Review

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Breakthroughs in therapies for NASH and remaining challenges

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Summary

Initially a condition that received limited recognition and whose clinical impact was controversial, non-alcoholic steatohepatitis (NASH) has become a leading cause of chronic liver disease. Although there are no approved therapies, major breakthroughs, which will be reviewed here, have paved the way for future therapeutic successes. The unmet medical need in NASH is no longer disputed, and progress in the understanding of its pathogenesis has resulted in the identification of many pharmacological targets. Key surrogate outcomes for therapeutic trials are now accepted by regulatory agencies, thus creating a path for drug registration. A set of non-invasive measurements enabled early-stage trials to be conducted expeditiously, thus providing early indications on the biological and possibly clinical actions of therapeutic candidates. This generated efficacy results for a number of highly promising compounds that are now in late-stage development. Intense research aimed at further improving the assessment of histological endpoints and in developing non-invasive predictive biomarkers is underway. This will help improve the design and feasibility of successful trials, ultimately providing patients with therapeutic options that can change the course of the disease.

Keywords: steatohepatitis; fibrosis; randomized controlled trials; non-invasive diagnosis; digital pathology; artificial intelligence; trial end-points; diabetes; obesity; cirrhosis; hepatocellular carcinoma.

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Introduction

Once deadly, liver diseases are now to a large extent treatable. Decisive and often spectacular therapeutic advances over the past three decades have forever changed the fate of patients with liver diseases. The cure of HCV infection led to a sharp reduction in mortality due to chronic hepatitis C, and to a major prognostic improvement, even in patients treated at an advanced stage. Powerful antivirals can now control HBV replication and turn this severe disease into a stable, chronic, condition. Several lines of therapy for hepatocellular carcinoma (HCC) provide prolonged remission or non-negligible survival gains. Patients with cirrhosis are now kept alive longer as we better understand how to manage or prevent the most severe complications of this condition. When everything else fails, liver transplantation is the last chance for cure, and is now associated with acceptable patient quality of life and manageable side effects from lifelong immunosuppression. The time has come, in 2022, to add non-alcoholic steatohepatitis (NASH) to this list of breakthroughs in hepatology. Unfortunately, and largely due to the lack of approved treatments, therapeutic progress in NASH appears far less impressive than for other liver diseases. Herein, we would like to argue that, although less spectacular, the progress in the field of NASH has been significant and probably even decisive for future developments. We will describe the major advances that have made it possible to successfully test some highly promising drugs, and the remaining challenges that must be overcome in the near future.

Building the scaffold for therapeutic success

A fully recognised unmet therapeutic need

NASH is now recognised as a major cause of cirrhosis[1] and end-stage liver disease.[2] The impact on liver transplantation has been steadily increasing[3] and while Europe lags behind the US,[4] it is following a similar path.[5] Recognising the mortality and morbidity burden associated with NASH has been key in acknowledging the major unmet clinical need it presents, especially regarding patient identification and management. The early view of NASH as an "incidentaloma" no longer stands.[6–8] Particularly worrisome are reports that the increasing presence of non-alcoholic fatty liver disease (NAFLD) in adolescence precipitates the occurrence of end-stage liver disease much earlier in adulthood than classical descriptions of the disease have suggested.[9,10] While the epidemiological link between NAFLD and HCC is less well understood, two observations are cause for concern. One is that exposure to metabolic risk factors clearly increases the risk of HCC occurrence.[11,12] The other is that in patients with NAFLD, HCC can occur before the cirrhotic stage.[13,14] While in both cases the risk of HCC is low (and probably very low in non-cirrhotic NASH), the population attributable fraction might be considerable given

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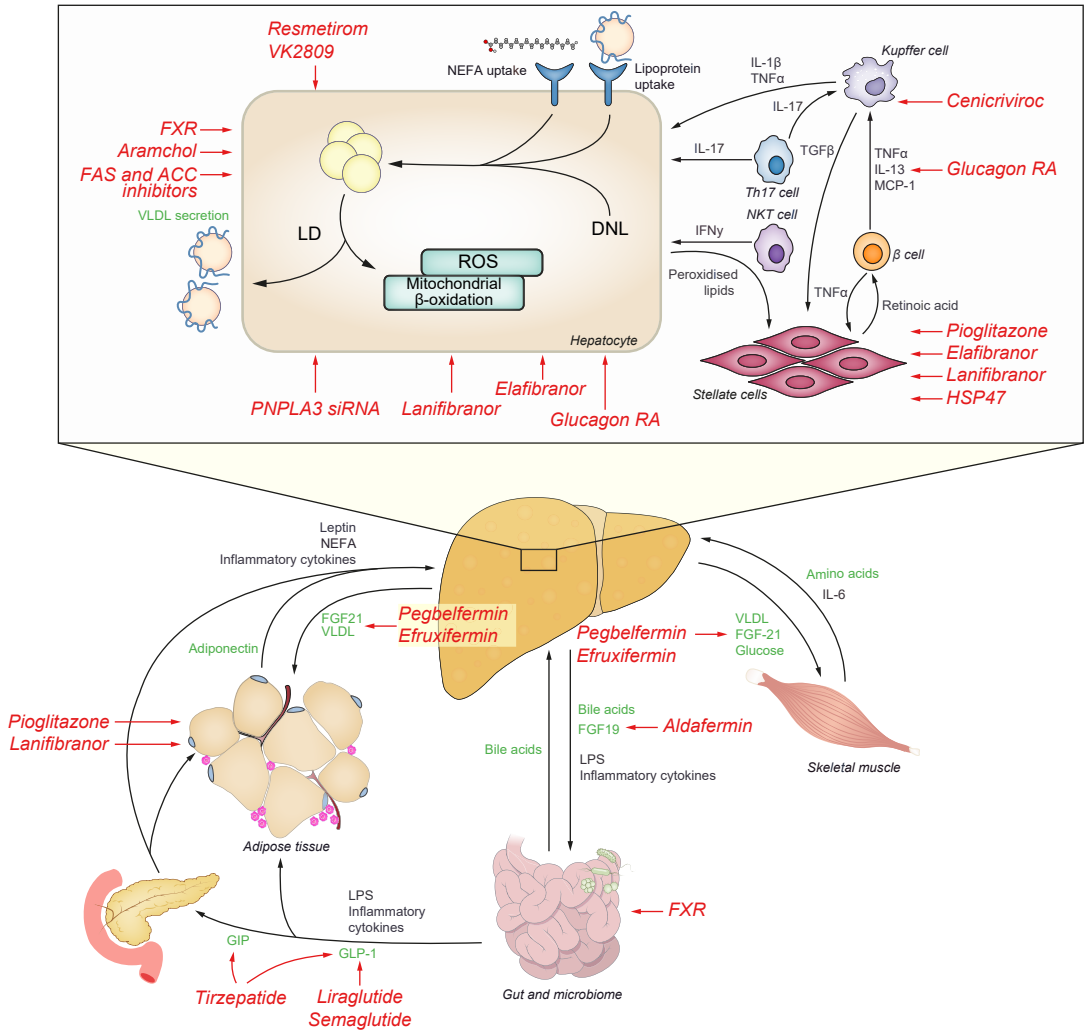

