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Beyond M1/M2: The role of reactive oxygen species in liver fibrosis and immune modulation

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ABSTRACT

Liver fibrosis is a progressive condition resulting from chronic liver injury and characterized by excessive extracellular matrix deposition and architectural disruption. Reactive oxygen species (ROS), long considered merely cytotoxic, are now recognized as key modulators of immune responses and fibrogenic pathways. In this context, macrophages play a central role. Traditionally classified into M1 (pro-inflammatory) and M2 (anti-inflammatory) phenotypes, macrophages exhibit greater heterogeneity and plasticity than this binary model suggests. This review critically re-evaluates the role of ROS in liver fibrosis, with emphasis on their dual functions as signaling mediators and inducers of oxidative stress. Particular attention is given to how ROS influence macrophage polarization, activation, and function within the fibrotic liver microenvironment, and how this crosstalk contributes to both fibrogenesis and resolution. By addressing the complexity of redox-immunological interactions, new opportunities emerge for more effective and personalized antifibrotic interventions, including targeted modulation of ROS and immune cell reprogramming. This integrative perspective will help guide the development of more effective and personalized antifibrotic treatments.

1. Introduction

Liver fibrosis is a pathological condition marked by the excessive accumulation of extracellular matrix proteins, resulting in progressive scarring and disruption of normal liver architecture [1–3]. This process is primarily driven by chronic liver injuries, such as persistent viral infections (e.g., hepatitis B and C) [4], excessive alcohol consumption [5], non-alcoholic fatty liver disease (NAFLD) [6], and metabolic disorders [7]. The underlying mechanism involves continuous liver cell damage and inflammation, which activate hepatic stellate cells (HSCs) [8–10]. Upon activation, HSCs transdifferentiate into myofibroblast-like cells, producing collagen and other matrix components, disrupting normal hepatic architecture. Over time, this fibrogenesis impairs liver function, leading to symptoms like fatigue, weight loss, and abdominal discomfort. Fibrosis severity is often assessed with staging systems like the METAVIR score, which evaluates necroinflammatory activity and fibrosis extent. Originally developed for chronic hepatitis C [11], METAVIR remains a standard tool in viral hepatitis but is less applicable in metabolic liver diseases such as NAFLD/NASH, where alternative systems (e.g., NAS, SAF score, or FLIP algorithm) are typically used.

Liver fibrosis is widely recognized as the key prognostic factor in chronic liver diseases. According to the landmark study by Angulo et al. [12], liver fibrosis stage is the only histologic feature independently associated with overall mortality and liver-related outcomes in patients

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with NAFLD [12]. The clinical significance of liver fibrosis lies in its potential to progress to more severe hepatic conditions. If not effectively managed, fibrosis can advance to cirrhosis, characterized by extensive scarring and impaired liver function [13–15]. Cirrhosis leads to complications such as portal hypertension, variceal bleeding, ascites, and hepatic encephalopathy, and markedly increases the risk of hepatocellular carcinoma (HCC). While progression from chronic liver disease to cirrhosis and then to HCC is often stepwise, some HCCs arise in non-cirrhotic livers, reflecting a more complex pathogenesis [16–19]. Early detection and intervention are crucial to halt or reverse the fibrotic process, preventing cirrhosis and reducing progression risk. Currently, therapeutic options to reverse liver fibrosis are limited, highlighting the need for continued research and development of effective anti-fibrotic treatments (Fig. 1) [20–22].

In the onset and progression of liver fibrosis, reactive oxygen species (ROS) play a dual role mediating hepatocyte damage and inflammation and modulating fibrosis resolution. At low concentrations, ROS act as vital second messengers in physiological signaling, a state commonly referred to as oxidative eustress, which denotes the controlled and beneficial redox signaling required for normal cellular function. By contrast, when ROS levels rise uncontrollably, they induce oxidative distress, a condition characterized by disruption of the oxidant/antioxidant balance that culminates in oxidative damage to lipids, proteins, and DNA, thereby promoting tissue injury. In the case of liver, these oxidative stress leads to damage of lipids, proteins, and DNA within hepatocytes, disrupting normal liver function and trigger immune responses primarily through resident Kupffer cells and the recruitment of circulating monocyte-derived macrophages [23,24]. The accumulation of ROS in the liver not only causes direct cellular injury but also activates various signalling pathways that contribute to inflammation and fibrosis progression. As mentioned, ROS can activate HSCs, which are central to the development of liver fibrosis [25,26]. Moreover, ROS-induced activation of inflammatory pathways involves the release of pro-inflammatory cytokines and recruitment of immune cells to the liver, exacerbating tissue injury and promoting fibrosis [27].

Macrophages, integral components of the liver's immune system, exhibit remarkable plasticity, adapting their functions in response to various microenvironmental signals [28–31]. Traditionally, macrophage activation has been categorized into two primary states: M1 and M2. M1 macrophages, often referred to as classically activated or "pro-inflammatory" macrophages, are typically induced by stimuli such as interferon-gamma (IFN-γ) or lipopolysaccharide (LPS). They are characterized by their production of pro-inflammatory cytokines, ROS, and their role in promoting Th1 immune responses. In the context of liver fibrosis, M1 macrophages contribute to tissue injury by exacerbating inflammation and activating HSCs, leading to increased collagen deposition and fibrosis progression [32].

In contrast, M2 macrophages, known as alternatively activated or "reparative" macrophages, are induced by stimuli such as interleukins IL-4 and IL-13 [33,34]. They are associated with anti-inflammatory responses, tissue repair, and resolution of inflammation. In liver fibrosis, M2 macrophages can attenuate injury by secreting anti-inflammatory cytokines, promoting extracellular matrix degradation, and inducing autophagy in HSCs, thereby mitigating fibrosis. However, an imbalance favouring M2 polarization has also been implicated in promoting fibrosis, as excessive M2 activity can lead to excessive extracellular matrix deposition [35].

In this context, recent studies have highlighted macrophage plasticity and the identification of diverse subtypes, such as TREM2+ lipid-associated macrophages (LAMs) and senescence-associated macrophages (SAMs), which contribute to both fibrosis progression and resolution. [30]. This review emphasizes the dynamic nature of macrophage polarization, offering a broader understanding of their roles in liver fibrosis beyond the traditional M1/M2 framework. The conventional classification of macrophages into M1 and M2 subtypes fails to capture the full spectrum of macrophage functions in liver fibrosis, and

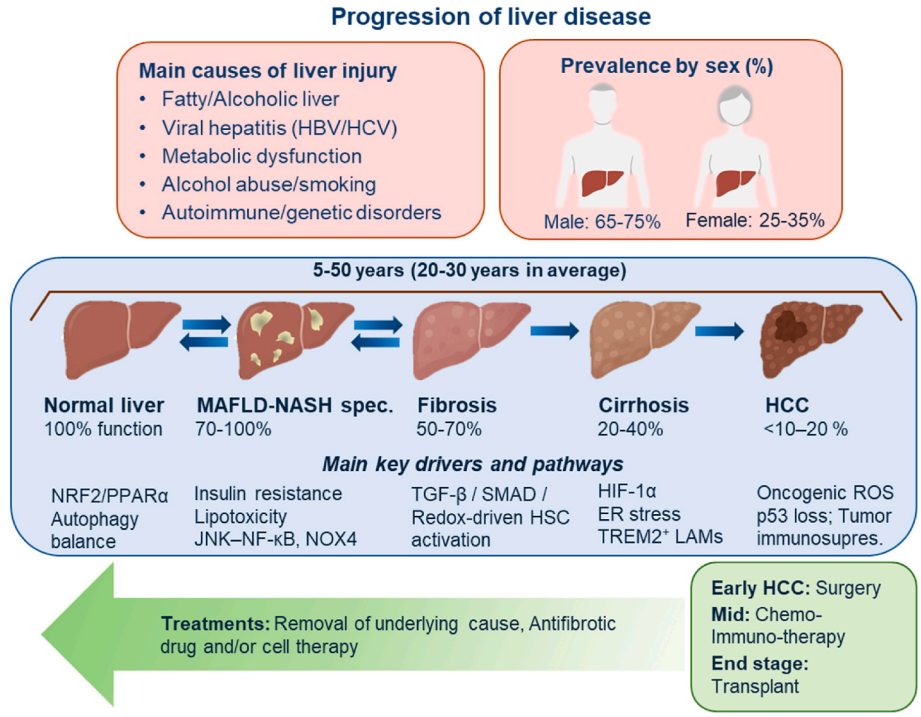

Fig. 1. Progression of chronic liver disease from normal to malignant stages. Illustration summarizing the progression from healthy liver homeostasis to HCC. Main causes of liver injury include viral hepatitis (HBV/HCV), metabolic dysfunction (MAFLD), alcohol abuse, and autoimmune or genetic disorders. The average progression spans 20–30 years, with higher prevalence in males (65–75 %). Each stage is characterized by distinct molecular drivers and pathways: redox and metabolic balance in healthy liver; insulin resistance and inflammatory signaling (JNK–NF-κB, NOX4) in MAFLD–NASH spectrum; TGF-β/SMAD activation and oxidative imbalance in fibrosis; hypoxia and macrophage remodelling (HIF-1α, TREM2^{+} LAMs) in cirrhosis; and oncogenic ROS and p53 loss driving HCC. Treatment options vary by stage, from antifibrotic or cell-based therapies to surgery, immunotherapy, or transplantation.

different studies have revealed intermediate and highly plastic macrophage states that dynamically respond to microenvironmental cues. By exploring the complex interplay between ROS and macrophages in the hepatic microenvironment, this review aims to provide a refined understanding of their role in liver pathology. It also synthesizes recent discoveries on the evolving roles of ROS in liver fibrosis, going beyond their traditional cytotoxic effects. While ROS are linked to cellular damage and inflammation, evidence shows they also modulate macrophage behavior, affecting fibrotic progression and resolution. These recent findings reevaluate the traditional M1/M2 macrophage polarization model, highlighting the complexity of macrophage activation. The review also discusses how ROS impact macrophage activation, underlying signaling pathways, and the therapeutic potential of targeting redox signaling and macrophage plasticity in liver fibrosis, with emerging strategies for improving diagnosis and treatment.

