The future R&D landscape in non-alcoholic steatohepatitis (NASH)

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Nonalcoholic steatohepatitis (NASH) is emerging as a major public health issue for the 21st century and is associated with significant liver-related morbidity and mortality. At present, there are no approved drug therapies for NASH. Consequently, NASH has become the focus of significant public and private research and development. In this review, we highlight the research and development (R&D) challenges and opportunities in this emerging therapeutic area. In particular, we consider the impact of the development of new biomarker strategies on clinical trial execution and design, and the positioning of single and combination therapies in future approaches to the treatment of NASH.

Introduction

Here, by imagining the future disease and therapeutic landscape, we provide insights into upcoming R&D challenges associated with developing therapies for the treatment of NASH. We review how new therapies could be positioned and differentiated from molecules currently in late-stage development; and recent advances in biomarker development that will support this. Finally, we consider the potential role of combination therapies in the treatment of NASH.

With an estimated global prevalence of 24%, nonalcoholic fatty liver disease (NAFLD) is a major cause of liver-related morbidity and mortality in Western societies [1]. Treatment guidelines in the USA define NAFLD as having accumulation of fat in the liver (steatosis) with the absence of alcohol, drug, or viral-induced fat accumulation [2]. Currently, there are no approved drug treatments for NASH. However, there are new therapies nearing the end of Phase 3 development, with the expectation of (conditional) approval by 2020.

NAFLD encompasses two main phenotypes, defined by histology following biopsy: (i) non-alcoholic fatty liver (NAFL); and (ii) NASH. Here, we focus on the NASH component of NAFLD. NASH represents a growing challenge in public health, with an increased prevalence of cardiovascular- and cancer- as well as liver-related deaths in patients with the disease. It is recognized that approximately one-third of patients with NASH will die from liver disease [1,3].

Several mechanistic disease drivers have been identified. Although these can be defined at the molecular, cellular, and system levels, the overall pathophysiology of the disease remains unclear. However, it is clear that metabolic dysfunction drives the disease, which is maintained by inflammation and liver injury, ultimately leading to fibrosis [4–6].

The mechanistic processes driving NASH are differentially active according to the stage of disease. Therefore, developing a single therapy for NASH will prove challenging and new therapies (individual or combination) are likely to be specific to disease stage (s). This will drive the development of stage-specific diagnostic biomarkers, and the rational design and development of new and specific therapies. Taken together, progress in both aspects will enable specific positioning of new therapies for NASH, which are not achievable with currently available research tools.

There are currently four molecules, with different modes of action, in Phase 3 clinical trials with expected clinical entry from 2019/20 onwards (Table 1). As would be expected in an emerging therapy area, each trial has different inclusion and/or exclusion criteria and primary endpoints, making the direct comparison of

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TABLE 1

Compounds in late development (Phase 3) for the treatment of NASH[a]

Compound (company); MoA	Phase 3 trials	Primary endpoint (efficacy)	Main inclusion criteria[2]	Timelines	Comments
Elafibranor (Genfit); PPAR-α and -δ agonist	RESOLVE-IT (clinicaltrials.gov, NCT02704403)	Resolution of NASH without worsening of fibrosis All-cause mortality, cirrhosis, and liver-related clinical outcomes	Liver biopsy proven NAS score ≥4 Fibrosis stage of 1 or greater but below 4	Data expected for end of 2019 MA approval planned for 2020	In November 2017, Drug Safety Monitoring Board recommended continuing trial (no safety issues)
OCA (Intercept); farnesoid X receptor agonist	REGENERATE (clinicaltrials.gov, NCT02548351)	One stage improvement in liver fibrosis score with no worsening of NASH, or NASH resolution with no worsening of liver fibrosis All-cause mortality and liver-related clinical outcomes	Liver biopsy Fibrosis stage 2 or 3 or fibrosis stage 1a or 1b if accompanied by ≥1 of defined risk factors	Data from interim analysis suitable for submission expected first half of 2019	Launched for primary biliary cholangitis (PBC) Black Box Warning to reinforce appropriate dosing in PBC (Feb 2018)
	REVERSE (clinicaltrials.gov, NCT03439254)	Improvement in fibrosis by >1 stage with no worsening of NASH	Confirmed diagnosis of NASH and a fibrosis score of 4	estimated primary completion date: July 2020	
Cenicriviroc (Allergan); CCR2/CCR5 antagonist	AURORA (clinicaltrials.gov, NCT03028740)	Improvement in fibrosis by >1 stage and no worsening of steatohepatitis Composite of histopathological progression to cirrhosis, liver-related clinical outcomes, and all-cause mortality	Liver biopsy Histological evidence of Stage 2–3 liver fibrosis	Launch expected 2021	
Selonsertib (Gilead Sciences); apoptosis signal-regulating kinase 1 (ASK-1) inhibitor	STELLAR 3 (clinicaltrials.gov, NCT03053050)	≥1-stage improvement in fibrosis without worsening of NASH Event-free survival at week 240 as assessed by time to the first clinical event	Liver biopsy consistent with NASH and bridging (F3 fibrosis)	Data from Phase 3 studies expected in first half of 2019	STELLAR-3 and STELLAR-4 studies have completed enrollment ahead of schedule Ongoing Phase 2 combination studies (NCT02781584, NCT03449446 clinicaltrials.gov)
	STELLAR 4 (clinicaltrials.gov, NCT03053063)		Liver biopsy consistent with NASH and cirrhosis (F4 fibrosis)		

