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Editorials

# Implications of liver fibrosis in clinical trials

## 1. Introduction

Liver fibrosis is characterized by a progressive scarring process that results from liver cell injury and chronic inflammation [1]. As a pivotal pathological step towards cirrhosis and liver failure, understanding fibrosis is critical to guiding clinical trials for liver disease. In this editorial, we explore the multifaceted implications of liver fibrosis on clinical trials, focusing on participant selection, safety protocols, endpoint selection, and regulatory challenges. Addressing these factors is crucial for advancing therapeutic strategies and improving patient outcomes in liver disease management.

## 2. Current strategies to reverse fibrosis

Liver fibrosis is the main predictor of liver-related mortality [2]. Also, patients with advanced fibrosis are at an increased risk of developing serious complications, such as variceal bleeding, ascites, and hepatic encephalopathy. Consequently, improvement in liver fibrosis is one of the main goals for any pharmacological treatment for liver disease. Currently, most strategies to reverse fibrosis focus on halting the underlying drivers of fibrogenesis and promoting tissue repair. The foundational approach involves controlling or curing the primary disease-such as eliminating viral infections, reducing metabolic injury, or abstaining from alcohol-to prevent ongoing tissue damage [1,3]. However, beyond addressing the triggering cause, therapeutic efforts are increasingly aimed at modulating molecular pathways involved in fibrosis. This includes targeting receptor-ligand interactions and intracellular signaling cascades that activate fibrogenic cells like hepatic stellate cells (HSCs). Additionally, direct inhibition of fibrogenesis through agents that interfere with collagen production, extracellular matrix deposition, or pro-fibrotic cytokines offers a promising avenue to not only halt but potentially reverse established fibrosis [1].

## 3. Challenges in clinical trials involving liver fibrosis

### 3.1. Patient selection and assessment of liver fibrosis

The heterogeneity of liver fibrosis, influenced by diverse etiologies such as viral hepatitis, alcohol use, and metabolic dysfunction-associated steatohepatitis (MASH), presents a primary challenge in clinical trial design. Accurate staging of liver fibrosis is crucial because the disease's progression directly impacts therapeutic efficacy. Liver biopsy is considered the gold standard for identifying and staging liver fibrosis, but it is an expensive and invasive procedure that carries potential risks, including abdominal pain, bleeding, and, in rare cases, death [4]. In addition, the interpretation and scoring of liver biopsy results are subject to significant inter- and intra-observer variability, and the procedure assesses only a small liver sample [5]. Given the known spatial heterogeneity of diffuse liver disease, limited sampling can lead to significant errors in determining diagnosis, the disease stage, and longitudinal evolution [6].

Advanced biochemical markers and imaging techniques have supplemented traditional liver biopsies, yet each diagnostic tool comes with limitations regarding sensitivity, specificity, and applicability across different patient populations [7]. Liver fibrosis results from an imbalance between extracellular matrix production (fibrogenesis) and degradation (fibrolysis). Consequently, the use of fibrogenic markers like PRO—C3 (a neoepitope of type III procollagen) reflects active collagen synthesis, while fibrolytic markers such as CTX-III (a fragment of cross-linked type III collagen) indicate collagen breakdown. High fibrogenesis marker levels often denote "active" fibrotic disease, making them attractive options to estimate and monitor liver fibrosis in clinical trials. For example, PRO—C3 and Enhanced Liver Fibrosis (ELF) have shown good performance in predicting liver fibrosis stage and progression over time [7]. Several imaging techniques, including vibration-controlled transient elastography (VCTE), 2D shear wave elastography (2D SWE), and magnetic resonance elastography (MRE), have also demonstrated adequate performance in staging liver fibrosis and predicting liver-related events [8]. Both serum and imaging biomarkers are safe, widely available, and facilitate the identification of targeted populations for inclusion in clinical trials (e.g., F2-F3 fibrosis or cirrhosis).

Choosing appropriate endpoints in liver fibrosis trials involves a delicate balance between clinical relevance and methodological feasibility. While the reversal or stabilization of fibrosis is the ultimate goal in clinical trials involving patients with F2-F3, the slow progression of the disease and the invasive nature of liver biopsies pose significant challenges. Conversely, the main goal for clinical trials involving patients with compensated cirrhosis will be decompensation-free survival or liver transplant-free survival. Although integrating non-invasive tests (NITs) and serum biomarkers is reshaping endpoint selection by providing safer, repeatable, and less invasive options, these methods require validation against long-term clinical outcomes to ensure they truly reflect changes in liver health and can predict patient prognosis.

