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Advances and emerging therapies in the treatment of NASH

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Abstract

Non-alcoholic steatohepatitis (NASH) now represents one of the most prevalent forms of cirrhosis and hepatocellular carcinoma. There have been a number of treatment agents which have undergone assessment in humans, following promising results in animal models. Presently, about 50 therapeutic agents are in various stages of development. Recently, however, there have been a number of exciting positive developments in this landscape, although there are inherent challenges ahead. In this piece, we review the aetiological and pathological basis of NASH progression and putative targets for current therapies. We also discuss some of the likely future directions and difficulties around this complex and growing challenging paradigm.

Introduction

Non-alcoholic fatty liver disease (NAFLD) which impacts an estimated 25% of the world's adult population[1–3], is the principal cause of chronic liver disease globally. NAFLD as a whole represents a pathological spectrum of liver injury, spanning from simple steatosis to non-alcoholic steatohepatitis (NASH) and liver fibrosis, with an evolutionary course to cirrhosis and risk of hepatocellular carcinoma.

Within the continuum, NAFLD develops into non-alcoholic steatohepatitis (NASH) in 20% of cases,[2] with NASH being a leading cause of further progression to liver cirrhosis and cancer,[4] and the second major cause of years of life lost among all cancers.

The association between NAFLD development and obesity, insulin resistance and type 2 diabetes mellitus, is well established.[5] Given the increasing prevalence of these related conditions, the incidence of NAFLD is projected to increase with data suggesting a 56% rise over the next decade.[2] Although NAFLD is typically associated with a western lifestyle, data demonstrates a rapid increase in disease burden in developing counties.

The pathogenesis of NAFLD is complex and thought to be dependent on 'multiple parallel hits' on a background of genetic susceptibility. NAFLD progression is best considered a dynamic two-way process relating to repetitive bouts of metabolic stress and inflammation, interspersed with endogenous anti-inflammatory reparative responses. Recent advances in deciphering the pathogenesis of NAFLD, including predisposing genetic determinants (PNPLA3, TM6SF2, HSD17b, MOAT7)[6] and identification and validation of involved biomarkers,[7] have improved our understanding of this disease, in addition to developing tools to stratify disease severity and

prognosis.[8,9] However, there remains significant knowledge gaps relating to susceptibility and progression variability between individuals.

Despite numerous clinical trials, there remains no licensed pharmacological intervention for NAFLD. In the absence of approved drug treatments, lifestyle interventions remain pivotal in the management of NAFLD across its entire disease continuum. There is a strong correlation between weight loss and the resolution of NAFLD, including fibrosis regression, and therefore therapies that induce weight loss are an obviously attractive drug target.[10]

Given emerging insights in NAFLD pathogenesis, it is possible that multiple tangential pathways are engaged to successfully alter the natural history of the disease.[11] While there are presently no licensed therapeutics for NAFLD, there are pharmacological agents for other components of the Metabolic syndrome (MetS). Despite biological plausibility, and some preliminary suggestions around efficacy, none of these have unequivocally achieved prerequisite endpoints.[12] The optimal combination of these therapies is again likely subject to various metabolic, genetic, and gene-environment considerations.

An interesting novel approach to some of the challenges within the area relate to the plausibility of the variability in liver homeostasis as influenced by the circadian clock. This evolutionarily conserved physiological mechanism controls highly coordinated aspects of metabolism including fatty acid synthesis, signalling of farnesoid X receptor (FXR), fibroblast growth factor 19 and 21 and peroxisome proliferator-activated receptor (PPAR) α and γ, glucagon like peptide 1 (GLP-1) and thyroid hormone receptor.[13] This has significant implications for targeted dosing regimens as part of potential clinical trials. The evidence for this is too extensive for the purpose of this

review, however, we would direct readers to an excellent review by Marjot[13] and colleagues on the topic.

In this review, we highlight some of the novel therapeutic targets for NASH currently undergoing clinical trials. A brief outline of these targets and associated compounds are outlined in table 1. An overview of the most important pathways is provided in Fig 1.

Fig 1: Overview of putative pathways implicated in NASH pathogenesis and molecular targets (from Konerman MA *et al*)[14]**Fig 1:** *Overview of putative pathways implicated in NASH pathogenesis and molecular targets (from Konerman MA et al*Click or tap here to enter text.