2. Mechanisms of ROS in liver fibrosis

2.1. Historical perspective

The understanding of ROS in liver fibrosis has evolved significantly over time, moving from initial observations of their cytotoxic effects to a more detailed recognition of their role as dynamic signalling molecules (Fig. 2). The impact of oxidative stress on liver pathology began to be recognized in the mid-20th century when Comporti et al. identified elevated lipid peroxidation levels in rats with carbon tetrachloride (CCl_4)-induced hepatic injury, establishing a link between ROS and liver damage [36,37]. Subsequent studies between the 1960s and the 1980s suggested a causative role for ROS in inducing liver damage. Research during this period demonstrated that ROS damage lipids, proteins, and DNA, leading to hepatocyte necrosis and apoptosis, which in turn amplify inflammation and activate HSCs to initiate fibrosis.

In the 1990s, research started to highlight ethanol-induced ROS formation in fatty liver and fibrosis, highlighting the oxidative stress's key role in alcohol-related liver diseases [38–40]. And the early 21st century brought deeper molecular insights into ROS in liver fibrosis. NADPH oxidases (NOXs) were identified as crucial sources of ROS, impacting HSC activation and hepatocyte apoptosis, which opened new avenues for targeted therapies to modulate NOX activity [41–43]. Studies also explored the potential of antioxidants and mitochondrial-targeted therapies to mitigate oxidative stress as promising strategies for preventing or reversing liver fibrosis [44].

More recently, research has expanded the focus from direct ROS-induced cellular damage to the complex interplay between oxidative stress, inflammation, and fibrosis. Studies have highlighted the role of hypoxia-induced ROS in organ fibrosis, suggesting that targeting hypoxia-related pathways could provide novel therapeutic approaches [45]. Furthermore, the traditional M1/M2 macrophage classification started to be reevaluated in the last decade in the seminal work of Murray et al. [46], and others (e.g. MacParland et al. [47]), emphasizing the need for a more comprehensive understanding of macrophage polarization in liver fibrosis. As a consequence of all these advances, nowadays ROS are no longer viewed simply as damaging byproducts of cellular metabolism but as key signaling molecules within complex biological processes. In the case of liver, their multifaceted involvement extends from the initiation of hepatocellular injury to the propagation of inflammation and the activation of fibrogenic cells. Understanding the precise mechanisms of ROS generation, their cellular targets, and the downstream signaling cascades where they act is crucial for developing effective anti-fibrotic strategies.

2.2. Sources and production of ROS in the fibrotic liver

The liver, due to its central role in metabolism and detoxification, is a significant site of ROS production, with various cellular and subcellular compartments contributing to the overall redox imbalance observed in fibrosis. ROS include free radicals like superoxide anion ($O_2\bullet-$), hydroxyl radical (•OH), alkoxyl radical (RO•), and peroxyl radical (ROO•),

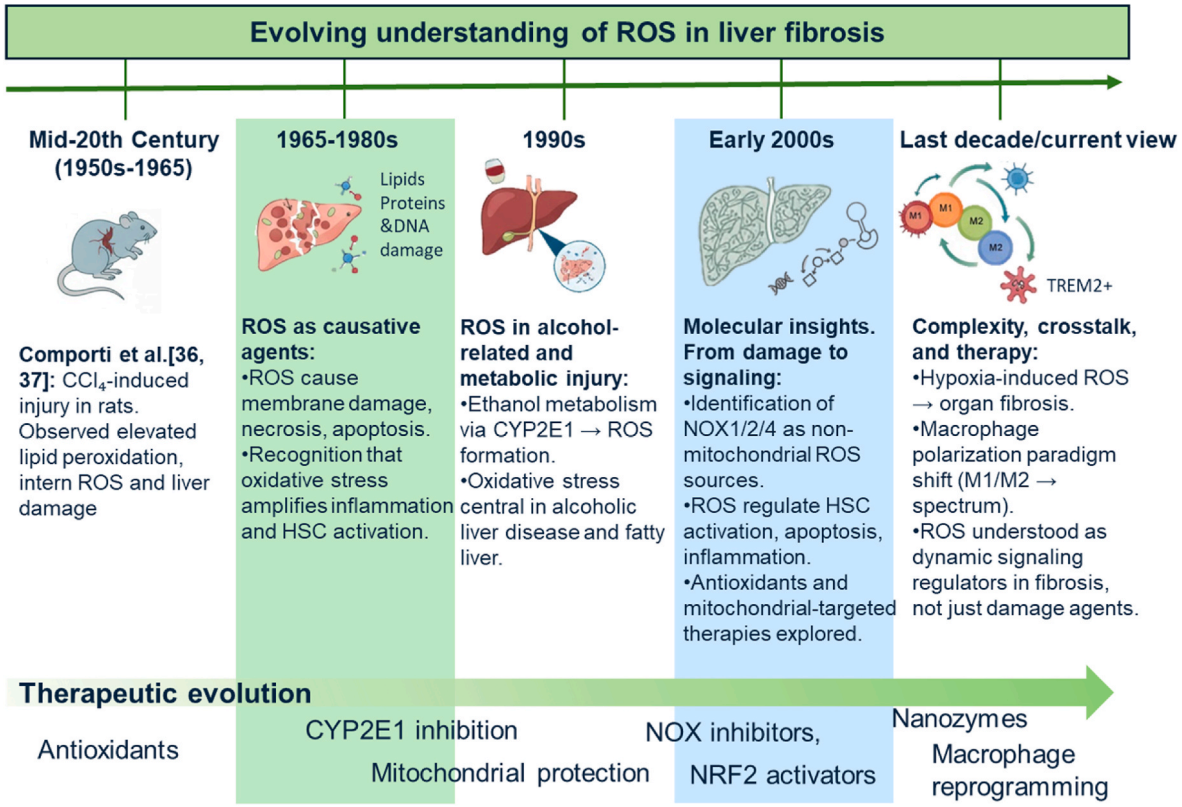

Fig. 2. Evolution of the understanding of reactive oxygen species (ROS) in liver fibrosis.

as well as non-radical species such as hydrogen peroxide (H_2O_2). The main cellular sources and roles of these ROS species are summarized in Table 1.

Hepatocytes: As the primary functional cells, hepatocytes are substantial producers of ROS, especially under pathological conditions such as viral infections, alcohol consumption, and NAFLD. In vitro studies using primary human hepatocytes and hepatoma cell lines have shown that cytochrome P450 enzymes (e.g., CYP2E1 during alcohol metabolism) generate ROS during the metabolism of various xenobiotics and endogenous compounds. In vivo, both murine models of chronic alcohol intake and patients with alcoholic liver disease demonstrate cytochrome P450 2E1 (CYP2E1) overactivation and associated ROS accumulation. Mitochondrial dysfunction, observed in murine models of NAFLD/NASH and confirmed in human biopsy samples, significantly contributes to ROS production through impaired oxidative phosphorylation in the electron transport chain [50]. This dysfunction can exacerbate ROS generation and even disrupt the mitochondrial permeability transition pore (MPTP), as demonstrated in hepatocyte culture systems, leading to mtDNA leakage and further inflammation. The accumulation of these hepatocyte-derived ROS initiates direct cellular damage to membranes, proteins, and DNA, triggering subsequent inflammatory responses and contributing to forms of cell death like ferroptosis and pyroptosis via NLRP3 inflammasome activation [48,49,58].

In addition to these pathways, hepatocytes express NADPH oxidases (NOXs), particularly NOX1, NOX2, and NOX4, which represent key non-mitochondrial ROS sources. Unlike mitochondrial ROS, NOX-derived ROS are generated in a more controlled but spatially localized manner at the plasma membrane and intracellular organelles. In hepatocytes, excessive activation of NOXs amplifies hepatocellular stress by promoting lipid peroxidation, protein carbonylation, and DNA oxidation, which in turn trigger cell death pathways such as apoptosis, pyroptosis, and ferroptosis. Moreover, NOX-derived ROS serve as upstream activators of the NLRP3 inflammasome, enhancing the maturation of IL-1β and IL-18 and thereby establishing a strong pro-inflammatory milieu. Crosstalk with cytokine signaling further aggravates this process: TGF-β, a central mediator of liver fibrosis, upregulates NOX4 expression in hepatocytes, leading to sustained ROS production. This increase in oxidative stress not only perpetuates hepatocyte injury but also provides paracrine signals that activate neighbouring Kupffer cells and hepatic stellate cells, linking hepatocyte-derived ROS to both inflammatory amplification and fibrogenic progression [41,42].

Hepatic Stellate Cells (HSCs): While primarily known for their role in extracellular matrix (ECM) production upon activation, HSCs themselves can be a source of ROS, particularly through the activation of NADPH oxidases (NOXs) like NOX1, NOX2, and NOX4. Evidence from cultured primary rat and human HSCs shows that ROS derived from neighbouring damaged hepatocytes or infiltrating immune cells also serve as potent activators of quiescent HSCs, driving their transdifferentiation into myofibroblast-like cells. Notably, NOX4 predominantly generates H_2O_2 instead of $O_2\bullet-$. This ROS-mediated activation stimulates HSCs to synthesize excessive amounts of collagen and other ECM components, directly contributing to fibrotic tissue accumulation [51]. The self-sustaining loop where activated HSCs produce ROS that further promotes their own activation is a critical aspect of fibrogenesis.

