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**Combination Strategies for Pharmacologic Treatment of Non-Alcoholic Steatohepatitis**

Combination Drug Therapy for NASH

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Abstract
Non-alcoholic steatohepatitis (NASH) is defined as hepatic steatosis, inflammation, and hepatocyte injury with or without fibrosis. It has emerged as the second leading indication for liver transplantation with a rising death rate in the non-transplantable population. While there are many drugs in evaluation, currently no approved therapies are on the market for this condition. Given this importance, the Food and Drug Administration (FDA) has provided formal guidance regarding drug development for stopping or reversing NASH or NASH associated fibrosis. The complex pathogenesis of NASH and its bidirectional relationship with metabolic syndrome has highlighted multiple drugs of interest that address metabolic, inflammatory, and fibrotic factors. A few promising liver specific targets include farnesoid X receptor (FXR) agonists and peroxisome proliferator-activated receptor (PPAR) agonists. Previously studied drug classes such as glucagon-like peptide-1 (GLP-1) analogs or sodium/glucose transport protein 2 (SGLT2) inhibitors have also demonstrated ability to improve hepatic steatosis. Here we discuss current rationale, scientific work, and preliminary data in combining multiple drugs for the purposes of a multimodal attack on the pathogenesis of NASH. We highlight multiple Phase 2 and Phase 3 studies that demonstrate the potential to achieve a response rate higher than previously assessed monotherapies for this condition. Ultimately, one of these combination strategies may rise above in its safety and efficacy to become a part of a standardized approach to NASH.

Key Words: Non-alcoholic Steatohepatitis; Fatty Liver; Combination Treatment; Drug Therapy; Pharmacologic Treatment; Clinical Trials

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Core Tip: Multimodal combination approaches targeting two or more molecular pathways contributing to steatohepatitis and liver fibrosis are needed to augment
efficacy of novel investigational drug regimens to achieve NASH resolution and NASH fibrosis improvement.

**INTRODUCTION**

Non-alcoholic steatohepatitis (NASH) is defined as the presence of ≥ 5% hepatic steatosis and inflammation with hepatocyte injury with or without fibrosis. Non-alcoholic fatty liver disease (NAFLD) represents a spectrum of pathology encompassing hepatic steatosis, steatohepatitis (NASH), and liver fibrosis, and poses a significant challenge to the medical community as there are currently no Food and Drug Administration (FDA) approved therapies available on the market. The definition of NAFLD includes the lack of secondary causes of hepatic steatosis such as alcohol consumption, steatogenic medication or hereditary liver disease. With NAFLD-associated death rates on the rise and NASH emerging as the second most common indication for liver transplantation, there has been heightened urgency to target various disease pathways in NASH development with the hopes of controlling the global impact of this disease[^1,^2^]. With this rising importance, the FDA has published formal guidance regarding drug development aimed at stopping or reversing NASH and NASH fibrosis. The current drug development pipeline contains many mono-therapeutic options which address a wide range of metabolic, inflammatory, and fibrosis target pathways associated with NASH pathogenesis.

The pathophysiology of NASH is based on a bidirectional relationship between Type 2 diabetes mellitus (T2DM), hypertension, obesity and dyslipidemia - or metabolic syndrome. This relationship contributes to excess free fatty acids generated from lipolysis and de novo lipogenesis in the liver, which creates lipotoxic species which induce oxidative stress, inflammasome activation, and fibrinogenesis[^3^]. Liver specific targets aimed at decreasing histologic inflammation or fibrosis such as farnesoid X receptor (FXR) agonists or peroxisome proliferator-activated receptor (PPAR) agonists are currently being evaluated for the treatment of NASH. These are in addition to drug classes such as glucagon-like peptide-1 (GLP-1) agonists and sodium/glucose transport
protein 2 (SGLT2) inhibitors that were initially approved for treatment of diabetes but have demonstrated the ability to decrease liver fat content[^4,^5]. While individually these agents have shown promise in early trials, there has been growing interest in pursuing a multimodal combination approach targeting two or more molecular targets/pathways responsible for NASH and NASH-associated liver fibrosis, particularly in context of modest effects of single agent strategies on histologic endpoints, with fewer than 50% of patients achieving either NASH resolution or fibrosis improvement of one stage or greater[^6]. Therefore, this mini review will succinctly summarize the current efforts to examine combination strategies of drugs which may further augment therapeutic response in patients with NASH.

