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

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Abstract

Non-alcoholic steatohepatitis (NASH) may 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 strong rationale for drug combinations to boost efficacy, and precision medicine strategies targeting key genetic modifiers of NASH are emerging. In this Perspective, we consider why antifibrotic effects observed in NASH pharmacotherapy trials have been underwhelming and outline potential approaches to improve the likelihood of future clinical success.

Introduction

Nonalcoholic fatty liver disease (NAFLD) is characterised by fat accumulation within the liver (hepatic steatosis) in the absence of secondary causes including significant 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 consequent risk of hepatic decompensation and hepatocellular carcinoma (HCC; 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 – characterised by the presence of two or more of insulin resistance/type 2 diabetes mellitus (T2DM), obesity, hypertension and hypercholesterolaemia, although a “lean” NAFLD variant phenotype is also recognised.[3] Notably, there is currently a vigorous debate and international Delphi consensus process about re-naming NAFLD, using terminology perceived as less stigmatising 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 recent systematic review and meta-analysis suggested an overall worldwide prevalence of 30[6]-32.4%[7], although there is significant geographical variation. Additionally, strong evidence is emerging linking NAFLD with social deprivation and food insecurity.[8] Between 1990-2017 the number of cases of decompensated NAFLD cirrhosis doubled (although relabelling of previous “cryptogenic” cirrhosis cases as NAFLD may have contributed) and it is the most rapidly increasing indication for liver

transplantation in the United States (US).[9,10] These observations point to 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). In contrast, two antifibrotic drugs are approved for the treatment of idiopathic pulmonary fibrosis (nintedanib and pirfenidone) and both may reduce the decline in lung function.[11] Here we reflect on the current landscape in NAFLD and offer our perspective on the challenges that stand in the way of achieving that goal and propose possible solutions that take advantage of new and emerging technologies.

Why is fibrosis important in NASH?

In general, progression of fibrosis to cirrhosis and adverse liver-related outcomes in NASH is slow and unpredictable, with the true fibrosis progression rate (FPR) uncertain. Nevertheless, the FPR appears to be significantly higher in NASH compared to isolated steatosis, corresponding to the progression of fibrosis by one-stage over 7 years and 14 years, respectively.[12] An FPR of only 0.03 stages was recently calculated from 1,419 placebo-treated participants undergoing per protocol repeat biopsy in 35 randomised controlled trials in NASH.[13] However, the generalisability 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 in biopsies taken a year apart compared to those progressing[14], although the inherent sampling error of liver biopsies may 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 (e.g., decompensation events, HCC), and liver-related and all-cause mortality. It is therefore alarming that a recent prospective study conducted in Southern California showed that 14% of patients with T2DM aged ≥ 50 years have advanced fibrosis and 6% have cirrhosis.[18] Furthermore, in a large meta-analysis of 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, 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 may lead to improvements in mortality independent of a reduction in liver-related events; proving this will require well conducted, long term (>5 years at least) clinical trials.

Contrastingly, data from two large (negative) drug trials in compensated NAFLD cirrhosis recently indicated that a reduction in fibrosis may be associated with

improved clinical outcomes. Specifically, after a median follow-up of 16.6 months, patients whose fibrosis regressed had a six-fold 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 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 reduction is foundational therapy for NAFLD, but is 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% body weight,[24,25] although most find this difficult to achieve and sustain. Numerous diets have been promoted for NAFLD with systematic reviews and randomised controlled trials favouring a Mediterranean diet,[26] which has proven benefits on liver, metabolic and cardiovascular health.[27] Physical activity has multisystem health benefits, even in the absence of weight loss, but recommendations regarding the type, intensity and frequency are unclear. Moreover, when counselling patients it may be best to discuss simple strategies to increase “movement” rather than “exercise”, given that age and co-morbidities (obesity, cardiovascular disease, osteoarthritis) may be limiting and investment in equipment/gym memberships may be unrealistic or off-putting. Even moderate physical activity could reprogramme key pathophysiological mechanisms.[28] Indeed, observational data has shown that insufficient physical activity is an independent predictor of fibrosis in NAFLD.[29,30] However, liver inflammation and fibrosis endpoints have largely been overlooked in lifestyle intervention studies. Notably, in a single-arm 16-week intervention study, diet and moderate-intensity exercise not only reduced body weight but also decreased hepatic venous pressure gradient in overweight patients with cirrhosis and portal hypertension.[31] 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, but it may lead to substantial weight loss (between 14-25%) and durable improvements in histological NASH and fibrosis[32], as well as reduced risk of major adverse liver and cardiovascular outcomes[33] and many cancers.[34] However, delivering such a complex intervention requires substantial resource and is therefore inaccessible to the vast majority of the global NAFLD population. Additionally, emerging data may suggest that bariatric surgery is associated with an increased prevalence of alcohol use disorder (AUD) and alcohol-related liver disease (ArLD), and potential candidates should be rigorously assessed prior to undertaking.[35]

