, chimeric antigen receptor; CCL, C–C motif chemokine ligand; CCR, C–C motif chemokine receptor; cDC, conventional dendritic cell; CXCR, C-X-C motif chemokine receptor; DCs, dendritic cells; FGF, fibroblast growth factor; FXR, farnesoid X receptor; GLP-1, glucagon-like peptide 1; HCC, hepatocellular carcinoma; HSCs, hepatic stellate cells; IL-, interleukin-; ILCs, innate lymphoid cells; LAMs, lipid-associated macrophages; LPS, lipopolysaccharide; LXRs, liver X receptors; MAIT, mucosal associated-invariant T; MMPs, matrix metalloproteinases; MSCs, mesenchymal stromal cells; NAFL, non-alcoholic fatty liver; NAFLD, non-alcoholic fatty liver disease; NK, natural killer; NKT, natural killer T; NLRP3, NLR family pyrin domain containing 3; PDGF, platelet-derived growth factor; PNPLA3, patatin-like phospholipase domain-containing protein 3; PPARs, peroxisome proliferator-activated receptors; SAMs, scar-associated macrophages; scRNA-seq, single-cell RNA-sequencing; TGF, transforming growth factor; THR, thyroid hormone receptor; TNF, tumour necrosis factor.

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Conflict of interest

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Please refer to the accompanying ICMJE disclosure forms for further details.

Authors' contributions

FT wrote the first draft of the manuscript, TP designed the figures, RL and SLF provided critical intellectual input. All authors read and edited the final version of the manuscript.

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Supplementary data

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