**FDA APPROVAL PATHWAY**

The FDA generally has two pathways for drug approval. The traditional pathway focuses on clinical benefit endpoints (i.e., morbidity and mortality) and requires long-term data. A brief review is provided in Figure 1. The accelerated approval pathway is intended to expedite the process for serious medical conditions with unmet needs. This pathway relies on short-term surrogate markers that would reliably predict long-term clinical outcomes to support drug approval. To inform clinical trial design for investigational drugs under evaluation for NASH, industry guidance was issued by the FDA in 2018 with a focus on patients with non-cirrhotic NASH with stage 2-3 Liver fibrosis[^7]. Although histologic endpoints were reinforced as required for assessment of surrogate endpoints for NASH and liver fibrosis, the agency encouraged the development and validation of noninvasive biomarkers in clinical trials to accelerate drug development. NASH was defined as a NAFLD activity score (NAS) greater than or equal to 4 with at least 1 point each in inflammation and ballooning degeneration, plus a NASH Clinical Research Network (CRN) fibrosis score greater than stage 1 fibrosis but less than stage 4 for enrollment in these trials. Lastly, the primary regulatory endpoints required to support accelerated approval include: 1) NASH resolution on histology (NAS less than 4 with individual components scores of 0 for
ballooning degeneration and 0-1 for inflammation) without worsening fibrosis; or 2) improvement in liver fibrosis greater than or equal to one stage without worsening NASH; or 3) NASH resolution and improvement in fibrosis by one stage or greater. Clinical benefit for these drugs was defined as superiority to placebo in delayed disease progression measured by a composite endpoint including progression to cirrhosis, hepatic decompensation, change in MELD score, liver transplantation, or all-cause mortality.

**DRUGS IN DEVELOPMENT - PHASE 2**

Key phase 2 trials for NASH therapeutics are summarized in Table 1. In brief, one major class being pursued is fibroblast growth factor 21 (FGF21) agonists such as pegbelfermin. FGF21 is endogenously produced by the liver and has a pleiotropic effect on metabolism that may benefit patients with NASH. Endogenous FGF21 concentrations are elevated as much as 10-fold in patients with obesity, NAFLD or NASH, leading to the hypothesis that these may represent an FGF21-resistant state which may benefit from exogenous stimulation to improve insulin sensitivity and lipid metabolism[8]. GLP-1 is an incretin hormone made by intestinal cells post prandially for which receptors are predominantly in the pancreas, adipose tissue and brain. It regulates plasma glucose by stimulating glucose release and inhibiting glucagon secretion. GLP-1 agonists have previously shown to improve hepatic steatosis, decrease liver inflammation, and ameliorate insulin resistance in murine models of fatty liver disease. Semaglutide and liraglutide have shown promising results with statistically significant NASH improvement or resolution compared to placebo [9,10]. Ursodeoxycholic acid is an orally administered side chain-shortened homologue of ursodeoxycholic acid that undergoes hepatic enrichment with hepatoprotective, anti-inflammatory, and antifibrotic activity. It has shown significant reduction of serum alanine aminotransferase (ALT) within 12 wk of treatment when compared with placebo, encouraging further investigation [11]. Aldafermin is an analogue of fibroblast
growth factor 19 (FGF19) which regulates bile acid metabolism and fat storage in the liver. FGF19 Levels are lower in patients with NAFLD and insulin resistance. Activation of the FGF19 pathway has been shown to improve insulin sensitivity and liver steatosis. In a 24-week placebo-controlled trial, the aldafermin group experienced a significant reduction in absolute liver fat content compared with placebo (P = 0.002) and fibrosis improvement of at least 1 stage (38% vs. 18%, P = 0.10)\textsuperscript{[13]}. The currently ongoing phase 2b ALPINE 4 study is designed to assess the efficacy, safety and tolerability of this agent (NCT04210245).