A less invasive approach is endoscopic intragastric balloon (IGB) insertion (using adjustable fluid-filled balloon), which reduces stomach capacity, and delays gastric emptying, thereby inducing weight loss. An open-label study of IGB placement, in combination with a prescribed diet and exercise programme, induced significant weight loss (mean reduction 11.7%), metabolic improvements, and ameliorated histological NASH (in 80%) and fibrosis (in 15%).[36] However, this study was limited by its small size, the lack of a control group and 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 HSC (or cell lines) to study phenotypic effects and then employs animal (usually rodent) models to determine efficacy and toxicity. However, this framework has inherent limitations in replicating the complexity of human *in vivo* pathophysiology.

In vitro/ex vivo models

Simple cell culture models are limited by their non-physiological conditions, lacking both cell-cell and cell-substrate interactions, which influence innate cellular responses in healthy and diseased milieux. 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-, bi-, or even, tri-cell culture systems are limited in their ability to predict drug-induced responses in complex, anatomically-organised multicellular tissues.

Self-assembling stem cell-derived, or organoid-derived tissues and bioprinted tissues may simulate some of the complexity of a liver tissue microenvironment; however, these platforms are still in their infancy.[37–39] Advances in the design of organoid-engineered human multicellular 3D NASH models have shown promise, recapitulating features of steatosis, inflammation and fibrosis, including a biophysical readout of organoid stiffening that reflects the fibrosis severity[40].[41] Moreover, co-culturing of pluripotent stem cell (PSC)-derived lineages may 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 and can be engineered from parenchymal and non-parenchymal cells to recapitulate anatomical features of the liver such as hepatic zonation and lobe-like structures.[42] Exposing LoCs containing multiple cell types to lipids has been reported to induce steatosis, hepatocyte ballooning, expression of TNF and alpha-smooth muscle actin indicative of a NASH phenotype[43] Moreover, therapeutic effects of several NASH drug candidates have been demonstrated using these models.[44]

A caveat of LoCs is the need to dissociate, and purify individual cell types from human liver tissues prior to their reassembly into synthetic liver structures by bioprinting. 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 with the use of iPSC lines as a reproducible standardised source of different cell lineages.

Moreover, despite impressive advances LoCs do not yet fully recapitulate the full physiological, anatomical, and cellular complexity of liver tissue. Alternatively, human *ex vivo* precision-cut liver slice (PCLS) systems have been shown to successfully model fibrogenesis and demonstrate efficacy of antifibrotic therapies.[45] They retain not only important architectural/zonal spatial contexts of parenchymal and non-parenchymal liver cells, but also resident Kupffer cells and lymphocytes, PCLS may also represent a valuable tool to study human innate immunity.[46]

Animal models

Rodent (mostly mouse) 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)[47] provides the closest approximation of human disease. Genetic models may complicate drug discovery as they will not recapitulate mechanisms in patients. A recent systematic review catalogued a total of 3,920 NAFLD models (including dietary, chemical, genetic and combination models) across 4,540 published studies[48] and showed inconsistencies in terminology and study design, and substantial heterogeneity in replicating human NAFLD phenotypic characteristics which, when further confounded by inter-lab variability,[49] severely compromises the reproducibility of *in vivo* experiments. NAFLD models incorporating a baseline/pre-treatment liver biopsy more accurately reflect clinical trial design, as they control for phenotypic variation through stratification of fibrosis severity and balance treatment allocation, whilst also allowing assessment of drug response in individual animals.[50]