Peroxisome Proliferator-Activated Receptors (PPARs)

Peroxisomes are intrinsically implicated in normal fatty acid (FA) catabolism, in addition to contributing to normal energy metabolism via the pentose phosphate pathway.[15] PPAR signalling characteristically involve multiple cellular organelles, including mitochondria, with pleiotropic effects, thereby influencing glucose metabolism, inflammatory processes and fibrogenesis.[16] Three distinct PPAR isotypes have been well characterised, α, β/δ and γ – which exhibit differential expression and actions dependant on isotype, organ and intra-organ cell type.[16]

Pioglitazone (with Vitamin E) has historically demonstrated histological improvements in NASH across a number of RCTs, however, has not received FDA approval as a licenced treatment.[17,18] It is, however, licensed as a treatment for type II diabetes mellitus (TIIDM). Therefore, it can be used for persons with co-existent TIIDM and NAFLD. Pioglitazone is effective in improving glucose homeostasis, and mobilises visceral adipose tissue further influencing it's glucose-lowering potential. Similarly, it

has been shown to have potent modulatory effects in reducing inflammation in coronary vessels.[19]

Lanifibranor

As suggested, PPARs are nuclear receptors with an array of diverse regulatory functions including metabolic and inflammatory coordination, and regulation of fibrogenesis. [20] In preclinical models, the indole-sulfonamide derivative; lanifibranor (IVA337), a pan-PPAR agonist, improved insulin sensitivity and macrophage activation, with consequent reduction in liver fibrosis and inflammatory gene expression with higher efficacy than single or dual PPAR agonists.[20,21]

Lanifibranor was evaluated in a Phase IIb, double-blind, randomised, placebo-controlled trial in patients with non-cirrhotic, with severe active biopsy confirmed NASH (NATIVE study).[22] Randomisation occurred in a 1:1:1 ratio, whereby patients received placebo, lanifibranor 800mg or lanifibranor 1,200mg, once daily for 24 weeks. Type 2 diabetes mellitus (T2DM), a strong determinant in NASH pathogenesis, is a stratification factor applied to balance the assignment of patients to the 3 arms. Design of the NATIVE study rationale and outline was described previously.[23] The statistical plan hypothesised rate of response would be 10% in placebo group and an excess rate of 20% for any dose of investigational medicinal product (IMP), thereby necessitating 72 patients per arm.

The primary end point was a reduction of at least 2 points in the SAF-A component of the Steatosis, Activity, Fibrosis (SAF) scoring system. Exploratory secondary end points included regression of fibrosis or resolution of NASH. There were 247 patients randomised in total, with 188 (76%) having moderate to advanced fibrosis. 55% of those allocated to 1,200mg of lanifibranor met the primary end point versus 33% of

placebo (p=0.007), however, 800mg vs placebo did not achieve statistical significance (48% vs 33%; p=0.07). Results also favoured by the 1200mg and 800mg doses of lanifibranor in achieving improvement in fibrosis stage of at least 1 without worsening of NASH (48% and 34% respectively, vs 9% in placebo). Similarly, there was associated improvement in liver enzymes and lipid, inflammation and fibrosis biomarkers in the treatment cohorts.

Clearly, PPAR modulation represents a promising target in NASH, given the relative success of PPARγ effects noted from the PIVENS trial and other longitudinal datasets[18] , with the suggestion that pan-PPAR agonism likely demonstrating true clinical benefit across all major accepted primary and secondary endpoints. Importantly, diabetes mellitus was a strong adjusted for in the stratification allocation of patients across representative cohorts.

Thyroid Hormone Receptor β (THRβ)

There is evolving evidence to suggest that NASH may in part be a consequence of diminished liver thyroid hormone levels or as a variant of functional hepatic hypothyroidism. This has been extrapolated from studies which demonstrate a higher incidence of hypothyroidism in NAFLD/NASH patients relative to population age-sex matched controls[24], in addition to a putative molecular pathway. [25]

In NASH, selectivity for THR-β may provide metabolic benefits of thyroid hormone mediated by the liver, including modulating hepatic steatosis, reducing atherogenic lipids (low-density lipoprotein–cholesterol (LDL-C), triglycerides), and lipoproteins (apolipoprotein B (ApoB), lipoprotein[a] [Lp(a)], Apo CIII), while minimising systemic sequelae related to excess exogenous thyroid hormone administration, particularly relating to cardiac and bone effects, which are principally mediated via THR-α.[24]

Resmetirom

Resmetirom (MGL-3196) acts as a selective thyroid hormone receptor-β (THR) agonist, which is demonstrates a 28-fold higher affinity than triiodothyronine (T3) for THR-β than the co-expressed THR-α receptor.[26,27] It is inherently liver specific, being highly protein bound (99%) and has poor tissue penetration outside of hepatic parenchyma.[28]