Fig. 1. Therapeutic targets in the complex pathophysiology of NASH. NASH is the result of a complex interplay of metabolic, inflammatory and fibrogenic processes. Within the liver, hepatocytes and several of their intracellular organelles, most notably mitochondria, play an important role, alongside stellate cells and several resident and infiltrating immune cells of different populations. Furthermore, NASH results from and impacts on an important crosstalk between the liver, the adipose tissue, the gut (including the gut microbiome), the muscle and the pancreas. The cardiovascular system is also involved (not depicted, see reference PMID: 2709179); Numerous mediators are involved. Drugs that have been tested in NASH or that are under development have differential targets inside and outside the liver, but ultimately aim to improve steatohepatitis and/or fibrosis. ACC, acetyl-CoA carboxylase; DNL, *de novo* lipogenesis; FASN, fatty acid synthase; FGF19, fibroblast growth factor 19; FGF21, fibroblast growth factor 21; FXR, farnesoid X receptor; GIP, glucose-dependent insulinotropic polypeptide; GLP-1, glucagon-like peptide 1; IFNγ, interferon-γ; IL-, interleukin-; LD, lipid droplets; LPS, lipopolysaccharide; MCP-1, monocyte chemoattractant protein 1; NEFA, non-esterified fatty acid; NKT cell, natural killer T cell; PNPLA3, patatin-like phospholipase domain-containing protein 3; RA, receptor agonist; ROS, reactive oxygen species; siRNA, small-interfering RNA; Th17, T helper 17 cell; TGF-β, tumour growth factor-β; TNF-α, tumour necrosis factor-α. Figure adapted from PMID: 26667070 (courtesy of J. Haas) and PMID: 30888594.

the increasing prevalence of NAFLD.[15] Increased healthcare utilisation and expenditure directed at people with metabolic fatty liver have been modelled[16] or derived from real-world data,[17,18] and show increased costs associated with NAFLD care. Interestingly, these data have been corroborated by studies of the impact of NAFLD on hospital admissions and death, specifically in diabetic populations. While in non-diabetic patients, alcohol-related liver disease was the leading cause of hospital admission, in diabetic patients, alcohol was the second and NAFLD the leading cause of admission.[19] It is a fact that a large proportion of patients with NAFLD do not or only occasionally drink alcohol.[20] But it is also true that the two entities, alcohol and metabolic fatty liver often coexist. At one extreme, in populations of heavy drinkers, obesity clearly increases the risk of cirrhosis.[21] At the other extreme, in the general population, the presence of metabolic risk factors such as diabetes, dyslipidaemia, insulin resistance or visceral obesity increase the risk of severe liver complications,[22] and this increase is often higher in people that drink moderate amounts of alcohol.[22]

From pathogenesis to druggable therapeutic targets

The acknowledgment of the potential clinical severity of NASH paved the way for myriad pathogenic studies aimed at identifying druggable targets (Fig. 1). Best understood is the metabolic root of the condition, in terms of its association with insulin resistance, adipose tissue dysfunction, lipid flux in the liver, d*e novo* lipogenesis and imbalance between energy intake and energy expenditure. This has generated multiple drug candidates that have been tested in patients with NASH, including: pioglitazone[23,24] or related-insulin sensitizers,[25] as well as dual or triple peroxisome proliferator activated receptor (PPAR) agonists[26–28]; glucagon-like peptide 1 (GLP-1) receptor agonists[29,30] and co-agonists with glucose-dependent insulinotropic polypeptide (GIP)[31,32] or glucagon agonists,[33] or even triple agonists[34]; fibroblast growth factor 21 analogues[35,36]; thyromimetics such as resmetirom[37]; and lipogenesis inhibitors, such as acetyl-CoA carboxylase,[38] stearoyl CoA desaturase 1,[39] fatty acid synthase[40] or diacylglycerol O-acyltransferase I[41] inhibitors. When such drugs induce massive weight loss, hepatic improvement (at least of steatohepatitis) has been documented.[30] When other drugs have additional, direct, hepatic effects, histological improvement is to be expected.[27,37] The key question is whether improving metabolic dysfunction and insulin sensitivity without inducing weight-loss[25,42] will be sufficient to trigger hepatic improvement, without targeted action at the site of liver injury. A second, highly diverse category of NASH drugs in development aim at controlling mechanisms of hepatic cell death and inflammation that are associated with lipotoxicity. Despite the appeal of directly targeting mechanisms of liver damage in steatohepatitis rather than the upstream cause, some of these have failed to demonstrate clear benefits, such as chemokine antagonists,[43,44] anti-apoptotics[45,46] or VAP1 (also known as AOC3) inhibitors (NCT04897594), or have been discontinued, such as c-Jun N-terminal kinase inhibitors (NCT04048876). Therefore, this approach raises questions regarding the efficacy of selectively inhibiting one pathway of injury alone. The overall contribution of a specific pathway may be modest, and compensatory mechanisms can attenuate a drug's effect.[47] Alternatively, pathway redundancy due to cross-reactivity between receptors from the same family and alternate ligands[48] may additionally limit the efficacy of a given compound. It is hoped that many compounds, such as farnesoid X receptor (FXR) agonists, PPAR agonists and ASK1 (apoptosis signal-regulating kinase 1) inhibitors have pluripotent effects; the efficacy of some of these compounds has been confirmed in phase II[27,49] or III trials.[50] Another category of potential NASH therapies are antifibrotic agents, particularly those that directly interfere with the fibrogenic process (rather than modulating fibrosis triggers). So far, most of these agents have failed, including simtuzumab,[51] belapectin[52] and emricasan.[46] One possible reason for failure is that they were tested in patients with cirrhosis,[45] who are at a very late stage in the fibrotic process. A more general concern may be that antifibrotic drugs with moderate – as opposed to strong – antifibrotic action may not be sufficient if steatohepatitis, the upstream driver of fibrogenesis, is not being controlled simultaneously.

There has clearly been tremendous progress in identifying the pathogenic mechanisms at play in fibrotic steatohepatitis.[53] This has led to a highly diverse spectrum of potential NASH agents, currently in clinical trials, in a field that is as vibrant as the one of HCV a decade ago. Some of the conceptual challenges for each class of agents, as outlined, could be solved by combining agents with pleiotropic modes of action or those that act at different levels of the disease process. While this holds theoretical promise, the choice of which molecules to combine will be critical, as early results have only generated tepid enthusiasm.[54,55]

Key point

Recognition of the unmet need for treatments for advanced NASH has helped define a regulatory framework for drug approval which allowed many therapeutic trials to be conducted.

A regulatory framework to guide therapeutic trials

The availability of candidate pharmacological agents and the flurry of NASH trials have provided the impetus for drug regulatory agencies to define a regulatory framework for drug approval in NASH[56] (Fig. 2). Several major advances have catalysed this activity. The first was the recognition of fibrotic NASH as a serious and life-threatening condition, thus justifying an accelerated approval pathway.[57] This allows a drug to be given conditional approval, while awaiting the evidence of clinical benefit required for definitive approval (Fig. 3). The rationale is to ensure faster patient access to potentially useful drugs in an area of unmet clinical need. The second major advance was the definition of surrogate endpoints for conditional approval: regression of fibrosis or resolution of NASH. These histological changes are achievable within a 12-18-month time-frame[30,50] and are therefore feasible within a trial context. Whether meeting these surrogate endpoints will result in clinical benefit has been questioned, though their use has been supported by regulatory agencies.[57] While no prospective demonstration is available, numerous retrospective studies have shown that fibrosis stage is associated with liver-related mortality and liver-related events,[58] while fibrosis stage reversal can even benefit patients with cirrhosis.[59] An important observation is that steatohepatitis itself increases the risk of liver-related events more than steatosis alone, even in the absence of fibrosis.[60] Moreover, changes in steatohepatitis status[61] (and, more widely speaking, in activity grade[62]) are positively associated with changes in fibrosis: improvement in

