New Therapeutic Targets in Alcoholic Hepatitis

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Abstract

Alcoholic liver disease (ALD) is a leading cause of liver related morbidity and mortality worldwide. ALD encompasses a spectrum of disorders including asymptomatic steatosis, steatohepatitis, fibrosis, cirrhosis and its related complications, and the acute on chronic state of alcoholic hepatitis. While multidisciplinary efforts continue to be aimed at curbing progression of this spectrum of disorders, there is an urgent need to focus our efforts on effective therapeutic interventions for alcoholic hepatitis (AH), the most severe form of ALD. AH is characterized by an abrupt development of jaundice and complications related to liver insufficiency and portal hypertension in patients with heavy alcohol intake. The mortality of patients with severe AH is very high (20–50% at 3 months). The current therapeutic regimens are limited. The development of new therapies requires translational studies in human samples and suitable animal models that reproduce clinical and histological features of human AH. This review article summarizes the clinical syndrome, pre-clinical translational tools, and pathogenesis of AH at a molecular and cellular level, with the aim to identify new targets of potential therapeutic intervention.

1. Introduction

Alcoholic Liver Disease is the leading cause of liver-related morbidity and mortality worldwide and is a major cause of death among adults with prolonged alcohol abuse[1]. According to WHO, 3.3 million deaths occur worldwide every year due to the harmful use of alcohol, representing 5.9% of all deaths. Alcohol is the third leading preventable cause of death in the US accounting for about 88,000 deaths per year[2,3]. This review highlights the natural history, pathogenesis, molecular and cellular targets, as well as the current and new therapies being investigated for Alcoholic Hepatitis.

Alcoholic liver disease encompasses different stages of liver disease as a consequence of susceptibility factors and duration of alcohol consumption; listed from least to most severe, are steatosis, alcoholic steatohepatitis (ASH), progressive fibrosis, end stage cirrhosis, decompensated cirrhosis, and superimposed hepatocellular carcinoma (HCC).

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Alcoholic hepatitis is an acute on chronic condition, diagnosed clinically by new-onset jaundice and/or ascites in the setting of ongoing alcohol abuse and underlying ALD. Severe forms of AH have very high short term mortality and represent one of the deadliest diseases in clinical hepatology, with a mortality rate of 30–50% at 3 months[4].

The true incidence of AH is not well known; population based studies estimate approximately 4.5 hospitalizations for AH per 100,000 persons per year[5]. Patients with ALD can present with acute episodes of jaundice and liver decompensation from other reasons, such as sepsis, biliary obstruction, diffuse HCC, drug-induced liver injury and ischemic hepatitis. All the etiologies stated above present with a similar clinical picture and there is a lack of biomarkers or other laboratory tests to distinguish these acute entities. Where diagnosis is unclear, transjugular liver biopsies in patients hospitalized for acute hepatitis with underlying ALD to confirm the existence of AH is important.

2. Environmental and Genetic Risk Factors

There is a positive correlation between cumulative alcohol intake and degree of liver fibrosis; however extensive variability in the histological response to alcohol abuse exists in individuals. At similar levels of ethanol consumption, some patients only develop fatty liver or macrovesicular steatosis, while others progress to fibrosis and cirrhosis. Several risk factors for the susceptibility of ALD have been identified including sex, obesity, drinking patterns, dietary factors, non-sex-linked genetic factors, and cigarette smoking[1,6,7](Figure 1).

Epidemiological studies suggest that several genetic factors influence the severity of steatosis and oxidative stress, and that the cytokine milieu, the magnitude of the immune response, and the severity of fibrosis also modulate an individual's propensity to progress to advanced ALD. The genetic factors that influence an individual's susceptibility to develop advanced ALD are largely unknown. Variations in genes encoding antioxidant enzymes, cytokines, other inflammatory mediators, and alcohol-metabolizing enzymes seem to play a role[7].

Owing to its fibrogenic potential, variations in the rate of generation of acetaldehyde could explain the differences in the susceptibility of individuals to ALD after abusive alcohol consumption. Although polymorphisms in the genes encoding the main alcohol-metabolizing enzymes such as ADH, ALDH and CYP2E1 are accepted to be involved in an individual's susceptibility to alcoholism, their role in the progression of ALD remains controversial[8].

Also, recent studies indicate that variations in the patatin-like phospholipase domain-containing protein 3 (PNPLA3) strongly influence the development of cirrhosis in alcoholic Caucasians and Mexicans. PNPLA3 polymorphisms can be considered as the only confirmed and replicated genetic risk factor for ALD. However deletion of the PNPLA3 gene did not affect obesity-associated fatty liver or liver enzyme elevation in animal studies[9–11].

Polymorphisms in genes that encode pro-inflammatory cytokines like TNF, IRAK-M, interleukin-1β, IL-1 receptor antagonists, IL-2, IL-6 and IL-10, involved in the pathogenesis

of ALD, have also been examined[12]. Moreover, studies have also investigated the role of genetic variation in factors involved in lipopolysaccharide induced intracellular pathways, including CD14 and toll-like receptor 4 (TLR4), as potential risk factors for ALD[13].

Despite the large number of studies that have assessed the role of genetic variation in susceptibility to ALD, a large-scale, well-designed, genome-wide association study of factors linked to the development of ALD remains to be performed. Consequently, a genetic test capable of identifying which patients are susceptible to advanced ALD is yet to be developed. Such a test could be useful in clinical settings, as it would help to identify the main genetic determinants of ALD, which could potentially assist in the development of future therapies[14].

3. Current treatment in management of alcoholic hepatitis

Patients that develop severe AH usually require hospitalization for initial management, a summary of current interventions is listed in Table 1. Primary prevention is aimed at alcohol abstinence; active management of alcohol use disorders is critical to achieving continued abstinence. For the successful management of these patients, a multidisciplinary team composed of hepatologists, psychologists, psychiatrists and social workers is highly recommended[15]. Significant protein calorie malnutrition is a common finding in alcoholics, as are deficiencies in a number of vitamins and trace minerals, including vitamin A, vitamin D, thiamine, folate, pyridoxine, and zinc[1,16, 17]. Nutritional support improves liver function and short-term follow-up studies suggest that improved nutrition might improve survival times and histological findings in patients with AH[18]. Most patients improve spontaneously with abstinence and supportive care. Medical treatment is considered for patients who present with a very severe clinical picture or continue to deteriorate (Figure 2).

