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Antifibrotic therapy in nonalcoholic steatohepatitis: time for a human-centric approach

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Abstract

Nonalcoholic steatohepatitis (NASH) might soon become the leading cause of end-stage liver disease and indication for liver transplantation worldwide. Fibrosis severity is the only histological predictor of liver-related morbidity and mortality in NASH identified to date. Moreover, fibrosis regression is associated with improved clinical outcomes. However, despite numerous clinical trials of plausible drug candidates, an approved antifibrotic therapy remains elusive. Increased understanding of NASH susceptibility and pathogenesis, emerging human multiomics profiling, integration of electronic health record data and modern pharmacology techniques hold enormous promise in delivering a paradigm shift in antifibrotic drug development in NASH. There is a strong rationale for drug combinations to boost efficacy, and precision medicine strategies targeting key genetic modifiers of NASH are emerging. In this Perspective, we discuss why antifibrotic effects observed in NASH pharmacotherapy trials have been underwhelming and outline potential approaches to improve the likelihood of future clinical success.

Sections

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Introduction

Nonalcoholic fatty liver disease (NAFLD) is characterized by fat accumulation within the liver (hepatic steatosis) without secondary causes, including substantial alcohol intake, medications or inherited metabolic conditions[1]. In some individuals, steatosis is associated with cellular injury (ballooned hepatocytes) and lobular inflammation — termed nonalcoholic steatohepatitis (NASH). NASH can lead to progressive fibrosis and sometimes cirrhosis with a consequent risk of hepatic decompensation and hepatocellular carcinoma (HCC) (global incidence 0.5–2.6% per year)[2]. HCC can also occur in the presence of non-cirrhotic NAFLD, but this is rare (0.1–1.3 per 1,000 patient-years)[2]. NAFLD most commonly occurs in the context of the metabolic syndrome that is characterized by the presence of two or more of the following conditions: insulin resistance and type 2 diabetes mellitus (T2DM), obesity, hypertension and hypercholesterolaemia (although a 'lean' NAFLD variant phenotype is also recognized)[3]. Notably, there is currently a vigorous debate and an international Delphi consensus process about renaming NAFLD, using terminology perceived as less stigmatizing whilst ensuring positive diagnosis and patient engagement[4,5]. The global burden of NAFLD (regardless of the definition) is increasing at an alarming rate[5]. A systematic review and meta-analysis published in 2022 suggested an overall worldwide prevalence of 30%[6] to 32.4%[7], although there is marked geographical variation. Additionally, strong evidence is emerging linking NAFLD with social deprivation and food insecurity[8]. Between 1990 and 2017, the number of patients with decompensated NAFLD cirrhosis doubled (although the relabelling of previous 'cryptogenic' cirrhosis cases as NAFLD might have contributed), and it is the most rapidly increasing indication for liver transplantation in the USA[9,10]. These observations pinpoint the urgent need to develop effective therapeutic interventions to stem the rising tide of NAFLD-associated morbidity and mortality.

Although the past four decades have witnessed an explosion in the biological understanding of NAFLD and the mechanisms driving progression to cirrhosis, this has not translated to an approved therapy that can directly modulate fibrosis and improve clinical outcomes in patients (Box 1). By contrast, two antifibrotic drugs are approved for treating idiopathic pulmonary fibrosis (nintedanib and pirfenidone) that can reduce the decline in lung function[11]. Here, we reflect on the current therapeutic landscape in NAFLD, offer our perspective on the challenges that stand in the way of developing an effective antifibrotic and propose possible solutions that take advantage of new and emerging technologies.

Why is fibrosis important in NASH?

The stages of NASH-related fibrosis range from absent (stage F0) to cirrhosis (stage F4). In general, fibrosis progression to cirrhosis and adverse liver-related outcomes in NASH is slow and unpredictable, with the actual fibrosis progression rate (FPR) uncertain. Nevertheless, the FPR seems to be substantially higher in NASH than in isolated steatosis, corresponding to fibrosis progression by one stage over 7 years and 14 years, respectively[12]. An FPR of only 0.03 stages was recently calculated from 1,419 participants treated with placebo undergoing per protocol repeat biopsy in 35 randomized controlled trials in NASH[13]. However, the generalizability of using clinical trial datasets for natural history insights is limited by selection bias and rigid entry criteria. Analysis of paired biopsy series suggests that NASH is a dynamic, bidirectional disease with almost as many patients regressing by up to two stages of fibrosis as seen in biopsy samples taken a year apart as patients progressing[14], although the inherent sampling error of liver biopsies might explain this.

Nevertheless, the importance of fibrosis (but no other histological features) in predicting outcomes in NAFLD has been highlighted by several studies[15–17], with advancing fibrosis stage heightening the risk of future liver-related morbidity (for example, decompensation events and HCC) and liver-related and all-cause mortality. It is, therefore, alarming that in a prospective study published in 2022, conducted in Southern California, 14% of 501 patients with T2DM aged ≥50 years had advanced fibrosis and 6% had cirrhosis[18]. Furthermore, in a large meta-analysis including 12 cohort studies involving 25,252 patients with established cardiovascular disease, higher levels of non-invasive fibrosis biomarker tests were related to an increased risk of cardiovascular events, cardiovascular mortality and all-cause mortality[19]. The association of cardiovascular diseases with liver fibrosis is also observed in the general population, although direct mechanistic pathways are not defined[20,21]. Interestingly, a decision-analytic model simulating the natural history of NAFLD showed that among patients aged 65 years, an estimated 10-year non-liver-related mortality was higher than liver-related mortality in all fibrosis stages[22]. This raises the intriguing possibility that targeting liver fibrosis might lead to improvements in mortality independent of a reduction in liver-related

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Box 1

How have we arrived where we are in fibrosis therapeutics?