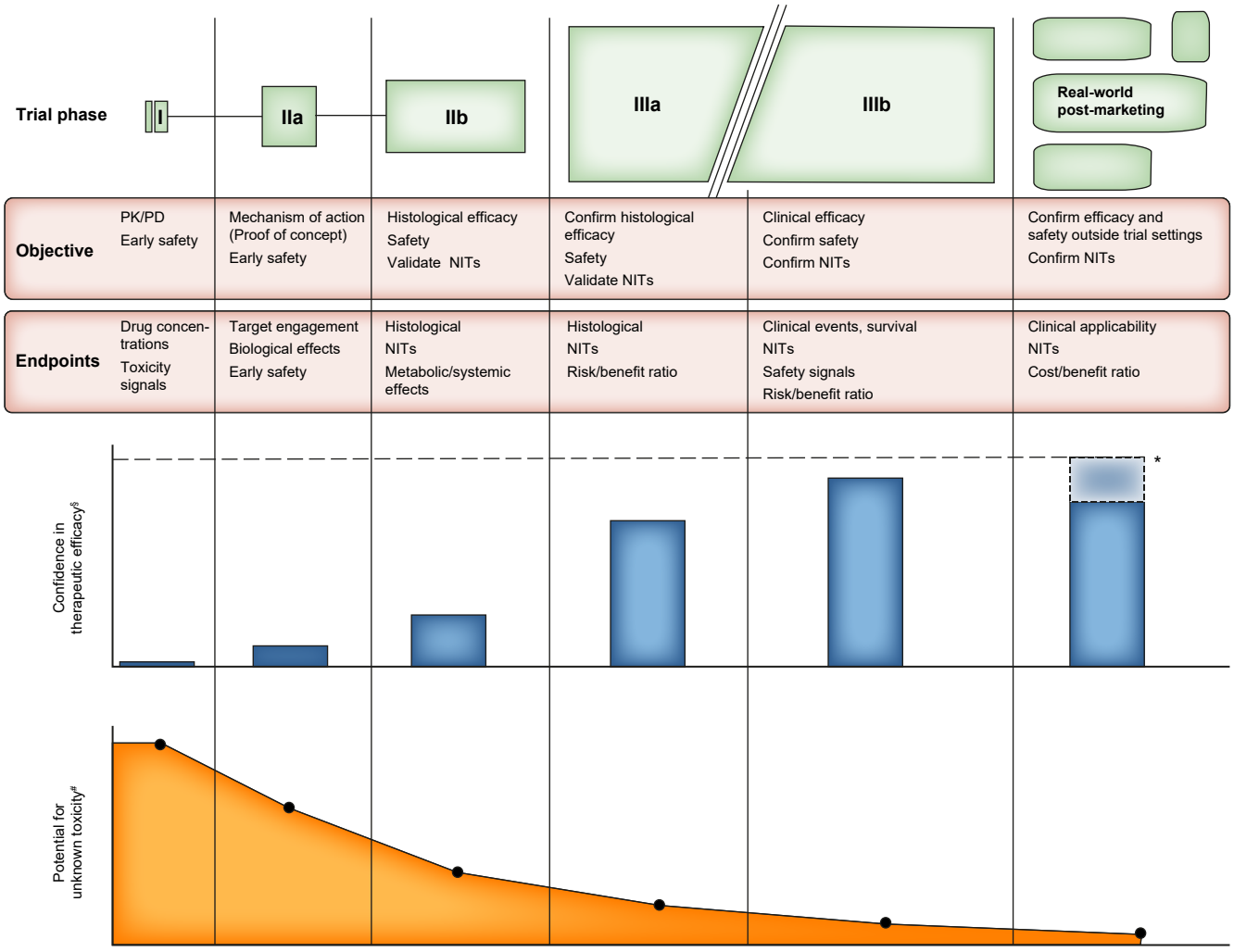

Fig. 2. Objectives and outcomes of sequential therapeutic trials for newly developed NASH compounds. §Level of confidence in efficacy of drug based on observed data; dashed line represents maximum confidence. *Variability due to differences in real-world settings. #Relative scale, level of x-axis represents zero-risk, bullet points represent risk at beginning of each respective trial phase. NASH, non-alcoholic steatohepatitis; NIT, non-invasive test; PD, pharmacodynamics; PK, pharmacokinetics.

Key point

Considerable progress in identifying pathways of injury resulted in the discovery of numerous pharmacological targets with several in-class compounds for a few of them.

fibrosis stage is often seen in patients with improved or resolved steatohepatitis, while worsening of steatohepatitis leads to worsening of fibrosis stage. This has been shown both in studies of natural history and pharmacological intervention trials[61,63] and is supported by a strong set of experimental data.[64] Thus, the chosen surrogates seem appropriate because they are achievable and have prognostic value. There are, however, caveats. Requiring complete NASH resolution could be unnecessarily strict given the aforementioned relationship between changes in activity and changes in fibrosis. More importantly, documenting the disappearance of steatohepatitis, as defined by the absence of ballooned hepatocytes, can be very challenging, as even expert pathologists have difficulty assessing hepatocyte ballooning.[65] Finally, there are differences between European and American regulatory agencies regarding which combination of surrogate histological endpoints are acceptable.[66]

A third important advance in the regulatory framework was the definition of the clinical benefit required for definitive approval (Fig. 3). This is typically tested in large long-term outcome trials, some of which are underway.[67] The regulators agreed that reducing the rate of progression to cirrhosis should be seen as a hard-clinical outcome, along with mortality, liver transplantation and the occurrence of cirrhotic complications. While clinical events may have a very low annual incidence, particularly in trials including patients with stage 2 fibrosis, histological progression to cirrhosis certainly occurs at a much higher rate, which increases the probability of success. Thus, defining efficacy endpoints that are achievable within the timeframe of a phase III trial enabled the development of a regulatory framework for drug approval in NASH. However, the final decision relies not only on efficacy parameters but also on a complex assessment of the risk-benefit balance in the wider context of competing comorbidities in

patients with NASH. This seems reasonable given that the chronic nature of the illness requires long-term therapy. However, it is the authors' opinion that NASH drugs should aim primarily to improve the liver disease; any added extrahepatic clinical benefit should not be seen as mandatory.