[a] Information obtained from www.clinicaltrials.gov, company websites or press releases. Scoring is based on the NASH CRN scoring system [40].

results difficult. Although each molecule has shown evidence of efficacy in a Phase 2 trial, there is considerable uncertainty about how strategic positioning will be achieved, and how differentiation against other more advanced but still to-be-developed molecules can be shown.

The variability seen in recent clinical trials, if replicated in Phase 3, suggests that head-to-head comparison of new entities with established therapy will be impractical and patient stratification will become essential. At the same time, the complexity of the disease and multiple pathways involved indicates that combination therapies are likely to predominate. Therefore, it is essential that these factors are understood when defining development strategies for future therapies in liver fibrosis.

Differentiation and positioning

The pathogenesis of NASH is complex. A 'dual hit' hypothesis is commonly proposed (Fig. 1). The first hit (e.g., insulin resistance or steatosis) serves as permissive factor, followed by a second insult (e.g., overnutrition-driven liver stress or inflammation) [7–9]. Additionally, genetic susceptibility can predispose the liver for the disease [10]. Metabolic dysfunction is believed to then drive the disease, which, consequently, is maintained by inflammation and liver injury. Especially in mild disease (NAFLD and/or 'early NASH'), the disease can stagnate without progression for years, or even resolve [11]. Importantly, chronic inflammation is linked to NASH progression. Processes that contribute to the inflammatory component include: disturbed bile acid metabolism; insulin resistance, which promotes adipose tissue dysfunction, increased adipokines and lipogenesis; the accumulation of lipids plus lipotoxicity; and the concomitant release of inflammatory mediators from adipose tissue and the liver [12–14]. As the disease progresses, fibrosis results from the lipotoxicity and inflammatory processes, which causes activation of hepatic stellate cells, their differentiation into myofibroblasts, and the subsequent deposition of extracellular matrix. The underlying profibrotic pathways are driven by multiple growth factors, primarily transforming growth factor beta (TGFβ), and involve intracellular signaling via nuclear hormone receptors, such as farnesoid X receptor (FXR) and peroxisome proliferator-activated receptors (PPAR) [15–17].

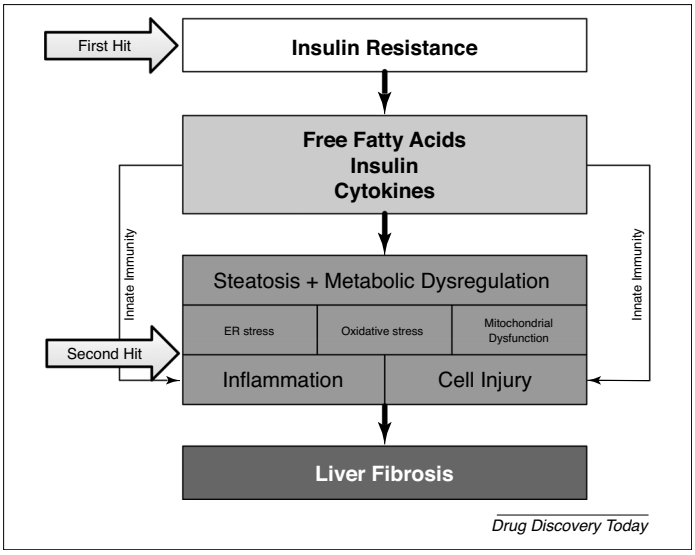

FIGURE 1

The 'two-hit hypothesis' for the pathogenesis of liver fibrosis [37,38]. Adapted from Ref. [39]. Abbreviations: ER, endoplasmic reticulum.