Arguably the beginning of contemporary fibrosis biology was the successful isolation of cells that were then described as 'hepatic lipocytes' — and later as hepatic stellate cells (HSCs) — reported by Friedman and colleagues in 1985 (ref. 136). Activation of HSCs into a myofibroblast-like cell is now the accepted pivotal process leading to excessive production of fibrotic extracellular matrix[137,138]. The central role of HSCs in fibrosis was confirmed using in vivo mouse models of liver injury, leading to work that showed how apoptosis of activated HSCs causes fibrosis regression[139–141]. At the time (1998), the dogma was that fibrosis was irreversible, which was a barrier to fibrosis drug discovery. A new paradigm of fibrosis as a dynamic process, with the potential to both progress and regress, has stimulated drug discovery and development. However, despite the subsequent 37 years of research progress since Friedman et al. described how to purify and culture HSCs, we are still waiting for an approved medicine to treat liver fibrosis. The rising global prevalence of nonalcoholic steatohepatitis (NASH) has provided further stimulus and investment for accelerating discovery of drugs to treat fibrosis and advancing antifibrotic candidates to clinical trials[5]. Unfortunately, most pharmacological studies in NASH-related fibrosis, particularly cirrhosis, are not fulfilling their preclinical promise, leading to numerous drug programmes being terminated (the so-called NASH graveyard extensively discussed in current reviews[79,80,85]). As we approach the 40th anniversary of the start of intensive liver fibrosis research, it is time to ask whether we require a refocus to ensure the field remains galvanized with the aim of delivering effective antifibrotics.

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events, which will require well-conducted, long-term (>5 years at least) clinical trials.

Contrastingly, data from two large (negative) drug trials including 1,135 patients with compensated NAFLD cirrhosis indicated that a reduction in fibrosis is associated with improved clinical outcomes. Specifically, after a median follow-up of 16.6 months, patients whose fibrosis regressed had a sixfold reduction in risk of liver-related events[23]. Moreover, the strong concordance between histological evidence of cirrhosis regression with decreases in non-invasive measures of fibrosis burden in this study, such as the Enhanced Liver Fibrosis (ELF) score and liver stiffness by transient elastography, underscores the potential of non-invasive tests for longitudinal disease monitoring.

Given that fibrosis progression and regression determine prognosis in NASH, there is an urgent unmet medical need (and multibillion dollar market) for effective antifibrotic therapies.

Weight loss is the cornerstone of NAFLD therapy but hard to achieve and sustain

Weight reduction achieved through lifestyle intervention leads to histological improvements in NASH. Fibrosis regression occurs in patients who manage to lose ≥10% of their body weight[24,25], although most find this difficult to achieve and sustain. Numerous diets have been promoted for NAFLD with systematic reviews and randomized controlled trials favouring a Mediterranean diet[26], which has been proven to benefit liver, metabolic and cardiovascular health[27]. Physical activity has multisystem health benefit[28] even without weight loss, but recommendations regarding the type, intensity and frequency are less clear. Moreover, when counselling patients, it might be best to discuss simple strategies to increase 'movement' rather than 'exercise', given that age and comorbidities (obesity, cardiovascular disease, osteoarthritis) might be limiting, and investment in equipment or gym memberships might be unrealistic or off-putting. Even moderate physical activity could reprogramme key pathophysiological mechanisms[29]. Indeed, observational data have shown that insufficient physical activity is an independent predictor of fibrosis in NAFLD[30,31]. However, liver inflammation and fibrosis end points have largely been overlooked in lifestyle intervention studies. Notably, in a single-arm 16-week intervention study, diet and moderate-intensity exercise reduced body weight and decreased hepatic venous pressure gradient (HVPG) in 50 patients with overweight, cirrhosis and portal hypertension[32]. Notwithstanding, the limited success of volitional lifestyle measures in real-world practice has provided a strong rationale for developing disease-modifying therapies.

Currently, bariatric surgery is only an option for a minority of selected patients with severe obesity. However, it can lead to substantial weight loss (between 14% and 25%), durable improvements in histological NASH and fibrosis[33], and reduced risk of major adverse liver and cardiovascular outcomes[34] and many cancers[35]. However, delivering such a complex intervention requires substantial resources and is therefore inaccessible to most of the global NAFLD population. Additionally, emerging data suggest that bariatric surgery is associated with an increased prevalence of alcohol use disorder and alcohol-related liver disease; potential candidates should be rigorously assessed before undergoing such surgery[36].

