Non-alcoholic Steatohepatitis (NASH) and Hepatic Fibrosis: Emerging Therapies

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NASH: A chronic progressive liver disease

- Non-alcoholic steatohepatitis (NASH) is a chronic and progressive liver disease, and is considered the progressive phenotype of nonalcoholic fatty liver disease (NAFLD), the most prevalent chronic liver disease worldwide
- NASH is characterized by the accumulation of fat in the liver (steatosis), inflammation and liver damage, which can progress to high-burden conditions such as fibrosis, cirrhosis, and hepatocellular carcinoma (HCC)
- An estimated 3%-7% of the adult population develop NASH, of which approximately 15%-20% progress to advanced fibrosis, namely bridging fibrosis (F3) or cirrhosis (F4)
- NASH frequently progresses undetected due to the early nonspecific symptoms of the disease, leading to serious patient consequences, such as end-stage liver disease (ESLD), an increased need for liver transplantation (LT), and mortality[1]
- Several investigators observe a positive relationship between the increasing incidence of NAFLD and sedentary lifestyle habits. Validating this, the prevalence of NAFLD and NASH is reported to be 80-90% in obese adults, 30-50% in diabetic cases, and up to 90% in individuals with hyperlipidemia
- Further, common risk factors observed are male gender, age, obesity, insulin resistance, and the cardiometabolic alterations defining the metabolic syndrome.[2]

The complex pathophysiology of NASH

- The initially proposed theories for NASH include a two-hit hypothesis, based on appearance of steatosis (first hit), followed by a second hit leading to inflammation, hepatocyte damage, and fibrosis, was proposed for the development of NASH
- While accumulation of triglycerides is necessary for the development of NASH, they may actually have a protective role against hepatocytes lipotoxicity, which is mainly induced by fatty acids and derived metabolites such as diacylglycerols, acylcarnitines or ceramides
- It also remains elusive, whether NASH develops sequentially, on the grounds of a fatty liver, or it is rather a de novo response to a lipotoxic environment
- The multi-parallel hypothesis proposed has suggested that NASH is the result of numerous conditions acting in a collateral fashion, including genetic predisposition, abnormal lipid metabolism, oxidative stress, lipotoxicity, mitochondrial dysfunction, altered production of cytokines and adipokines, gut dysbiosis and endoplasmic reticulum stress. The hypothesis shows that hepatic inflammation in NASH may even precede steatosis
- Number of other contributing factors are also being defined that have all together put forth a more complex picture of NASH pathogenesis and its future therapeutics (Figure 1).[3]

Emerging therapies in NASH: Based on its complex pathophysiology

- NASH is a progressive inflammatory phenotype of liver disorder. Presently apart from lifestyle interventions no other effective treatment available for NASH management
- Multiple pathogenic pathways are involved in NASH progression, and targets for intervention have been identified
- These targets mediate glucose, lipid, and bile acid metabolism; inflammation; apoptosis; and fibrosis
- Novel therapeutic agents are being developed in each of these pathways, and several have shown promise in early phase testing and other under approval.[4]