Non-invasive read-outs for early trial success

With a recognised indication, many pharmacological candidates ready to enter the clinic and a well-defined registrational path, the remaining obstacle was the selection of the best agents to be tested in humans. This is typically studied in early-phase trials, including proof-of concept trials. For obvious reasons this selection could not be made on histological grounds. The slowly evolving nature of histological lesions, uncertainties around pathological assessment, and short treatment durations rendering repetition of biopsies largely unacceptable for trial participants, all argue for alternative ways of assessing potential efficacy. Moreover, large sample sizes would be necessary, given sampling variability and non-quantitative histological scales. Therefore, the use of non-invasive biomarkers in early development was another area of recent and significant progress. Imaging studies, in particular a precise and quantitative measurement of liver fat content by MRI, have enabled the assessment of the anti-steatogenic effect of drugs[68] (Fig. 4). In some studies, a 30% decrease in MRI-proton-density fat fraction (PDFF) correlated with NASH resolution.[68] While this parameter may not be predictive for all mechanisms of action,[69] it clearly has value for some drugs in which the magnitude of liver fat reduction is loosely proportional to the rate of NASH resolution.[70] Corrected T1, another MRI parameter that increases with hepatic inflammation and fibrosis,[71,72] is being increasingly used as an early indicator of histological improvement (Fig. 5). cT1 could be predictive of clinical outcomes,[73] although more data are needed, particularly to understand the value of short-term changes, like those seen in early trials. Many metabolic parameters assessing glucose homeostasis, lipid metabolism, hepatic lipogenesis, insulin resistance and systemic inflammation are being used to confirm the biological actions of NASH drugs in early-phase trials. A list of biomarkers, albeit shorter, also exists for the early assessment of hepatic anti-inflammatory activity: aminotransferases,[74] gamma glutamyltransferase[75] and cytokeratin-18 and possibly cT1. The anti-fibrotic effect, on the other hand, is more difficult to measure. Despite major advances in measuring liver stiffness through operator dependent and independent technologies (such as magnetic resonance elastography, Fig. 4)[76] and despite the availability of many serum-based biomarkers[77] there are still gaps in understanding how sensitive and specific to change these biomarkers are and whether early changes relate to a genuine antifibrotic effect. Nonetheless, the availability of numerous and diverse biomarkers in early-phase trials has led to tremendous progress in the transition of new drugs to the clinical trial arena over the past decade.

Feasibility of NASH trials: a clear, empirical demonstration

Last but not least, a major concern was that large clinical trials requiring liver biopsy for selection and repeat biopsies to assess efficacy would not be feasible. Despite a high screening failure rate, at least 5 large phase III trials are now fully enrolled. Long-term trial retention seems to be good as well. The demonstration of feasibility of these late-phase NASH trials clearly removes a major roadblock towards the final accessibility of NASH therapies.

Promising agents and encouraging trial results

Over the past 15 years, many compounds have been tested as potential therapies for NASH. While some have failed, others are currently in development, with new targets and approaches holding clear promise (Fig. 1). Herein, we will briefly review the major advances in classes of drugs that are already in phase III registrational trials. A more comprehensive discussion of compounds in development is available elsewhere.[78]

FXR agonists

FXR plays an important role in bile acid metabolism but also in metabolic, inflammatory and fibrogenic pathways.[79] Obeticholic acid (OCA) is a first-in-class, potent and selective FXR agonist which initially demonstrated an insulin-sensitising effect in patients with type 2 diabetes.[80] In two NASH studies,[49,50] OCA at 25 mg daily was significantly more likely to induce histological regression of fibrosis than placebo. The remarkable aspect of this result is that the efficacy seen in the smaller phase II trial was confirmed in a large, international, phase III trial.[49] This is, for the moment, a unique example of such consistency, as for many other compounds, subsequent registration trials have failed to confirm the optimistic preliminary results from earlier trials.[26,43,81–83] In the FLINT trial,[49] OCA also induced resolution of NASH at a higher rate than placebo, which was not confirmed at the interim analysis of the REGENERATE trial.[50] However there probably is a real effect of OCA on steatohepatitis since the drug reduces hepatocyte ballooning and inflammation[50] – the two main histological lesions defining steatohepatitis. When considering the whole randomised population with NASH and fibrosis (stages 1 to 3), OCA led to resolution of steatohepatitis as it did when using an overall pathological definition rather than a score-based definition.[50] OCA is now being tested in a large phase III trial of patients with NASH cirrhosis, an indication supported by experimental

data showing that besides their antifibrotic effect,[84] FXR agonists may modulate factors that determine complications of cirrhosis such as portal hypertension and bacterial translocation.[85–87] If OCA does indeed induce histological improvement in NASH, it is surprising that another FXR agonist, tropifexor, failed to elicit histological improvement on conventional histology.[88] Other than the obvious explanation of different lengths of therapy (12 *vs.* 18 months) or statistical power, the reason for these discordant results is unknown.

Key point

An FXR agonist was the first to successfully complete a phase III trial; thyromimetics, GLP1-receptor agonists and other compounds in late development have demonstrated highly promising clinical results.

Since OCA induces side effects such as pruritus and increases in LDL, many second generation FXR agonists have been developed on the premise that a non-bile acid pharmacological structure may alleviate some of these effects.[88–92] While this seems to be true for LDL increases, pruritus is clearly a class effect with a quite obvious dose-response relationship. It remains to be seen if these new FXR agonists can maintain histological efficacy at doses that minimise the occurrence of pruritus.

Thyromimetics

The use of thyromimetics to treat NASH derives from both epidemiological and biological arguments. Subclinical hypothyroidism has been documented in cohort studies of patients with metabolic steatosis.[93,94] In the liver, an imbalance of deiodinase activity favours the synthesis of inactive thyroid hormone T3 thus leading to a state of cellular hypothyroidism.[95] Thyroid hormones have many beneficial functions, such as inducing lipophagy and mitochondrial biogenesis which contributes to the removal of liver fat. However, a NASH drug would need to be highly selective for the beta isoform of the thyroid hormone receptor in order to avoid unwanted extrahepatic side effects. Resmetirom is the first such oral, liver-directed THR-β1-selective agonist demonstrating marked effects on lipid parameters.[96] In a phase II trial, resmetirom had a potent anti-steatogenic effect and improved atherogenic dyslipidaemia in patients with NASH, while weight and glycaemic parameters were unaffected.[37] NASH resolution was achieved in a subset of patients with control liver biopsies, although a much larger trial would be required to observe an ensuing effect on liver fibrosis by conventional histology. Such a registrational trial is underway (MAESTRO-NASH, NCT03900429) as well as two other phase III trials (NCT04951219, NCT04197479). The very good tolerability profile, if confirmed, will certainly be a major asset of this promising drug. Another compound with liver-specific thyromimetic properties, VK2809, is currently under investigation (NCT04173065).

Incretins and other metabolic hormones

Another highly promising approach is related to incretins and other hormones that are mainly known to handle body energy homeostasis and hence regulate glucose and lipid metabolism. GLP-1 is secreted by intestinal L-cells after exposure to nutrients. This short-lived hormone stimulates insulin secretion and impacts on satiety by acting on the central nervous system and slowing down gastric emptying and intestinal transit.[97] GLP-1 receptor agonists are indicated for obesity and type 2 diabetes and have also demonstrated cardiovascular[98] and renal[99] protective effects. Two trials in NASH have already been completed[29,30] and semaglutide, the leading compound in this class, not only leads to metabolic improvement, but also improves features of steatohepatitis. Despite these positive results, two important questions remain unanswered: the first relates to the surprising lack of fibrosis reversal despite massive weight loss, high proportion of steatohepatitis resolution and a trial duration of 18 months,[30] identical to the REGENERATE trial at the interim analysis.[50] The second one is whether all hepatic effects are mediated through weight loss or whether there are weight loss-independent effects. The lack of hepatic receptors for GLP-1 suggests that the major effects are exerted centrally and possibly through actions on adipose tissue, as studies of the acute effects of GLP-1 receptor agonists have shown.[100]

Several dual or even triple agonists are in development, associating GLP-1 with GIP agonism (tirzepatide[101]) or GLP-1 with glucagon agonism (cotadutide[33,102]), or even triple agonists.[34] Combinations of GLP-1 receptor agonists and long-acting amylin analogues[103] are being tested as well. Trials of these compounds in NASH are underway and combinations of incretins seem to induce weight loss of an even higher magnitude than GLP-1 receptor agonists alone.[104]