A less-invasive approach is insertion of an endoscopic intragastric balloon (IGB) (using an adjustable fluid-filled balloon), which reduces stomach capacity, and delays gastric emptying, thereby inducing weight loss[37]. An open-label study of IGB placement in 21 patients with fibrotic NASH, in combination with a prescribed diet and exercise programme, induced significant weight loss (mean difference −14.4 ± 7.9 kg; $P = 0.01$) and metabolic improvements (for example, mean difference in haemoglobin A_{1c} −1.2 ± 0.5; $P = 0.02$). Although histological NASH improved in 80% of participants (median baseline NAFLD activity score (NAS) of 4 versus median follow-up NAS of 1; $P < 0.001$) 6 months after IGB placement, effects on hepatic fibrosis were variable[38]. This study was limited by its small size, lack of a control group and the short duration of follow-up; further studies are clearly needed.

What are the limitations of our current approach to drug development?
Are our preclinical models fit for purpose?
Traditional liver fibrosis drug development starts with basic in vitro assays using isolated hepatic stellate cells (HSCs) (or cell lines) to study phenotypic effects and then employs animal (usually mouse) models to determine efficacy and toxicity. However, this framework has inherent limitations in replicating the complexity of human pathophysiology in vivo.

In vitro and ex vivo models. Simple cell culture models are limited by their non-physiological conditions in lacking both cell–cell and cell–substrate interactions, which influence innate cellular responses in healthy and diseased milieux[39]. Additionally, primary cells adapt in vitro to favour their optimal phenotypic state for survival in culture conditions, drifting from their normal physiological behaviours in native tissue. Hence mono-cell, bi-cell or even tri-cell culture systems are limited in predicting drug-induced responses in complex, anatomically organized multicellular tissues.

Self-assembling stem cell-derived or organoid-derived tissues and bioprinted tissues simulate some of the complexity of a liver tissue microenvironment; however, these platforms are still in their infancy[40–42]. Advances in designing organoid-engineered human multicellular three-dimensional NASH models have shown promise, recapitulating features of steatosis, inflammation and fibrosis, including a biophysical readout of organoid stiffening that reflects the fibrosis severity[43]. Moreover, co-culturing pluripotent stem cell (PSC)-derived lineages might provide greater reproducibility in constructing liver-like microstructures. However, these models lack a physiologically relevant vasculature and the immune components of NASH.

Liver-on-a-chip (LoC) systems overcome some of the limitations of organoids. They can be engineered from parenchymal and non-parenchymal cells to recapitulate anatomical features of the liver, such as hepatic zonation and lobe-like structures[44]. Exposing LoCs containing multiple cell types to lipids has been reported to induce steatosis, hepatocyte ballooning, tumour necrosis factor (TNF) and α-smooth muscle actin expression, indicative of a NASH phenotype[45]. Moreover, the therapeutic effects of several NASH drug candidates have been demonstrated using these models[46].

A caveat in the use of LoCs is the need to dissociate and purify individual cell types from human liver tissues before their reassembly into synthetic liver structures[47]. This processing inevitably introduces epigenetic alterations and activation of stress pathways that alter the biology of the different cellular constituents. There are also challenges with batch-to-batch variability of LoCs, which might be overcome by using induced PSC lines as a reproducible, standardized source of different cell lineages. Moreover, despite impressive advances, LoCs do not fully recapitulate the physiological, anatomical and cellular complexity of the liver tissue. Alternatively, human ex vivo precision-cut

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liver slice (PCLSs) systems successfully model fibrogenesis and have demonstrated antifibrotic therapy efficacy[48]. PCLS retain architectural and zonal spatial contexts of parenchymal and non-parenchymal liver cells, resident Kupffer cells and lymphocytes, and they might represent a valuable tool for studying human innate immunity[49].

Animal models. Mouse or rat models offer the advantage that the biological actions, pharmacology, efficacy and toxicity of drugs can be determined in a living animal. However, there is a lack of agreement on which NAFLD model (if any)[50] provides the closest approximation of human disease. Genetic models might complicate drug discovery as they will not recapitulate mechanisms in patients. A systematic review catalogued a total of 3,920 NAFLD models (including dietary, chemical, genetic and combination models) across 4,540 published studies[51], and found inconsistencies in terminology and study design. In addition, the study demonstrated substantial heterogeneity in replicating human NAFLD phenotypic characteristics, which, when further confounded by interlaboratory variability[52], severely compromises the reproducibility of in vivo experiments. NAFLD models incorporating a baseline (pretreatment) liver biopsy resemble clinical trial design, as they control for phenotypic variation through stratification of fibrosis severity and balance treatment allocation whilst enabling assessment of drug response in individual animals[53].