To overcome this uncertainty the field should agree on a standardised model that can be adopted by industry, with continued iterative improvements advised from the academic community. As well as providing metabolic, immune, fibrogenic, carcinogenic and ideally angiogenic characteristics of human NASH, an ideal model would also pose the relevant clinical challenges of late-stage disease (e.g., addressing the association between hepatic fibrosis and increased cardiac-related mortality). Inherent differences between mouse and human immune systems, including variation in innate and adaptive immune compartments, means that mice will not accurately mimic the immunological components of human NASH. The translational value and reproducibility of laboratory mouse models is also limited by their sanitised environment and divergent microbiota. This was circumvented by implanting lab-strain embryos into wild mice to restore natural microbiota and pathogens.[51] The resulting "wilding" mice more faithfully replicated human disease and accurately predicted two (non-NASH) clinical trial failures (anti-CD28 monoclonal antibody for T-cell expansion, and an anti–TNFα treatment during septic shock), where 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. Humanised mice offer a partial solution and can include human hepatic[52] as well as partial reconstitution of a human immune system; however, they are technically challenging and expensive.

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

Are we targeting the right mechanism(s), at the right time, for fibrosis regression?

In addition to the technical challenges discussed above, does the current reductionist approach, positioning HSC as the central instigators of (NASH) fibrosis, remain valid? We still lack concrete evidence that HSC are the pivotal fibrogenic cells in human NASH; indeed, the cross-tissue heterogeneity of fibroblasts in perturbed states, including fibrosis and cancer, is increasingly recognised.[53] 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.

Recent discoveries concerning the role of cell-cell crosstalk between epithelial, myeloid and mesenchymal cells in fibrosis also underscore the limitations of focusing on individual cell types and suggest that inhibiting key intercellular networks that trigger or promote disease progression may be tractable treatment targets in NASH-related fibrosis.[54] The functional heterogeneity and dynamic plasticity of cell lineages in the liver, including HSC[55], highlights 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. One fundamental question remains unanswered however - are we targeting the correct mechanisms underpinning fibrogenic NASH?

Hepatocellular senescence appears to be a key stimulator of steatosis and fibrosis, however, senolytic drugs have not yet advanced to clinical development. A planned early-phase trial evaluating the combination of the tyrosine kinase inhibitor dasatinib and the antioxidant quercetin, which decreased senescent cells in diabetic kidney disease[56], will provide important proof-of-principle in fibrotic NASH.[57–59] Future work will clarify the importance of other fibrogenic mechanisms in NASH[60], including extrahepatic drivers (e.g., the microbiome/gut-liver axis) and direct hepatic triggers (e.g., proinflammatory modes of hepatocyte cell death such as ferroptosis[61]) to establish the safest, and most potent approaches.

Another key question is when in the disease process is best to intervene with antifibrotics (and when is it simply too late)? Certain architectural changes that are likely to be irreversible (e.g., vascularised septae)[62,63] plus the reduced regenerative capacity in advanced cirrhosis, argues for pre-cirrhosis as the optimal initial stage at which antifibrotics should be clinically utilised; otherwise, as evidenced by the multiple trial failures in NAFLD-related cirrhosis, the likelihood of success is low.. As a histological surrogate endpoint is not established for drug trials in NAFLD cirrhosis, a long-term composite clinical outcome endpoint (including all-cause mortality) is required.[64] Although a decrease in portal hypertension (using hepatic venous pressure gradient (HVPG)) is associated with improved clinical outcomes in patients with both compensated and decompensated cirrhosis,[65,66] variability is a potential issue, although possibly overstated,[65] however, it is not yet accepted by regulatory agencies. This is despite having been evaluated as a primary endpoint in several large NAFLD cirrhosis studies.[67–69]

How can we improve the fibrosis drug discovery process?