### 3.2. Regulatory considerations

Regulatory agencies usually require a reduction in all-cause mortality, cirrhosis, hepatic decompensation events, and Model for End-

*Abbreviations:* 2D SWE, 2D shear wave elastography; AASLD, American Association for the Study of Liver Diseases; CTGF, Connective tissue growth factor; ELF, Enhanced Liver Fibrosis; EMA, European Medicines Agency; FDA, Food and Drug Administration; FFA, Free Fatty Acids; FGF-21, Fibroblast Growth Factor 21; FXR, Farnesoid X Receptor; HCV, Hepatitis C virus; HIV, Human immunodeficiency virus; HSCs, Hepatic stellate cells; MASH, Metabolic dysfunction-associated steatohepatitis; MELD, Model For End-Stage Liver Disease; MRE, Magnetic resonance elastography; NIT, Non-invasive test; OCA, Obeticholic Acid; PK, Pharmacokinetic; PPARs, Peroxisome Proliferator-Activated Receptors; TGF-$\beta$, Transforming growth factor-beta; THR-ß, Thyroid Hormone Receptor Beta; VCTE, Vibration-controlled transient elastography

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Stage Liver Disease (MELD) score for long-term approval in patients with chronic liver disease, including cirrhosis. However, these endpoints usually take several years to be achieved. Intermediate or surrogate endpoints could help obtain an accelerated or early approval of a pharmacological agent. However, the Food and Drug Administration (FDA) and European Medicines Agency (EMA) surrogate endpoints substantially vary. For example, in non-cirrhotic MASH, the FDA requires the resolution of MASH or improvement in fibrosis, while the EMA requires both coprimary endpoints for its approval [9]. Other surrogate measures closely linked to liver-related outcomes, such as hepatic venous pressure gradient (HVPG) and fibrosis scores, can also enrich study populations and maximize the ability to demonstrate clinical benefits.

The FDA supports the use of NITs in MASH clinical trials, particularly for early-phase studies and screening participants in Phase 3 trials to identify those at high risk for fibrosis prior to liver biopsy. However, while NITs such as serologic biomarkers (e.g., Fibrosis-4 [FIB-4], ELF score) and imaging modalities (e.g., VCTE, 2D-SWE, or MRE) are increasingly used, these may be influenced by changes in alcohol intake and/or body mass index. For example, active drinkers typically exhibit elevated aspartate aminotransferase levels, while some of them may have lower platelet counts due to bone marrow suppression rather than portal hypertension, diminishing the accuracy of markers such as FIB-4 [10,11]. They also tend to present with higher liver stiffness measurements, likely driven by inflammation, which can decline within one month of abstinence [12]. In VCTE, this inflammatory effect could be particularly pronounced in individuals with elevated transaminases or cholestatic injury. Similarly, VCTE correlates with fibrosis stages in autoimmune hepatitis, but may be less accurate within the first three months of treatment due to confounding by inflammation. Finally, while FIB-4 has shown excellent accuracy in predicting cirrhosis in patients with chronic hepatitis B and C, VCTE is effective in detecting advanced fibrosis in these patients [13]. Thus, an appropriate selection of NITs according to the underlying liver disease and setting is key to obtaining reliable results.

### 3.3. Adaptive designs

Adaptive trial designs are particularly suited to the challenges of liver fibrosis due to the disease's variable progression and the need for long-term data. These designs allow for modifications based on interim data, including changes to the treatment dosages or inclusion criteria. For example, group-sequential designs enable early stopping for futility or efficacy, which is critical in fibrosis trials where progression may take years, thus avoiding unnecessary follow-up and exposure to ineffective therapies. Sample size re-estimation is useful when there is uncertainty in effect size or variance due to fibrosis stage heterogeneity. When recruitment is challenging and patient populations are diverse, adaptive randomization can shift allocation toward better-performing arms, increasing the probability that participants receive the most promising therapy. Seamless phase II/III designs, which combine safety and efficacy assessments, can accelerate the development timeline, avoiding delays between phases and reducing logistical and regulatory burdens. This is highly relevant for antifibrotic therapies, where early signal detection and refinement of target populations are crucial. Ultimately, adaptive designs may enhance efficiency, reduce patient burden, and support earlier access to effective treatments in a field where trial timelines are historically long and costly.