Several clinical scoring models have been developed to help predict outcomes of patients with AH and to guide therapy, including the Maddrey Discriminant Function (DF); Glasgow Alcoholic Hepatitis Score (GAHS); Mayo End-stage Liver Disease (MELD); Age, Bilirubin, INR, Creatinine (ABIC); MELD-Na, UK End-stage Liver Disease (UKELD), and three scores of corticosteroid response at 1 week: an Early Change in Bilirubin Levels (ECBL), a 25% fall in bilirubin, and the Lille score. The MELD, DF, GAHS, ABIC and scores of corticosteroid response prove to be valid in an independent cohort of biopsy-proven alcoholic hepatitis[19]. Combining features of various scoring models, for example, from the DF, ABIC, MELD and Lille, may prove to be a better prognosis indicator[20].

Corticosteroids have been used in the treatment of AH for more than 40 years. Prednisolone is widely considered the first line therapy for severe AH. Both the AASLD and EASL practice guidelines recommend the use of corticosteroids (i.e. prednisolone 40 mg daily for 4 weeks) for patients with severe AH, defined by Maddrey's discriminant function >32 or the presence of hepatic encephalopathy [1,21]. Clinical trials conducted, to determine efficacy of steroid administration, so far suffer from heterogeneity and a high risk of bias. A recent meta-analysis from individual data however showed improved survival in patients with a high DF. In this study patients were categorized as complete responders, partial responders, or null responders and was able to predict the 6-month survival of each group using 2 new

cut-offs of the Lille score [22]. In another study, the response to prednisolone was assessed based on the change in bilirubin after one week of therapy and quantified using the Lille score. The Lille score was calculated after 7 days of initiation of therapy and it was determined that corticosteroids can be discontinued in non-responders, defined by a Lille score >0.45[20]. A Lille score greater than 0.45 predicts a 6-month survival rate of less than 25% [23]. Clinical practice guidelines recommend stopping corticosteroids after one week in those with an unfavorable Lille score, as the risks of continued therapy likely outweigh the benefits. The contraindications for the use of corticosteroids are not well defined. When considering treatment with corticosteroids, patients require careful monitoring for evidence of present or developing infections and/or active GI bleeding.

Pentoxifylline is a phosphodiesterase inhibitor that inhibits the synthesis of tumor necrosis factor, which is increased in patients with AH. In practice, pentoxifylline is typically reserved as a second-line agent for patients with contraindications to corticosteroid therapy. A large trial, STOPAH, comparing prednisolone and pentoxifylline is underway and should prove to be a definitive study for assessing the efficacy of these drugs for AH[24]. Current consensus regarding Pentoxifylline is that it is not effective rescue therapy in patients who do not respond to corticosteroids[25].

Infliximab and etanercept, anti-TNF agents, were also investigated as potential therapies for patients with AH. Rationale for their use was similar to pentoxifylline; TNF-α was implicated as a key culprit in the potentiation of hepatocyte inflammation. The use of these agents should theoretically benefit patients hospitalized with AH. However translational studies did not support the hypothesis[26,27]. Other larger studies resulted in adverse side effects such as increased rates of infection and increased mortality. Presently anti-TNF-α agents are not recommended for treatment of AH[28].

N-acetylcysteine is known to replenish glutathione in damaged hepatocytes and prevent cell death in ALD. A recent randomized trial showed that the combination of N-acetylcysteine with prednisolone showed a clear trend to improve survival, by reducing 1-month mortality (8% vs. 24%) and reduce incidence of hepatorenal syndrome and infection, although the study was underpowered to reach statistical significance. In addition, it was found to have no effect on six-month survival[29]. The favorable safety profile of N-acetylcysteine makes it a potential option, in combination with corticosteroids, for patients with severe disease.

4. Role of transplantation in Alcoholic Hepatitis

ALD is the second most common indication for liver transplantation (LT) for chronic liver disease after HCV cirrhosis. Despite this, it is estimated that as many as 95% of patients with end-stage liver disease related to alcohol are never formally evaluated for candidacy for liver transplantation.

An important issue that is still unresolved is the role of LT in patients with alcoholic hepatitis, who are generally excluded from transplant. Patients with severe AH who do not respond to medical are unlikely to survive the "mandatory" 6-month abstinence period as their risk of mortality is quite high[30,31]. However post-transplant outcomes appear to be

good for highly selected patients with severe AH unresponsive to medical therapy with low rates of alcohol relapse[32,33]. Salvage liver transplantation in highly selected patients has been shown to improve survival significantly, but is not available in the vast majority of transplant centers[32]. The availability of living donor transplantation and extended criteria donor liver transplantation are likely to heighten the debate on this issue.

5. Translation research in ALD: integrating animal and human studies

While animal models serve as the cornerstone of many research studies, their use in exploring potential novel targets for the treatment of alcoholic liver disease, particularly alcoholic hepatitis, is limited. Many rodent models produce some features of chronic alcoholic liver disease, but it is difficult to mimic acute-on-chronic liver injury as seen in patients with alcoholic hepatitis, though improved models are emerging.

While many models exist to mimic mild to moderate levels of hepatocyte damage, a better model of alcoholic hepatitis, is the chronic-plus-binge feeding model. As the name implies, it models the chronic drinking behaviors with intermittent binges seen as in patients presenting with alcoholic hepatitis. This model causes hepatocyte damage, disruption of mitochondrial function, and oxidative stress, resulting in moderate rises in AST and ALT, but in addition to mimicking steatosis, liver injury, and early fibrogenic response, it was also able to demonstrate hepatic neutrophil infiltration as seen in early stages of alcoholic hepatitis[34], though the hepatocellular damage and inflammation were noted to be transient. Given the prevalence of obesity, rodent models have been developed to encompass both non-alcoholic steatohepatitis and alcoholic liver disease. The hybrid model with high-fat and high cholesterol plus chronic and binge ethanol feeding involves a high-fat and high cholesterol diet comprising 40% of the caloric intake, while chronic intragastric ethanol feeding comprised 60% of the caloric intake of these mice, and was supplemented by weekly binges of ethanol. This model represents moderate to severe alcoholic hepatitis, causing significant liver inflammation, by reproducing chronic alcoholic steatohepatitis characterized by balloon cell degeneration, macrophage activation and infiltration, and progression of liver fibrosis. It closely mimics alcoholic hepatitis histologically, with findings of neutrophil infiltration, but also clinically represents this acute entity as mice develop splenomegaly, hypoalbuminemia, and hyperbilirubinemia[35]. Models to show advanced fibrosis and cholestasis, as observed in alcoholic hepatitis, do not yet exist. "Second-hit" or "Multiple-hit" models exist in which use chronic ethanol feeding to induce hepatic susceptibility to other agents including nutritional modification or pharmacologic agents to achieve the acute on chronic disease state have shown some promising pathologic similarities to human alcoholic hepatitis including coagulative necrosis and inflammation [36], but often as a consequence of the second agent used, and thus calls into question their clinical relevance.