To mitigate against failing we must look critically at (1) how we are selecting drug targets/combinations; (2) whether we are utilising preclinical models (and readouts used to determine drug efficacy) with sufficient proximity to patients; and (3) optimising 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 metabolic factors could pinpoint the key fibrogenic cell interactions (and best potential therapeutic targets) in NASH.[70] The evolution of artificial intelligence (AI) and high-performance computing can now combine with carefully archived biopsy samples to 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[71] could transform drug target discovery and validation. Moreover, the enormous repertoire of human tissue within clinical trial biobanks offers previously unimaginable opportunities, including the ability to interrogate these samples using state-of-art single-cell 'omics and AI to understand how drugs that have failed in trials may have in fact influenced liver cell biology (e.g., HSC activation, hepatocyte function), far beyond the typical endpoints mandated by regulators and trialists. Such approaches can re-evaluate our pharmacological theory, identify more/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 such that they represent the transitions of NAFLD from steatosis through to F1 to F4 fibrosis may 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 can emerge novel molecular targets 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.[72] However, in the opinion of the authors, human 3D precision cut liver slices (PCLS) models currently offer the closest replicant of a human liver, with the caveat that they lack a peripheral blood supply and whole-body context.[73] An important advantage of PCLS is that variables influencing drug efficacy and toxicity such as ageing, gender, 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 so as to avoid hypoxic damage during the collection, transport and processing of resected tissues. For scalability, the PCLS platform requires miniaturisation to at least 96-well format and the design of fibrosis assays that lend themselves to automation. [74]

How can we improve clinical trials and enhance their outputs?

A positive outcome on a pre-specified endpoint (either NASH resolution or fibrosis improvement) in a well-designed placebo-controlled phase 2 trial is likely to increase the probability of a successful phase 3 study (as illustrated by phase 2 studies of

obeticholic acid and resmetirom that were predictive of statistically significant effects on fibrosis and NASH resolution, respectively, at phase 3). Nevertheless, multiple clinical factors have been identified as potential obstacles to successful drug development in NASH.[75,76] Key issues include: a high placebo response rate (estimated at 22%),[77] 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 simply as a result of being observed); and the lack of a standardised approach to diet and exercise across trials.[78] 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 may vary in responsiveness to a specific drug further complicate evaluation of new candidates. There also remains an unknown hierarchy of targets, such that certain mechanisms of action may be too far "downstream". Finally, undisclosed alcohol use, which has recently been highlighted in patients presumed to have 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 (PEth) in blood, or ethyl glucuronide in hair (hEtG) and urine (uEtG).[79,80]

Refinement of clinical trial design

Given the track record of drug failures in NASH, lengthy development time, difficulty in recruiting participants, complex disease biology with multiple potential therapeutic targets and 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 endpoint.

Another aspect involves optimisation of participant selection that could involve enrichment of patients harbouring known genetic polymorphisms associated with an accelerated disease course and adverse outcomes. Adopting new trial designs (e.g., platform trials) to efficiently evaluate multiple drugs through adaptive randomisation has recently been proposed in NASH.[81] However, unlike in oncology, platform trials will be inherently more challenging to implement in a highly heterogeneous and slowly progressive condition such as NASH, where molecular subtypes (suitable for specific targeted therapies) have not yet been defined and robust endpoints (e.g., survival) generally do not apply.

Finally, NASH drugs are currently assessed in near-ideal test conditions in highly selected non-diverse, often less deprived, patient populations. This is not representative of routine clinical practice. In contrast, pragmatic effectiveness trials measure the benefit a treatment produces in patients in everyday "real-world" settings. Recent data showing high rates of advanced fibrosis in older T2DM patients creates a potential opportunity for real-world studies to determine whether metabolic interventions prevent progression to cirrhosis.[82]

Histological assessment of fibrosis in NAFLD

Medicines regulatory authorities currently require pathologist staging of biopsies using ordinal scales.[83] 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. Observer-defined scoring also introduces unavoidable intra- and inter-observer variation of any histological features and the negative impact of this on NAFLD clinical trials has been specifically demonstrated.[84] Digital quantification ought to offer an objective alternative and solution to observer-related error. However, whilst an observer is easily able to compensate for inherent variation in stain quality and intensity, such variation represents a significant challenge for current computational methods and routinely encountered artefact is also troublesome.[85] 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.[86]