Immune Cells: Tissue-resident Kupffer cells and monocyte-derived macrophages both contribute to the hepatic macrophage pool during fibrosis, but they differ in origin, longevity, and function. Kupffer cells originate from yolk sac progenitors and maintain themselves through local proliferation under homeostatic conditions. In contrast, monocyte-derived macrophages are derived from circulating monocytes recruited during liver injury via chemokine signaling (e.g., CCL2–CCR2 axis). While Kupffer cells are the first responders to injury and often initiate inflammatory signaling, monocyte-derived macrophages are more plastic and can acquire diverse activation states, participating in both fibrogenic and resolution phases [30].

These cells, upon activation by damage-associated molecular patterns (DAMPs) or pathogen-associated molecular patterns (PAMPs) often via Toll-like receptor 4 (TLR4), produce substantial amounts of ROS via NADPH oxidase (NOX) pathways. These ROS contribute to hepatocellular damage and HSC activation. Neutrophils, recruited early

Table 1
Main ROS-generating sources and fibrogenic signalling pathways in liver fibrosis.

Component	ROS Sources/Pathways	Key Effects in Fibrosis	Signalling Pathways/Mechanisms
Hepatocytes	- Cytochrome P450 enzymes (e.g., CYP2E1) [48,49]. - Mitochondrial ETC dysfunction [50]. - Peroxisomes, xanthine oxidase [51] - NADPH oxidases (NOX1, NOX2, NOX4) [51].	- Lipid, protein, DNA damage - Mitochondrial permeability transition - DAMP release, pyroptosis, ferroptosis	- NLRP3 inflammasome activation - NF-κB and JAK/STAT1 signaling
Hepatic Stellate Cells (HSCs)	- ROS from hepatocytes/macrophages [41,42]. - Upregulation by TGF-β [52,53].	- Activation and transdifferentiation - ECM protein synthesis (e.g., collagen) - Positive feedback loop of ROS production	- TGF-β/SMAD2/3 pathway - Suppression of SMAD7 (inhibitor)
Immune Cells (Kupffer cells, monocytes, neutrophils)	- NOX2-dependent ROS burst [41]. - Respiratory burst in neutrophils [54, 55] - TLR4-PAMP/DAMP interactions e	- Cytokine production (IL-1β, IL-6, TNF-α) - Neutrophil extracellular traps - Immune cell recruitment	- NF-κB, MAPKs (JNK, p38) - IL-17 from Th17 cells via STAT3 - Impaired Treg function under high ROS
Liver Microenvironment	- Hypoxia (HIF-1α) [45,56]. - Excess nutrients (e.g., free fatty acids, glucose) [56]. - Cytokine-rich milieu [57].	- Redox imbalance - Amplified inflammation - HSC priming and immune activation	- Indirect modulation of multiple pathways - Chemokines (CCL2), adhesion molecules (VCAM-1, ICAM-1)
Macrophages	- Redox-sensitive phenotype shifts. - ROS from surrounding cells and intrinsic NOX4 [42,51].	- Inflammatory traits in high ROS conditions (e.g. NF-κB/STAT1) - Resolving traits moderate-to-low ROS conditions (e.g., STAT6/TGF-β) - High plasticity and spatiotemporal heterogeneity	- sEV - Mediated phenotype modulation - ROS regulate polarization and function via NRF2, NOX4

Abbreviations: CL2, C–C motif chemokine ligand 2; CYP2E1, cytochrome P450 2E1; DAMP, damage-associated molecular pattern; ECM, extracellular matrix; ETC, electron transport chain; HIF-1α, hypoxia-inducible factor 1 alpha; HSC, hepatic stellate cell; ICAM-1, intercellular adhesion molecule 1; IL, interleukin; JAK, Janus kinase; MAPK, mitogen-activated protein kinase; NF-κB, nuclear factor kappa-light-chain-enhancer of activated B cells; NOX, NADPH oxidase; NRF2, nuclear factor erythroid 2–related factor 2; NLRP3, NOD-like receptor family pyrin domain-containing 3; PAMP, pathogen-associated molecular pattern; ROS, reactive oxygen species; sEV, small extracellular vesicle; STAT, signal transducer and activator of transcription; TGF-β, transforming growth factor beta; Th17, T helper 17; TLR4, toll-like receptor 4; TNF-α, tumor necrosis factor alpha; Treg, regulatory T cell; VCAM-1, vascular cell adhesion molecule 1.

during inflammation, release large quantities of ROS through their "respiratory burst," exacerbating tissue injury and promoting the formation of neutrophil extracellular traps, which further drive inflammation and fibrosis [59,60]. The dynamic interplay between these immune cells and their ROS production capacity dictates the inflammatory tone of the fibrotic liver.

Liver Microenvironment: Beyond specific cell types, the overall hepatic microenvironment significantly influences ROS dynamics. Other non-mitochondrial sources of ROS include peroxisomes (involved in fatty acid β-oxidation) and xanthine oxidases [51]. In fibrotic tissue, chronic hypoxia caused by sinusoidal capillarization and impaired perfusion enhances mitochondrial ROS generation and stabilizes hypoxia-inducible factor 1 alpha (HIF-1α), which upregulates glycolytic enzymes and NOX isoforms, further promoting oxidative stress. Altered nutrient metabolism, as seen in NAFLD and alcoholic liver disease, contributes additional sources of ROS: excess free fatty acids and glucose overload impair β-oxidation and oxidative phosphorylation, leading to electron transport chain dysfunction and increased mitochondrial ROS leakage [56].

In parallel, inflammatory cytokines such as tumor necrosis factor alpha (TNF-α), interleukin 1β (IL-1β), and transforming growth factor beta (TGF-β) not only stimulate NOX activity in hepatocytes, immune cells, and HSCs but also suppress antioxidant defenses, including NRF2-mediated transcription of superoxide dismutase (SOD), catalase, and glutathione synthesis enzymes. The convergence of hypoxia, metabolic reprogramming, and cytokine-driven signaling thus creates a self-reinforcing pro-oxidative environment in which ROS production overwhelms the liver's antioxidant capacity, perpetuating both cellular damage and profibrotic signaling [61]. This comprehensive view of the microenvironment highlights how systemic and local factors converge to create a pro-oxidative and pro-fibrotic milieu.

2.3. ROS as modulators of fibrotic signalling and immune modulation

Mechanistically, ROS exert pleiotropic effects that link inflammatory and fibrogenic pathways. (Fig. 3). In macrophages, ROS derived from mitochondria and NADPH oxidases activate the IkB kinase (IKK) complex, which phosphorylates IκB and promotes its proteasomal degradation, liberating the nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB) from cytoplasmic sequestration [57]. Once liberated, NF-κB translocates to the nucleus, where it induces the transcription of pro-inflammatory cytokines such as TNF-α, IL-1β, and IL-6, along with chemokines like C–C motif chemokine ligand 2 (CCL2) that recruit immune cells. These signals attract monocytes and macrophages, which adopt pro-inflammatory phenotypes and amplify liver injury while simultaneously activating HSCs. In parallel, ROS trigger the activation of the NOD-like receptor family pyrin domain-containing 3 (NLRP3) inflammasome through the thioredoxin-interacting protein (TXNIP pathway), leading to IL-1β and IL-18 maturation and initiating pyroptosis, a form of inflammatory cell death that further contributes to fibrogenesis [62].

Beyond these immune effects, ROS also act directly on hepatic stellate cells (HSCs), enhancing TGF-β/SMAD and mitogen-activated protein kinase (MAPK) signaling and thereby promoting extracellular matrix production and myofibroblast differentiation. Crosstalk between these processes establishes a feed-forward loop in which inflammatory cytokines reinforce fibrogenic activation, while activated HSCs generate additional ROS, perpetuating both inflammation and fibrosis in a coordinated manner. TGF-β is a central mediator of this process. ROS increase TGF-β expression in hepatocytes and immune cells and facilitate its activation by promoting oxidative modifications of latency-associated peptides, which release active TGF-β from its latent complex. In HSCs, binding of the TGF-β with its receptor induces phosphorylation and nuclear translocation of SMAD2/3, initiating the transcription of fibrogenic genes like the collagen type I alpha 1 chain (COL1A1) and alpha-smooth muscle actin (α-SMA = . ROS additionally