The traditional approach of identifying molecular drivers in animal models and translating this work to human disease poses many challenges. In addition, the the challenges of finding appropriate models to mimic histologic findings in human alcoholic liver disease and incorporate co-morbidities faced by patients with alcoholic liver disease and those presenting with alcoholic hepatitis, remains difficult to do.

A systematic rational approach to designing translational studies to examine alcoholic hepatitis begins by first determining the phenotype of the patient, disease severity and comorbidities that contribute to the acute disease state, with careful consideration given to related overlapping syndromes. Disease severity and patient prognosis can be computed using a variety of scoring systems as discussed previously. The second step involves obtaining anthropometrical, clinical, and histological data, though liver biopsy, and collection of ancillary biospecimens including suprahepatic and peripheral blood, PMNs, and stool for genomic, proteomic, and metabolomic studies. The third step involves the correlation of gene and protein expression in biospecimens obtained with clinical or histological features observed in patients to identify potential molecular drivers of disease. Finally, the fourth step involves testing hypothetical relations in in vitro and in vivo models in carefully designed animal studies, with the eventual goal in mind of identifying druggable molecular targets to reverse or abort progression of this disease (Figure 3).

6. Molecular pathogenesis of Alcoholic Liver Disease and potential targets for intervention

An understanding of the spectrum of disease states comprising ALD and the pathogenic mechanisms at work is imperative to our understanding of the development of alcoholic hepatitis as an acute on chronic state, and provides ample opportunity to halt progression in early disease stages. Table 2 summarizes some of the key molecular and cellular markers that are potential new targets for therapeutic intervention. Chronic alcohol ingestion leads to steatosis, an asymptomatic state reversible on cessation of alcohol consumption. Prolonged alcohol consumption can lead to alcoholic steatohepatitis, and inflammatory stated marked my neutrophil infiltration and hepatocellular damage. Histological evaluation demonstrates fat accumulation, hepatocyte ballooning, neutrophil infiltration, and even early signs of pericellular and sinusoidal fibrosis. Fibrosis can then progress to cirrhosis complicated by poor synthetic function portal hypertension and its associated complications. Alcoholic hepatitis, as previously mentioned, is an acute on chronic disease state, in which the majority of patients have underlying advanced fibrosis, and in addition to a marked inflammatory response, histologically, canalicular and lobular bilirubinostasis may be present[37]. The interplay of molecular and cellular markers contributing to disease is complex (Figure 4) and is discussed in detail below.

Steatosis as a result of decreased fatty acid oxidation

Steatosis evolves from the intrahepatic accumulation of fats, mainly triglycerides, phospholipids, and cholesterol esters, which is aggravated by excessive alcohol intake through disruption of fatty acid oxidation, and increase of fatty acid and triglyceride synthesis and uptake. Alcohol intake increases the NADH/NAD+ ratio in hepatocytes, which disrupts mitochondrial β-oxidation of fatty acids resulting in accumulation and steatosis[38]. Excessive alcohol consumption is also responsible for decreasing β-oxidation of fatty acids through it's metabolite, acetaldehyde, which directly inhibits the DNA-binding ability and transcriptional activation ability of peroxisome proliferator-activated receptor-α (PPAR-α)[38], a nuclear hormone receptor that regulates transcription of many genes involved in free fatty acid transport and oxidation[39,40]. Ethanol can also indirectly inhibit the function of

PPAR-α through various mechanisms. The first is by up-regulating cytochrome P450 2E1 (CYP2E1) and hepatic oxidative stress, which in turn inhibits oxidation of fatty acids by preventing up-regulation of PPAR-α as demonstrated in wild-type mice as compared to CYP2E1 knock-out mice[41]. The second and third include down regulation of adiponectin, and zinc, both of which have been demonstrated to down-regulate PPAR-α[42,43].

Steatosis as a result of increased fatty acid uptake and synthesis

It is well known that alcohol consumption increases hepatic influx of free fatty acids from adipose tissue and chylomicrons from the intestinal mucosa[38]. Fatty acid synthesis is increased through up-regulation of lipogenic enzymes. This is mainly accomplished directly through up-regulation of sterol regulatory element-binding protein-1c (SREBP-1c), a master transcription factor that up-regulates the expression of genes encoding lipogenic enzymes, or indirectly through inhibition of factors that inhibit SREBP-1c. Acetaldehyde promotes transcription of SREBP-1c, which in turn up-regulates expression of lipogenic enzyme genes, contributing to increased fatty acid synthesis[44]. SREBP-1c is also up-regulated through multiple other processes including ethanol-induced hepatocyte ER stress[45], production and binding of adenosine to A1 receptors[46], and through endocannnabinoids[47]. Endocannabimoids mediate alcoholic liver injury through signaling through either of two cannabinoid receptors, cannabinoid receptor 1 (CB1) and cannabinoid receptor 2 (CB2)[48]. Mouse studies have demonstrated that signaling through the CB1 cannabinoid receptor worsens alcoholic liver injury while signaling through the CB2 confers a protective role in the development of steatosis and alcoholic liver injury[47]. This suggests that CB1 receptor antagonists and CB2 receptor agonists could serve as potential therapeutic agents in preventing steatosis and alcoholic liver disease. CB1 receptor antagonists have their limitations, due to their neuropsychiatric side effects, however, peripherally acting CB1 antagonists are currently being explored[48]. SREBP-1c is upregulated indirectly though down-regulation of various factors that reduce its expression, such as ethanol-mediated AMP-activated protein kinase (AMPK) inhibition[49]. Studies in rat hepatoma cells demonstrated that activation of SREBP-1c by ethanol is also mediated by mammalian sirtuin 1 (SIRT1), a NAD(+)-dependent class III protein deacetylase; ethanol exposure induced SREBP-1c lysine acetylation and SREBP-1c transcriptional activity, which was reversed by adding a SIRT1, suggesting that ethanol must have an inhibitory effect on SIRT1, inhibiting its inhibitory effect on SREBP-1c[50]. This finding suggests that SIRT1 agonists could be potentially be used to curtail the effects of ethanol on steatosis. To extend this further, it has been shown that adiponectin confers protection against alcoholic fatty liver via modulation of complex hepatic signaling pathways through central regulatory system, SIRT1-AMPK axis [51].