A double-blind, randomised, placebo-controlled study of 84 patients (and 41 controls) with biopsy-confirmed NASH using Resmetirom for 36 weeks was undertaken across 25 sites in the United States.[29]

Within this trial patients had a presumptive diagnosis suggestive of NASH, based on the presence of the metabolic syndrome, plus a vibration controlled transient elastography (VCTE) consistent with liver fibrosis, and steatosis based on a controlled attenuation parameter (CAP), or metabolic syndrome plus a previous liver biopsy consistent with NASH with non-cirrhotic fibrosis. Additionally, patients required a minimum of 10% hepatic fat on a screening MRI-PDFF, before being eligible for liver biopsy to confirm prerequisite criteria for enrolment. Biopsy criteria required evidence of stage 1-3 fibrosis, with NAS score of ≥4, including fulfilling each component of the score (i.e. ≥1 of each; steatosis, balloon degeneration and lobular inflammation).

Patients were assigned on a randomised basis of 2:1 by a computer-based system to receive resmetirom 80 mg, or matched placebo, orally once daily. Serial hepatic fat measurements were obtained at weeks 12 and 36, and a second liver biopsy was obtained at week 36. The primary endpoint was relative change in MRI-PDFF determined hepatic fat vs placebo at week 12 in patients, in patients who had underwent a baseline and week 12 MRI-PDFF. Resmetirom-treated patients (n=78)

demonstrated relative reduction of hepatic fat when compared with placebo (n=38) at week 12 (−32·9% resmetirom *vs* −10·4% placebo; p<0·0001) and week 36 (−37·3% resmetirom [n=74] *vs* −8·5 placebo [n=34]; p<0·0001). Those adverse events reported were predominantly mild or moderate, and were equally distributed across both groups. A phase III, 52-Week, open-label, active treatment extension study to evaluate safety and tolerability of once daily administration of Resmetirom (MGL-3196) is ongoing (MAESTRO-NAFLD-OLE)[30] in parallel with the double-blind randomised, controlled trial of Resmetirom in ~2,000 patients using 80mg or 100mg daily vs placebo (MAESTRO-NAFLD-1). [31]

These results highlight the clear potential of THRβ modulation using Resmetirom, which on the whole was remarkably well tolerated. There is some topline data to suggest that MAESTRO-NAFLD has achieved requisite endpoints, and that the last component of the trial series, MAESTRO-NASH[32], which contains serial histological assessments should read out shortly. The method in which Resmetirom has undergone assessment through a series of parallel, composite trials has maximised recruitment potential and explored the variation across both NAFLD and NASH, is to be applauded and minimising variability in prescriptive allocation to each cohort. It is possible that Resmetirom may be the 1st licensed treatment for individuals with NASH.

Fibroblast Growth Factor (FGF)

The Fibroblast Growth Factor (FGF) 19 subfamily, comprises FGF19, FGF21 and FGF23. Fibroblast growth factor 21 is predominantly secreted by the liver, with a broad continuum of tissue-specific autocrine, paracrine, and endocrine mediated metabolic pathways.[33] Of note, FGF21 induces production and secretion of adiponectin through

PPARγ in adipose tissue and is capable of inducing PGC1α.[34] Circulating levels of FGF21 and FGF21 mRNA expression are increased in individuals with NAFLD.

Pegbelfermin

Pegbelfermin (BMS-986036), a PEGylated human fibroblast growth factor 21 (FGF21) analogue, which previously improved markers of liver fibrosis in obese patients with type 2 diabetes was the subject of a phase II double blind, randomised clinical trial.[35] Patients with NASH with fibrosis staging 1-3, were allocated to 10mg pegbelfermin once daily (n=25), 20mg pegbelfermin (n=24) once weekly, and placebo (n=26), stratified in 1:1:1 ratio, adjusted for diabetes status.

Within the trial there as a significant improvement in absolute hepatic fat fraction in both treatment groups [10mg pegbelfermin (daily) vs placebo (-6.8% vs 1.3%; p=0.0004; 20mg pegbelfermin (weekly) vs placebo (-5.2% vs -1.3%; p=0.008)]. The trial did not assess histological changes at the end of treatment. A further Phase 2B Randomized Double-Blind, Placebo-Controlled Study Evaluating the Safety and Efficacy of BMS-986036 (PEG-FGF21) in adults with NASH and Stage 3 liver fibrosis (FALCON1) is presently in active follow up and should report in the near future.