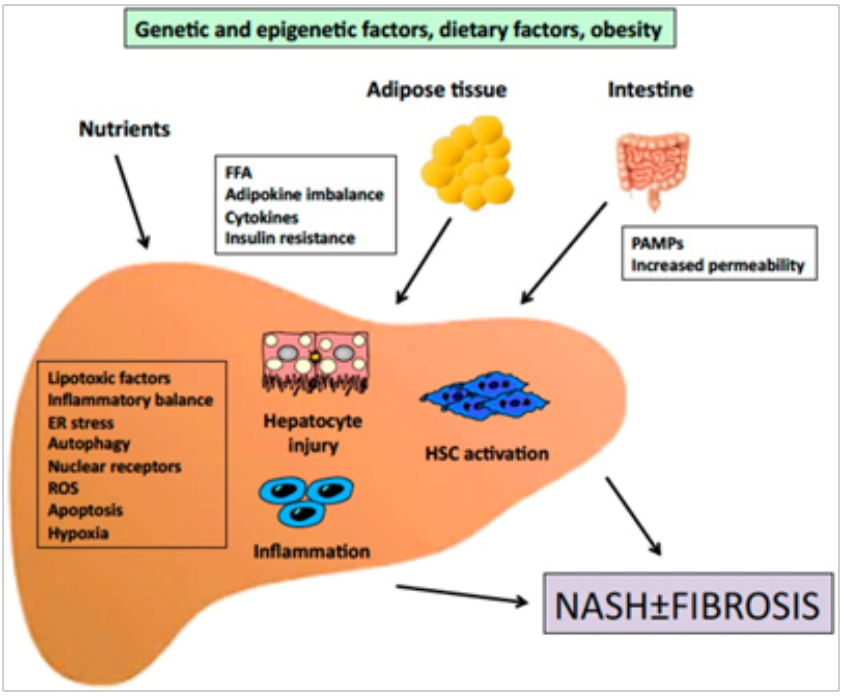
1. Deranged Lipid Metabolism

Abnormal accumulation of lipids in hepatocytes is an essential aspect of NASH. These lipids derive from the systemic circulation, denovosynthesis, and reduced degradation or export from hepatocytes. As lipotoxicity promotes cell death and inflammation, many interventions target toxic lipid buildup.[5]

Lipid-altering agents

- Stearoyl-CoA desaturase (SCD) is an enzyme that catalyzes the rate-limiting step in the synthesis of monounsaturated

Figure 1: The complex pathogenesis of NASH: Paving way for future therapeutic3



Sources: Caligiuri A, Gentilini A, Marra F. Molecular Pathogenesis of NASH. *Int J Mol Sci*. 2016;17(9):1575. Published 2016 Sep 20

fatty acids such as oleic acid. Located in the endoplasmic reticulum, experimental studies have shown that a deficiency (or inhibition) of the SCD-1 isoform is associated with decreased liver steatosis as well as improved insulin sensitivity. Obese subjects with NASH appear to have greater stearoyl-CoA desaturase 1 activity (SCD1, $p < 0.002$) than those with a normal liver, which is also reflected at the level of gene expression.

- Aramchol, an SCD-1 inhibitor, is a cholic acid-arachidic acid conjugate that was evaluated in a double-blind, placebo-controlled trial of 60 patients with biopsy-proven NAFLD (only 6 had NASH) who were randomized assigned to daily 100mg or 300mg Aramchol versus placebo (n=20 per group). The drug was associated with reduced hepatic fat content when administered as a 300mg/day regimen versus placebo for 3 months.Aramchol is currently being evaluated in a multicenter phase IIb trial to determine its effectiveness in NASH patients.
- Statins are HMG-CoA reductase inhibitors that are extensively used in both primary and secondary prevention of cardiovascular disease due to their effective plasma lipid-lowering abilities. Dyslipidemia is a common feature of both the metabolic syndrome and NAFLD, placing patients at increased risk of cardiovascular events. Moreover, NAFLD alone is an independent risk factor for cardiovascular disease. However, statins have been found to be underused for this condition, even though they are considered to be generally safe at moderate doses in patients with chronic liver disease and are found to provide protection from steatosis, steatohepatitis and stage F2-F4 fibrosis.[5,6]

PPAR agonists

Obesity and insulin resistance create metabolic stress that result in lipotoxicity. Insulin reduces fatty acids (FA) release from adipocytes. Because insulin resistance can overwhelm the liver with adipose-derived FA, diabetes treatments are potential therapies for NASH[5]

- Peroxisome proliferator-activator receptors (PPARs) are a group of nuclear receptors that are expressed in the liver, adipose tissue, heart, skeletal muscle and kidney and transcriptionally regulate multiple metabolic processes including B-oxidation, lipid transport and gluconeogenesis
- Pioglitazone,a peroxisome proliferator-activated receptor (PPAR)**γ** agonist, improved NASH but has side effects such asweight gain
- The dual PPARα/δ agonist, elafibranorhas shown to improve both hepatic and peripheral insulin sensitivity in abdominally obese individuals in a liver-targeted fashion and also shown to resolve NASH in a phase IIb, randomized double-blind placebo controlled trial (GOLDEN-505)

- A dual PPARα/γ agonist, saroglitazar, which has been approved to treat diabetic dyslipidemia in India has also been shown to result in a significant decrease in ALT levels in subjects with NAFLD and biopsy-proven NASH. Further clinical studies are needed in this drug to confirm this initial success.[5,6]
- Theglucagon-like peptide 1 (GLP-1) analogue liraglutide improved NASH but adversely affected liver fibrosis.[5]