Direct effects of ethanol on enzymes involved in fatty acid metabolism

In addition to regulating transcription factors involved in the regulation of genes involved in fatty acid metabolism, ethanol can also directly affect the activities of these enzymes through it's inhibition of AMPK, a serine-threonine kinase. This was demonstrated through animal models of concomitant obesity and alcohol consumption, in which these mice demonstrated defective adiponectin-AMPK signaling even in the presence of increased adiponectin downstream of p-AMPK, suggesting a role for AMPK in steatosis[52]. AMPK

functions to inactivate the rate-limiting enzyme acetyl-CoA carboxylase (ACC) in fatty acid synthesis. In turn, inactivation of ACC leads to a reduction in the levels of malonyl Co-A, a precursor in fatty acid synthesis, and an inhibitor of the rate-limiting enzyme carnitine palmitoyltransferase 1 (CPT1), in fatty acid oxidation, resulting in less fatty acid synthesis and increased oxidation[53].

In addition, AMPK directly phosphorylates and inhibits SREBP activity in hepatocytes, thereby attenuating steatosis. In this manner, AMPK inhibits fatty acid synthesis but promotes fatty acid oxidation via the inactivation of ACC enzyme activity[54]. Alcohol consumption inhibits AMPK activity in the liver, leading to increased activity of ACC and decreased activity of CPT1 contributing to hepatic fatty acid accumulation and progression to steatosis[49].

6. Cellular pathogenesis of alcoholic liver disease

The role of autophagy in steatosis

Autophagy removes lipid droplets from hepatocytes[55]. The role of alcohol intake on the autophagic process depends greatly on the chronicity or acuity of intake. Chronic alcohol consumption inhibits autophagy, and thus leads to fat accumulation and steatosis[56,57]. Mouse studies have demonstrated that acute ethanol consumption conversely activates autophagy through the production of reactive oxygen species (ROS) and inhibition of the mammalian target of rapamycin (mTOR) signaling pathway, inhibiting progression to steatosis[58].

The role of the innate immune system in development of steatohepatitis

The innate immune system plays a large role in propagation of inflammation in steatohepatitis and the development of fibrosis. Excessive alcohol intake results in the mass production of ROS, which react with nucleic acids, fatty acids, proteins, and structural cell components to form adducts. These adducts are known to be potent activators of the innate immune system[59].

The contribution of bacterial-immune system interplay has received more attention in many gastrointestinal disorders. Kupffer cells, the livers native phagocytes, serve as the first line of innate immune system defense. It is known that alcohol increases gut permeability resulting in translocation of bacterial products such as LPS into portal circulation. This results in activation of Kupffer cells through TLR4 signaling through MyD88-dependent and independent (TRIF/IRF-3) pathways[60] leading to production and release of pro-inflammatory cytokines, including TNF-α [61–63]. TLR antagonists have been proposed as potential therapeutic agents for the management of alcoholic liver disease. Kupffer cells are also activated through binding of complement proteins, C3 and C5, induced through ethanol activation of the complement cascade[64,65].

While the innate inflammatory response is generally believed to propagate liver injury, Kupffer cells also have mechanisms in place to slow down and halt progression of inflammation through secretion of anti-inflammatory cytokines including IL-6 and IL-10.

Through activation of STAT3, these cytokines curb inflammation and slow progression of liver injury[67, 68].

Neutrophil infiltration is cytokine-mediated, through IL-17; IL-17 also stimulates hepatic stellate cells (HSCs) to produce IL-8 and CXCL1, these chemokines in turn, recruit more neutrophils, completing the cycle. Additional mediators that assist with neutrophil recruitment include IL-1, osteopontin, CXCL4, CXCL5, and CXCL6; these also activate macrophages during liver injury[68,69].

The role of the innate immune system in development of fibrosis

Hepatic fibrosis is characterized by the excessive accumulation of collagen and other extracellular matrix proteins. The extensive hepatocellular damage that occurs at this stage of liver injury, results in the production of cytokines, neuroendocrine factors, and angiogenic factors, leading to activations HSCs. Portal and bone marrow-derived fibroblasts also contribute to fibrosis[70,71].

The ethanol metabolite, acetaldehyde plays an important role in the instigation and maintenance of fibrosis, through HSC activation and maintenance of the activated phenotype. Through its rapid reaction with cellular components to form adducts, acetaldehyde and its adducts, malondialdehyde, 4-hydroxynonenal, and malondialdehyde-acetaldehyde, act upon HSCs to keep them in the perpetually activated "on" state[72].

In a similar manner, ROS can cause activation of HSCs. ROS also directly stimulate fibrosis through stimulating production of collagen though stimulation of pro-fibrogenic signaling pathways in HSCs including ERK1, ERK2, phosphinositide 3 kinase-Akt and JNK[73]. ROS also propagate collagen accumulation by preventing collagen degradation, first through direct inhibition of metalloproteinases which degrade collagen, and the secondly, through upregulation of the tissue inhibitor of metalloproteinases[73].

Extrinsic propagators of fibrosis include transluminal translocation of bacterial LPS. LPS activates the TLR4 on HSCs directly inducing HSC activation[74], it activates TLR4 signaling pathways on hepatic sinusoidal endothelial cells promoting angiogenesis and subsequent fibrosis[75]. Finally, TLR4 indirectly stimulates fibrosis through activation of Kupffer cells, which in exchange, release ROS and other pro-fibrogenic cytokines, causing activation of HSCs[76,77].