Pegozafermin

A Phase 1b/2a proof-of-concept study evaluating pegozafermin (formerly BIO89-100) for the treatment of NASH has just been reported. [36]

This biopsy-confirmed, single-arm cohort for patients with fibrosis stage F2 and F3 treated patients with pegozafermin 27mg once weekly for 20 weeks. Approximately 65% of patients at baseline were F3 staged. The cohort comprised 20 patients, 19 received an end of treatment biopsy to allow histological assessment in addition to non-invasive biomarkers.

The primary endpoint was 2-point or greater improvement in NAS without worsening of fibrosis, which was achieved in 63%, with 47% having NASH resolution or fibrosis improvement. Exploratory outcomes of non-invasive tests included MRI-PDFF (-64% mean change from baseline), FAST score (-76% mean change from baseline) and transient elastography of -31% mean change from index assessment.

Clearly, there are inherent biases within a single cohort study, however, there is clearly suggestion that pegozafermin has potential efficacy, with further RCTs necessary. ENLIVEN is a phase 2 randomised, double-blind, placebo-controlled study looking to evaluate the efficacy, safety and tolerability f BIO89-100 in a cohort of 216 patients with NASH and will look to complete in 2023s. The primary endpoints will include histological resolution of NASH without worsening of fibrosis, and those with ≥1 stage decrease in fibrosis with no worsening of NASH staging.

Aldafermin

Aldafermin is an analogue of fibroblast growth factor 19, which acts through inhibition of bile acid synthesis and regulates metabolic homeostasis. Recently, Harrison and colleagues[37] report results from a 24-week, phase 2 study, which utilised serial liver biopsies as an outcome in patients with NASH.

Within this trial, 78 patients with NAS score ≥4, stage 2 or 3 fibrosis by NASH CRN classification, and absolute liver fat content >8% were recruited. Patients were allocated in a 2:1 ratio; to Aldafermin 1mg (n = 53) once daily or placebo (n = 25) for 24 weeks.

The primary outcome was absolute improvement in liver fat content from index scan to that achieved at week 24. Exploratory secondary outcomes examined serum biomarkers, and specific histologic measures of fibrosis improvement, including NASH

resolution. At conclusion of the trial, the Aldafermin group met the primary endpoint (7.7% fat reduction, compared with placebo 2.7%; p=0.002), with significant changes noted in other biochemical markers including 7α-hydroxy-4-cholesten-3-one, bile acids (BAs), aminotransferases, and neoepitope-specific N-terminal pro-peptide of type III collagen (PRO-C3) in the treatment cohort. Histological improvements were less impressive, with fibrosis improvement of ≥1 stage with no worsening of fibrosis achieved in 38% of those receiving aldafermin vs 18% of the placebo group (p =0.10). Similarly, NASH resolution failed to achieve desired significance. A similarly designed Phase 2b/3 study (ALPINE), recruited 171 patients, and examined additional dose scheduling (0.3mg, 1mg and 3mg) compared to placebo. The primary endpoint was again improvement of liver fibrosis by ≥1 stage with no worsening of NASH at 24 weeks. Again, unfortunately, this endpoint was not achieved according to a top-line data release, although full processing of the results is awaited.[38,39] Again, it appears that multiple secondary endpoints were achieved in the treatment groups, including reduction in hepatic steatosis as measured by MRI-PDFF, transaminase and PRO-C3 levels.

Herein, we see the potent mechanism of the FGF19 and 21 analogues in reducing hepatic steatosis. However, this hepatic fat reduction clearly does not uniformly offset the other pathogenic elements potentiating NASH, and aldafermin failed to demonstrate the requisite resolution endpoints on histological assessment. Aldafermin is unlikely to be pursued again as a potential strategy, whilst the FGF21 analogues pegbelfermin and pegozafermin may yet yield positive outcomes.

Glucagon-like Peptide-1 (GLP-1) Agonists

GLP-1 agonists were originally licenced for treatment in type II diabetes mellitus. Hepatocytes lack GLP-1 receptor expression[40], therefore, the potential mechanisms through which GLP-1 agonists exert an effect in NASH likely relate to improvements in weight and insulin resistance, coupled with mitochondrial dysfunction, pro-inflammatory mediators and lipotoxicity. [40–42]

Semaglutide

Semaglutide looked to build upon the encouraging early signs noted within the LEAN trial.[43] A 72-week double-blind, placebo-controlled, phase II trial with biopsy-confirmed NASH was undertaken by Newsome and colleagues.[44] Within the trial cohort were histological grades F1-F3, with those randomised to treatment receiving either 0.1, 0.2, or 0.4mg of subcutaneous simaglutide or placebo.