FXR-bile acid axis

Bile acids (BAs) contribute to NASH pathogenesis by facilitating dietary fat absorption and acting as hormones. After conjugation by small-intestinal bacteria, secondary BAs are absorbed into the systemic circulation and bind to farnesoid X receptor (FXR) and other receptors to regulate tissue insulin sensitivity and lipid synthesis. Hence BA metabolism is a potential NASH therapeutic target.[5]

- FGF-19 is a hormone that is transcriptionally regulated via FXR activation in response to the postprandial influx of bile acids in the terminal ileum. FGF-19 then binds to the FGFR4/β-klotho receptor complex on the hepatocyte cell membrane, thus suppressing gluconeogenesis while promoting glycogen synthesis. It has been suggested that FGFR4 activation could have a cancer-promoting effect on hepatocytes. Thus non-tumorigenic variants have been developed with some success in initial studies. NGM-282 is considered to be one non-cancer-promoting agent and it is currently in a phase II trial. NGM282, a fibroblast growth factor 19 (FGF-19) variant, regulates BA synthesis and found to reduce hepatic steatosis and aminotransferases in a recently completed phase II trial
- Volixibat, an inhibitor of the ileal BA transporter, is currently in a phase II trial
- Several FXR agonists are under study alone or in combination. One such drug, obeticholic acid, or OCA, is a synthetic bile acid derivative and FXR agonist that was recently studied in biopsy-proven NASH patients without cirrhosis in the FLINT trial. It improved fibrosis and NASH in a large phase II study and is now in phase III.[5,6]

2. Cell injury or death (apoptosis)

Once hepatocytes are injured by the toxic build-up of lipids, oxidative and endoplasmic reticulum stress pathways trigger cell death. Downstream mediators of these pathways try to reestablish tissue homeostasis by initiating repair responses. Striking a balance between injury and repair determines whether NASH progresses or resolves. Repetitive injury and/or dysregulated repair promote neoplasia of repair-related cells, progressive scarring, andincreased risk for liver cancer and cirrhosis.[5]

- Caspase-regulate programmed cell death (apoptosis) in NASH. Emricasan is a pan-caspase inhibitor that was initially studied in patients with chronic liver diseases of diverse etiologies, and was found to lower ALT levels, in particular in patients with hepatitis C and with NASH. The efficacy of Emricasan is being evaluated in a phase IIb trial in patients with NASH and fibrosis
- Apoptosis related kinase-1 (ASK-1) is an ASK that is activated by oxidative stress. Selonsertib, an ASK-1 inhibitor, reduced fibrosis in a small phase II study and is now under investigation for NASH with advanced fibrosis
- Pentoxifylline (PTX) is a methylxanthine derivative that has an inhibitory effect on phosphodiesterase as well as on TNFα, and thereby modulate the functions of other inflammatory cytokines. Histologic improvement has been found with PTX in patients with biopsy-proven NASH. PTX helps in reduction in lipid peroxidation, through this agents oxygen radical scavenging properties and glutathione-replenishing ability. Though, large robust studies are needed to confirm the efficacy of PTX in the treatment of NASH
- Vitamin E, with its known antioxidant effects is found to be effective in NASHin patients without diabetes.[5,6]

3. Inflammation

Inflammation is necessary to repair liver injury, but recovery depends on the type and number of inflammatory cells that accumulate. Diet, adiposity, metabolic syndrome, and the intestinal microbiome modulate the inflammatory response. The gut innate immune system is critically involved in NASH pathogenesis because it regulates trafficking of bacteria and bacterial products through the portal circulation.[5]

- Endotoxin, or lipopolysaccharide (LPS), is a key proinflammatory molecule. Anti- LPS antibodies contained in hyperimmune bovine colostrum (IMM124E) impact inflammation and insulin resistance and are being studied in NASH
- Toll-like receptors (TLRs) recognize LPS, and JKB-121, an inhibitor of TLR-4, is also being evaluated
- Chemokines that direct inflammation are also promising therapeutic targets for NASH. During liver injury, hepatocytes and other liver cell types release inflammatory chemokines such as CCL2 (MCP1) and CCL5 (RANTES) that help recruit macrophages and other inflammatory cells to the site. CCL2 and CCL5 act via their respective receptors, CCR2 and CCR5, located on a host of inflammatory cell types to elicit migration. Interestingly, CCL2 and CCL5 are two of 17 up-regulated genes in NASH patients. Cenicriviroc is a CCR2/CCR5 antagonist and the results of a phase IIb trial (CENTAUR) with this drug are awaited in NASH patients.
- Tipelukast, an anti-inflammatory agent that inhibits leukotriene production, is being studied in pulmonary fibrosis and NASH.[5,6]