7. Cellular targets for therapeutic management of alcoholic hepatitis

Cell death via apoptosis

Massive hepatocyte cell death is a prominent feature of alcoholic hepatitis, and as previously discussed, apoptosis is a prominent feature of many of the preceding stages of alcoholic liver disease. Since caspase inhibitors are known to inhibit apoptosis, animal studies have been done in models of chronic liver injury from viral hepatitis secondary to hepatitis C infection, and non-alcoholic steatohepatitis, and caspase inhibitors have shown promising results in ameliorating liver injury and impeding progression to fibrosis[78–80]. It is reasonable to think such an approach would work in alcoholic liver disease, in particular in alcoholic hepatitis.

Role of Innate Immune System

Studies from other models of liver disease suggest that following activation, neutrophils undergo transmigration into the liver parenchyma where they destroy damaged hepatocytes through the release of ROS and proteases, supporting their prominent role in ALD[81]. IL-17 is increased in patients with alcoholic hepatitis and directly induces neutrophil recruitment, but also indirectly promotes neutrophil recruitment by stimulating HSCs to secrete IL-8 and CXCL1[82,83]. This suggests that the modification of these chemokines, or their precursors or activators, may mediate neutrophil infiltration and perhaps attenuate alcoholic hepatitis. Translational studies have examined the role of the CXCL family of chemokines, and found elevated levels correlate with severity of disease, degree of portal hypertension, and patient survival[84,85]. Given these promising findings, therapeutic agents that target CXCL chemokines may be considered in the treatment of AH. Osteopontin is an extracellular matrix protein that is markedly upregulated in alcoholic hepatitis, similar to other CXCL chemokines[86]. Agents that inhibit osteopontin, therefore, are also attractive in considering new therapeutic agents. The redundancy of chemokines and their receptors makes the development of targeted therapeutics challenging.

Instigators of inflammation are also thought to play an important role. Sources of inflammatory mediators can be classified as sterile, originating from intracellular sources, or microbiological, from bacterial translocation in the gut. Damage-associated molecular patterns (DAMPs) are intracellular molecules released by dying cells that trigger the innate immune system[87]. Among the DAMPs, high mobility group box-1 (HMGB-1) has been implicated in the development of alcoholic steatohepatitis[88], and likely also has a role in alcoholic hepatitis. Gut-derived bacterial products belong to the class of pathogen-associated molecular patterns (PAMPs). These PAMPs circulate trough the portal circulation and induce an inflammatory response through activation of HSCs and Kupffer cells[88,89]. Inhibition of gut leakage could be a potential target for therapy aimed at preventing the initiation of the innate immune response in alcoholic hepatitis.

Role of the Adaptive Immune System

While the role of the innate immune system has been widely explored, the role of the adaptive immune system in hepatocellular injury and propagation of alcoholic hepatitis leaves many questions unanswered. It is well-known that the adaptive immune system responds to oxidative stress and peroxidation adducts, but it's role in hepatocellular damage and inflammation in alcoholic hepatitis remains unknown. As previously described, increased alcohol consumption generates ROS through multiple mechanisms and leads to adduct formation; protein adducts have altered conformation and function, and are relatively immunogenic. Patients with alcoholic hepatitis have been found to have circulating T cells with antibodies to these adducts, enforcing that the adaptive immune response likely plays a large, yet undiscovered role in AH [90–93].

Targeting dysbiosis

Alterations in the gut microbiome has unique implications on the development of alcoholic hepatitis, this was first suggested in the intragastric mouse feeding model in which elevated serum ethanol levels were maintained, treated mouse populations were observed to have

both microbial translocation and dysbiosis[94]. In studies involving patients with chronic alcoholic liver disease, administration of probiotics appeared to improve liver function in this patient group, further supporting that the intestinal bacterial milieu is of great importance[95]. Work examining the applicability of probiotics in patients with alcoholic hepatitis is still underway. Other studies which included genomic and metabolomic analyses of intestinal bacteria revealed low levels of lactobacilli and reduced production of saturated long chain fatty acids (LCFA). In this model, supplementation with LCFA restored eubiosis, intestinal barrier function, and reduced liver injury in mice[96], suggesting a role for potential supplementation of LCFA in this patient group.

The role of hepatocyte proliferation and regeneration

Hepatic regeneration in the healthy liver results from expansion of the remaining healthy hepatocytes. In the diseased state, in which hepatocyte proliferation is inhibited, pluripotent liver progenitor cells, also referred to as oval cells, or ductal hepatocytes, proliferate and differentiate to repopulate hepatocytes or biliary epithelial cells[99]. In the rodent model, alcohol attenuates regeneration of hepatocytes following partial surgical hepatectomy[100], so though human studies lack, it is reasonable to hypothesize that alcohol not only causes hepatocellular injury and death, but also prevents regeneration. While histologically, the presence of bilirubinostasis and severe fibrosis are associated with a poorer prognosis in alcoholic hepatitis, the presence of proliferating hepatocytes is associated with better prognosis[101]. In addition, intense neutrophilic infiltrate was also associated with better prognosis[99], suggesting that cytokines released by neutrophils likely play a role in hepatic regeneration following cessation of alcohol, and that resolving inflammation may actually have a beneficial, rather than detrimental role in alcoholic liver disease, contributing to hepatic regeneration. Severe alcoholic hepatitis is marked by a failure of liver progenitor cells to progress past massive proliferation to maturation into mature hepatocytes[102], the mechanism for this remains to be elucidated. Potential therapeutic agents to promote hepatic regeneration are being explored.