The primary endpoint was the resolution of NASH with no progression of fibrosis, with secondary endpoint of improvement of fibrosis staging, with no increased histological NASH activity. The secondary endpoint relating to regression of fibrosis was limited to those with F2/F3 disease accordingly.

320 patients (230 with F2 or F3 fibrosis) were randomised to receive simaglutide 0.1mg (n=80), 0.2mg (N=78), or 0.4mg (n=82) versus those receiving placebo (n=80).

NASH resolution was achieved, with no worsening of fibrosis in 40% (0.1mg), 36% (0.2mg), 59% (0.4mg) of those treated with semaglutide, versus 17% in the placebo group ($p<0.001$ semaglutide 0.4mg vs, placebo). In terms of achieving improvement in overall histological fibrosis, there was no appreciable difference between groups, or between those receiving treatment or placebo (0.4mg semaglutide cohort 43% vs 33%

placebo; p=.048). In terms of secondary outcomes, there was significant weight loss in the 0.4mg group (13%) compared to 1% in the placebo group.

There are some interesting analyses relating to the failure of this trial, some of which mirror the PIVENS study[17], which also demonstrated a high level of fibrosis regression in the placebo cohort (31%), both of which are considerably higher than other similar trials.[44] A phase III trial of semaglutide in NASH is now being planned.

More recently, the diabetes trial; STEP 2[45] has demonstrated that patients randomised to semaglutide 2.4mg (once weekly) resulted in -9.6% mean bodyweight reduction from baseline at week 68 versus -3.4% with placebo. Again, highlighting the weight-related improvements associated with semaglutide usage.

Liraglutide

The LEAN study was a multicentre (4 UK centres), double-blind, randomised, placebo-controlled phase II trial assessed the efficacy of subcutaneous liraglutide (1.8mg daily) compared to placebo.

The trial cohort included those with relative obesity and histological evidence of NASH. The trial design incorporated a randomisation minimisation of 1:1, stratified by trial centre and diabetes status, whereby 26 patients received liraglutide and 23 placebo. In those patients who underwent end-of-therapy liver biopsy; 9 of 23 (39%) patients in the treatment group and 2 of 22 (9%) of the placebo group achieved resolution of NASH (relative risk 4.3 (95% CI 1.9-17.7) p=0.019). Contrastingly, 9% (2/23) in treatment group versus 36% (8/22) of placebo patients demonstrated clear progression of fibrosis (0.2 (0.1-1.0); p=0.04).

Whilst the numbers within the trial were small, the encouraging signals provided a tantalising insight into potential biological potential, which subsequently formed the basis for the semaglutide trial. There are no current NASH trials looking to extend on the use of liraglutide.

There are some interesting similarities between this trial and the PIVENS study[17], which both demonstrated a high level of fibrosis regression in the placebo cohort (31%), both of which are considerably higher than other similar trials.[44] The reasons for this significant improvement within the control populace are not immediately apparent, however, selection criterion are increasingly recognised as fundamental pitfalls in NASH trial design, which we discuss later. A phase III trial of semaglutide in NASH is presently being planned.

More recently, the diabetes trial; STEP 2[45] has demonstrated that patients randomised to semaglutide 2.4mg (once weekly) resulted in -9.6% mean bodyweight reduction from baseline at week 68 versus -3.4% with placebo. Again, highlighting the weight-related improvements associated with semaglutide usage. As has been suggested previously, it remains to be seen whether the weight loss associated with the use of GLP-1 is powerful enough to impact on relevant histological features of NASH.

Farnesoid X Receptor (FXR) Agonists

The Farnesoid X Receptor (FXR) exists as two entities within humans; FXRα and FXRβ, albeit the latter is a pseudogene. As a member of the nuclear receptor (NR) family, FXR acts as a ligand-modulated transcription factor whose role is to increase or decrease the transcriptional activity of regulated promoters in a coordinated fashion.

FXR is a metabolic nuclear receptor and is activated by primary bile acids such as chenodeoxycholic acid (CDCA), cholic acid (CA), and, synthetic agonists such as obeticholic acid (OCA). FXR plays crucial roles in regulating cholesterol homeostasis, lipid metabolism, glucose metabolism, and the microbiome all of which likely relate to NASH pathogenesis.[46]

Obeticholic Acid (OCA)

6-ethylchenodeoxycholic acid (obeticholic acid) is a bile acid derivative, which is a potent activator of the farnesoid X nuclear receptor, that can reduce liver fat and fibrosis in animal models of NAFLD. The FLINT trial[47] assessed the efficacy of obeticholic acid (OCA) in patients with biopsy proven NASH. FLINT categorically assessed response to treatment for non-cirrhotic, non-alcoholic steatohepatitis to assess treatment with obeticholic acid given orally (25 mg daily) or placebo for 72 weeks, with patients stratified to a 1:1 allocation ratio by centre or diabetes status.