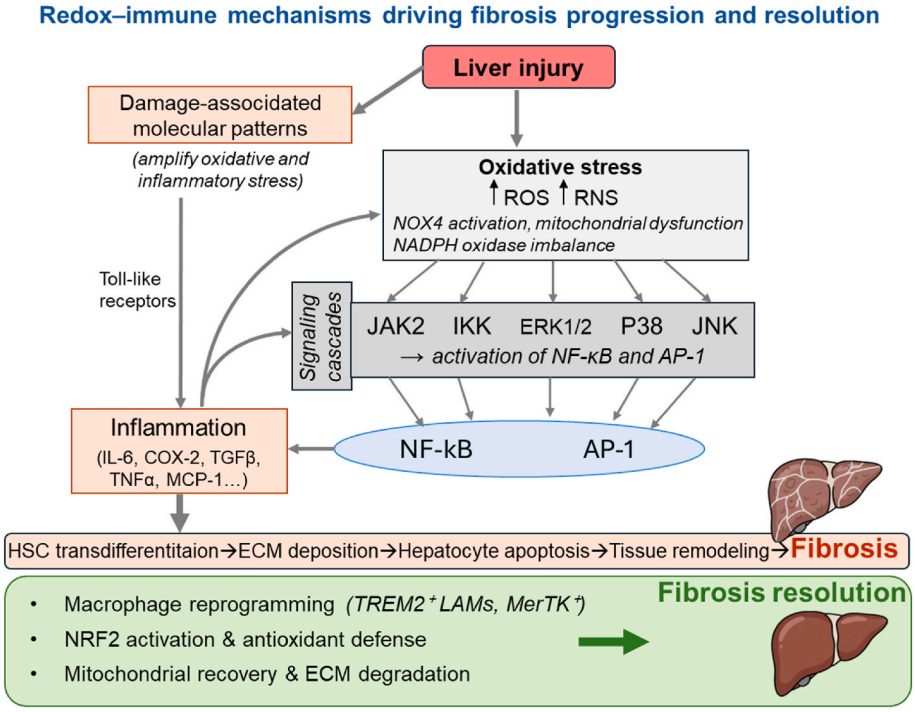

Fig. 3. Schematic representation of the main signalling pathways involved in oxidative stress-mediated inflammation in liver disease, along with therapeutic strategies for intervention. **Abbreviations:** AP-1, activator protein 1; COX-2, cyclooxygenase-2; ERK1/2, extracellular signal–regulated kinase 1/2; IKK, IκB kinase; IL-6, interleukin-6; iNOS, inducible nitric oxide synthase; JAK2, Janus kinase 2; JNK, c-Jun N-terminal kinase; MCP-1, monocyte chemoattractant protein-1; NF-κB, nuclear factor kappa-light-chain-enhancer of activated B cells; NRF-2, nuclear factor erythroid 2–related factor 2; p38, p38 mitogen-activated protein kinase; RNS, reactive nitrogen species; ROS, reactive oxygen species; TGF-β, transforming growth factor beta; TNF-α, tumor necrosis factor alpha.

suppress SMAD7, a natural inhibitor of the pathway, reinforcing a positive feedback loop that sustains HSC activation and promotes fibrosis progression [52,53].

In addition to enhancing SMAD-dependent transcription, TGF-β also regulates NADPH oxidase activity. In both hepatocytes and HSCs, TGF-β induces NOX4 expression, which elevates intracellular ROS levels and sustains a profibrotic state. ROS, in turn, can further activate latent TGF-β, establishing a positive feedback loop that drives chronic fibrosis [42,51]. This redox–cytokine interplay highlights NOX enzymes as nodal points where TGF-β signaling converges with oxidative stress pathways to perpetuate liver injury.

ROS also influence the MAPK pathways, including extracellular signal–regulated kinase 1/2 (ERK1/2), c-Jun N-terminal kinase (JNK), and p38 MAPK (p38) [63,64]. In murine models of CCl_4-induced fibrosis and hepatocyte culture systems, these signalling cascades are activated either directly or through upstream kinases such as MEK and ASK1. JNK signalling contributes to hepatocyte apoptosis and the release of DAMPs, which activate immune cells and HSCs. p38 MAPK has been shown in both rodent models and human liver samples to drive the expression of inflammatory cytokines and matrix metalloproteinases (MMPs), facilitating immune cell infiltration and ECM remodelling. Meanwhile, ERK1/2 signalling supports the proliferation and survival of HSCs, further enhancing fibrogenic responses.

In addition, the Janus kinase/signal transducer and activator of transcription (JAK/STAT) pathway is intricately modulated by oxidative stress [65]. ROS can oxidize cysteine residues on JAKs and STATs, directly altering their activity, while also regulating the expression of cytokines such as IL-6 and interferons. IL-6-driven STAT3 activation, for example, promotes hepatocyte survival in early injury but induces fibrogenic gene expression in HSCs when chronically activated. At the same time, ROS enhance STAT1 signalling in macrophages and T cells, reinforcing pro-inflammatory activity [66,67]. These context-dependent effects of ROS on JAK/STAT signalling highlight the complexity of redox regulation in liver disease.

In fact, the influence of ROS extends beyond signalling pathways to the recruitment and phenotypic modulation of immune cells. In murine models of steatohepatitis and in human endothelial cell cultures, oxidative stress has been shown to drive the production of chemokines like CCL2 and upregulates adhesion molecules such as the intercellular adhesion molecule 1(ICAM-1) and; the vascular cell adhesion molecule 1 (VCAM-1) on endothelial cells, enabling monocyte and neutrophil infiltration into the liver. The oxidative microenvironment itself also acts as a chemotactic signal, reinforcing inflammation and immune cell accumulation in a self-perpetuating loop [55,68].

Macrophages are particularly sensitive to redox cues. Elevated ROS levels favor M1-like polarization through NF-κB and STAT1 activation, promoting the release of pro-inflammatory cytokines that exacerbate liver injury in both CCl_4-induced fibrosis models and human monocyte-derived macrophage cultures. Conversely, moderate ROS or compartmentalized redox changes can support M2-like or pro-resolving phenotypes via STAT6 and TGF-β signalling [69]. Interestingly, recent evidence shows that small extracellular vesicles (sEVs) derived from activated HSCs can modulate macrophage phenotype depending on the activation stage. Short-term HSC-sEVs promote M1 polarization in resident Kupffer cells, while long-term HSC-sEVs induce M2-like features in bone marrow–derived macrophages [70,71], evidencing the context-dependent nature of macrophage responses in fibrosis.

ROS also shape the behaviour of other immune cells. Neutrophils, major producers of ROS through NADPH oxidase, contribute to liver injury not only by releasing ROS but also by forming neutrophil extracellular traps, which have been implicated in promoting fibrotic responses. Th17 cells are driven toward differentiation by ROS-mediated STAT3 activation, leading to IL-17 production that stimulates HSCs and perpetuates fibrogenesis [54,72]. Meanwhile, regulatory T cells (Tregs) are particularly vulnerable to high oxidative stress, which impairs their suppressive function and shifts the immune balance toward chronic inflammation. Dendritic cells are similarly modulated by ROS during maturation, enhancing their antigen-presenting capacity and cytokine production, further promoting T cell activation and fibrosis.

At the tissue level, ROS shape the hepatic microenvironment by inducing hepatocyte death, mitochondrial dysfunction, and senescence. These effects reinforce inflammation, immune cell recruitment, and HSC activation, creating a pro-fibrotic niche. Moreover, the plasticity of immune responses, particularly among macrophages, depends heavily on redox conditions, emphasizing the importance of spatial and temporal dynamics in understanding fibrotic progression.

3. Macrophage polarization in liver fibrosis

3.1. Limitations of the classic M1/M2 polarization model

Undoubtedly, macrophages play a key role in the initiation, progression, and resolution of liver fibrosis. Traditionally, their behaviour has been interpreted through the M1/M2 polarization framework [73], adapted from Th1/Th2 immunology [74,75]. M1 macrophages, induced by stimuli such as IFN-γ and LPS, are pro-inflammatory and characterized by markers like cluster of differentiation 68 (CD68), CD86, inducible nitric oxide synthase (iNOS), and TNF-α. In early liver injury (whether viral, metabolic, or toxic) Kupffer cells and monocyte-derived macrophages adopt an M1 phenotype that amplifies inflammation through the NOD-like receptor family pyrin domain-containing 3 (NLRP3) inflammasome activation and ROS-dependent NF-κB signalling. These macrophages recruit neutrophils via IL-1β and IL-6 and activate HSCs through TGF-β1 and platelet-derived growth factor (PDGF) signaling loops.

As fibrosis progresses, a phenotypic shift to the M2 state occurs, induced by IL-4 and IL-13 released by Th2 cells and apoptotic hepatocytes [34,76]. M2 macrophages ($CD163^+$, $CD206^+$, $Arg1^+$, $YM1^+$) were initially thought to facilitate fibrosis resolution by secreting matrix-degrading enzymes like MMP-9 [77] and anti-inflammatory cytokines such as IL-10 [78,79]. However, some M2 subsets, for example, promote fibrosis through galectin-3-mediated HSC activation [80], and others, like $TREM2^+$ LAMs, exhibit hybrid pro-fibrotic and pro-resolving properties [81]. Moreover, spatial heterogeneity has been observed: periportal macrophages often display stronger M1 characteristics, whereas pericentral ones lean toward an M2 phenotype, likely influenced by hypoxia gradients [47].

Therefore, the classic M1/M2 dichotomy is increasingly regarded as insufficient (Fig. 4). Key limitations include: (1) Artificial dichotomy: Single-cell RNA sequencing has identified more than ten distinct macrophage clusters in fibrotic livers, many of which express both M1- and M2-associated markers [47,82,83]; (2) Context-dependence: M2-like macrophages can either exacerbate fibrosis (e.g., via CCL2-mediated chemotaxis of HSCs) or promote resolution through MER proto-oncogene tyrosine kinase (MERTK)-dependent efferocytosis, a process by which macrophages clear apoptotic cells, helping resolve inflammation, depending on ROS levels [84,85]; (3) Temporal dynamics: For instance, fate-mapping studies have shown that macrophages can reversibly switch phenotypes during fibrosis regression [30]. Furthermore, disease-specific subsets have been identified that fall outside the M1/M2 framework. These include senescence-associated macrophages (SAMs) in NASH and $CX3CR1^+$ restorative macrophages in viral hepatitis [86,87].