To overcome this uncertainty, experts should agree on a standardized model that the industry can adopt, with continued iterative improvements advised by the academic community. An ideal model (besides providing metabolic, immune, fibrogenic, carcinogenic and ideally angiogenic characteristics of human NASH) would pose the relevant clinical challenges of late-stage disease (for example, addressing the association between hepatic fibrosis and increased cardiac-related mortality). Inherent differences between mouse and human immune systems, including variations in innate and adaptive immune compartments, mean that mice will not accurately mimic the immunological components of human NASH. In addition, the sanitized environment and divergent microbiota of laboratory mouse models limit the translational value and reproducibility of the models. This was circumvented by implanting laboratory-strain embryos into wild (non-laboratory) mice to restore natural microbiota and pathogens[54]. The resulting 'wilding' mice replicated human disease more faithfully than conventional models. Moreover, they accurately predicted two (non-NASH) clinical trial failures (an anti-CD28 monoclonal antibody for T cell expansion and an anti-TNF treatment during septic shock), in which conventional mouse studies had predicted success. A further factor is ageing, which increases the risk of fibrosis; most mouse studies use very young animals in which tissue repair and immunity are more robust, and a lifetime of exposure to environmental stressors is not modelled. Humanized mice offer a potential solution and can include human hepatic cells[55] and partial reconstitution of a human immune system; however, they are technically challenging and expensive.

Additional limitations of mouse models relate to the lack of consideration of biological sex and genetic and ethnic variations in the human population that influence disease progression. When combined, these limitations considerably affect our faith in animal models to predict drug mechanisms and efficacy, and argue strongly for using human-based models early in the drug development process.

Are we targeting the right mechanisms for fibrosis regression at the right time?

In addition to the technical challenges discussed above, does the current reductionist approach, positioning HSCs as the central instigators of (NASH) fibrosis, remain valid? We still lack concrete evidence that HSCs are the pivotal fibrogenic cells in human NASH; indeed, the cross-tissue heterogeneity of fibroblasts in perturbed states, including fibrosis and cancer, is increasingly recognized[56]. Moreover, given that fibrosis is a highly conserved wound-healing response, manipulation of HSC activation could lead to unanticipated effects such as impaired hepatic regeneration.

Discoveries concerning the role of cell–cell crosstalk between epithelial, myeloid and mesenchymal cells in fibrosis underscore the limitations of focusing on individual cell types, and suggest that key intercellular networks that trigger or promote disease progression might be tractable treatment targets to inhibit in NASH-related fibrosis[57]. The functional heterogeneity and dynamic plasticity of cell lineages in the liver, including HSCs[58], highlight yet further levels of complexity that are not yet accounted for in our preclinical drug discovery platforms.

Table 1 outlines the current prospective single-agent pharmacotherapy approaches under evaluation. However, one fundamental question remains: are we targeting the correct mechanisms underpinning fibrogenic NASH?

Hepatocellular senescence seems to be a key stimulator of steatosis and fibrosis; however, senolytic drugs have not yet advanced to clinical development. An early-phase trial (NCT05506488) evaluating the combination of the tyrosine kinase inhibitor dasatinib and the antioxidant quercetin, which decreases senescent cells in diabetic kidney disease[59], will provide important proof-of-principle for their use in fibrotic NASH[60–62]. Future work will clarify the importance of other fibrogenic mechanisms in NASH[63], including extrahepatic drivers (for example, the microbiome and gut–liver axis) and direct hepatic triggers (for example, pro-inflammatory modes of hepatocyte cell death such as ferroptosis[64]) to establish the safest and most potent approaches.

Another key question is when in the disease process is it best to intervene with antifibrotics (and when is it simply too late)? Certain architectural changes, that are likely to be irreversible (for example, vascularized septae)[65,66] plus the reduced regenerative capacity in advanced cirrhosis, argue for pre-cirrhosis as the optimal initial stage at which antifibrotics should be clinically utilized. Otherwise, as evidenced by the multiple trial failures in NAFLD-related cirrhosis, the likelihood of success is low[67]. As a histological surrogate end point is not established for drug trials in NAFLD-related cirrhosis, a long-term composite clinical outcome end point (including all-cause mortality) is required[68]. Although decreased portal hypertension (using HVPG) is associated with improved clinical outcomes in patients with both compensated and decompensated cirrhosis[69,70], variability is a potential issue, although possibly over-stated[69]. However, it is not yet accepted by regulatory agencies despite being evaluated as a primary end point in several large studies in NAFLD-related cirrhosis[71–73].

How can we improve the fibrosis drug discovery process?

To mitigate against failure, we must look critically at: (1) how we are selecting drug targets and/or combinations; (2) whether we are utilizing preclinical models (and readouts used to determine drug efficacy) with sufficient proximity to patients; and (3) optimizing the discovery biology that can be extracted from human data, including existing clinical trial materials and electronic health record data (Fig. 1). The precision and depth of quantitative biological information generated from single-cell omics technologies, which includes spatial analyses in intact tissues for transcripts, proteins, post-translational modifications and

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Table 1 | Investigational monotherapy compounds in NASH-related fibrosis or cirrhosis clinical trials