Irrespective of the method of evaluation of a needle core biopsy, the wisdom of judging the condition of the whole organ from a minutel sample is questionable, particularly where all histological features of disease (such as NASH) are known to be inhomogeneously distributed.[87] For example, in a study of paired biopsies from 51 patients taken at the same time, the discordance rate for the hepatocyte ballooning score was 18%, and ballooning would have been missed completely in 24% of patients if only a single biopsy had been undertaken. Further, there was discordance in fibrosis stage between paired biopsies in 41% of patients. Ultimately, where 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, alternative methods that non-invasively and dynamically assess the architecture of the whole organ (e.g., MRI) are an imperative.[88] Indeed, a 30% reduction in MRI-PDFF is associated with five-times higher odds of NASH resolution.[89] Given the challenges associated with liver biopsy, both the European Medicines Agency (EMA) and US Food and Drug Administration (FDA) support the development of non-invasive biomarkers to potentially replace histology as less burdensome and more reliable endpoints in future trials.[90]

Future therapeutic considerations

Combination therapies

Many drug monotherapies continue to be evaluated in phase 2 trials, with specific compounds targeting a range of disease mechanisms including insulin resistance, lipid metabolism, lipotoxicity/oxidative stress, inflammation, cell death and fibrosis.[91] Five agents have progressed to phase 3 (aramchol (stearoyl-CoA desaturase-1 inhibitor), resmetirom (thyroid hormone receptor-β agonist), obeticholic acid (farnesoid X receptor (FXR) agonist) and, lanifibranor (pan-PPAR agonist) in non-cirrhotic NASH and belapectin (galectin-3 inhibitor) in NASH-related cirrhosis (although the primary endpoint is prevention of oesophageal varices rather than improvement in fibrosis)).

To date, the effect of single agents on histological fibrosis have been modest. Although a recent press-release from the pivotal phase 3 MAESTRO-NASH trial of resmetirom reported positive topline data for NASH and fibrosis primary efficacy endpoints, the field is increasingly shifting focus 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[92,93], but will inform future development. 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 (i.e., overlapping, outlasting or additive), and navigating a more complex route to regulatory approval. [94] .Combinations may also be utilised strategically to mitigate unwanted effects, such as dyslipidaemia associated with FXR agonists and acetyl-CoA carboxylase (ACC) inhibitors. These iatrogenic effects may potentially increase cardiovascular risk in an already atherogenic patient population[95,96].

A precision medicine paradigm for NASH therapy

As our ability to assimilate a truly 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, including variants associated with all-cause cirrhosis (e.g., *PNPLA3*, *TM6SF2, HSD17B13*, and *MARC1*)[97,98]. For example, loss-of-function variants in HSD17B13 are associated with reduced risk of progression to NASH and cirrhosis[99] and hepatocyte-targeted 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[100] and early studies of an investigational ASO medicine (AZD2693) in pre-cirrhotic NASH patients homozygous for the PNPLA3 148M risk allele are ongoing.

Cell-specific therapies for fibrosis

The next generation of therapeutics for fibrosis in NASH may also include cell-specific approaches such as vitamin A-coupled lipid nanoparticles delivering siRNA against HSC heat shock protein 47 (HSP47, a collagen chaperone) which reversed advanced fibrosis in mouse models[101] and showed initial antifibrotic effects in patients with successfully eradicated hepatitis C and advanced fibrosis.[102] The investigational agent (BMS-986263) is now being evaluated in NAFLD-related cirrhosis.

Additionally, advances in immunotherapy offers increasingly targeted approaches; as an example CAR T cells may 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 (uPAR)-specific CAR T cells that ablated senescent HSCs, and reduced fibrosis in a mouse NASH model.[103] Moreover, *transient* antifibrotic CAR T cells were recently generated *in vivo* by delivering modified mRNA in T cell-targeted lipid nanoparticles.[104] When administered to mice with cardiac fibrosis, these *in vivo*-reprogrammed CAR T cells, which were designed to bind to fibroblast activation protein (FAP) on activated cardiac fibroblasts (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, as well as the ability to titrate dosing and to re-dose as needed.