3.2. Macrophage plasticity and functional diversity: Beyond the M1/M2 paradigm

Macrophage polarization is increasingly recognized as a dynamic continuum rather than a strict M1/M2 dichotomy, with redox cues serving as key modulators of this spectrum. High and sustained ROS exposure (particularly NOX4-derived H_2O_2 in hepatic stellate cells and macrophages) promotes pro-inflammatory and profibrotic activation via

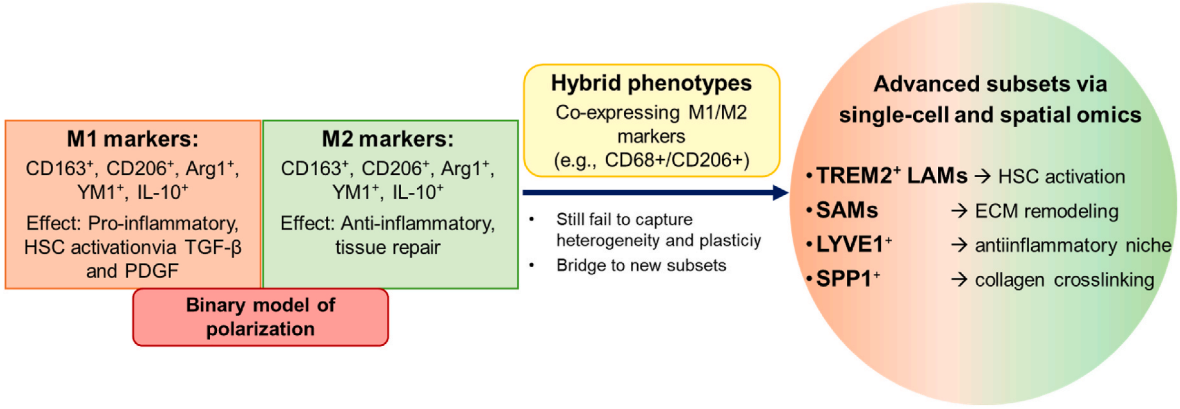

Fig. 4. From M1/M2 dichotomy to macrophage heterogeneity in liver fibrosis. The classical binary M1/M2 model (left) provides a simplified view of macrophage polarization. Hybrid phenotypes (center) highlight their plasticity and context-dependent behavior. Advances in single-cell and spatial omics (right) have identified specialized subsets such as TREM2$^+$ lipid-associated macrophages (LAMs), senescence-associated macrophages (SAMs), LYVE1$^+$ periportal macrophages, and SPP1$^+$ macrophages, which define pro-fibrotic and reparative niches. This transition beyond the M1/M2 dichotomy highlights the complexity and therapeutic relevance of macrophage heterogeneity in liver fibrosis.

NF-κB, STAT1, and TGF-β/SMAD signalling. Conversely, moderate or compartmentalized ROS levels favor reparative polarization through NRF2-driven antioxidant programs and STAT6/TGF-β signalling, enabling efferocytosis and matrix resolution. Transient or spatially restricted ROS bursts can generate hybrid phenotypes, such as TREM2$^+$-LAMs, that simultaneously engage in efferocytosis while sustaining stellate cell activation. A summary of this functional diversity is presented in Table 2, and the dynamic, redox-sensitive nature of macrophage plasticity is schematically illustrated in Fig. 5. These hybrid and redox-sensitive phenotypes illustrate the limitations of the M1/M2 dichotomy and set the stage for the current exploration of macrophage heterogeneity.

Recent advances in single-cell RNA sequencing, fate-mapping, and spatial transcriptomics have uncovered a rich diversity of macrophage subsets that defy the classic classification, including hybrid phenotypes co-expressing both pro-inflammatory (e.g., IL-1β, TNF-α) and anti-inflammatory (e.g., CD206, Arg1) markers [47,88]. These transitional states are further shaped by local environmental factors such as extracellular matrix stiffness, redox gradients, and metabolic cues (Fig. 6). Importantly, these technologies have revealed spatially organized immune niches, where distinct macrophage subsets localize to fibrotic septa, periportal regions, or areas of hepatocyte damage, thereby influencing disease trajectories in a context-dependent manner. Such findings highlight that macrophage identity is not only plastic but also dynamically imprinted by microenvironmental context, allowing cells to switch roles in the different stages of fibrosis progression and resolution.

Functionally specialized macrophage populations have been described in various fibrotic contexts. In NASH, TREM2$^+$-LAMs exhibit both reparative and pro-fibrotic features, engaging in efferocytosis via MERTK while sustaining HSC activation through osteopetrosis-associated transmembrane protein 1(OSTM1) secretion [81]. Similarly, senescence-associated macrophages (SAMs), identified by CD36 and p16INK4a, contribute to ECM remodelling by producing MMP-12, despite their role in clearing senescent HSCs [90,91]. Spatial transcriptomic analyses have revealed zonal heterogeneity, with LYVE1$^+$

Table 2
Macrophage polarization, biomarkers, and functional diversity in liver fibrosis.

Aspect	Description	Key Features/Markers	Implications in Liver Fibrosis
Classical M1/M2 Model	Binary polarization framework adapted from Th1/Th2 [73,74].	- M1: CD68$^+$, CD80$^+$, CD86$^+$, iNOS$^+$, TNF-α$^+$, IL-1β$^+$. - M2: CD163$^+$, CD206$^+$, Arg1$^+$, YM1$^+$, IL-10$^+$.	- M1: Amplifies inflammation and activates HSCs via TGF-β and PDGF. - M2: Resolves inflammation, but some subtypes promote fibrosis (e.g., via Galectin-3)
Limitations of M1/M2	Oversimplified, fails to capture in vivo [88].	- Mixed/hybrid phenotypes co-expressing M1 and M2 markers (e.g., CD68$^+$/CD206$^+$).	Misrepresents macrophage heterogeneity and temporal transitions during fibrosis progression/regression.
Key Subsets Beyond M1/M2	Macrophage states identified via single-cell and spatial omics [19,47].	- TREM2$^+$ LAMs: TREM2$^+$, CD9$^+$+ efferocytosis + HSC activation - SAMs: CD36$^+$, p16, INK4a$^+$, MMP-12$^+$ [89–91]. - LYVE1$^+$ (periportal): LYVE1$^+$, MRC1$^+$, IL-10$^+$ [92,93]. - SPP1$^+$ (fibrotic septa): [92,93].	Define pro-fibrotic and reparative niches: LAMs and SPP1$^+$ macrophages promote ECM deposition; LYVE1$^+$ macrophages exert anti-inflammatory effects; SAMs remodel ECM via MMP12.
Regulatory Mechanisms	Pathways and signals driving macrophage phenotype and plasticity.	- HIF-1α (glycolysis, M1-like), OXPHOS (M2-like) [56,94]. - Epigenetics: MJD3 (H3K27 demethylation, reparative) [95,96]. - ROS: low ROS linked to NRF2 (anti-inflammatory), high ROS linked to NOX4 (pro-fibrotic) [97].	Identify key drivers of macrophage reprogramming; potential therapeutic targets for ROS and metabolism-based interventions.
Therapeutic Challenges	Translating macrophage heterogeneity into interventions	Lack of exclusive markers distinguishing resident (Kupffer) vs. monocyte-derived macrophages.	Highlights the need for spatiotemporal profiling and targeted delivery systems in antifibrotic therapy.

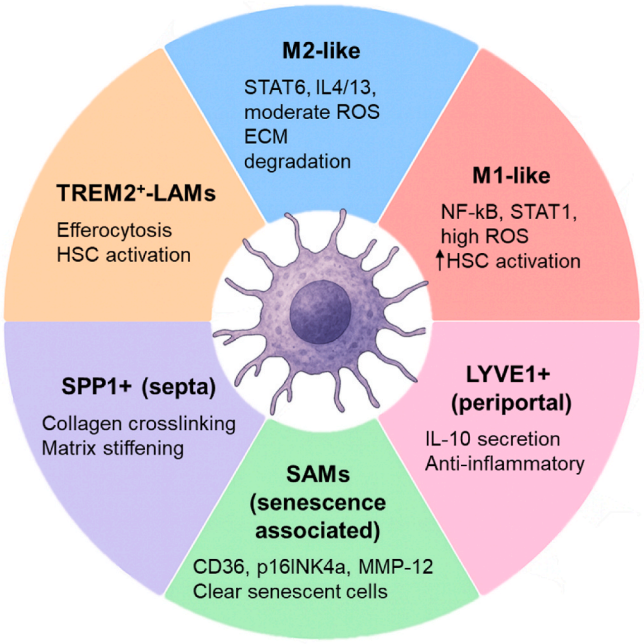

Fig. 5. Schematic representation of macrophage plasticity. The surrounding wedges depict diverse macrophage subsets, including M1-like, M2-like, TREM2$^+$ lipid-associated macrophages (LAMs), senescence-associated macrophages (SAMs), SPP1$^+$, and LYVE1$^+$ populations, highlighting their key features and functional roles.

macrophages in periportal areas exerting anti-inflammatory effects through IL-10, in contrast to SPP1$^+$ macrophages in fibrotic septa that enhance collagen crosslinking [92]. Such plasticity allows macrophages to shift between inflammatory (e.g., CCR2$^+$) and restorative (e.g., GPNMB$^+$) phenotypes in response to changing disease states.

To provide a conceptual framework, macrophage states in the fibrotic liver can be envisioned along a redox-sensitive continuum rather than a binary M1/M2 model. High and sustained ROS exposure (particularly NOX4-derived H_2O_2 in hepatic stellate cells and macrophages) promotes pro-inflammatory and profibrotic activation via NF-κB, STAT1, and TGF-β/SMAD signaling. Moderate or compartmentalized ROS levels, in contrast, favor reparative polarization through NRF2-driven antioxidant programs and STAT6/TGF-β signaling, enabling efferocytosis and matrix resolution.