Primary outcome was improvement in liver histology, defined as decrease in NAS of at least 2 points, with no deterioration of fibrosis staging. The trial included a pre-planned interim analysis of biochemical markers, supporting continuation of the trial. Within the trial, 141 patients were randomised to OCA, while 142 received placebo. 50 (45%) of 110 persons within the treatment cohort, and 23 (23%) of 109 in placebo group who underwent liver biopsy at baseline and again at 72 weeks demonstrated improved liver histology (relative risk 2.2, 95% CI 1.4-3.3; p=0.0002). Unfortunately, there was the unexpected consequence of increased cholesterol and decreased in HDL. This sequelae is more likely due to the fact that functional farnesoid X receptor activation reduces bile acid synthesis by inhibiting the conversion of cholesterol to bile acids. This is a key regulatory step in cholesterol homeostasis. The Flint trial therefore

demonstrated improved histological features of NASH, but long-term safety and utility required further clarification.

Recently, obeticholic acid has achieved the interim histological endpoint of fibrosis improvement (1,968 patients, 311 placebo; 312 OCA 10mg; 308 OCA 25mg) with no worsening of NASH in the phase 3 REGENERATE study.[48] The NASH resolution endpoint was unfortunately not achieved (25 [8%] placebo; 35 [11%] OCA 10mg; 71 [23%] OCA 25mg). The results from this planned interim analysis identify clinically significant histological improvement, that is likely to translate to clinical benefit. This study is ongoing to assess clinical outcomes and is likely to complete in 2025.

While the REGENERATE study failed to definitively dispel any lingering concerns around the efficacy of OCA in NASH resolution, there is clearly positive signals from what was a well-designed and well powered study. With regards to concerns around dyslipidaemic features with OCA treatment, the CONTROL study demonstrated good safety, acceptability and LDL-C control with co-administration of atorvastatin with OCA which should provide confidence in this approach going forward.[49]

Chemokine Receptor Antagonists

The chemokine receptor 2 (CCR2) and receptor 5 (CCR5) are central orchestrators of leukocyte trafficking in inflammatory processes. Emerging evidence for the role of CCR2 and CCR5 receptors in human inflammatory diseases, arteriosclerosis and NASH has led to growing interest in developing CCR2- and CCR5-selective antagonists.[50]

Cenicriviroc

Cenicriviroc (CVC) is a dual C-C chemokine receptors; type 2 and 5 dual antagonist under investigation as a putative therapy for NASH.[51] Recently, year 1 primary analysis of the 2-year CENTAUR study demonstrated that CVC had an antifibrotic effect without impacting on degree or inducing regression of steatohepatitis.

The CENTAUR study was a randomized, controlled study of adults with NASH, NAS ≥4, and NASH Clinical Research Network stage 1-3 fibrosis. The innovative study design included a placebo to treatment cross-over schedule, with participants in arms A and C receiving CVC 150 mg or placebo, respectively, for 2 years; whilst patients in arm B received placebo in year 1 and switched to CVC in year 2. Histological assessment was performed with biopsy performed at baseline, year 1, and year 2. Of 289 randomized participants, data on 242 entering year 2 was available for analysis. At year 2, 24% of patients who converted to CVC, versus 17% who remained on placebo achieved ≥1-stage fibrosis improvement, with no worsening of NASH ($p = 0.37$). A significant proportion of patients on treatment who achieved fibrosis response at 1 year, maintained similar benefit at year 2 (60% arm A versus 30% arm C), including 86% on CVC who had stage 3 fibrosis at baseline histology. Unfortunately, following 2 years of investigation, a almost identical percentage of patients on CVC and placebo achieved ≥1-stage fibrosis improvement, again with no worsening of NASH (15% arm A versus 17% arm C). Exploratory endpoints of fibrosis assessment, demonstrated consistent reductions in levels of N-terminal type 3 collagen pro-peptide (PIIINP) and enhanced liver fibrosis (ELF) scores. Similarly, there were commensurate increases in aspartate aminotransferase-to-platelet ratio index (APRI), and Fibrosis-4 (FIB4) scores observed in apparent non-responders.