Further fine-tuning will be required to determine the best context-specific cell-surface targets, to minimise off-target effects and the disruption of effective tissue regeneration and repair. [103,104]

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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. [105]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, most NASH patients will die of cardiovascular disease or non-hepatic malignancy rather than liver disease, so individualised outcome and treatment prediction models are needed.[106,107]

While 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 for assessment of efficacy will likely be superseded by reliable non-invasive tests. Moving forward, we anticipate more efficient clinical trial design, including genotype-driven approaches, with 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) remains the elephant in the room.[108] The initial wave of new NASH drugs will face unchartered reimbursement territory and could encounter strict prior authorisation from payers, tied to the (histological) enrolment criteria of pivotal trials.

Finally, drug repurposing was recently at the forefront of efforts to identify effective therapies during the COVID-19 pandemic and highlighted the need for a standardised translational drug development platform[109]Several studies[110,111] indicate that similar approaches could deliver unexpected success in the face of a NAFLD pandemic.

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Tables

Phase	*Molecular target/mechanism*	*Compound*	*Trial*	*Fibrosis Stage*	*Treatment (n)*	*Ref*
Phase II	Long-acting Fibroblast Growth Factor (FGF) subclass analogues	Efruxifermin (FGF21)	HARMONY	F2-F3	24-96	[112]
			SYMMETRY	F4 (Comp)	36	[113]
			ENLIVEN	F2-F3	24	[114]
		Pegozafermin (FGF21)	ALPINE4	F4 (Comp)	48	[115]
		Aldafermin (FGF19)				
	Non-Peroxisome Proliferator-Activated Receptor-γ (PPAR-γ) active stereoisomer of pioglitazone	Deuterium-stabilized R-pioglitazone (PXL065)	NCT04321343	F1-F3	36	[116]

	Fatty Acid Synthase (FASN) inhibitor	Denifanstat	FASCINATE-2	F2-F3	52	[117]
	Cyclophilin inhibitor	Rencofilstat	AMBITION	F2-F3	4	[118]
Phase III						
	Peroxisome Proliferator-Activated Receptor-α/δ/γ (PPAR-α/δ/γ) agonist	Lanifibranor	NATiV3	F2-F3	72	[119]
	Farnesoid X Receptor (FXR) agonist	Obeticholic Acid	REGENERATE	F2-F3	~500	[120]
			REVERSE	F4 (Comp)	72	[121]
	Thyroid Hormone Receptor β (THRβ) agonist	Resmiterom	MAESTRO-NASH	F1-F3	52	[122]
			MAESTRO-NASH OUTCOMES	F4 (CTP-A)**	~156	[123]
	Glucagon-like Peptide-1 (GLP-1) agonist	Semaglutide	ESSENCE	F2-F3	260	[124]
	Stearoyl CoA Desaturase-1 (SCD) partial inhibitor/PPAR-γ-induction	Aramchol	ARMOR	F1-F3	72-120	[125]

*NCT number is provided for those trials without specified trial name. Comp, compensated cirrhosis. CTP-A, Child-Turcotte-Pugh Class A.

Table 1: *Investigational monotherapy compounds in NASH-related fibrosis or cirrhosis clinical trials.*

Phase	*Molecular target/mechanism*	*Compound*	*Trial*	*Fibrosis Stage*	*Treatment Duration (weeks)*
Phase I	Tyrosine Kinase inhibitor (TKi)	Dasatinib	TRUTH	F2-F3	21**
	Flavonoid (general antioxidant)	Quercetin			
Phase II	Diacylglycero O-acyltransferase 2 inhibitor (DGAT2i)	PF-06865571 (Ervogastat)	MIRNA	F2-F3	48
	Acetyl-CoA Carboxylase inhibitor (ACCi)	PF-05221304 (Clesacostat)			
	Non-bile acid Farnesoid X Receptor (FXR) agonist	Tropifexor	ELIVATE	F2-F3	48
	Sodium Glucose Co-transporter 1/2 (SGLT1/2) inhibitor	Licoglifozin			
	Peroxisome Proliferator Activated Receptor (PPAR)-α agonist	K-877-ER (Pemafibrate-ER)	NCT05327127	F1-F3	48
	Sodium Glucose Co-transporter-2 (SGLT/2) inhibitor	CSG452 (Tofogliflozin)			
	Glucagon-Like Peptide-1 (GLP-1) agonist	Semaglutide	NCT04971785	F4 (Comp)	72
	Acetyl-CoA Carboxylase (ACC) inhibitor	Firsocostat			
	Non-bile acid Farnesoid X Receptor (FXR) agonist	Cilofexor			

*NCT number is provided for those trials without specified trial name. **Alternate/interval dosing regimen – 3 days for 3 consecutive weeks/4 drug-free weeks, cycle is repeated 3 times. ER, Extended Release. Comp, compensated cirrhosis.