This redox-sensitive continuum explains how oxidative cues orchestrate macrophage interconversion during both fibrogenesis and regression. Elevated oxidative stress favors polarization toward M1-like and SPP1$^+$ macrophages, characterized by enhanced glycolysis, NOX4 activation, and pro-inflammatory cytokine secretion (TNF-α, IL-1β) that sustains HSC activation and ECM deposition [99]. As oxidative stress becomes modulated or spatially compartmentalized, macrophages can transition toward TREM2$^+$ LAMs and SAMs, which exhibit mixed or intermediate phenotypes contributing to debris clearance and tissue remodelling. Under controlled ROS generation, LYVE1$^+$ macrophages emerge, supporting anti-inflammatory and reparative functions through oxidative phosphorylation (OXPHOS) and nuclear factor erythroid 2–related factor 2 (NRF2) activation. These transitions are reversible and context-dependent, integrating ROS with cytokine, hypoxic, and metabolic cues to drive macrophage plasticity across a redox-sensitive spectrum in liver fibrosis [100,101].

Importantly, these redox-driven macrophage transitions are closely coupled to metabolic reprogramming. Pro-inflammatory, M1-like macrophages predominantly rely on HIF-1α–driven glycolysis, which ensures rapid ATP generation and supports biosynthetic and redox demands required for cytokine secretion and reactive oxygen species (ROS) production. In contrast, reparative or M2-like macrophages depend on OXPHOS and fatty acid oxidation, enabling sustained energy efficiency, redox balance, and synthesis of anti-inflammatory mediators. This metabolic dichotomy provides an energetic basis for the functional specialization of macrophage subsets within the fibrotic liver microenvironment, directly linking HIF-1α-induced glycolytic flux with inflammation and e.

The regulation of macrophage states is also governed by other interconnected mechanisms beyond metabolic reprogramming, related

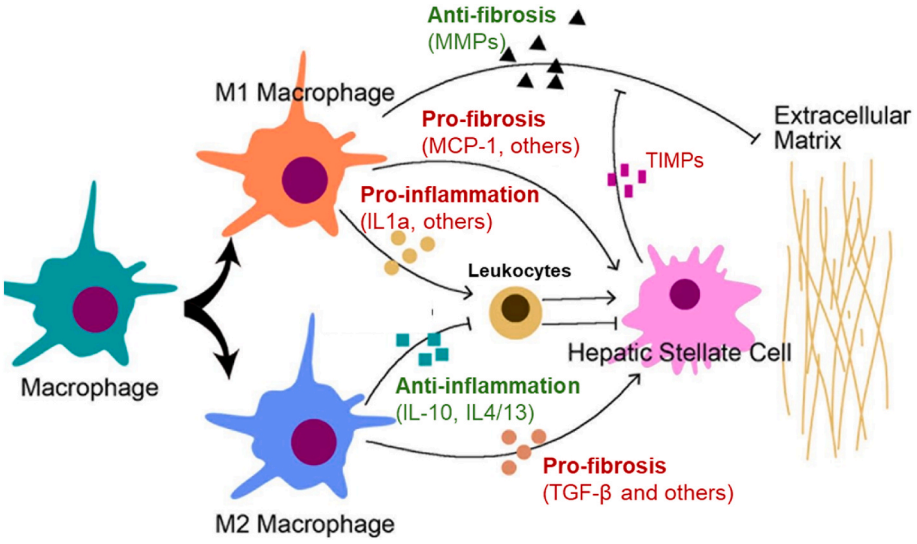

Fig. 6. Interconnected macrophage responses in liver fibrosis progression. Peripheral monocytes and resident macrophages differentiate into diverse macrophage populations, broadly categorized as M1 or M2 phenotypes, with potential for interconversion. M1 macrophages contribute to pro-inflammation (e.g., IL-1α), promote fibrosis (e.g., via MCP-1-mediated hepatic stellate cell activation), and can also exert anti-fibrotic effects through matrix metalloproteinases (MMPs). Conversely, M2 macrophages primarily mediate anti-inflammatory responses (e.g., IL-10), yet they also contribute to pro-fibrotic processes (e.g., via TGF-β affecting hepatic stellate cells) and influence extracellular matrix remodelling through inhibitors of MMPs (TIMPs). Both macrophage subsets dynamically interact with hepatic stellate cells and other leukocytes, illustrating the complex and interconnected pathways that govern the progression of liver fibrosis. Adapted from Wang, Z. et al. [98] International Immunopharmacology, 2023, 120, 110357.

toHIF-1α-driven glycolysis and OXPHOS [94,102,103]. Thus, epigenetic modulation, including JMJD3-mediated H3K27 demethylation promotes reparative polarization [95,96]. In addition, receptor signaling, exemplified by PPARγ-LXRα crosstalk, controls lipid metabolism. ROS further shape macrophage fate through redox-sensitive pathways: low levels of ROS activate NRF2, promoting antioxidant and anti-inflammatory programs, while elevated ROS drive NOX4-mediated pro-fibrotic activation [97].

Fig. 7 illustrates the most significant transitions above-mentioned and the interconnected mechanisms by which ROS regulate macrophage states in liver fibrosis. As mentioned, one the one side, although M1-like macrophages are traditionally linked with pro-inflammatory and fibrogenic functions, they can also exert antifibrotic activity by producing MMPs, specially MMP-9 and MMP-12. This typically occurs under conditions of fibrosis regression or following efferocytosis of apoptotic hepatocytes and neutrophils, where clearance of dying cells reprograms macrophages toward a pro-resolving phenotype. The resulting matrix metalloproteinase (MMP) activity contributes to extracellular matrix degradation and facilitates tissue remodelling [77,89,104,105]. On the other side, e, among others, SMAD2/3 phosphorylation and transcription of fibrogenic genes such as COL1A1 and α-SMA. In parallel, M2-secreted CCL2 recruits additional $CCR2^{+}$ monocytes from the circulation, thereby sustaining an inflammatory loop that fuels both immune cell infiltration and HSC activation [34,84,106].

Furthermore, NOX4-derived ROS represent a central driver of macrophage- and HSC-mediated profibrotic responses. TGF-β induces NOX4 expression, particularly in HSCs, resulting in sustained production of H_2O_2. These NOX4-derived ROS activate downstream SMAD2/3 and NF-κB signalling, which reinforce HSC activation and collagen deposition. Moreover, elevated oxidative stress can itself induce NOX4 expression, creating a feed-forward loop in which ROS and NOX4 synergize to perpetuate fibrosis [107–111]. Thus, while multiple ROS sources contribute to fibrosis, NOX4-derived ROS are specifically responsible for driving profibrotic activation in HSCs.

Taken together, these findings support a unified ROS level–macrophage functional spectrum model in liver fibrosis. Under conditions of high and sustained ROS or NOX4 activity, macrophages adopt pro-inflammatory and pro-fibrotic states (e.g., M1-like, $SPP1^{+}$), characterized by NF-κB and STAT1 activation, glycolytic metabolism, and secretion of TNF-α and TGF-β that sustain HSC activation. In contrast, moderate or compartmentalized ROS levels promote reparative and anti-inflammatory phenotypes (e.g., $LYVE1^{+}$ and M2-like macrophages), driven by NRF2 and STAT6 signalling and by enhanced oxidative phosphorylation that favours matrix resolution. Finally, dynamic or transient ROS fluctuations give rise to hybrid macrophage states, such as $TREM2^{+}$ LAMs and SAMs, which simultaneously perform efferocytosis, lipid handling, and limited pro-fibrotic signalling. This redox-dependent functional continuum positions ROS as a master regulatory switch integrating inflammatory, metabolic, and fibrogenic pathways, as illustrated in Fig. 6.

Despite these advances, challenges remain in identifying subset-specific surface markers and distinguishing tissue-resident from monocyte-derived macrophages. For instance, a next step would be integrating spatial multi-omics with in vivo lineage tracing, key to refining therapeutic targeting of functionally distinct macrophage populations while preserving homeostatic roles.

4. Therapeutic implications

Therapeutically, understanding macrophage plasticity is key to developing more effective and targeted approaches for treating liver fibrosis. For instance, given the pathogenic role of ROS in driving hepatic inflammation and fibrogenesis, nowadays research efforts are

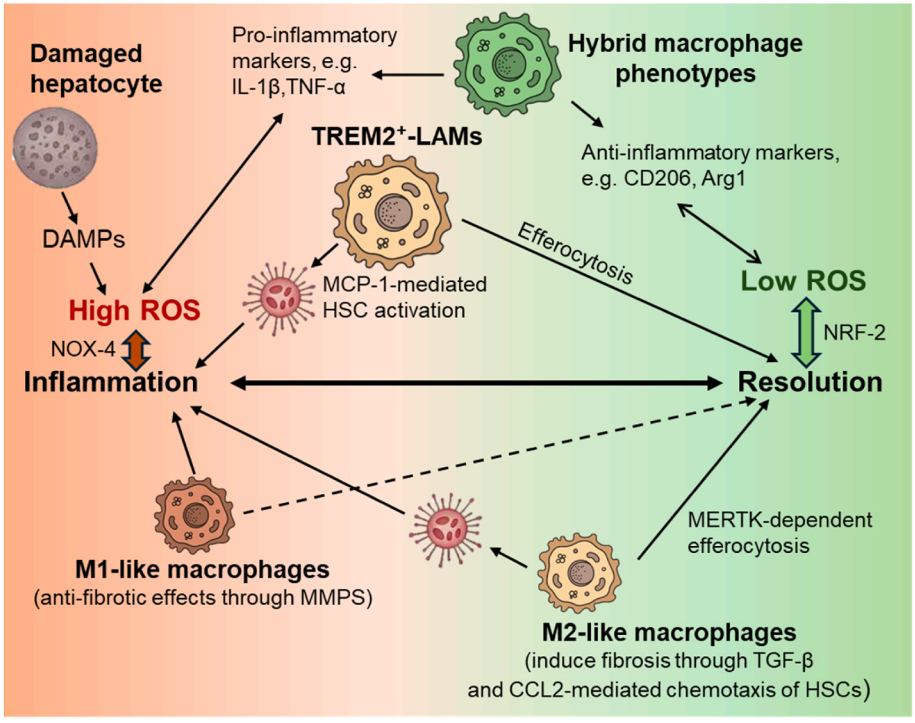

Fig. 7. Interconnected mechanisms regulating macrophage states in liver fibrosis. M1 macrophages not only drive inflammation but can also release anti-fibrotic MMPs (e.g., MMP-9, MMP-12) under regression conditions or after efferocytosis, contributing to extracellular matrix degradation. Conversely, M2 macrophages promote fibrosis through secretion of TGF-β, which activates HSCs via SMAD2/3 signalling, and CCL2, which recruits $CCR2^{+}$ monocytes to sustain inflammation. ROS are central regulators of these processes: moderate ROS activate NRF2, favouring anti-inflammatory programs, whereas NOX4-derived ROS drive profibrotic activation by sustaining TGF-β and NF-κB signalling. Together, these mechanisms highlight the dynamic, redox-sensitive plasticity of macrophages in the fibrotic liver microenvironment.