PPAR agonists

PPARs have pleiotropic actions as critical regulators of fatty acid metabolism, glucose metabolism, inflammation and fibrogenesis.[105] Three PPAR isotypes have been identified – α, β/δ and γ[106] – the expression and actions of which differ according to organ and intra-organ cell-type, resulting in a complex system of nuclear receptor-mediated inter-organ crosstalk.[107]

Mono PPARα agonists are ineffective in NASH and selective PPARδ agonists, such as seladelpar,[108] are currently being developed for primary biliary cholangitis[109] and tested in NASH.[110] Pioglitazone, a PPARγ agonist is associated with a broad spectrum of metabolic effects resulting from the restoration of adipose tissue biology[111,112] and a decrease in chronic systemic inflammation.[113,114] In patients with NASH, these changes are associated with improvements in liver histology.[115] There are now several phase IIb trials assessing the histological efficacy of pioglitazone, all however stopping short of a robust demonstration of fibrosis

reversal.[23,24,116,117] It is important to note that a formal demonstration of pioglitazone's histological efficacy in a large phase III trial is not available. Well-known side effects of pioglitazone[118] – *e.g.* weight gain, fluid retention and bone loss[119] with risk of fractures – have reduced enthusiasm around its long-term use in NASH. Several attempts to dissociate the metabolic effects of thiazolidinediones from their unwanted side effects have been undertaken,[120–123] though the histological benefit of this approach is yet to be proven.[25]

Possibly a more efficient attempt to mitigate the side effects of thiazolidinediones would be the induction of combined PPAR agonism using dual or triple agonists. The most succesful story so far has been with lanifibranor,[124] a pan-PPAR agonist with a higher potency for improving experimental NASH than individual PPAR agonists.[125] A phase IIb trial has shown improvement across the whole range of histological lesions, including, remarkably, a higher proportion of patients reaching both resolution of steatohepatitis and fibrosis improvement.[27] Saroglitazar, a dual α-γ agonist in development for primary biliary cholangitis[126] and approved in India for NASH, has also shown promising results in Western trials.[127,128] Meanwhile, a phase III trial of elafibranor, a dual β/δ agonist also in development for primary biliary cholangitis,[129] did not confirm the earlier positive results[26] in NASH.

Lipogenesis inhibitors

De novo lipogenesis is an important source of liver fat in patients with NAFLD[130,131] especially under dietary stress from high-fructose intake.[132,133] As mentioned, several lipogenesis inhibitors are in development[38,40,41] with one of them, aramchol, already in a phase III trial (NCT04104321). Aramchol is a fatty acid/bile acid conjugate which is a partial inhibitor of hepatic steroyl-CoA desaturase-1,[134–136] a rate-limiting enzyme in the biosynthesis of monounsaturated fatty acids[137] which regulates body adiposity, energy expenditure, fatty acid β-oxidation in liver[138] and insulin sensitivity.[139] In experimental models, aramchol improved inflammation, oxidative stress and fibrosis.[134,136] Phase II studies in humans have confirmed the reduction in liver fat[39,140] and indicated histological improvements in steatohepatitis and fibrosis.[39]

Remaining challenges and opportunities

For all the progress in understanding the clinical burden of NASH and ensuing efforts to develop pharmacological therapies, many grey areas remain. Some of these may explain failures of recent trials. Heterogeneity of the disease is probably real, but a clear way to classify patients into separate groups that might benefit from distinct therapeutic approaches is still elusive.[141] No data yet exist demonstrating that different genetic polymorphisms condition response to therapy.[142] However, recent studies have suggested that among patients with NAFLD, categories of genetic NAFLD and metabolic NAFLD have strikingly different pathogenic mechanisms, with only the latter relying on insulin resistance[143] – one of the main targets of drugs currently in development. Another caveat is that we are largely unable to predict which patients will progress clinically. Trials with similar inclusion criteria and with identical follow-up reported markedly different rates of clinical progression.[51,144] This speaks to the inability of semiquantitative histological staging

Key point

Early proof-of-concept trials have greatly benefited from imaging and blood-based biomarkers while the feasibility of large, late-stage trials, has been empirically demonstrated.

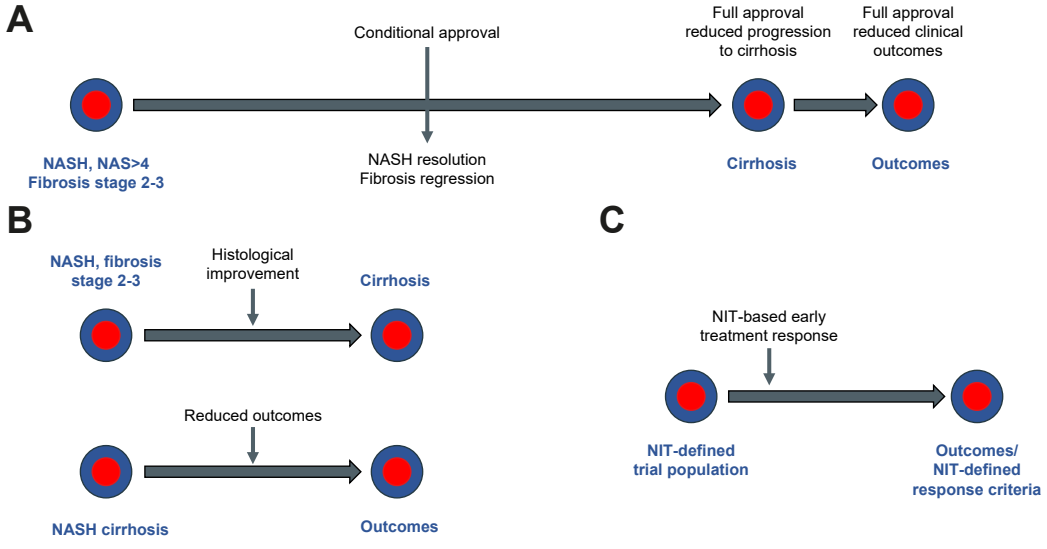

Fig. 3. Current and possible future regulatory requirements for approval of NASH medications. (A) Traditional histological efficacy and longer-term clinical outcome single trial in a population of non-cirrhotic NASH. (B) Alternative pathway of two trials of shorter duration in non-cirrhotic and cirrhotic populations. (C) Pending qualification of future trials based on NITs may eliminate the need for liver biopsy at inclusion and histological changes as an outcome. NAS, non-alcoholic fatty liver disease activity score; NASH, non-alcoholic steatohepatitis; NIT, non-invasive test.