The AURORA study is a dual phase randomised, double-blind trial of cenicriveroc utilising surrogate endpoints of fibrosis stage improvement of ≥1 (NASH CRN) and no worsening of steatohepatitis at month 12.[52] A second phase of the study enrolled additional participants to determine long-term clinical outcomes including histopathological progression to cirrhosis, liver-related clinical outcomes, and all-cause mortality. Patients were randomised to receive cenicriveroc 150mg OD or placebo for 40 months, randomised in a 2:1 ratio respectively. Within these groups, the primary outcome was achieved in 22.3% (95% CI 19.6-25.2) in cenicriveroc cohort vs 25.5% (95% CI 21.5-29.9) in the placebo arm. None of the additional secondary endpoints were achieved within the reported study outcomes, although not all outcomes have been definitively reported at this point.[52]

Unfortunately, it appears that Cenicriveroc is unlikely to form the basis of any further trials in NASH going forward, although there may as yet be some benefit in relation to cardiovascular sequelae, this is unlikely to be explored in a pure NASH population. While some of the initial data appeared promising, particularly with respect to non-invasive markers of fibrosis, there was poor correlation to histological outcomes in their trial cohort.

Metabolic Enzyme Modulators

This is a class of related compounds that target specific aspects of lipogenesis and triglyceride synthesis. It includes Acetyl Co-A carboxylase (ACC) inhibitors, Steroyl-CoA desaturase-1 inhibitors (SCD-1), and Diacylglycerol acyltransferase-2 inhibitors.

Acetyl Co A Carboxylase Inhibitors (ACCi)

Hepatic de-novo lipogenesis (DNL) is a potentiator of NAFLD, which may result in an increased triglyceride burden within hepatocytes.[53,54] A promising approach involves targeting ACC; which catalyses the initial reaction in the DNL pathway whereby acetyl-CoA is converted to malonyl CoA. Within DNL homeostasis, malonyl CoA is an essential basic substrate whilst also functioning as a potent allosteric inhibitor of carnitine palmitoyltransferase-1. Carnitine palmitoyltransferase-1 plays a vital capacitance in the co-localization of long-chain fatty acyl CoA across the mitochondrial membrane where it undergoes β-oxidation._[55,56]The dimerization is catalyzed in a stepwise manner, involving both a biotin carboxylase (BC) reaction and a carboxyltransferase (CT) reaction.[57]

Diacylglycerol Acyltransferase -2 (DGAT-2) Inhibitors

Active inhibition of DGAT2 induces a reactive downregulation of SREBP-1, (a potent mediator of glycolysis and inducer of lipogenesis); which suppresses downstream lipogenic modulators and upregulates alternative oxidative processes.[58] Furthermore, DGAT2 is central to the esterification of FAs with DAG, producing triglycerides. Previous studies in patients with NAFLD have shown beneficial effects on triglyceride-lowering and ameliorating hepatic steatosis._[59,60]

A recent trial has examined the possibility of exploiting the potential utility of another novel ACC inhibitor (PF -05221304) (2, 10, 25, and 50mg) compared to placebo with an evaluation of relative liver fat fraction at 16 weeks.[61] A parallel component of the study explored the putative benefit of adding a DGAT2 inhibitor (PF-06865571 – 300mg BD) since it may additionally offset the potential for hypertriglyceridemia experienced in ACC inhibitors. Dose-dependent reductions in liver fat were achieved

using PF-05221304 and PF-06865571 monotherapy from index MRI to week 6. Placebo-adjusted changes were -44.5% ($p<0.0001$) and -3.4% ($p = 0.0007$) respectively. Co-administration lowered steatosis by -44.6% which was relatively equivalent to PF-05221304 monotherapy, however, a greater proportion of patients receiving both therapies achieved >30 or >50% reduction in liver fat burden. While this combination approach provides some tantalizing insights, robust, long-term data with added histological considerations are needed to verify this preliminary data.

Firsocostat

Firsocostat (GS-0976) is another highly liver-specific, small molecule that binds avidly to the BC regulatory terminal, thereby inhibiting downstream dimerization and consequent ACC activation. Firsocostat is uniquely hepatocyte-specific, as it was developed as a substrate for hepatic organic anion-transporting polypeptide (OATP) transporters.[62] This results in exclusive hepatic biodistribution of compound delivery which has favorable therapeutic potency. In a recent open-label trial[63], Firsocostat was combined with semaglutide +/- cilofexor (FXR agonist), it demonstrated encouraging signals of enhanced liver steatosis resolution (as measured by MRI-PDFF) despite no additional benefit on weight loss (7-10%) versus semaglutide monotherapy. Tolerability overall seemed good with predominant GI upset AEs. It will continue to be evaluated through a number of upcoming trials.