Phase	Molecular target and/or mechanism	Compound	Trial	Fibrosis stage	Participants (n)	Refs.
II	Long-acting FGF subclass analogues	Efruxifermin (FGF21)	HARMONY	F2–F3	24–96	116–119
		Pegozafermin (FGF21)	SYMMETRY	F4 (comp)	36	
		Aldafermin (FGF19)	ENLIVEN	F2–F3	24	
			ALPINE4	F4 (comp)	48	
	Non-PPARγ-active stereoisomer of pioglitazone	Deuterium-stabilized *R*-pioglitazone (PXL065)	NCT04321343[a]	F1–F3	36	120
	FASN inhibitor	Denifanstat	FASCINATE-2	F2–F3	52	121
	Cyclophilin inhibitor	Rencofilstat	AMBITION	F2–F3	4	122
III	PPARα/δ/γ agonist	Lanifibranor	NATiV3	F2–F3	72	123
	FXR agonist	Obeticholic acid	REGENERATE	F2–F3	~500	124,125
			REVERSE	F4 (comp)	72	
	THRβ agonist	Resmetirom	MAESTRO-NASH	F1–F3	52	126,127
			MAESTRO-NASH OUTCOMES	F4 (CTP-A)	~156	
	GLP1 agonist	Semaglutide	ESSENCE	F2–F3	260	128
	SCD partial inhibitor/PPARγ induction	Aramchol	ARMOR	F1–F3	72–120	129
	Galectin 3 inhibitor	Belapectin	NAVIGATE	F4	357	130

comp, compensated cirrhosis; CTP-A, Child–Turcotte–Pugh class A; FASN, fatty acid synthase; FGF, fibroblast growth factor; FXR, farnesoid X receptor; GLP1, glucagon-like peptide 1; NASH, nonalcoholic steatohepatitis; PPAR, peroxisome proliferator-activated receptor; SCD, stearoyl CoA desaturase 1; THRβ, thyroid hormone receptor-β. [a]Trial without a specified trial name.
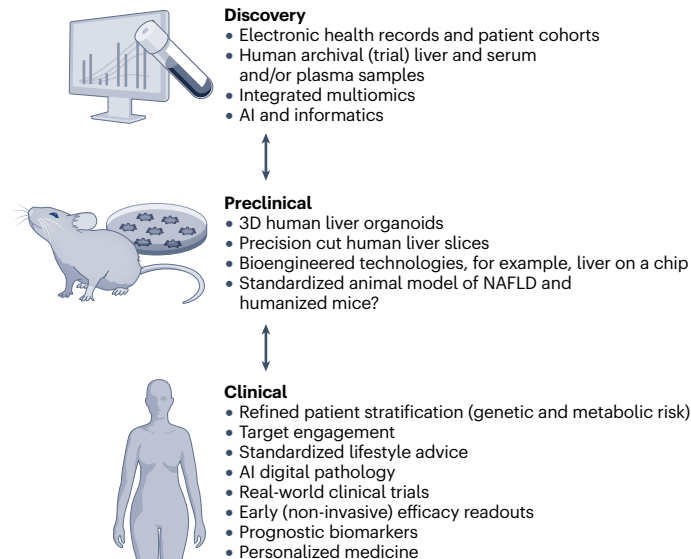
metabolic factors, could pinpoint the key fibrogenic cell interactions (and best potential therapeutic targets) in NASH[74]. The evolution of artificial intelligence and high-performance computing can now combine carefully archived biopsy and serum or plasma samples with clinical data and focus the drug discovery process on the patient rather than on laboratory-based experiments. Indeed, integrated multimodal multiscale human resources such as the national-level SteatoSITE NAFLD Data Commons could transform drug target discovery and validation. Moreover, the enormous repertoire of human tissue within clinical trial biobanks offers previously unimaginable opportunities, such as the interrogation of those samples using state-of-the-art single-cell omics and artificial intelligence. This process will enable investigators to understand how drugs that have failed in trials might have influenced liver cell biology (for example, HSC activation and hepatocyte function) far beyond the typical end points mandated by regulators and trialists. Such approaches can re-evaluate our pharmacological theory, identify more or less susceptible patient subgroups and uncover new avenues towards a more stratified approach to drug development. The application of advanced techniques to serial biopsy samples (selected to represent the transitions of NAFLD from steatosis to F1–F4 fibrosis stage) might generate surprises regarding the cellular triggers and mediators of fibrosis and the signalling events that influence disease progression and regression. From these serial biopsy studies, novel molecular targets can emerge for which we can have a high degree of confidence in designing drugs with precision for suppressing fibrosis at a specific disease stage.

Preclinical drug testing should then ideally employ human tissue models that, as accurately as possible, reflect the pathobiology of fibrosis. Innovations in modelling whole-body physiology (using advanced tissue chip systems to recapitulate interdependent organ systems linked by vascular flow) offer a potential glimpse of the future[75]. However, in the opinion of the authors, human three-dimensional PCLS models are currently the closest replicants of a human liver, with the caveat that they lack a peripheral blood supply and whole-body context[48]. An essential advantage of PCLS is that variables influencing drug efficacy and toxicity, such as ageing, biological sex, lifestyle, ethnicity and genetics, can be accounted for at the preclinical stage of drug development. Notwithstanding, establishing a PCLS platform is challenging, requiring a laboratory with proximity to hospital operating theatres to avoid hypoxic damage during the collection, transport and processing of resected tissues. For scalability, the PCLS platform requires miniaturization to at least 96-well format, and the design of fibrosis assays that lend themselves to automation[76].

How can we improve clinical trials and enhance their outputs?