A clear and simple pathophysiological trajectory cannot be defined, but different processes are active in driving the disease at different stages. Therefore, emerging therapies might be stage specific, aiming to reduce stage-relevant disease drivers, symptoms, and prevent further progression (Fig. 2). Given that inflammation is a key disease driver, and fibrosis is currently an irreversible pathological outcome, key goals are to control inflammation and to slow down, prevent, or eventually reverse fibrosis. These requirements facilitate both differentiation between individual therapies and their rational combination into individualized treatments. Translating these concepts to drug development requires distinct mechanistic approaches to therapy, specific preclinical models, different clinical development strategies, and diversified regulatory pathways to approval.

At least seven distinct pathways and corresponding mechanistic approaches to treatment can be identified (Table 2). Broadly speaking, metabolic dysfunction, glucose metabolism, and lipid metabolism are important as early drivers. These could all form the 'first hit'. Bile acid signaling, liver injury, and inflammatory processes have a role in disease maintenance and progression. Fibrosis is the key pathology of end-stage disease. We argue that the relevance of individual pathways and, therefore, the potential of corresponding target mechanisms to be efficacious in treating NASH differs, both between patients and according to the stage of disease. As such, therapeutic approaches can be more or less specific for a distinct pathway and/or mechanism and, consequently, stage of the disease.

Strategies aiming at the prevention or treatment of NASH address early and late disease and, therefore, are not limited to liver targeting and hepatic outcomes alone. There is a need for preclinical mechanistic models that reflect the pathogenesis and pathophysiology at different stages of human disease. Attempts to recapitulate human disease often involve the use of nonphysiological drivers [e.g., carbon tetrachloride (CCl_4), bile duct ligation, or a methionine-choline-deficient (MCD) diet], which can produce a liver pathology phenotypically similar to human NASH, but not reflect the human metabolic and systemic drivers. Genetic obesity models display steatosis and insulin resistance, but are largely protected from the development of inflammation and fibrosis. By contrast, high-calorie diet-driven models should in principle mirror the human pathology, but liver pathology in these models often does not resemble the human situation [18–20]. In the unlikely event that any model will recapitulate every stage of human disease, it is important that the predominant mechanisms are understood, and the use of the model aligned with the appropriate stage.

In clinical development, it is necessary to consider optimal positioning for each molecule to take into account (or advantage of) the target mechanism(s) with a dominant role at different disease stages (Fig. 2). The current frontrunning compounds, obeticholic acid (OCA) and elafibranor, target bile acid signaling and glucose metabolism, respectively. Both aim for a 'broad' center regulatory label with medium NAFLD Activity Score (NAS; i.e., scoring of at least 1 for each of inflammation, steatosis, and ballooning), and medium fibrosis scores (i.e., scoring of at least 1 but below 4). With more compounds targeting a variety of mechanisms, clinically meaningful differentiation will only be