8. Conclusion

ALD is a leading cause of liver related morbidity and mortality, encompassing a spectrum of disorders ranging from asymptomatic steatosis, steatohepatitis, fibrosis, cirrhosis and its related complications, as well as the the acute on chronic state of AH. While multidisciplinary efforts continue to be aimed at curbing progression of this spectrum of disorders, there is an urgent need for effective therapeutic interventions for AH given it's high mortality rate and limitations of current treatment regimens. Corticosteroids and pentoxyfylline, though used extensively, offer only a modest survival benefit. Anti-TNF agents, including infliximab and etanercept, did not prove to be safe or effective, due to their increased rates of infection and increased mortality observed in larger studies. Adjunctive therapies, such as N-acetylcysteine, which is known to replenish glutathione in damaged hepatocytes and prevent cell death in ALD, was also found to have no effect on six-month survival. Liver transplantation continues to be limited for patients with ALD, and though risk of associated complications is not higher among this group, there remains a hesitancy and concern among providers, and it is estimated that as many as 95% of patients with end-

stage liver disease related to alcohol are never formally evaluated for candidacy for LT. This drives our need to find better therapeutic targets for ALD with focus on AH. While our understanding of the molecular and cellular mechanisms of disease stems from animal models, there remains a dire need for translational research in this field to aid in bridging our gaps in understanding. Recent work in discovering the molecular and cellular basis of disease progression in ALD has helped uncover potential new targets for therapeutic intervention. In early stages of ALD, particularly in steatosis, many molecular targets involved in modifying transcription of genes responsible in fatty acid synthesis and accumulation have been explored as potential targets of therapeutic intervention including SREBP-1c, and the cannabinoid receptors CB1, and CB2. Similar pathways, including activation of the receptor PPAR-α have been explored in their role in fatty acid oxidation as it relates to progression to steatosis. The role of various inflammatory pathways in progression to steatohepatitis have been explored as potential targets including inhibition of translocation of bacterial products, such as LPS, which are known to trigger an inflammatory response, inhibition of TLR signaling pathways, and release of pro-inflammatory cytokines including IL-6, IL-10. Inhibition of profibrogenic pathways in HSCs, including ERK1, ERK2, phosphinositide 3 kinase-Akt and JNK, have also been explored and are attractive candidates for therapeutic intervention to prevent progression to fibrosis. Much work remains to elucidate the role of the cellular responses of the innate and adaptive immune systems in AH in uncovering potential targets for intervention. The role of IL-17 in inducing neutrophil recruitment by stimulating HSCs to secrete IL-8 and CXCL1 has been explored, with the thought that modification of these chemokines may mediate neutrophil infiltration and attenuate alcoholic hepatitis. The role of osteopontin has also been explored as it contributes to neutrophillic recruitment in alcoholic hepatitis, but much work remains ahead. Similarly, the mechanisms have not yet been elucidated, but it is well known that the adaptive immune response plays an important role in progression to AH, as antibodies to adducts generated through free radical oxidation have been found on circulating T cells, though potential targets for therapeutic intervention remain to be discovered.

Acknowledgments

Abbreviations

AASLD	American association for the Study of Liver Disease
ALD	alcoholic liver disease
HCC	hepatocellular carcinoma
ASH	alcoholic steatohepatitis
AH	alcoholic hepatitis
ADH	alcohol dehydrogenase

ALDH	acetaldehyde dehydrogenase
CYP2E1	Cytochrome P450 2E1
EASL	European Association for the Study of the Liver
ER	endoplasmic reticulum
IRAK-M	Interleukin-1 receptor-associated kinase
MELD	model for end-stage liver disease
DF	Maddrey Discriminant Function
GAHS	Glasgow Alcoholic Hepatitis Score
ABIC	Age, Bilirubin, INR, Creatinine
UKELD	UK End-stage Liver Disease
IL	interleukin
LPS	lipopolysaccharide
LT	liver transplant
TLR	toll-like receptor
SREBP-1c	sterol regulatory element-binding protein 1c
TLR4	toll-like receptor 4
TNF	tumor necrosis factor
AMPK	AMP-activated protein kinase
PNPLA3	patatin-like phospholipase domain-containing protein 3
PPAR	peroxisome proliferator-activated receptor
ROS	ROS
HSC	hepatic stellate cell
NK	natural killer
IFN	interferon
SAMe	S-adenosylmethionine
NIAA	National Institute of Alcohol Abuse and Alcoholism
PMN	Polymorphonuclear leukocytes
WHO	World Health Organization

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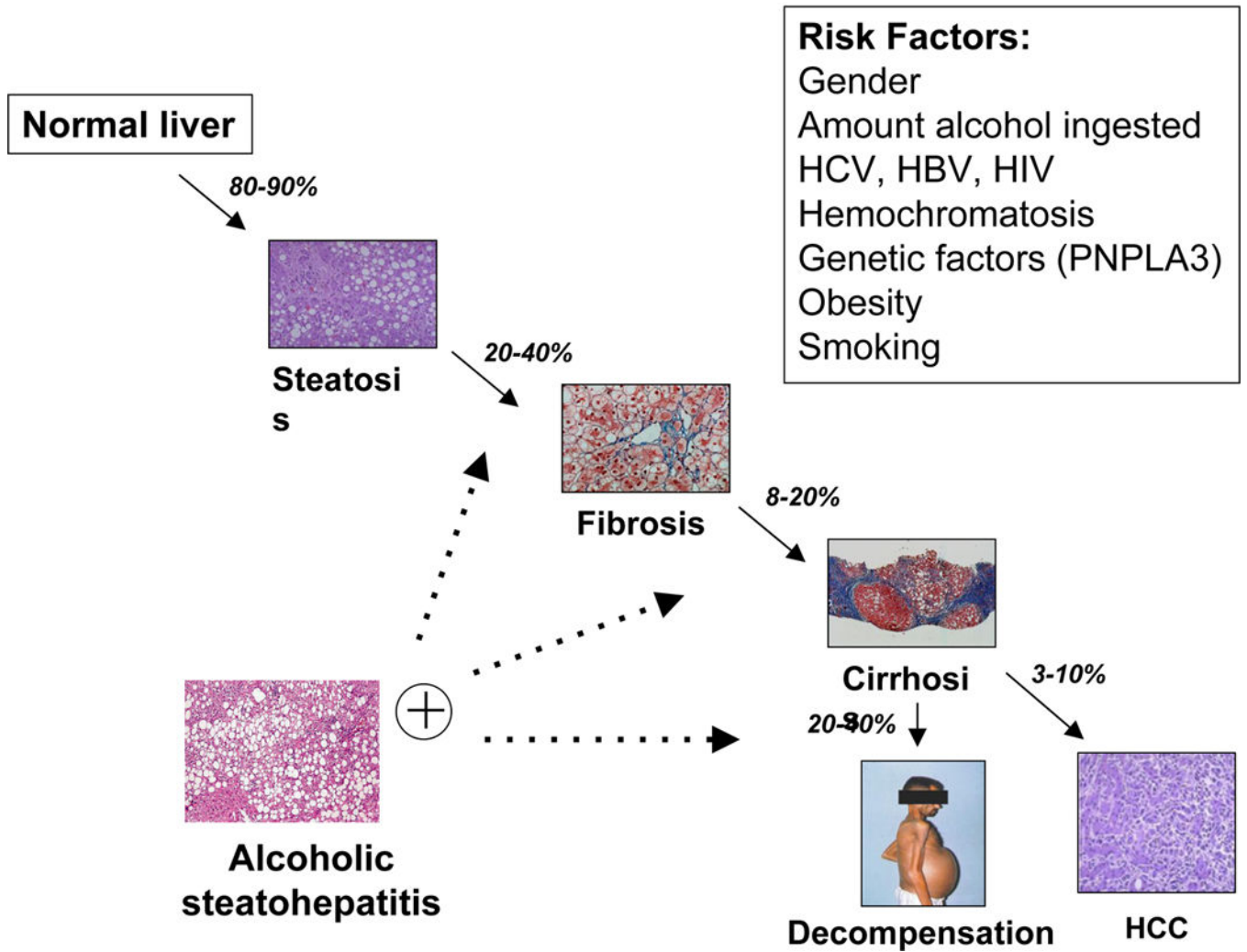
Figure 1.
Natural progression along the spectrum of ALD, from steatosis, to the inflammatory state of steatohepatitis, to progressive fibrosis and cirrhosis, and finally, to decompensated cirrhosis and HCC. Exacerbations of AH occur at many of the later stages of disease. Predisposing risk factors to accelerated progression are listed.