## 4. Therapeutic targets, mechanisms, and drug metabolism

Emerging treatments targeting molecular pathways involved in the activation of HSCs, the principal fibrogenic cells in the liver, are being tested in clinical trials (Fig. 1). Therapies that inhibit the action of transforming growth factor-beta (TGF-$\beta$) or downregulate connective tissue growth factor (CTGF) have shown promise [14,15]. Additionally, antifibrotic drugs that disrupt the extracellular matrix or modify the immune response to hepatic injury are under investigation [16]. Each potential therapy requires clinical trials designed around these specific mechanisms, necessitating precise and innovative methodologies to assess their efficacy and safety (Fig. 1).

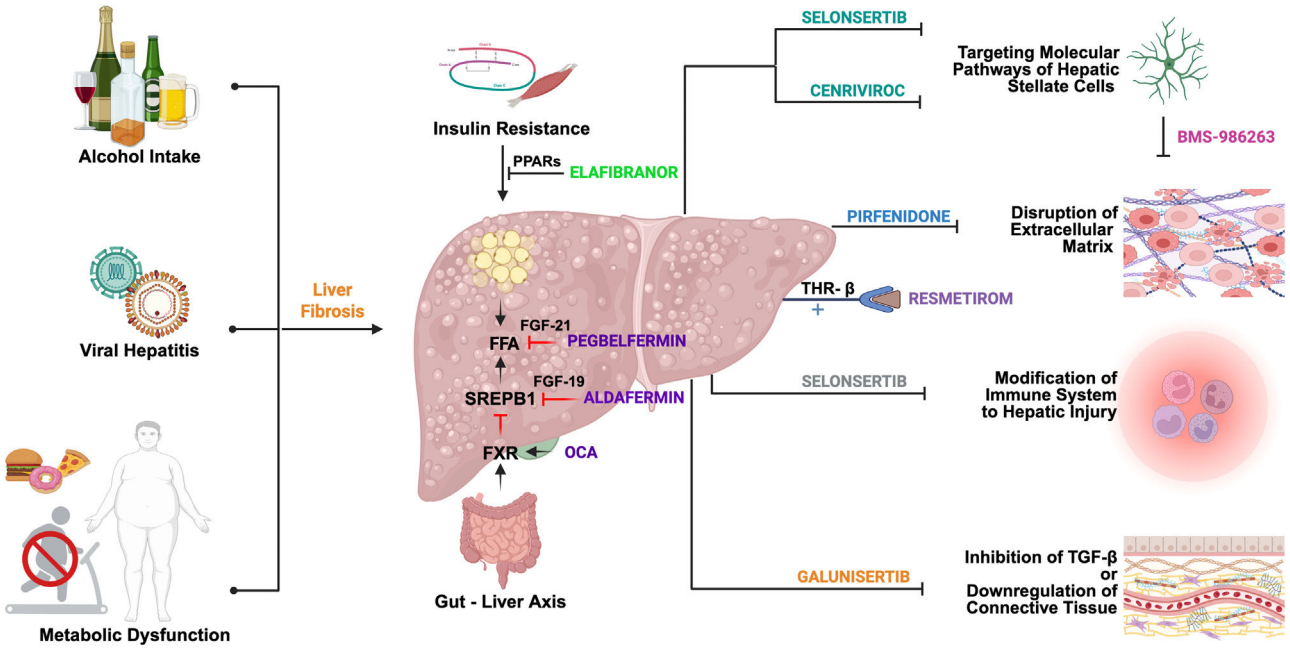


**Fig. 1.** Therapeutic targets for liver fibrosis with their corresponding mechanisms. PPARs, Peroxisome Proliferator-Activated Receptors; FFA, Free Fatty Acids; FGF-21, Fibroblast Growth Factor 21; FXR, Farnesoid X Receptor; OCA, Obeticholic Acid; THR-$\beta$, Thyroid Hormone Receptor Beta.