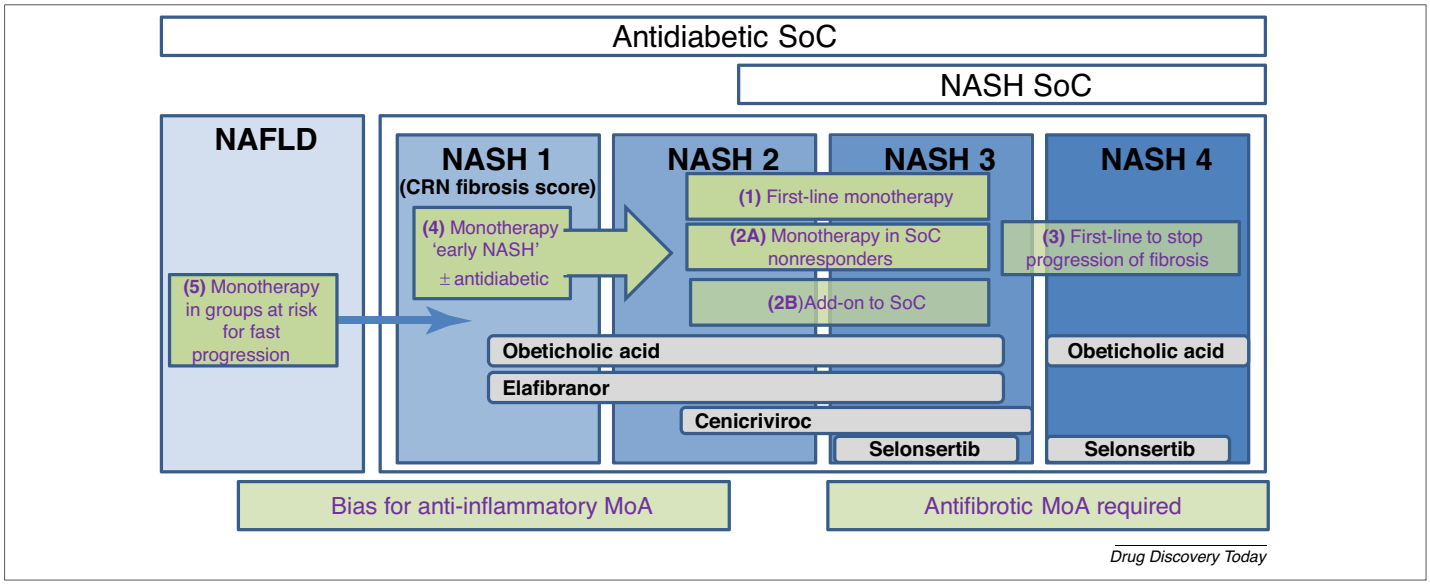

FIGURE 2

Potential disease landscape and drug positioning for nonalcoholic steatohepatitis (NASH) in 2020 (current understanding). Six potential positioning opportunities are illustrated, as follows: (1) Ideally a drug will have multiple mechanisms of action (MoAs; 'dirty drugs'); (2A) drug MoA should be different from standard of care [SoC; e.g., obeticholic acid (OCA) or elafibranor] with different MoAs required; (2B) MoA should be different from SoC and include antifibrotic activity. This is the only positioning where a single mechanism asset might be considered; (3) antifibrotic MoA required to stop progression, but have additional MoAs to reverse NASH; (4) MoA should include anti-inflammatory activity (±antifibrotic activity); (5) MoA should include anti-inflammatory activity (±antifibrotic activity).

possible if each compound is developed in its 'sweet spot'. This holds true, especially from a scientific view on differentiation, with the caveat that regulators and payers might require more specific data to recognize differentiation between larger patient populations potentially comprising niches where single compounds might show varying efficacy. Therefore, and as reflected in more recent trials (Table 1 and Fig. 2), it should also be directed at the population that is likely to show superior efficacy because of their specific disease stage (or a specific pathophysiological situation), and where the targeted pathway has a dominant role. Inevitably, this approach will lead to segmentation of the NASH patient population. It is likely to occur throughout the NASH patient journey, starting with healthy populations at risk of developing NASH all the way through to the fibrotic disease stage (albeit with some, especially early, segments potentially not providing worthwhile opportunities for drug development programs in the absence of biomarkers identifying 'at risk' populations). A second opportunity for segmentation will be available once approved drugs are on the market, creating patient populations that are nonresponders to any given drug. Both dimensions will require diagnostic markers to be developed to define these populations.

In line with nuanced strategies for clinical development, it can be expected that compounds launched later than 2025 will pursue different labels and, therefore, need to use adapted regulatory strategies. For these molecules, there is an increasing emphasis on pathways that either directly target inflammation and immune systems or liver fibrosis for late stage disease. In summary, an increased awareness and understanding of different drug development strategies in NASH is leading to novel commercial opportunities and, of course, risks.