underway to develop therapies that modulate oxidative stress beyond traditional antioxidants [112–116]. These traditional antioxidants, such as N-acetylcysteine (NAC) and vitamin E, have shown some promise in experimental models and early clinical trials by reducing oxidative damage and improving liver histology. However, their lack of specificity and uncertain long-term efficacy limit clinical utility [112,117–121].

More advanced strategies involve direct modulation of ROS-generating enzymes and redox-sensitive pathways. For example, NOX inhibitors like GKT137831 (Setanaxib), which specifically targets NOX1 and NOX4, have demonstrated antifibrotic effects in animal models by attenuating ROS-driven TGF-β and NF-κB signaling [107,110,111,122,123]. Setanaxib has advanced into phase II clinical trials for primary biliary cholangitis and liver fibrosis associated with NASH, indicating translational progress [123]. In addition, mitochondria-targeted antioxidants (e.g., MitoQ, SkQ1) have been explored for over a decade as promising approaches to counteract hepatocyte oxidative stress at its source [124–126]. While these therapeutic advances are encouraging, it should be acknowledged that most of these data derive from murine models, which only partially mimic the complexity of human liver fibrosis. Thus, translational studies using humanized models and patient-derived systems are essential to confirm the relevance and safety of these ROS-targeting and macrophage-reprogramming strategies in clinical contexts.

Given the high degree of macrophage plasticity in the liver, therapeutic efforts are increasingly focused on reprogramming their phenotypes rather than simply suppressing their activity. For instance, a promising target is the redox-sensitive transcription factor NRF2, which regulates antioxidant gene expression. NRF2 activation not only reduces ROS but also modulates inflammatory responses, delivering a combined anti-inflammatory and antifibrotic benefit [127]. This immunoregulatory principle is further exemplified by cytokines such as IL-10, which promote tissue repair and fibrosis resolution through macrophage-mediated pathways [78]. Pharmacological activators of NRF2 (e.g., bardoxolone methyl, sulforaphane) are also being investigated as means to restore redox balance and favor anti-inflammatory polarization [128], and have been evaluated in early-phase trials for chronic liver and kidney diseases due to their dual antioxidant and anti-inflammatory effects [129]. Other examples include therapeutic agents such as peroxisome proliferator-activated receptor gamma (PPARγ) agonists (e.g., pioglitazone) and adenosine monophosphate–activated protein kinase (AMPK) activators, which have been shown to shift macrophages toward anti-inflammatory states and reduce fibrosis [130].

Another promising direction involves newly developed drug delivery systems such as exosome-based carriers to transport siRNAs or miRNAs to macrophages, reprogramming their activity [28,131] reducing, for instance, CCL2/TGF-β signalling [132,133] or nanoparticle-mediated approaches designed to selectively target hepatic macrophages with polarizing agents, improving specificity and minimizing off-target effects [112,121,134]. Among nanomedicine-enabled therapies for redox-based interventions, nanozymes, engineered nanomaterials with intrinsic enzyme-like activities, are gaining increasing attention. These materials can mimic the activity of natural antioxidant enzymes such as SOD, catalase, and peroxidases, neutralizing excessive ROS and restoring redox homeostasis in the fibrotic liver microenvironment [112,120,135].

Remarkably, beyond their antioxidant role, nanozymes can exert profound immunomodulatory effects [136,137]. For instance, by reducing ROS-driven activation of NF-κB and NLRP3 inflammasome pathways, they limit pro-inflammatory cytokine release and prevent sustained M1 polarization and, at the same time, favoring the transition of macrophages toward reparative phenotypes, enhancing IL-10 production and matrix resolution [138]. Moreover, when combined with drug delivery or surface functionalization strategies, nanozymes can be tailored to selectively target hepatic macrophages, acting not only as ROS scavengers but also as precision reprogramming tools for macrophage-mediated fibrosis resolution. This dual ability to modulate redox balance and immune function positions nanozymes as a promising therapeutic platform to address the complexity of ROS-immune-fibrotic interactions, as depicted in Fig. 8.

In parallel, epigenetic and metabolic reprogramming is being explored to drive reparative macrophage functions. Modulation of histone acetylation, DNA methylation, and cellular metabolism (e.g., glycolysis vs. oxidative phosphorylation) may provide novel levers for therapeutic control [139–141]. Supporting these strategies, Colony Stimulating Factor 1 Receptor (CSF1R) inhibitors have been shown to selectively deplete profibrotic $CCR2^{+}$ macrophages while sparing $GATA6^{+}$ peritoneal-derived subsets critical for fibrosis resolution [142,143]. Other promising approaches, mentioned in section 2, include immune checkpoint blockade, such as anti-TREM2 antibodies that target LAMs [47], and metabolic interventions like ACOD1 inhibition, which prevents itaconate-mediated immunosuppression [144].

Due to the multifactorial nature of liver fibrosis, these above-described monotherapies can be complemented with combination therapies that simultaneously target oxidative stress and immune modulation. For instance, combining antioxidants with macrophage-polarizing agents may offer synergistic benefits by reducing ROS while promoting fibrosis resolution. Integrating antifibrotic agents (e.g., TGF-

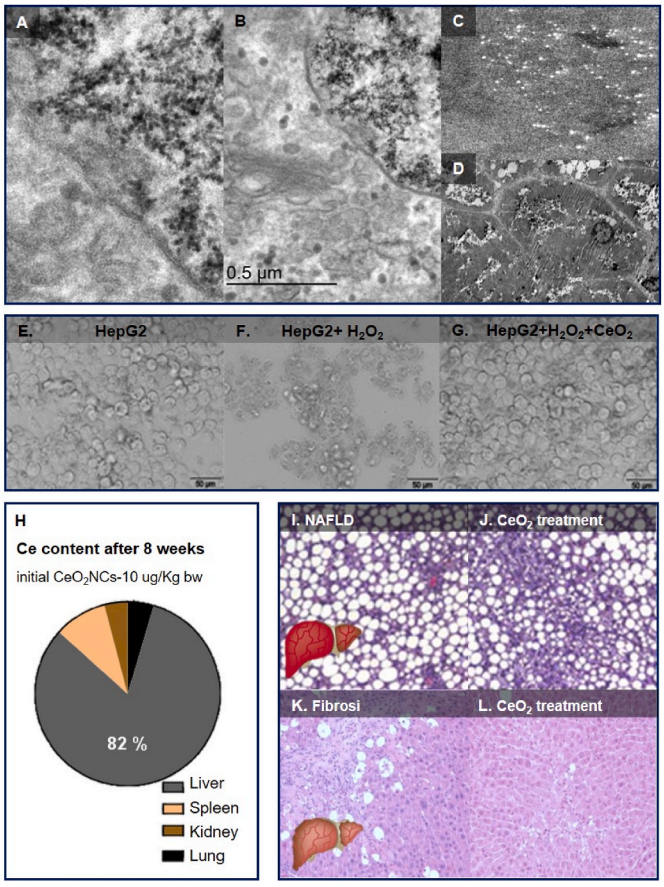

Fig. 8. The therapeutic potential of nanozymes (Cerium Oxide nanoparticles, CeO_2) as a new tool for addressing liver diseases. A, B, C, D. Transmission electron microscopy (TEM) and dark-field imaging confirm CeO2NPs' morphology and cytoplasmic localization within human HepG2 hepatic cells. **E-G.** Phase-contrast microscopy reveals CeO_2NPs protect HepG2 cells from hydrogen peroxide-induced oxidative stress. **H.** Assessment of the organ distribution of CeO_2NPs after 8 weeks in vivo. **I-L.** Furthermore, CeO_2NPs demonstrate protective effects in rat models of NAFLD (induced by methionine and choline deficient diet, MCDD) and liver fibrosis (induced by CCl4). Reproduced with permission from Casals, E. et al. [112], Small, 2020, 16(20). Copyright © 2020 WILEY-VCH Verlag GmbH & Co. KGaA, Weinheim.

β inhibitors, anti-HSC therapies) with redox modulators and immune-targeting compounds could disrupt the feedback loops that sustain chronic inflammation and matrix deposition. And with recent computational advances enabling the analysis of large-scale datasets, personalized medicine approaches hold great promise. Patient-derived omics data, including transcriptomics, redox profiles, and immune signatures, can guide tailored therapeutic regimens that combine ROS modulation with macrophage reprogramming, ultimately improving outcomes in specific patient subgroups.