Management of Alcoholic Hepatits

INITIAL EVALUATION

Clinical Signs and Symptoms	Laboratory Markers
Recent-onset jaundice, malaise, ascites, edema, pruritus, fever, confusion/lethargy/agitation, asterixis, tender hepatomegaly, splenomegaly, pedal edema	Abrupt rise in Tbil (usually > 5 mg/dl), AST>ALT (usually >2X upper limit), GGT >100 U/mL, Albumin <3.0 g/L, INR >1.5, Platelet count <150,000

ASSESS AND TREAT PRECIPITATING FACTORS OF DECOMPENSATION

Hepatic Encepalopathy	Alchohol Withdrawal	Infection	Renal Insufficiency
• Assess for precipitant: GI bleed, infection, medication non-compliance • Treat underlying precipitant: add Lactulose, Rifaxamine, Zinc	• Moderate: Baclofen in USA, or Clometiazol in Europe • Severe: benzodiazepines; if intubation needed, Propofol for sedation.	• Rule-out pneumonia, cellulitis, SBP, UTI, meningitis based on clinical signs/symptoms • Pan-culture, Chest X-Ray • Broad-spectrum antibiotics if indicated	• Early-detection and close monitoring • Volume expansion with albumin • Consider terlipressin or NE if progressive HRS-1

ASSESS OTHER HEPATIC CAUSES OF JAUNDICE

Mechanical Obstrucion	Drug-induced Liver Injury	Viral Hepatitis	Auto-immune Hepatitis	Ischemic Hepatitis
• Rule out HCC/biliary obstruction • Perform doppler abdominal US and if indicated, MRI	• Review detailed history of medications, supplements, pharmacy records • http://livertox.nih.gov	• Rule out Hepatitis A, B, C, especially if first episode, or high clinical suspicion	• Rule out severe autoimmune hepatitis if first episode and/or clinical suspicion (ANA, ASMA, IgG).	• Presence of hypotension, septic shock, massive bleeding or recent cocaine use?

ROLE FOR TRANSJUGULAR LIVER BIOPSY?

☐ Non-invasive diagnostic measures have yielded inconclusive results
☐ Uncertain alcohol assessment.
☐ Use of any potential hepatotoxic substance in the last 3 months.
☐ Possible indication of a salvage liver transplantation.

All negative — Any positive

Clinically diagnosed AH — **Biopsy-proven AH** ← **Biopsy recommended** → Not full criteria Alc Hep / Other diagnosis

Maddrey DF >32
 ABIC >6.7
 or MELD >21 → Prednisolone 40 mg → Supportive therapy / Specific therapy of the finding