TABLE 2

Pathways and target mechanisms in NASH

Pathway	Target mechanism (examples)
Metabolic dysfunction	Upstream regulators impacting lipid and glucose metabolism
Glucose homeostasis	Insulin sensitivity, glucose uptake, and metabolism
Lipid metabolism	Production, update, and recycling of lipids
Bile acid signalling	Lipid homeostasis, carbohydrate homeostasis, inflammatory, and immune pathways
Liver injury	Hepatocyte apoptosis, cell regeneration, and oxidative stress
Inflammation and immune system	Proinflammatory cells, soluble mediators, and cell adhesion
Fibrosis	Stellate cell activation, myofibroblast invasion and function, and extracellular matrix deposition and degradation

Biomarkers and development

The ultimate goal of NASH therapy is the prevention of fibrosis, which, in the absence of future potential therapies, is an irreversible pathology responsible for morbidity in patients. This should relate directly to defined outcomes of clinical trials (e.g., reduction of liver-related deaths, reduced incidence of hepatocellular carcinoma or reduced need for transplantation). These outcomes might also be relevant for pricing and reimbursement. However, in the context of clinical development, there are more nuanced approaches that will depend on the drug development stage [21,22].

Endpoints of the currently ongoing Phase 3 trials are based on the NAS and histological assessment of fibrosis in liver biopsies. With late-stage liver fibrosis being mostly irreversible and an established cause for liver-related mortality, it is expected that regulators will grant approval if drugs can stabilize or ideally reduce either disease activity (NAS), fibrotic stage, or both [23]. Currently, determination of NAS and liver fibrosis requires a liver biopsy and pathological assessment, introducing key challenges both for clinical trial conduct and disease staging (NAS and fibrosis grade). First, liver biopsies come with a procedure-related risk of adverse events [24]. Being an invasive procedure, biopsies impair patient recruitment and retention into trials [25]. Second, a liver biopsy allows histopathological assessment of only the tissue specimen extracted. given that the pathological processes in NASH can vary in activity in different parts of the liver, there is a risk that the specimen obtained for analysis is not representative of the disease stage [24]. Finally, while specific standardized guidelines exist on how to grade NAS and fibrosis stage, elaborate processes are often established at a specific site (e.g., multiple histopathological assessments of the same sample by different pathologists), adding cost and complexity to trials. Nevertheless, currently this is the only option to satisfy regulatory requirements.

There is more flexibility in earlier (Phase 2) clinical trials, where surrogate end points (biomarkers) can be used to establish efficacy. These can come with the risk of progressing compounds that will ultimately prove to be ineffective on biopsy-assessed outcomes. However, ongoing and future work might increase confidence in one or more of these surrogate biomarker outcomes and establish them as validated endpoints.

Three distinct types of desirable NASH biomarker, based on modality, can be distinguished: (i) functional biomarkers designed to assess liver function (arguably the important parameter). For example, this can be measured by the intake of a paracetamol premetabolite and assessment of metabolites in the exhaled air [26]. However, the usefulness of this method is limited because of the large functional reserve of the liver (i.e., functional liver assessments can give a normal result even if large parts of the liver have been damaged, e.g., by fibrotic remodeling); (ii) blood-borne biomarkers allowing the assessment of many aspects of NASH (e.g., metabolism, inflammation, liver health, or fibrosis). They have the advantage of easy acquisition and analysis [27]. However, their specificity and sensitivity might not yet be sufficiently high to generate the confidence required to progress. Also, this is a systemic assessment that could also reflect processes that are not NASH or even liver related. Markers of inflammation include leptin, adiponectin, C-reactive protein (CRP), interleukin-6 (IL-6), IL-8, tumor necrosis factor α (TNFα), monocyte chemotactic protein 1 (MCP1) levels, and cytokeratin 18 (CK18). Others, such as hyaluronic acid, leptin, laminin, tissue inhibitor of metalloproteinase 1 (TIMP-1), and fibronectin, are established as markers of fibrosis; and (iii) several imaging biomarkers are proposed [28,29]. Magnetic resonance imaging (MRI) diffusion can distinguish non-fibrotic liver (F0) from advanced fibrosis (F3, F4), but it does not distinguish between the intermediate stages of fibrosis (F1, F2) and has limitations in advanced liver disease. Conversely, Fibroscan stiffness can differentiate between early and intermediate (F0, F1, F2) and advanced (F3, F4) fibrosis, but has limitations in obese patients, patients with narrow intercostal spaces, congestive heart failure, and extrahepatic cholestasis. Typically, imaging biomarkers are reflecting local (tissue) changes. However, these non-invasive procedures come with potentially high set-up cost (e.g., Fibroscan or elastography) or are not easily accessible (e.g., MRI). Their key advantages are that they provide unique data, can, theoretically, be performed frequently, and give specific liver readouts. However, the relationship of their readouts to disease activity (NAS) or fibrosis is not yet fully clear, and neither are currently accepted in place of a biopsy.