Table 2: *Investigational combination therapy regimens in NASH-related fibrosis or cirrhosis clinical trials.*

Figure legends

Fig. 1. Strategies to increase the chances of successful drug development in NASH.

At all stages of the drug development process (discovery, preclinical testing, clinical evaluation), a plethora of patient-based approaches are now available to help pinpoint the most effective drug candidates for NASH. AI, artificial intelligence.

Box 1: How have we arrived where we are in fibrosis therapeutics?

Arguably the beginning of contemporary fibrosis biology was the successful isolation of what were then described as "hepatic lipocytes" - and later as hepatic stellate cells (HSC) - which was reported by Friedman and colleagues in 1985.[131] Activation of HSC into a myofibroblast-like cell is now the accepted pivotal process leading to excessive production of fibrotic extracellular matrix.[132,133] The central role of HSC in fibrosis was confirmed using *in vivo* mouse models of liver injury, leading to work that described how apoptosis of activated HSCs caused fibrosis regression.[134–136] 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 showed us how to purify and culture HSCs, we are still waiting for an approved medicine to treat liver fibrosis. The rising global prevalence of NASH has provided a further stimulus and investment for accelerating fibrosis drug discovery and advancing antifibrotic candidates to clinical trials.[137] Unfortunately, most pharmacological studies in NASH-related fibrosis, and particularly cirrhosis, are not fulfilling their preclinical promise, leading to numerous drug programmes being terminated (the so-called "NASH graveyard" extensively discussed in recent reviews.[138–140] As we approach the 40th anniversary of the start of intensive liver fibrosis research, it is time to ask if we require a refocus to ensure the field remains galvanised to the aim of delivering effective antifibrotics.

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JAF, DAM and PNB wrote the initial draft of the article. All authors contributed substantially to discussion of the content and reviewed and/or edited the manuscript before submission.

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Ethics declarations

Competing interests

PNB has received educational honoraria from Takeda. PNB also served as a consultant and is employed by Resolution Therapeutics. JAF serves as a consultant or advisory board member for Ipsen, Sosei Heptares, Redx Pharma, River 2 Renal

Corp., Stimuliver and Galecto Biotech. JAF has received research funding from Genentech and Intercept Pharmaceuticals. TJK serves as a consultant or received speaker fees from Resolution Therapeutics, Clinovate Health, Perspectum Diagnostics, and Incyte Corporation. AME serves as a consultant or received speaker fees from GSK, Gilead and SOBI. DAM is a director, major shareholder and salaried CSO of FibroFind Ltd. He has received research funding from GSK and collaborates with AstraZeneca on novel treatments for advanced hepatocellular carcinoma. RL serves as a consultant to Aardvark Therapeutics, Altimmune, Anylam/Regeneron, Amgen, Arrowhead Pharmaceuticals, AstraZeneca, Bristol-Myer Squibb, CohBar, Eli Lilly, Galmed, Gilead, Glympse bio, Hightide, Inipharma, Intercept, Inventiva, Ionis, Janssen Inc., Madrigal, Metacrine, Inc., NGM Biopharmaceuticals, Novartis, Novo Nordisk, Merck, Pfizer, Sagimet, Theratechnologies, 89 bio, Terns Pharmaceuticals and Viking Therapeutics. In addition, RL's institutions received research grants from Arrowhead Pharmaceuticals, AstraZeneca, Boehringer-Ingelheim, Bristol-Myers Squibb, Eli Lilly, Galectin Therapeutics, Galmed Pharmaceuticals, Gilead, Intercept, Hanmi, Intercept, Inventiva, Ionis, Janssen, Madrigal Pharmaceuticals, Merck, NGM Biopharmaceuticals, Novo Nordisk, Merck, Pfizer, Sonic Incytes and Terns Pharmaceuticals. RL is also co-founder of LipoNexus Inc.

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