Overall, therapies targeting both ROS and macrophage behaviour are transforming the treatment landscape for liver fibrosis. While challenges remain in achieving cell-specific targeting, ensuring delivery efficiency, and maintaining long-term efficacy, integrative strategies that address the dynamic interplay between redox balance and immune regulation are poised to enable more effective and durable therapeutic solutions.

5. Conclusion and future directions

ROS are now recognized as central regulators of liver fibrosis, integrating signals from hepatocyte injury, immune activation, and HSC *trans*-differentiation. Rather than being viewed as passive cytotoxic by-products, ROS have been identified as dynamic modulators of the immune–fibrotic crosstalk. Through e.g. NADPH oxidases and TGF-β–dependent pathways, they influence macrophage polarization and sustain HSC activation, thereby linking oxidative stress to fibrosis progression.

Liver fibrosis thus arises from intricate interactions between immune and non-immune cells within a redox-sensitive microenvironment. Acting through cascades such as NF-κB, HIF-1α, STAT3, MAPKs, and JAK/STAT, ROS drives inflammation, apoptosis, immune cell recruitment, and extracellular matrix remodelling. These processes shape macrophage phenotypes along a continuum that transcends the classical M1/M2 dichotomy, giving rise to hybrid populations with context-dependent functions. ROS not only regulate macrophages but also influence other immune populations, including neutrophils, Th17 cells, and dendritic cells, thus embedding fibrogenesis within a broader immune network.

In addition, ROS also impact non-immune cells. In hepatocytes, oxidative stress induces mitochondrial dysfunction and DNA damage, promoting inflammation and DAMP release that stimulates immune activation. ROS also directly activate HSCs, driving their *trans*-differentiation and enhancing ECM stiffness, thereby perpetuating fibrosis. This revised perspective, supported by insights from single-cell and spatial omics, reveals liver fibrosis as a complex, redox-governed immunological landscape, rather than a linear inflammatory process.

Therapeutically, targeting ROS offers multiple entry points. While traditional antioxidants have shown limited clinical success due to lack of specificity, emerging strategies target ROS-generating enzymes, activate protective redox programs like NRF2, or reprogram macrophages through metabolic and epigenetic interventions. Advanced delivery platforms, such as nanoparticle-based carriers, further enhance the precision of these therapies. The convergence of redox modulation and immune reprogramming suggests that combination therapies hold the greatest promise for disrupting the vicious cycles of chronic liver injury and fibrogenesis.

It is important to note that most preclinical studies investigating ROS modulation and macrophage polarization in liver fibrosis here reviewed, have been conducted in murine models. While these models are instrumental in elucidating mechanistic pathways, it is important to note that they only partially reproduce the heterogeneity, chronicity, and immune-metabolic complexity of human liver disease and other diseases. Differences in macrophage subpopulations, redox regulation, and fibrogenic signalling between species may limit the direct translation of experimental findings to clinical settings.

Therefore, future research should prioritize the validation of redox-immunomodulatory mechanisms in humanized models, ex vivo organoid systems, and patient-derived samples to bridge this translational gap and improve the predictive value of preclinical studies.In addition to this, to efficiently develop these novel antifibrotic therapies, it is essential to recognize the functional diversity and plasticity of liver macrophages, moving beyond the traditional M1/M2 paradigm (Box 1). Distinct macrophage subsets, including TREM2^{+} lipid-associated macrophages, SPP1^{+} fibrogenic macrophages, and senescence-associated macrophages (SAMs), display context-dependent pro-fibrotic or reparative roles. This has led to the development of redox-targeted strategies, such as NOX inhibitors (e.g., Setanaxib), mitochondria-targeted antioxidants (e.g., MitoQ), and NRF2 activators, some of which are now progressing through clinical trials. In parallel, macrophage reprogramming therapies using PPARγ agonists or nanoparticle-based delivery systems are showing promise in experimental models.

However, despite their promise, these strategies also face important limitations. For instance, Setanaxib, in a randomized, placebo-controlled trial in patients with Primary Biliary Cholangitis, was found to be well tolerated and showed potential for anti-cholestatic and anti-fibrotic effects, but it failed to meet its primary endpoint [123]. MitoQ and other mitochondria-targeted antioxidants demonstrate strong preclinical effects, yet their translation has been hampered by variable bioavailability and potential off-target interactions [145]. Similarly, pharmacological activation of NRF2 holds therapeutic potential, but long-term activation may carry risks of impaired host defense and tumorigenesis [128].

Despite these encouraging preclinical evidences, several factors have limited the clinical success of ROS-targeting therapies. The spatiotemporal dynamics of ROS in the liver microenvironment complicate precise pharmacological modulation, since levels fluctuate between cell types, compartments, and disease stages, as discussed in this review. Broad antioxidant strategies can also interfere with physiological redox signalling required for homeostasis, reducing specificity. In addition, many redox-active compounds are chemically unstable or rapidly neutralized by endogenous antioxidants, leading to only transient effects in vivo. In addition, the architectural and immunometabolic heterogeneity of fibrotic tissue hampers local drug delivery and bioavailability. Overcoming these barriers will likely require next-generation approaches such as redox-responsive nanocarriers with sustained activity [112,146] or spatially targeted delivery systems that preserve physiological signalling while selectively counteracting pathological ROS activity.

Recognizing these limitations points to the need for novel approaches. Combined strategies that simultaneously modulate oxidative stress and immune cell behavior may yield synergistic antifibrotic effects. Moreover, integrating redox and immune profiling with omics technologies and spatial diagnostics will facilitate the development of personalized therapies tailored to disease stage and patient-specific immune–redox signatures. To advance this integrative view and fully unlock its therapeutic potential, several key challenges still need to be addressed:

1. Context-specific modulation of ROS: Developing therapies that fine-tune ROS activity without indiscriminately suppressing it remains a major challenge. ROS serve both physiological and pathological roles depending on their concentration, location, and timing. Achieving therapeutic benefit requires a nuanced understanding of redox thresholds, signalling contexts, and cell-specific responses throughout the progression of liver fibrosis.
2. Enhancing cell- and tissue-specific targeting: Most antioxidants lack selectivity for key hepatic cell types involved in fibrogenesis, such as hepatic stellate cells and macrophage subsets. Despite many research efforts, advanced targeted delivery systems, such as nanoparticles, ligand-directed carriers, or engineered vesicles, are still needed to achieve targeted modulation of redox pathways while minimizing off-target effects and immune clearance.
3. Improving translational relevance of models: Many preclinical models fail to recapitulate the human liver microenvironment,

Box 1
Redox-Immunology in Liver Fibrosis: Key Implications for Clinical Practice

Fibrosis stage drives prognosis.

The severity of liver fibrosis remains the strongest predictor of liver-related outcomes across etiologies, including NAFLD, viral hepatitis, and ALD.

ROS: From cytotoxic agents to immune modulators.

ROS not only mediate hepatocyte injury but also influence macrophage polarization and HSC activation, shaping both progression and resolution of fibrosis.

Beyond M1/M2: Macrophage plasticity matters.

Functional diversity among liver macrophages (e.g., TREM2^{+}, SPP1^{+}, and SAMs) reveals pro-fibrotic and pro-resolving subsets that can be targeted for therapeutic benefit.

Emerging therapeutic strategies.

- **Redox-targeted agents**: NOX inhibitors, mitochondrial antioxidants (e.g., MitoQ), and NRF2 activators are advancing toward clinical translation.
- **Macrophage reprogramming**: Agents like PPARγ agonists and nanoparticle-based polarizing systems show promise in promoting tissue repair.
- **Combined approaches**: Co-targeting ROS and immune cells may yield synergistic antifibrotic effects.

Personalized antifibrotic care.

Integrating immune-redox profiling, omics technologies, and spatial diagnostics could support tailored treatments, improving outcomes in patients with liver fibrosis.

particularly in terms of immune heterogeneity and redox dynamics. New platforms like human liver organoids, liver-on-a-chip, and humanized mouse models offer improved physiological relevance but require broader adoption, standardization, and validation.

4. Identifying reliable redox and immune biomarkers: The lack of robust, non-invasive biomarkers that reflect hepatic ROS levels and immune cell states hinders early diagnosis and treatment monitoring. Omics-driven approaches, including redox proteomics, metabolomics, and single-cell transcriptomics, may uncover dynamic signatures useful for patient stratification and precision therapies.
5. Translating redox-immune insights into therapies: The plasticity of macrophages complicates therapeutic design, as immune reprogramming must preserve homeostatic functions. In parallel, clinical trials need better-defined endpoints for fibrosis regression and redox modulation. Regulatory consensus and personalized approaches based on immune-redox profiling will be critical for translating emerging strategies into real-world impact.

In summary, recognizing the multifaceted roles of ROS in liver fibrosis, not merely as agents of damage, but as key regulators of immune cell plasticity and fibrogenic signalling, opens the door to a new generation of antifibrotic therapies. By integrating redox biology with immune modulation and harnessing advances in mechanistic insight, omics technologies, and targeted delivery, we can begin to design interventions that are not only more effective, but also tailored to the dynamic, patient-specific nature of chronic liver disease. This systems-level approach has the potential to redefine how we diagnose, monitor, and treat liver fibrosis in the era of precision medicine.

CRediT authorship contribution statement

Huilun Lu: Writing – original draft, Writing – review & editing. **Jie Huang:** Writing – original draft, Writing – review & editing. **Ya-chao Wang:** Validation, Writing – review & editing. **Eudald Casals:** Visualization, Writing – original draft. **Gregori Casals:** Supervision, Validation, Writing – review & editing. **Muling Zeng:** Supervision, Validation, Writing – original draft, Writing – review & editing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Data availability

No data was used for the research described in the article.

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