Liver fibrosis significantly alters the pharmacokinetics and pharmacodynamics of many medications. As fibrosis progresses, architectural distortion of the hepatic parenchyma and sinusoidal capillarization lead to decreased hepatic blood flow, altered expression and function of drug-metabolizing enzymes (particularly cytochrome P450 isoforms), and reduced hepatic clearance. Depending on the drug's metabolic pathway, these changes can result in drug accumulation, prolonged half-lives, or reduced therapeutic efficacy. Given this, does individualization become a central consideration in clinical trials and routine clinical care for patients with liver fibrosis. Failing to adjust doses can lead to suboptimal treatment responses, increased adverse events, or toxicity, particularly in trials involving antifibrotic agents, immunomodulators, or metabolic regulators.

Population pharmacokinetic (PK) modeling and physiologically based pharmacokinetic (PBPK) simulations are increasingly used to predict drug behavior in fibrotic livers. These models incorporate patient-specific parameters like age, sex, liver volume, enzyme activity, and blood flow changes to simulate how drugs distribute and are metabolized in the context of hepatic disease. Such modeling not only supports safer dose recommendations but also enhances trial design efficiency.

For example, The MAESTRO—NASH and REGENERATE trials, among others, have included fibrosis-stratified cohorts or explored different dosing regimens for agents like resmetirom and obeticholic acid to ensure both safety and efficacy, respectively [17,18]. These trials often demonstrate differential responses based on fibrosis stage, with more pronounced benefits, or toxicities, emerging in patients with less hepatic reserve. Thus, higher doses of a drug may be well tolerated in patients with F1 or F2 fibrosis but cause cholestasis or hepatic decompensation in F3-F4 stages due to impaired bile acid handling or portal hypertension-related shunting.

## 5. Precision medicine and genomic markers

Emerging approaches in precision medicine offer the potential to further tailor drug dosing beyond clinical markers. For example, polymorphisms in genes encoding CYP enzymes (e.g., CYP3A4, CYP2C9) or drug transporters (e.g., SLCO1B1) may influence drug metabolism independently of fibrosis stage. Integrating pharmacogenomic screening into trials could help identify individuals at risk for altered drug exposure, allowing for personalized dosing strategies that enhance safety and optimize efficacy.

## 6. Quality of life and patient-reported outcomes

Fibrosis significantly impacts patients' quality of life (QoL), even before the onset of cirrhosis. As fibrosis progresses, patients may experience fatigue, abdominal discomfort, cognitive symptoms, pruritus, and reduced physical endurance - symptoms that impair emotional well-being, daily functioning, and social engagement. Incorporating patient-reported outcomes (PROs) into clinical trials offers critical insight into how antifibrotic therapies influence these symptoms, beyond what histology or liver function tests can capture. In some cases, treatments may halt fibrosis progression without reversing it, yet still improve patient symptoms and functionality. Capturing these nuances through systematic, longitudinal PRO assessments, ideally synchronized with imaging and clinical evaluations, allows researchers to assess the real-world impact of therapies. This patient-centered approach underscores the importance of not only targeting fibrosis biologically, but also improving how patients feel and function.

## 7. Conclusions

Clinical trials in liver fibrosis present both significant challenges and valuable opportunities to advance the treatment landscape for chronic liver disease. From the accurate assessment of fibrosis stage to tailoring drug dosing and integrating non-invasive endpoints, each aspect requires careful planning to optimize safety, efficacy, and relevance. Adaptive trial designs offer promising solutions to reduce time and cost, while patient-reported outcomes ensure that therapies improve not just histology but daily living. Advances in pharmacokinetics, precision medicine, and biomarker development further enable individualized approaches. Ultimately, thoughtful integration of these strategies can accelerate the development of effective antifibrotic therapies and improve outcomes for patients with chronic liver disease.

## Declaration of competing interests

None.

## Author contributions

**Vinay Jahagirdar:** Investigation, Writing – original draft, Writing – review & editing. **Kaanthi Rama:** Investigation, Writing – original draft, Writing – review & editing. **Daniel Cabrera:** Writing – review & editing. **Francisco Idalsoaga:** Writing – review & editing. **Luis Antonio Díaz:** Conceptualization, Writing – review & editing. **Marco Arrese:** Investigation, Writing – review & editing. **Juan Pablo Arab:** Investigation, Writing – review & editing.

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## Data availability statement

The datasets generated and analyzed during the current study are not publicly available but are available from the corresponding author upon reasonable request.

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