A positive outcome on a prespecified end point (either NASH resolution or fibrosis improvement) in a well-designed placebo-controlled phase II trial is likely to increase the probability of a successful phase III study. This was illustrated in phase II studies of obeticholic acid[77] and resmetirom[78] that were predictive of statistically significant effects on NASH resolution and fibrosis at phase III (REGENERATE trial (n = 1,968, NCT02548351), P = 0.0002 for improvement in fibrosis of one or more stages with 25 mg obeticholic acid versus placebo; and MAESTRO-NASH trial (n = 966, NCT03900429), P < 0.0001 for improvement of one or more stages in fibrosis with 100 mg resmetirom versus placebo)[77,78]. Nevertheless, multiple clinical factors have been identified as potential obstacles to successful development of drugs for the treatment of NASH[79,80]. Key issues include a high placebo response rate (estimated at 22%)[81], in part attributable to biopsy sampling variability and inexact assessment of fibrosis using standard histopathological metrics; regression to the mean (a statistical phenomenon that can affect data interpretation when the outcome measure has high variability); the Hawthorne effect (the tendency for study participants to change their behaviour

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Discovery
- Electronic health records and patient cohorts
- Human archival (trial) liver and serum and/or plasma samples
- Integrated multiomics
- AI and informatics

Preclinical
- 3D human liver organoids
- Precision cut human liver slices
- Bioengineered technologies, for example, liver on a chip
- Standardized animal model of NAFLD and humanized mice?

Clinical
- Refined patient stratification (genetic and metabolic risk)
- Target engagement
- Standardized lifestyle advice
- AI digital pathology
- Real-world clinical trials
- Early (non-invasive) efficacy readouts
- Prognostic biomarkers
- Personalized medicine

Fig. 1 | Strategies to increase the chances of successful drug development for NASH-related fibrosis. At all stages of the drug development process (discovery, preclinical testing and clinical evaluation), a plethora of patient-centric approaches are now available to pinpoint the most effective drug candidates for nonalcoholic steatohepatitis (NASH)-related fibrosis. Based on a priori biological knowledge, hypothesis-driven approaches are now further enhanced by multiomics analysis, artificial intelligence (AI) and machine learning methods to improve drug target discovery. Innovative clinical trial designs using hierarchical stratifications (for example, recruit-by-genotype, augmented digital pathology read-outs, and real-world outcomes and end points) offer great potential to enhance success. Together, these refined approaches are likely to yield a paradigm shift in the development and delivery of effective diagnostic tests and treatments to improve liver-related and extra-hepatic outcomes in patients with NASH. NAFLD, nonalcoholic fatty liver disease.

simply as a result of being observed); and the lack of a standardized approach to diet and exercise across trials[82]. Additionally, uncertainty around the optimal duration of drug exposure required for fibrosis improvement (especially if the mechanism of action is indirect) and the existence of multiple (undefined) disease subtypes that might vary in responsiveness to a specific drug further complicate the evaluation of new candidates. There also remains an unknown hierarchy of targets, such that certain mechanisms of action might be too far 'downstream'. Finally, undisclosed alcohol use, which has been highlighted in patients suspected of having NAFLD, is a potential confounder but could be routinely tested for using highly sensitive and specific direct markers of alcohol intake, such as phosphatidyl ethanol in the blood or ethyl glucuronide in the hair and urine[83,84].

Refinement of clinical trial design

Given the track record of drug failures in NASH (owing to lengthy development time, difficulty in recruiting participants, complex disease biology with multiple potential therapeutic targets and a bulging pipeline of early drug candidates worthy of evaluation) there is a strong argument for pursuing new approaches to streamline clinical trials. One approach involves the ongoing quest by large research consortia such as LITMUS and NIMBLE for suitable non-invasive fibrosis biomarkers that could supplant the need for liver biopsies in NASH trials. Biopsies

are a barrier to participant recruitment, largely account for the high and costly screening failure rate of trials (~60–70%) and represent an imperfect surrogate end point[85].

Another aspect involves optimization of the selection of participants, for example enriching studies for patients harbouring known genetic polymorphisms that are associated with an accelerated disease course and adverse outcomes. Adopting new trial designs (for example, platform trials) to efficiently evaluate multiple drugs through adaptive randomization has been proposed in NASH[86]. However, unlike in oncology, platform trials are inherently more challenging to implement in a highly heterogeneous and slowly progressive condition such as NASH, in which molecular subtypes (suitable for specific targeted therapies) have not yet been defined and robust end points (for example, survival) generally do not apply.

Finally, NASH drugs are currently assessed in near-ideal test conditions in highly selected non-diverse populations, often with low levels of deprivation. This approach does not represent routine clinical practice. By contrast, pragmatic effectiveness trials measure the benefit that a treatment produces in patients in everyday real-world settings. Current data showing high rates of advanced fibrosis in patients aged ≥50 years with T2DM create a potential opportunity for real-world studies to determine whether metabolic interventions prevent progression to cirrhosis[18].