Stearoyl-CoA Desaturase-1 (SCD-1) Inhibitors

Stearoyl-CoA Desaturase-1 catalyses monounsaturated fatty acids, preferentially stearoyl - (C18:0) and palmitoyl (C16:0) -Co-Ausing nicotinamide adenine dinucleotide phosphate (NADPH), cytochrome b5 and associated cytochrome b5 reductase to yield Oleic acid (C18:1), and palmitoleic acid (C16:1) respectively. [64,65]

3β-arachidyl amido cholanoic acid (Aramchol), is an oral, liver-specific bile acid derivative, that partially antagonises SCD1 expression within the hepatic parenchyma, thereby reducing liver triglyceride burden. Animal models have shown histological improvements in both steatohepatitis activity indices and fibrosis.[66]

ARREST was a 52 week, double-blind, placebo-controlled, phase 2b trial that sought to determine the efficacy of Aramchol 400mg, 600mg versus placebo in cohort of 247 patients with NASH.[67] The primary endpoint was a relative reduction in hepatic triglyceride concentration as measure using MR spectroscopy. Secondary endpoints of note included histological assessment and resolution of transaminases. Aramchol 600mg, unfortunately, failed to reach significance in relation to the primary outcome, thus making all additional analyses exploratory in nature. In determining histological endpoints, NASH resolution, without worsening fibrosis was noted in 16.7% of patients taking Aramchol 600mg vs 5% within the control population (OR – 4.74). Similarly, resolution of fibrosis by ≥1 stage without worsening steatohepatitis was noted in 29.5% versus 17.5% respectively.

Again, there appears to be benefit in further exploring the potential additive effects of these agents, particularly in tandem with compounds targetting synergistic pathways. Future trials are likely to employ this strategy to achieve requisite endpoint outcomes.

Conclusion

In the last decade there have been significant developments in our understanding of the pathophysiology of NASH, which consequently has led to the development of a number of promising therapeutic interventions. New molecules and pathways are being targeted, while we look to further improve our understanding around metabolomic and genomic contributors to the pathogenesis of NASH. Given the

complexity of the underlying pathophysiology, and the number of associated conditions it is likely that a personalised approach may be necessary in order to achieve specific desired endpoints, which may require multiple therapeutic agents.[11] Current trial reports have highlighted the challenges that exist around histology-based trial endpoints including variability in liver histology interpretation (especially evaluation of ballooning degeneration)[68], lack of matching of those with particular co-morbidities (e.g. diabetes) between phase II and III studies, and strict recording of dietary and exercise during follow up period beyond standard treatment timing.[69] Presently, there are a number of alternative modalities under investigation to determine whether they will prove robust surrogates to traditional histological-based outcomes. End-points based on Magnetic Resonance Imaging in particular as a non-invasive modality may prove effective, particularly for early study designs for drugs that influence hepatic[70,71] steatotic burden, rather than anti-inflammatory or anti-fibrotic modes of action. However, no biomarker or imaging modality has been fully approved as a replacement for histological assessment to date.

Whilst there have been enormous developments in the understanding of the pathogenesis of NASH, enabling the development of novel compounds that will hopefully prevent disease progression from NASH to cirrhosis and/ or hepatocellular carcinoma, the overriding emphasis should remain one of disease prevention. Population health strategies to reduce the prevalence of obesity and increase the number of individuals engaging in regular exercise are critical to address the rapidly developing challenges of obesity and other related conditions such as diabetes.

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Molecular target	Pharmaceutical agent
Peroxisome Proliferator-Activated Receptor (PPAR) agonists (Receptor specificity)	Bezafibrate (α) Fenofibrate (α) Pioglitazone (γ) Rosiglitazone (γ) Saroglitazar (α/γ) Elafibranor (α/δ) Lanifibranor (α/δ/γ)
Thyroid Hormone Receptor β (THRβ)	Resmiterom
Fibroblast Growth Factor (FGF) (subclass)	Aldafermin (FGF19 analogue) Pegozafermin (FGF21 analogue) Pegbelfermin (FGF21 analogue)
Glucagon-like Peptide -1 (GLP-1) Agonists	Liraglutide Semaglutide
Farnesoid X Receptor (FXR) agonists	Obeticholic Acid (OCA)
Chemokine Receptor (C-C) antagonist (Receptor specificity)	Cenicriveroc (CCR2, CCR5 dual antagonist)
Metabolic Enzyme Inhibitors (Specific Enzyme)	Firsocostat (ACCi) PF- 05221304 (ACCi) PF- 06865571 (DGAT2) Aramchol (SCD-1 inhibitor)

Table 1: *Overview of putative molecular targets and specific agents trialled in NASH*