Biomarkers could also be grouped according to which purpose they serve, especially in the context of clinical development. Groups include: (i) biomarkers indicating disease pathology and stage, both at time of initial diagnosis and during the course of the disease. These would allow assessment of therapeutic effectiveness, independently of the specific drug treatment; (ii) biomarkers indicating risk of progression to more advanced disease stages, or to fibrosis, or indicating the likelihood of remission. This type of biomarker could be used to stratify patients (e.g., selecting those at risk for progression). There is likely to be a different benefit versus risk assessment for drug therapy in these patients because they could respond better to the drug therapy under investigation; and (iii) mechanism-based biomarkers indicating a response to a specific drug, based on a defined mechanism. The effectiveness of a drug could be assessed before a definite change in disease stage occurs. This would allow for filtering of compounds in early clinical development, stopping ineffective drugs and only starting late-stage development for the most promising drug candidates.

Academic groups, independent laboratories, and pharmaceutical companies, as well as large public–private consortia are aiming to identify biomarkers that could facilitate the treatment of NASH and determine the efficacy of compounds in development. Examples of current studies in this area include the LITMUS consortium under the Innovative Medicines Initiative 2 in the EU, or as part of The Liver Forum/Collaborative Research in the USA.

Importantly, to maximize their usefulness for drug development, biomarkers in preclinical disease models should correlate to disease activity in the clinic. Serum biomarkers of collagen formation and degradation have been investigated [e.g., pro-collagen III N-terminal peptide (P3NP/Pro-C3) and MMP-9-mediated degradation of type III collagen (C3M)] [30]. Pro-C3 is a synthesis marker and C3M a resolution marker for Type III collagen, a major scar collagen that is deposited during fibrogenesis. Both markers have demonstrated potential as biomarkers related to disease progression in patients with liver fibrosis, and Pro-C3 levels have been shown to indicate active fibrogenesis and structural progression of fibrosis [31]. Therefore, it would make sense that the choice of preclinical disease models to investigate new therapies should be guided by the translatability of biomarkers. Some biomarkers could even be developable as an endpoint in early *in vitro* screening assays (e.g., fibroblast activation assay), providing an endpoint with potential to be used *in vitro, in vivo*, and in the clinic.

How would the availability of biomarkers change current or anticipated treatment paradigms and clinical development? First, liver biopsies would not be required, with surrogate biomarkers providing the necessary readouts for therapeutic utility or defining the initial diagnosis. Second, at-risk populations could be identified early during the course of the disease, leading to positive impacts on the benefit–risk assessment of drug therapy. Timely

confirmation of response to therapy would both streamline clinical development, enabling more robust and standardized trials with less patient burden, and reduced costs and timelines. Finally, response biomarkers would also support physicians in selecting the best treatment. One of the most significant benefits of a biomarker-led approach to NASH treatment would be the possibility to select: (i) the best drug (in terms of mode of action); for (ii) the specific pathophysiological drivers or mechanism; and (iii) for specific or defined patient groups.

However, two key questions remain. The first is what specifically would be required to validate a surrogate biomarker, and whether regulatory authorities would still additionally require 'hard endpoints' (i.e., liver biopsies, liver-related events, cirrhosis etc.) for full or unconditional approval of a new therapy, at least for a transition period. The second is whether biomarker-based endpoints are sufficient alone to support pricing at a level where pharmaceutical companies decide to risk investment. It is likely that acceptance will be at the end of a long process, requiring pharmaceutical companies, academics and regulatory authorities to work together to validate NASH biomarkers.