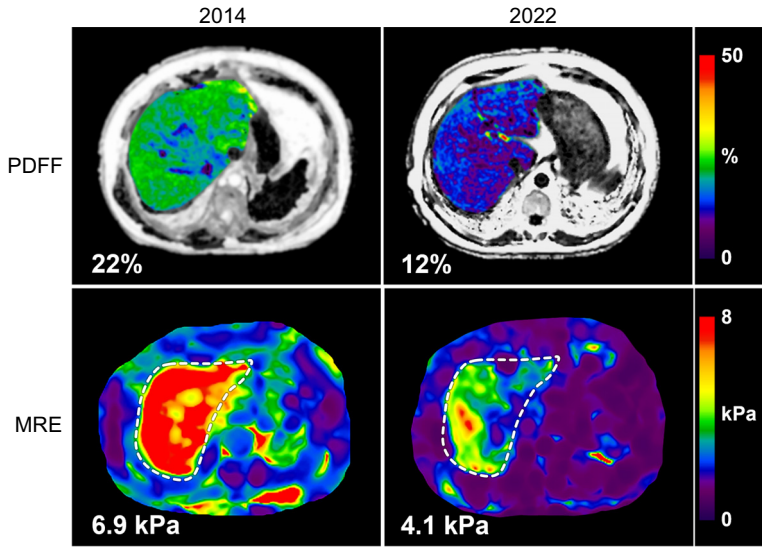

Fig. 4. Hepatic improvement in a 63-year-old man, diagnosed in 2014 with type 2 diabetes, obesity and hyperlipidemia, after antidiabetic therapy and successful implementation of dietary and lifestyle changes. Improvement documented by MRI for steatosis (PDFF row) and fibrosis (MRE row). Images courtesy of Dr. Richard Ehman and Dr. Meng Yin, Mayo Clinic, Rochester, MN, USA. MRE, magnetic resonance elastography; PDFF, proton-density fat fraction.

systems to optimally stratify patients at risk of disease progression. Some reports suggest that the area of fibrosis (as opposed to the fibrosis stage) may be a better indicator of future liver-related events.[144] Recent developments in the biomarker field could compensate for the lack of prognostic information provided by liver biopsy.

Innovations in the assessment of NAFLD in clinical trials and practice

Key point

The demonstration of histological improvement will be expanded and strengthened by digital image quantification and artificial intelligence-based assessment of parameters of fibrosis and inflammation, currently not measured by conventional pathology.

The development of knowledge regarding NAFLD has been anchored on histological assessment of liver biopsy specimens. However, this approach has many limitations that render it unsuitable for translation into routine clinical practice.[145] Perhaps the greatest limitation is the relative imprecision secondary to sampling variability, both intra- and inter-observer variability, in the assessment of histological findings that even limit their utility as research tools to establish the value of specific therapeutics.[62] These limitations have prompted intense efforts to develop and validate non-invasive tests (NITs) both for use in clinical trials and for routine clinical management of NAFLD.

Improving histological assessment of NASH in clinical trials

The limitations of subjective histological assessment using conventional scoring systems have spurred efforts to improve the repeatability and reliability of histological assessment of therapeutic benefits in clinical trials. These efforts have focused on both unique imaging methodologies and machine-learning approaches to enhance the precision and reproducibility of results. However, none of them are approved for this purpose and their use remains experimental.

One approach involved machine-learning-based identification of steatosis, ballooning, inflammation and fibrosis from annotated histological sections using conventional scoring systems.[54] The resulting ability to diagnose steatohepatitis was comparable to reading by well-trained hepato-pathologists. The fibrosis scores used by this system also correlated well with conventional staging. The limitations of these approaches remain the use of conventional scoring systems with their own drawbacks and the training of machines on findings annotated by conventional systems. Nevertheless, this approach is being applied in several trials to assist pathologists and enhance the reproducibility of results.

Second-harmonic generated images on unstained paraffin-embedded sections have been used to measure quantifiable collagen fibrillar parameters (qFP)[146] and develop a continuous fibrosis staging system with a vastly expanded dynamic range.[147] This has been adapted for commercial use and a qFP-based score for fibrosis has been validated in independent cohorts with NASH.[148] This method further varies from conventional reading by providing the score for central vein, peri-central (zone III), zone II sinusoidal, periportal (zone I) and portal areas (Fig. 6). Recently, additional parameters including septal areas, septal width and septal cellularity have been added to the evaluation. In a recently concluded clinical trial of tropifexor for NASH, differential movement of fibrosis was noted in different regions following therapy.[149] Further features of progressive *vs.* regressive fibrosis were identified which allowed for re-allocation of many individuals who did not have a full stage change in fibrosis to progressed and regressed categories. Other innovations using high-resolution scanning of conventionally stained slides have also been developed, with the advantage that the same histological section as the one reviewed by the pathologist undergoes machine-learning analysis of collagen properties or image-analysis quantification of histological activity (Fig. 7). These tools will enable early detection of changes in fibrosis and may be useful in phase II trials, where go-no go decisions have to be taken to move to more advanced trials.

Other histological biomarkers are also in development. In a landmark study, a group of pathologists annotated all ballooned cells in a set of liver biopsies.[65] While the majority identified biopsies with ballooned cells, there was only one cell that was identified by all pathologists to be ballooned. This has important implications for the field, which include the use of machine-learning-based identification of ballooned cells[150] as an aid to conventional histological reading. It also raises the potential for quantitative assessment of

ballooning instead of the current scoring system with its limited dynamic range. If validated, this may allow for the assessment of ballooning along an expanded dynamic range. Similar approaches to quantify steatosis are also being developed.[151] Sonic hedgehog staining is also in development as an independent histological biomarker linking activity to fibrosis. Overall, this remains a very active area of research and is likely to evolve over the next few years.

Use of NITs in clinical trials

The specific clinical purpose served by a NIT can include its use as a risk factor and as a diagnostic, prognostic, disease monitoring, predictive or treatment response assessment tool.[152] The evidence base required to support the full regulatory approval of a NIT for a specific intended use is thus quite substantial and unfortunately most NITs do not yet have the evidence base to support their qualification to completely replace histology for the assessment of therapeutic benefits in clinical trials. Two major efforts, the LITMUS and the NIMBLE initiatives[153,154] are currently attempting to bridge these data gaps and are expected to lead to qualification of both imaging and circulating biomarkers for NAFLD.

Despite the data gaps in the field, substantial evidence has also been generated regarding the use of NITs in NAFLD. From a diagnostic perspective, the key elements needed for clinical decision making include an assessment of disease activity and fibrosis. Steatosis, hepatocellular injury and ballooning have been proposed as markers of activity [61,155]; however, the lack of correlations between these markers and mortality have led to development of alternate paradigms where steatosis severity is considered separately from inflammation and ballooning injury.[156] MRI-PDFF has emerged as a powerful and well-validated measure of total hepatic triglyceride content which correlates with histological scoring of steatosis severity (Fig. 4).[157] The continuous attenuation parameter (CAP) obtained during vibration-controlled transient elastography (VCTE) represents another commonly available tool to evaluate the presence of pathological steatosis[158] but does not distinguish the grades of steatosis with great accuracy.[159] A new approach to the measurement of CAP, the continuous CAP measure has been introduced and high-quality data are awaited to see if this can provide better quantification of the steatosis burden. Other measures, such as the fatty liver index,[160] lack the sensitivity and specificity to be useful in the clinical trial setting.[161] Aspartate aminotransferase and alanine aminotransferase are conventional measures of liver injury but do not correlate well with ballooning or lobular inflammation.

Several simple laboratory-aids for assessment of fibrosis severity exist. Of these, fibrosis-4 (FIB-4), aspartate aminotransferase-to-platelet ratio index (APRI) and NAFLD fibrosis score are the most extensively validated tools. FIB-4 and the APRI were developed as tools to identify advanced fibrosis (stage 3 or higher); however, it is known that NASH with stage 2 fibrosis is associated with a higher rate of liver-related outcomes and death.[58,162] It is therefore relevant to consider the use of NITs to identify those with NASH, high activity and stage 2 or higher fibrosis, which is also referred to as "at-risk" NASH.[154]

The NIS-4 test, based on circulating levels of mir34a, alpha-2 macroglobulin, haemoglobin A1C and YKL-40, has been validated both in those with and without type 2 diabetes to identify those with "at-risk" NASH within a population of patients with risk factors for NASH.[163] In recent studies it also outperformed both alanine aminotransferase for the diagnosis of NASH and FIB-4 for the identification of stage 2 or higher fibrosis.[164] Fibrometer-VCTE is another NIT which utilises the liver stiffness measurement from VCTE and laboratory markers for its generation; it is superior to FIB-4 for the diagnosis of fibrosis stage 2 or greater.[164] The enhanced liver fibrosis test (ELF test) also has similar performance.[164] This raises the possibility that, in the future, the "to be treated" population of patients for clinical trials can be identified using these tools, with or without MRI-PDFF or CAP.