FXR agonists, which bind to the transcription factor FXR to help regulate bile acid metabolism are in multiple phases of clinical trial investigation. The FXR agonist tropifexor has demonstrated a robust and dose-dependent decrease in ALT, hepatic fat fractionation, and body weight with good safety and tolerability after 12 wk of treatment in a phase 2 trial \textsuperscript{[13]}. The PPAR\alpha, \beta/\delta and \gamma, play a central role in the regulation of glucose and lipid metabolism and of the inflammatory and fibrogenic pathways which contribute to NASH pathogenesis. Lanifibranor (IVA337), a pan-PPAR agonist, combines pharmacological effects that could improve fatty acid oxidation, dyslipidemia, and insulin sensitivity, and has demonstrated anti-inflammatory, antifibrotic and hepatoprotective effects in preclinical models and phase 1/2 trials. TVB-2640 is an orally bioavailable, first-in-class FASN (fatty acid synthase) inhibitor. FASN is a key enzyme in the de novo lipogenesis pathway that is responsible for the synthesis of excess fat and activation of fibrogenic and inflammatory mechanisms in the liver of patients with NASH. TVB-2640 demonstrated significant improvement in several NASH endpoints in the FASCINATE-1 trial as summarized in Table 1\textsuperscript{[14]}. Firsocostat (GS-0976) is an inhibitor of ACC (acetyl-coenzyme A carboxylase) which catalyzes de novo lipogenesis in the liver. In a randomized placebo-controlled trial, firsocostat 20 mg decreased hepatic steatosis and surrogate markers of fibrosis \textsuperscript{[15]}. VK2809 is a small molecule prodrug of a potent thyroid beta receptor agonist which has demonstrated favorable effects on lipid metabolism and biomarkers of hepatic steatosis and
steatohepatitis in a phase 2 trial, supporting its potential role in patients with NASH \cite{16}, MSDK-0602K is a novel insulin sensitizer designed to preferentially target the mitochondrial pyruvate carrier while minimizing direct binding to the transcriptional factor PPARγ. MSDK-0602K did not demonstrate statistically significant effects on primary and secondary histologic endpoints in a phase 2 trial, but favorable effects on liver cell injury and glucose metabolism support further investigation for in patients with type 2 diabetes \cite{17}.

**DRUGS IN DEVELOPMENT – PHASE 3**

Novel investigational agents which have completed are undergoing evaluation in phase 3 trials are summarized in Table 2. Obeticholic acid, an FXR agonist, has been shown to improve the histological features of NASH, with fibrosis improvement in 23% of patients treated with Obeticholic acid compared with 12% in placebo group \cite{18}. Elafibranor, a PPAR agonist, improves liver enzymes, lipids, glucose levels, and markers of systemic inflammation and is being tested in a phase 3 study (NCT02704403) \cite{19}. Aramchol inhibits steroyl-CoA desaturase 1 (SCD1), a key enzyme in hepatic lipogenesis that converts saturated fatty acids into monounsaturated fatty acid. In the phase 2b ARREST trial, aramchol demonstrated liver fat reduction, biochemical improvement, NASH resolution and fibrosis reduction in a dose response pattern \cite{20}. It has since been included in an ongoing phase 3 trial to test its safety and efficacy (NCT04104321). Cenicriviroc is an oral, dual antagonist of C-C motif chemokine receptor (CCR) types 2 and 5. It has shown anti-inflammatory and anti-fibrotic properties, which are mediated by CCR types 2 and 5 (CCR2/CCR5) blockade. In a randomized double-blind phase 2b study of 289 subjects, cenicriviroc was associated with a statistically significant improvement in NASH fibrosis of one stage or greater vs placebo (20% vs. 10%; \( P = 0.02 \)) \cite{21}. A phase 3 randomized, double-blind, placebo-controlled trial (AURORA) is currently ongoing with evaluation of 2000 adults with NASH who are treated with cenicriviroc or placebo for 52 wk \cite{22}. Resmetirom is a liver-directed, orally active, selective thyroid hormone receptor-β agonist designed to
improve NASH by increasing hepatic fat metabolism and reducing lipotoxicity. In a phase 2b study, resmetirom treated patients showed a relative reduction of hepatic fat compared with placebo with statistically significant NASH resolution vs placebo \textsuperscript{12}, and is currently under evaluation in a phase 3 registration trial (NCT03900429). GLE-MD-02 (belapectin), is an inhibitor of galectin 3 that reduces liver fibrosis and portal hypertension. In a phase 2 trial, belapectin was safe but not associated with significant reduction in hepatic venous pressure gradient (HVPG) or fibrosis. However, in a subgroup analysis of patients without esophageal varices, 2 mg/kg belapectin reduced HVPG and development of varices, suggesting a possible benefit in patients with NASH cirrhosis without esophageal varices\textsuperscript{[24]}. In the phase 3 NAVIGATE trial, the safety and efficacy of belapectin is under evaluation with primary clinical endpoints of development of varices and event-free survival (NCT04365868).