Combination therapies

With no approved standard of care (SoC) for NASH, coupled with the complexity of drug development in the indication, it could be considered premature to discuss combination therapy. However, an objective of this review is to imagine possible future treatment paradigms to guide on-going drug discovery and development in the field. Effective therapies in other fibrotic conditions, such as idiopathic pulmonary fibrosis (IPF) [32], are essentially combination therapies (i.e., fixed combinations targeting multiple mechanisms within a single molecule). It is recognized that the complexity of fibrosis, in settings such as IPF and NASH, means that targeting a single mechanism in isolation is unlikely to deliver the required level of efficacy. It is also clear from experience in IPF that, when one or more effective therapies emerge as SoC, new therapies face the increased hurdle of demonstrating clinically meaningful benefits as add-on to this SoC, without significant compromise of safety and tolerability. Availability of effective treatment(s) means that monotherapy development (active drug tested versus placebo), at least for an initial label, becomes difficult. As a result, the paradigm must shift to one of add-on (combination) of active plus SoC versus placebo plus SoC, with the attendant increased challenge this brings for clinical study size and design. What does this mean for (preclinical) drug discovery activities? It brings qualitative and quantitative guidance to rational selection of targets and molecules. The chosen mechanism of action (MoA) must be complementary to that of the SoC, and there can be no relaxation of potency, selectivity or drug metabolism and pharmacokinetics (DMPK) candidate selection criteria. However, there must be avoidance of additive or adjacent adverse effects or undesirable drug interactions. The safety and tolerability considerations will become more important moving from a rare fibrotic disease, such as IPF, to a more chronic and widespread disease, such as NASH. There is also the need to consider and assess the potential for broader interactions with drugs used for treating other conditions associated with patients with NASH.

How do the above considerations influence our thinking on potential combination therapies in NASH? The multifactorial nature of the disease has been discussed. This could offer a range of combination possibilities both within (e.g., combination of two antifibrotic mechanisms) and across (e.g., anti-inflammatory and antifibrotic) mechanisms. However, because of the temporal shift in key disease drivers as the disease progresses, certain combinations do not make practical sense and can be rejected (e.g., a mechanism targeting early inflammation plus that targeting late-stage fibrosis). To support a rational choice in the short-term, one should consider the likely future paradigm. Assuming it is likely that one or more therapies currently in Phase 3 (e.g., OCA or elafibranor) is successful, it will in turn mean that combination therapy (i.e., add-on to SoC) will be the initial route forward for subsequent new therapies [33,34].

Therefore, a new entity with a single MoA could be considered, but this would need to have a different mechanism to that of the future SoC to which it is to be added. Consequently, selection of targets and progression of associated programs now (in 2018) should prioritize those that are expected to deliver added benefit when added to either OCA or elafibranor (or other advanced programs). The ideal drug candidate would have properties allowing it to be combined with either agent, providing flexibility in anticipation of different treatment practices emerging.

As described earlier, the anticipated advances in the development of imaging and molecular biomarkers in NASH offer the possibility of identifying promising combinations early in clinical development. Analyses of shifts in biomarker profiles are already being deployed. For example, a recent proof of concept study [35] (https://clinicaltrials.gov/ct2/show/NCT02781584) explored combination of the apoptosis signal-regulating kinase 1 inhibitor, selonsertib, with either the FXR agonist, GS9674, or the acetyl-CoA carboxylase inhibitor, GS0976. This made use of the MRI proton density fat fraction technique to assess liver fat content, and several molecular biomarkers of fibrosis, including fractional synthesis rate of lumican. Importantly, this study was also preceded by a pharmacokinetic study [36] to confirm the absence of pharmacokinetic interactions between the combination components, to determine whether dose adjustments would be required.

In summary, it is crucial to consider the opportunities and challenges presented by potential combination therapies in NASH at an early stage, and to build this thinking into preclinical and early clinical evaluation and decision making.

Concluding remarks

It is clear that, as highlighted in Fig. 2, the emerging therapies for NASH will occupy different positions within the disease process. Equally, it is apparent that there will still be an unmet need and a requirement for therapies with different modes of action aligned to the stage of disease and with the ability to work in combination with emerging standards of care. This clearly represents a challenge to those working in or entering the field of NASH research. It also highlights a need for new approaches to basic research, target selection, preclinical models, biomarkers, patient stratification, clinical trial design, and other areas of drug development.

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