Histological assessment of fibrosis in NAFLD

Medicine regulatory authorities currently require a pathologist to stage biopsies using ordinal scales[87]. Despite widespread adoption and notionally clearly defined stages, interpretation remains necessary. The development of fibrosis is a dynamic and non-linear continuum. One limitation of ordinal fibrosis scales is that they impose a small number of scores on this continuum[88]. Observer-defined scoring also introduces an unavoidable intra-observer and interobserver variation of histological features − this negative effect on NAFLD clinical trials has been specifically demonstrated[89]. Digital quantification ought to offer an objective alternative and solution to observer-related error. However, although an observer can easily compensate for inherent variation in stain quality and intensity, such variation represents a marked challenge for current computational methods, and routinely encountered artefact is also troublesome[90]. Stain-free methods to quantify liver scarring and NASH features have also been developed and show promise in detecting drug-induced tissue changes that conventional scoring methods miss[91].

Irrespective of the method of evaluating a needle core biopsy, the validity of judging the condition of the whole organ from a minute sample is questionable, especially considering that all histological features of the disease (such as NASH) are known to be heterogeneously distributed[92]. A study in which two biopsies of the right liver lobe were obtained at the same time from 51 patients with a suspected diagnosis of NAFLD demonstrated discordance between the scores for histological features in the biopsy samples from the same patient assigned by a single pathologist. For example, hepatocyte ballooning was not present in one of the paired biopsy samples but present in the other in 9 of the 51 patients (18%). The effect of this histological intrahepatic heterogeneity on the overall identification of features required for a diagnosis of NASH was also examined; ballooning was absent from both biopsy samples from the same patient in 14 patients, present in both biopsies in 28 patients, and present in one biopsy alone in the remaining 9, such that ballooning would have been missed in 9 of the 37 patients (24%) if only a single biopsy had been obtained and assessed. Further, only 30 of the 51 patients had the same assigned NASH-CRN fibrosis score on both of the paired biopsy samples,

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with a difference of at least one scale point in 21 patients (41%) and two or more scale point differences in biopsy samples from 6 patients. Ultimately, alternative methods that non-invasively and dynamically assess the architecture of the whole organ (for example, MRI) are imperative as a gold standard expert subjective assessment of scarring using ordinal scales results in artefactual information loss and is inherently inexact, and 'objective' computational methods struggle with real-world conditions[93]. Indeed, a 30% reduction in MRI proton density fat fraction is associated with a five times higher odds of NASH resolution[94]. Given the challenges associated with liver biopsy, the European Medicines Agency and US Food and Drug Administration (FDA) support the development of non-invasive biomarkers to potentially replace histology as less burdensome and more reliable end points in future trials[87].

Future therapeutic considerations

Combination therapies. Many drug monotherapies continue to be evaluated in phase II trials, with specific compounds targeting a range of disease mechanisms, including insulin resistance, lipid metabolism, lipotoxicity and oxidative stress, inflammation, cell death and fibrosis[95]. The following agents are being tested for their effectiveness in improving NASH-related fibrosis in phase III trials: aramchol (stearoyl-CoA desaturase 1 inhibitor), resmetirom (thyroid hormone receptor-β agonist), obeticholic acid (farnesoid X receptor (FXR) agonist), lanifibranor (pan-peroxisome proliferator-activated receptor agonist) in non-cirrhotic NASH, and belapectin (galectin 3 inhibitor) in NASH-related cirrhosis (although the primary end point is the prevention of oesophageal varices rather than improvement in fibrosis). These compounds, including their mechanism of action, are listed in Table 1.

To date, the effect of single agents on histological fibrosis has been modest. Although a press release from the pivotal phase III MAESTRO-NASH trial of resmetirom (NCT04197479) reported positive topline data for NASH and fibrosis primary efficacy end points, the focus in the field is increasingly shifting towards combination therapies (Table 2).

An ideal drug combination should be safe, well tolerated and possess orthogonal activities to amplify treatment efficacy. So far, the choice of combination regimens has been serendipitous rather than underpinned by hard science; initial trials have only shown limited effects on non-invasive fibrosis biomarkers[96,97] but will inform future development. In addition, novel computational and high-throughput preclinical combinatorial screening methods could be employed to improve the likelihood of clinical success[75]. Key challenges will be identifying which patient subphenotypes are likely to respond best to certain combinations, defining the chronology of regimens (that is, overlapping, outlasting or additive), and navigating a more complex route to regulatory approval[98]. Combinations might also be utilized strategically to mitigate unwanted effects, such as dyslipidaemia associated with FXR agonists and acetyl-CoA carboxylase inhibitors. These iatrogenic effects might potentially increase cardiovascular risk in an already atherogenic patient population[99,100].

A precision medicine paradigm for NASH therapy. As our ability to assimilate a holistic picture of NAFLD evolves (based on demographics, comorbidities, disease staging, and detailed genetic and metabolic risk assessment — incorporating multiomics), treatment approaches could increasingly be tailored to individual patients. In parallel, genetic and molecular advances have paved the way for novel interventions, including precision medicines that can modulate the activity of specific genes associated with NASH. Genetically validated targets include polymorphisms associated with high-risk NASH phenotypes, such as variants associated with all-cause cirrhosis (for example, *PNPLA3*, *TM6SF2*, *HSD17B13* and *MARC1*)[101,102]. For example, loss-of-function variants in *HSD17B13* are associated with reduced risk of progression to NASH and cirrhosis[103] and hepatocyte-targeted small interfering RNA (siRNA)-mediated knockdown of HSD17B13, mimicking genetic loss of function, is currently being tested in early clinical trials in NASH-related fibrosis. ASO-mediated silencing of *Pnpla3* improved all features of NAFLD, including fibrosis, in mice fed a NASH-inducing diet[104] and early studies of an investigational ASO medicine (AZD2693) in patients with pre-cirrhotic NASH (NCT04483947), homozygous for the *PNPLA3*148M* risk allele, are ongoing.