A key goal in the design of trial populations is to identify those at risk of outcomes so that the impact of therapy is identifiable quickly. This requires measures of risk assessment or prognosis. Liver histology is a surrogate endpoint where the fibrosis stage is the best correlate of clinical outcomes.[58,162] The ELF test has recently been approved as a prognostic biomarker, with those with an ELF value >11.3 considered to be at high risk of liver-related outcomes. Population-based studies have also shown a link between the FIB-4 score and mortality and liver outcomes.[165] MRI-PDFF and liver stiffness measurement by VCTE have also been shown to provide prognostic

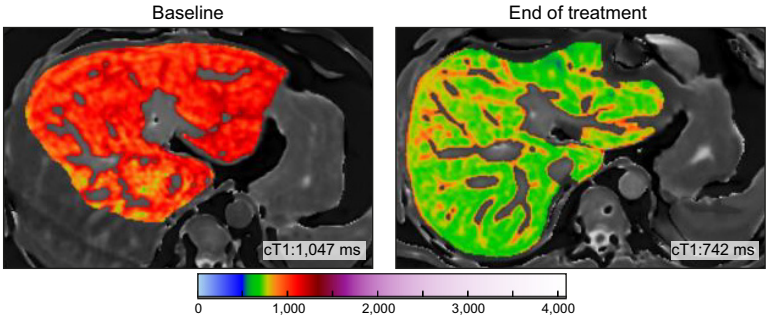

Fig. 5. Changes in Liver cT1 after a 3 month very low-calorie diet (800 calories per day) intervention in a patient with fibrotic NASH. Maps produced using LiverMultiscan™. The inserts show median cT1 values. Images courtesy of Dr Michael Pavlides and Dr Dimitrios Koutoukidis, Oxford NIHR Biomedical Research Centre, University of Oxford, Oxford, United Kingdom. NASH, non-alcoholic steatohepatitis.

Key point

Further validation of non-invasive diagnoses is expected to select patients for therapy, measure treatment effect in trials and guide future management of NASH in clinical practice.

information in NAFLD.[166,167] These tools, in conjunction with the diagnostic tests mentioned earlier, can be used to identify the population of interest in clinical trials. Specifically, the current data make it feasible to identify those with "at-risk" NASH and those with NASH and advanced fibrosis or cirrhosis for specifically targeted therapy.[168] It is anticipated that once these NITs are validated for these specific purposes, the field will pivot to their use to define trial populations in NASH.

Predictive biomarkers are those that identify individuals who will either respond to a specific treatment or not. These are critically important in order to avoid unnecessary exposure to drugs in those who are unlikely to benefit and to match patients with drugs that will work for them. Unfortunately, this remains a relatively underdeveloped area and a major unmet need in the field.

Another key need for NITs is to identify therapeutic response. While many NITs have been assessed as static markers of fibrosis burden, their sensitivity to change and the precise relationship of changes in biomarker values to changes in fibrosis stage is not established for almost any of them. The FIB-4 index has been shown to be sensitive to change in both directions but is not sufficiently sensitive to be clinically useful for this purpose.[169] The analysis of the REGENERATE phase III trial of OCA contributed key data in the effort to assess drug efficacy through non-invasive methods rather than through liver biopsy.[170] First, it reproduced the demonstration of the efficacy of the active drug over placebo by documenting improvements in NITs in the active arm but not in the placebo arm. Second, it showed that NIT changes tracked histological changes since histological responders were also NIT responders. Also, in a recent study of over 1,000 patients with cirrhosis who had liver biopsies a year apart, a subset was noted to have regression of fibrosis stage.[59] This was associated with concomitant improvement in several NITs and a decrease in risk of clinical outcomes. Additional data are now needed to further confirm that changes in a NIT not only correlate with changes in activity and fibrosis, depending on their use in a trial setting, but also with clinical outcomes in the long-term. Such data will be needed across multiple mechanisms of action to establish a surrogate endpoint for clinical trials that will allow for accelerated trials in a population defined non-invasively based on the diagnostic and prognostic utility of specific NITs.

Adequacy of success in clinical trials vs. its effectiveness

It is well known that the effectiveness of drugs is often poorer than the efficacy noted in clinical trials. Many factors contribute to this including the treatment of patients who were excluded from the trial, varying comorbidity profiles, compliance and the potential for drug-drug interactions not obvious in trial settings. There is an ethical and regulatory imperative to generate real-world data on the effectiveness of drugs approved based on the evidence generated in strictly controlled trial settings. Several real-world cohorts are currently being developed for this purpose. There have been remarkable advances in how controlled data can be

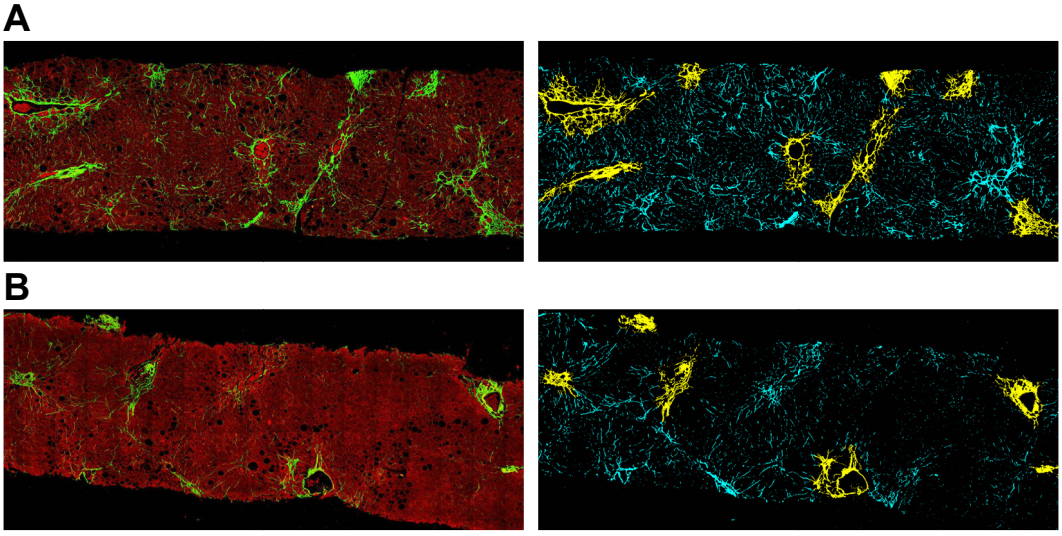

Fig. 6. Second harmonic generation/two-photon excitation fluorescence imaging of unstained slides by dual-photon microscope. Imaging shows a major reduction in perisinusoidal fibrosis and a lesser reduction in periportal fibrosis despite identical fibrosis staging by the NASH CRN classification (stage 2), (A) before and (B) after therapeutic intervention. This method enables the specific detection of fibrous collagen disposition (green) in hepatic parenchyma: collagen around the portal tract and central veins appears in yellow and perisinusoidal collagen in turquoise. Image courtesy of Dr. Dean Tai, HistoIndex, Singapore. NASH CRN, Non-Alcoholic Steatohepatitis Clinical Research Network.

collected in real-world settings including point of care virtual trials and efficacy-to-effectiveness trial designs.[171] There are current unmet needs in this space for NASH-specific designs to both assess the benefits and harms of treatment in real-world settings, but also to evaluate these in the context of the impact of treatments on associated comorbidities which share common biology linked to the insulin-resistant state.[172]

The future of therapeutics in NASH

Given the complexity of NASH pathogenesis, there is great interest in the development of combination therapies targeting different aspects of the disease. As this area of research develops, several elements will need to be considered.