Gut and Microbiome related therapies

Disturbed gut-liver barrier integrity has been reported to be essential in the pathogenesis of NAFLD and NASH since

bacteria and their products (especially lipopolysaccharide or LPS) can escape into the systemic circulation causing a massive inflammatory response from the liver. Thus, any therapies that prevent this bacterial or LPS 'translocation' to the systemic circulation and liver are of interest.[6]

- IMM-124e is an IgG-enhanced bovine-derived colostrum with favourable results in preliminary clinical studies including improved glycemic control, insulin resistance and lipid profile with more studies underway
- Orlistat is an FDA-approved lipase inhibitor to treat obesity whose action is located in the gut to reduce dietary fat absorption. It has shown to improve liver enzyme levels and liver content, its efficacy in NASH yet to defined
- Other agents targeting the microbiome include a macrolide antibiotic, solithromycin, which is currently in a phase II trial.[6]

4. Fibrosis

Cirrhosis, the most deleterious consequence of NASH, occurs because regenerative responses cannot keep pace with hepatocyte death, and thus perpetuate futile wound-healing responses that drive neoplasia and fibrosis.These defective regenerative efforts also promote liver cancer. Hepatic stellate cells (HSCs) are the major producers of fibrous matrix in liver. Mediators of HSC activation, such as transforming growth factor b (TGF-b), are important therapeutic targets.[5]

- Simtuzumab is a monoclonal antibody targeting lysyl oxidase-like 2 (LOXL2), a key matrix enzyme in collagen formation and is highly expressed in the liver. The drug is currently being studied in a phase II trial in both cirrhotic and non-cirrhotic subjects with NASH
- Other emerging monoclonal antibodies under way are fresolimumab, and pamrevlumab
- Galectin-3 is a protein expressed predominantly in immune cells that recognizes and binds to galactose residues. Galectin-3 is an essential protein in liver fibrogenesis and thus a good target of the inhibitor, GR-MD-02. This drug is currently in two phase II clinical trials in NASH patients with fibrosis/cirrhosis.[5,6]

Box 1 depicts the emerging therapies for NASH based on its various pathophysiological mechanisms.[5,6]

Box 1: Current and emerging therapies for NASH

Insulin resistance
- GLP-1 (liraglutide)
- PPAR (elafibranor, pioglitazone, saroglitazar)

Bile acid circulation
- FXR (obeticholic acid)
- FGF19 (NGM282)
- IBAT (Volixibat)

Gut microbiome
- Solithromycin

Inflammation
- Chemokines (cenicriviroc)
- Lipopolysaccharide (LPS) (IMM124E)
- Toll-like receptors (TLRs) (JBK-121)
- Tipelukast

Apoptosis/Cell death
- ASK-1 (emricasan, selonsertib)
- Vitamin E
- Pentoxifylline (PTX)

Fibrosis
- Lysyl oxidase-like 2 (LOXL2) (Simtuzumad)
- Monoclonal antibodies : Fresolimumab, and pamrevlumab
- Galectin-3 (GR-MD-02)

Sources: 1. Wegermann K, Diehl AM, Moylan CA. Disease pathways and molecular mechanisms of nonalcoholicsteatohepatitis. Clin Liver Dis (Hoboken). 2018 Apr 20;11(4):87-91. **2.** Oseini AM, Sanyal AJ. Therapies in non-alcoholic steatohepatitis (NASH). Liver Int. 2017;37Suppl 1(Suppl 1):97–103.

Conclusion

A number of molecular pathways interact to orchestrate the pathogenesis of NASH based on which a number of novel drugs and molecular targets are under way and awaiting FDA-approval. Earlier drug development in NASH has focused on biologically plausible, single-molecule targets that yielded disappointing results. The new interventions and novel approaches toward development of much needed effective drug therapies targeting the complex pathophysiology of this multifaceted disorder have paved the future way for an effective and well-structured pharmacological approach for NASH.

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