Cell-specific therapies for fibrosis. The next generation of therapeutics for fibrosis in NASH might also include cell-specific approaches such as vitamin A-coupled lipid nanoparticles delivering siRNA against HSC heat shock protein 47 (a collagen chaperone), which reversed advanced fibrosis in mouse models[105] and showed initial antifibrotic effects in patients with successfully eradicated hepatitis C and advanced fibrosis[106]. The investigational agent BMS-986263 is now being evaluated in NAFLD-related cirrhosis (NCT04267393).

Table 2 | Investigational combination therapy regimens in NASH-related fibrosis or cirrhosis clinical trials

Phase	Molecular target/mechanism	Compound	Trial	Fibrosis stage	Treatment duration (weeks)	Ref.
I	TKI Flavonoid (general antioxidant)	Dasatinib Quercetin	TRUTH	F2–F3	21[a]	131
II	DGAT2i ACC inhibitor	PF-06865571 (ervogastat) PF-05221304 (clesacostat)	MIRNA	F2–F3	48	132
	Non-bile acid FXR agonist SGLT1/2 inhibitor	Tropifexor Licoglifozin	ELIVATE	F2–F3	48	133
	PPARα agonist SGLT2 inhibitor	K-877-ER (pemafibrate-ER) CSG452 (tofogliflozin)	NCT05327127[b]	F1–F3	48	134
	GLP1 agonist ACC inhibitor Non-bile acid FXR agonist	Semaglutide Firsocostat Cilofexor	NCT04971785[b]	F4 (comp)	72	135

ACC, acetyl-CoA carboxylase; comp, compensated cirrhosis; DGAT2i, diacylglycerol O-acyltransferase 2 inhibitor; ER, extended release; FXR, farnesoid X receptor; GLP1, glucagon-like peptide 1; NASH, nonalcoholic steatohepatitis; PPAR, peroxisome proliferator-activated receptor; SGLT, sodium glucose co-transporter; TKI, tyrosine kinase inhibitor. [a]Alternate and interval dosing regimen: 3 days for three consecutive weeks/four drug-free weeks; cycle repeated three times. [b]Trials without a specified trial name.

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Additionally, advances in immunotherapy offer increasingly targeted approaches; for example, chimeric antigen receptor (CAR) T cells might act as 'guided missiles' to treat fibrotic diseases. These tools have been developed to engage specific receptor moieties such as urokinase-type plasminogen activator receptor-specific CAR T cells that ablated senescent HSCs and reduced fibrosis in a mouse NASH model[107]. Moreover, transient antifibrotic CAR T cells were generated in vivo by delivering modified mRNA in T cell-targeted lipid nanoparticles[108]. When administered to mice with cardiac fibrosis (these in vivo-reprogrammed CAR T cells were designed to bind to fibroblast activation protein on activated cardiac fibroblasts), these so-called FAPCAR T cells reduced cardiac fibrosis and improved cardiac function. This transient in vivo approach potentially circumvents the risk of persistent antifibrotic CAR T cells in the setting of future injuries and the ability to titrate dosing and re-dose as needed.

Further fine-tuning will be required to determine the best context-specific cell-surface targets, to minimize off-target effects and the disruption of effective tissue regeneration and repair[107,108].

Conclusions

In 2008 the renowned biologist Sydney Brenner said: "We don't have to look for a model organism anymore. Because we are the model organisms". These words have proved prescient, and now that NASH is giving up its pivotal secrets through ever more sophisticated human-based technologies and datasets, the future of drug development in NASH looks brighter[109]. However, in contrast to oncology, where several examples of FDA-approved drugs and companion diagnostics are embedded in clinical practice, navigating the path towards precision medicine for a complex disease like NASH is more challenging. Moreover, the cause of death in most patients with NASH is cardiovascular disease or non-hepatic malignancy rather than liver disease, so individualized outcome and treatment prediction models are needed[110,111].

Although advanced methods of liver tissue analysis will be useful for target identification, the need for liver biopsies to stage patients for initiation of treatment or assessment of efficacy is likely to be superseded by reliable non-invasive tests. Moving forward, we anticipate more efficient clinical trial design, including genotype-driven approaches, with the approval of new drug monotherapies or combination regimens for subgroups of patients with specific genetic or metabolic risk profiles. Meanwhile, the cost-effectiveness and affordability of future NASH therapies (especially when lined up against diet and exercise) remain the elephant in the room[112]. The initial wave of new NASH drugs will face unchartered reimbursement territory and could encounter strict prior authorization from payers tied to the (histological) enrolment criteria of pivotal trials.

Finally, drug repurposing was at the forefront of efforts to identify effective therapies during the COVID-19 pandemic and highlighted the need for a standardized translational drug development platform[113]. Several studies[114,115] indicate that similar approaches could deliver unexpected success in the face of a NAFLD pandemic.

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