First, increasing evidence of the benefits of GLP-1 agonists and the SGLT2 (also known as SLC5A2) inhibitor class on those with type 2 diabetes and cardiovascular and renal disease means that they are likely to be background therapy for many patients with NASH, regardless of the status of their liver disease. Furthermore, in those with high disease activity and fibrosis stage, *i.e.* the population most at risk, such therapies may benefit both activity and fibrosis. It is however likely that there will be a greater benefit on one than on the other given the heterogeneity of disease biology from individual to individual and the varying efficacy of individual drugs on activity and fibrosis. The implications for long-term disease evolution will need to be considered in this setting and specific approaches, such as disease remission induction and maintenance strategies *vs.* intermittent cycled therapy *vs.* other approaches, will be needed for successful development of combination strategies.

Finally, given the biological complexity and clinical heterogeneity of the disease and its comorbidities, in an ideal world, identification of the precise drivers of disease would aid the development of targeted therapeutics. Such precision medicine approaches will require large numbers of well phenotyped and genotyped cohorts. The use of polygenic risk scores to identify individuals with specific risk characteristics and pathways of liver injury will be important for successful implementation of precision medicine approaches. Recent data from the Million Veterans Program[173] represent important steps in this direction but only provide insights on the development of fatty liver.[174] There is a major unmet need to integrate genomic, phenomic and transcriptomic data to move the field towards specifically targeted therapeutics which will benefit the largest number of patients with NASH.

Abbreviations

APRI, aspartate aminotransferase-to-platelet ratio index; CAP, controlled attenuation parameter; ELF, enhanced liver fibrosis; FIB-4, fibrosis-4; FXR,

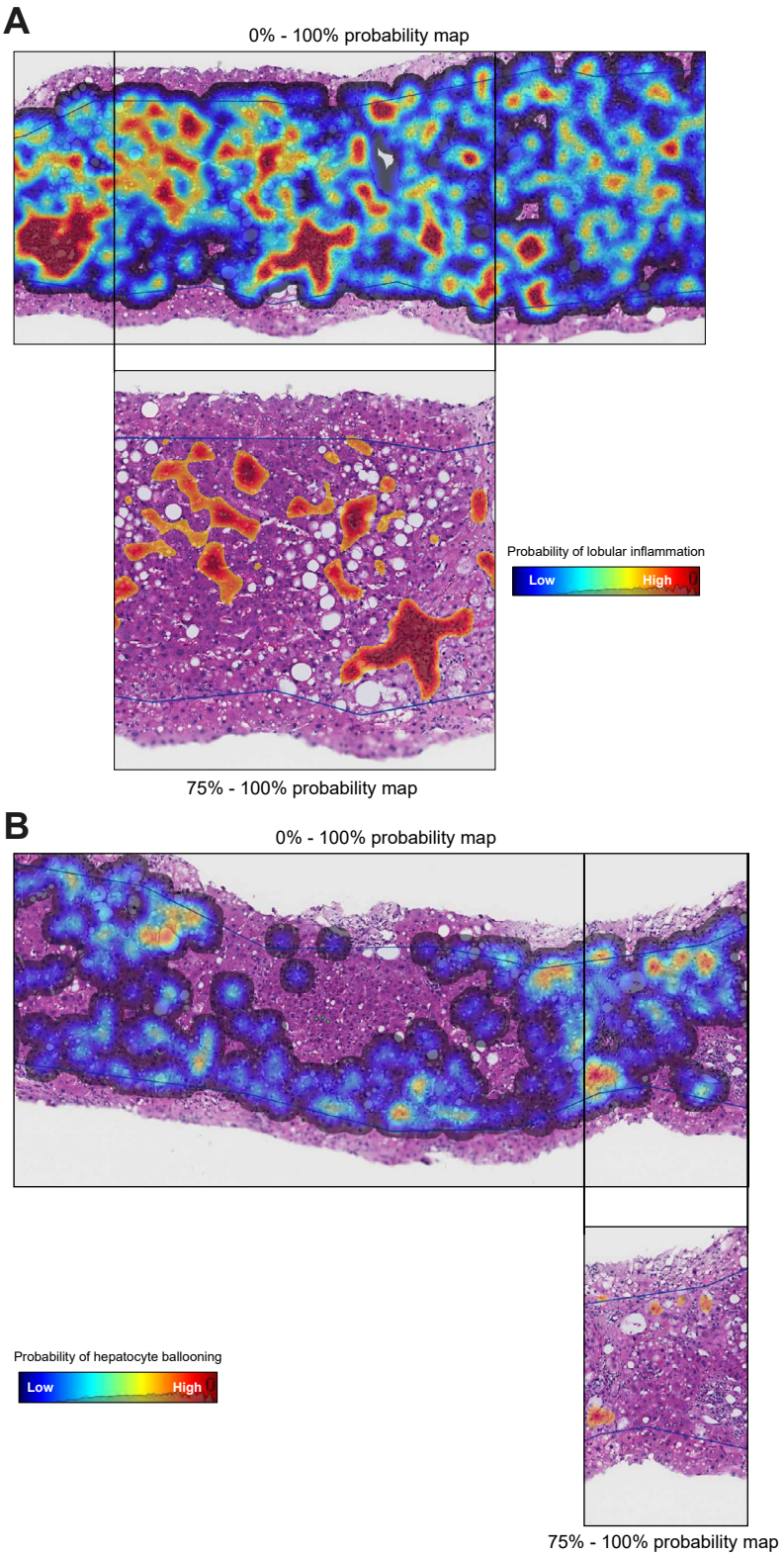

Fig. 7. Topological probability heatmaps. Topological probability heatmaps (machine-learning predictive model) of inflammation (A) and hepatocyte ballooning (B). Probability thresholds (75% here) are used to accept features, which are then classified and quantified for size, density, morphometry. The related parameters are combined to form continuous severity scores. (FibroNest™ image analysis platform on hematoxylin and eosin stained and digitized slides. Image courtesy of Dr Mathieu Petitjean, PharmaNest, Princeton, NJ, USA) NASH, non-alcoholic steatohepatitis.

farnesoid X receptor; GIP, glucose-dependent insulinotropic polypeptide; GLP-1, glucagon-like peptide 1; HCC, hepatocellular carcinoma; NAFLD, non-alcoholic fatty liver disease; NASH, non-alcoholic steatohepatitis; NIT, non-invasive test; OCA, obeticholic acid; PDFF, proton-density fat fraction; PPAR, peroxisome proliferator activated receptor; qFP, quantifiable collagen fibrillar parameters; VCTE, vibration-controlled transient elastography.

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Conflicts of interest
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Author names in bold designate shared co-first authorship

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