**COMBINATION THERAPEUTICS**

The rationale of combining two or more strategies for NASH therapy aims to augment rates of NASH resolution and NASH fibrosis improvement. By targeting the development of steatohepatitis, liver fibrosis as well as controlling metabolic syndrome, we may achieve response rates higher than 32\% as seen currently via trials of drugs as monotherapy \textsuperscript{[6]}. Table 3 is a collection of studies currently underway, and some completed, that evaluate multidrug regimens for the treatment of NASH. FXR agonists, which regulate bile acid metabolism, are one major class of drugs incorporated in many of these trials. Obeticholic acid, a promising drug in this class, has demonstrated the dose-dependent ability to improve liver fibrosis and steatohepatitis in NASH patients with stage F2/F3 fibrosis based on initial and secondary analysis of the REGENERATE trials \textsuperscript{[18,25]}. Cilofexor, another FXR agonist, has been tested in combination with firsocostat, an ACC inhibitor and selonsertib, an ASK1 inhibitor, in the phase 2b ATLAS study that demonstrated improvements in liver enzymes, fibrosis, NAS score on histology and improvements in liver elastography in the cilofexor/firsocostat group.
compared to placebo [26]. Ongoing studies with tropifexor include combinations with cenicriviroc and LYS0006 with early results still pending and waiting to be reviewed. By now it is well known that features of metabolic syndrome increase the risk of developing NAFLD. Type 2 diabetes, specifically, is a risk factor for NASH and its presence increases the risk of progression of NASH fibrosis [27,28]. Therefore, some of the ongoing trials in combination therapy for NASH include semaglutide, pioglitazone or licogliiflozin; from drug classes that traditionally have been utilized for the management of T2DM. In one randomized, placebo controlled trial in patients with biopsy proven NASH, a GLP-1 analog, liraglutide, was associated with greater improvement in steatohepatitis and lower progression of fibrosis[39]. A proof-of-concept trial is currently underway including semaglutide along with cilofexor and firsozostat. Licogliiflozin, which has shown benefit in lowering liver fat content, is being studied as part of a combination trial with tropifexor in the ELIVATE trial. Numerous trials have already established the benefit of pioglitazone in improving inflammation and fibrosis in patients with biopsy proven NASH and therefore it is included as recommended management for a select group of patients according to the most recent American Association for the Study of Liver Diseases (AASLD) guidelines on NAFLD [29].

Aside from the ability to decrease inflammation and fibrosis in NASH, allowing treatment with lower doses of various drugs in combination or improvement the side effect profile are two alternate motives for pursuing combination therapy. In Wister rat models for NASH, one group was able to demonstrate a synergistic therapeutic effect on inflammation and oxidative stress from combining elafibranor and obeticholic acid at lower doses than with each drug in monotherapy [30]. FXR agonists have been shown to increase low density lipoprotein (LDL) cholesterol concentrations. In the CONTROL study, the authors were able to lower the LDL concentration below baseline with the addition of atorvastatin [31]. In a separate phase 2, proof-of-concept trial, fenofibrate was tested in combination with the ACC inhibitor, firsozostat, to help lower triglyceride levels [32].
CONCLUSION

In this concise review, we have discussed numerous possible therapeutic options for cessation or reversal of NASH and associated fibrosis. It is clear that NASH and particularly NASH fibrosis or cirrhosis is a leading topic garnering much interest in the study of liver diseases today, which would be appropriate considering the clinical impact. Although some of the above targets may seem promising, there are still a few concerns regarding study of this particular topic. Firstly, it is evident by reviewing the endpoints of each of the studies listed above that there is much heterogeneity. This is partly because the gold standard of liver biopsy to prove effectiveness in this endeavor is cumbersome, costly, and generally not favored by patients. The use of surrogates for liver fibrosis and resolution including markers of turnover, inflammation, or non-invasive assessments of liver scarring have not universally been agreed upon in the use of clinical trials. Secondly, for those studies that have utilized liver biopsies are part of their endpoint assessment, there can be considerable inter-observer variability in interpretation of liver biopsy specimens, assuming they are of adequate quality. Additionally, it is apparent that many of the study developers use magnetic resonance imaging with proton density fat fraction (MRI-PDFF) for assessment of liver fat content while there are additional tools such as the controlled attenuation parameter (CAP) of transient elastography systems which might be more acceptable as a point of care test. Lastly, endoscopic and surgical bariatrics represent an emerging area of therapeutic development for the management of obesity in context of NASH. In one prospective study of 180 patients, bariatric surgery was associated with NASH resolution in 84% of patients with improvement in fibrosis in 70% of patients at the 5 year mark after surgery [33]. Separately, in a 6 mo multi-center study of 85 patients with T2DM undergoing duodenal mucosal resurfacing, not only did their A1c improve, but so did their ALT levels and FIB-4 scores hinting at the possible insulin sensitizing, lipid lowering, anti-inflammatory and antioxidant effects of this procedure [34]. In conclusion, this is an expanding area of study. There may be more questions than answers at this current time considering the heterogeneity of the NAFLD disease spectrum, variability
in who will progress in disease and what treatments the medical community will be able to recommend in ameliorating this disease burden. One of the answers however does seem to implicate combination therapy as the optimal approach.
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