Lille Model | 7 days

4 weeks corticoids followed by a 2 week taper — <0.45 — ≥0.45 — • Stop Prednisolone. • Consider OLT if approved in the center..
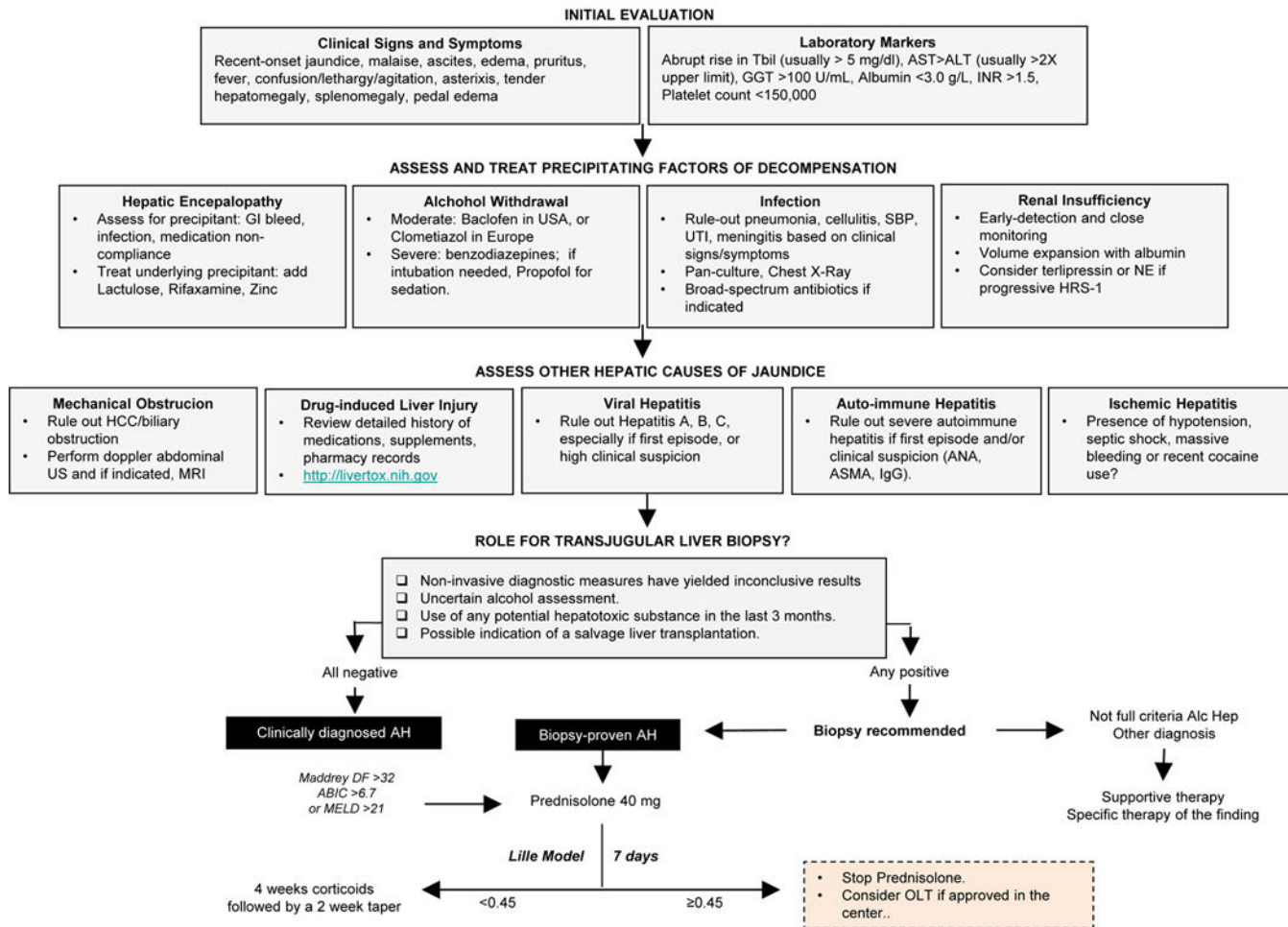
Figure 2.
Clinical evaluation for a patient with high suspicion of AH involves ruling out precipitating factors of decompensated liver disease and confounding illnesses. Role of transjugular liver biopsy is dependent on the care provider's confidence in the diagnosis. Initial treatment requires ongoing surveillance for improvement.
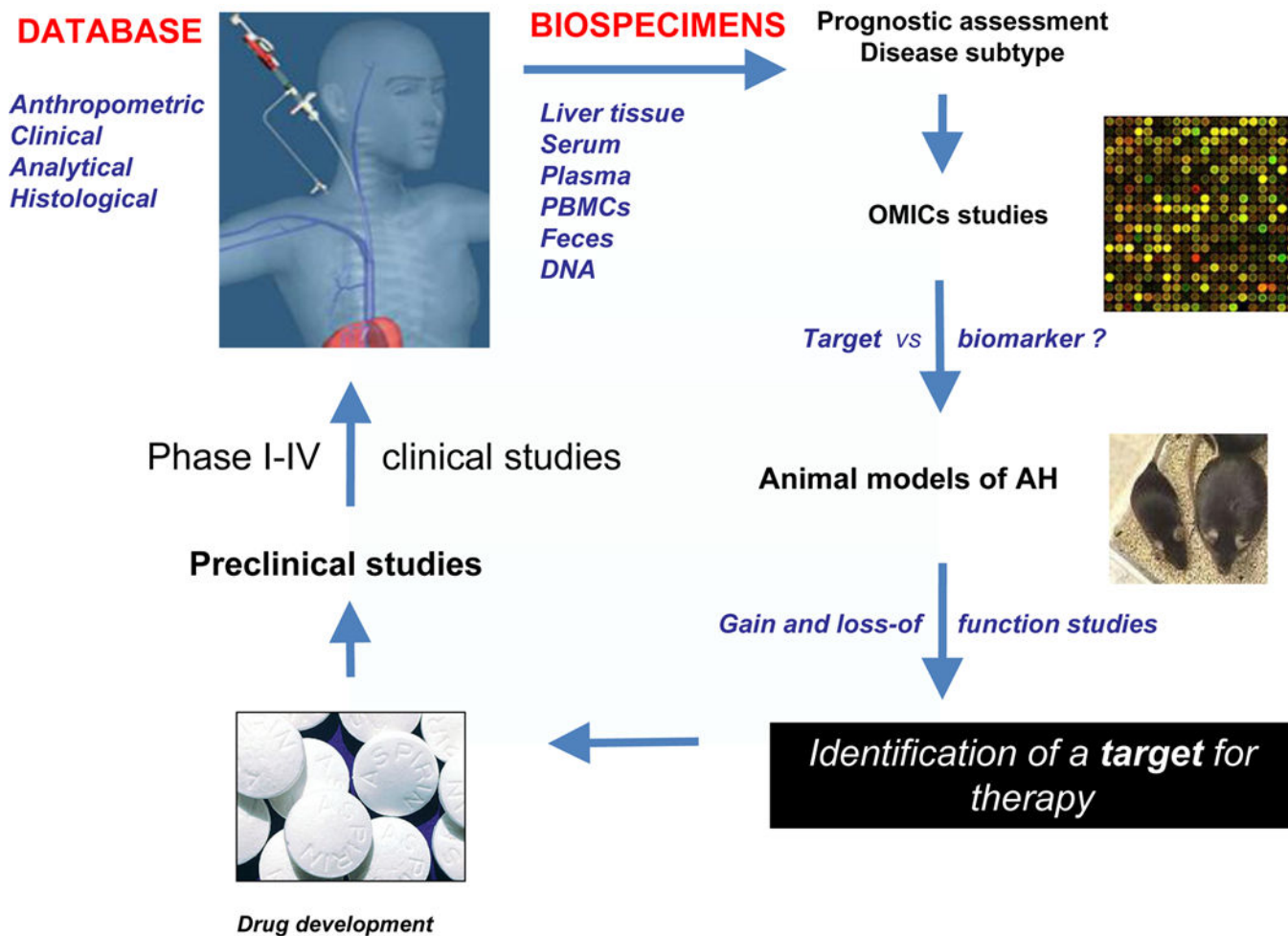
Figure 3.
Systematic approach to designing translational studies to evaluate important molecular markers and potential therapeutic targets in AH.
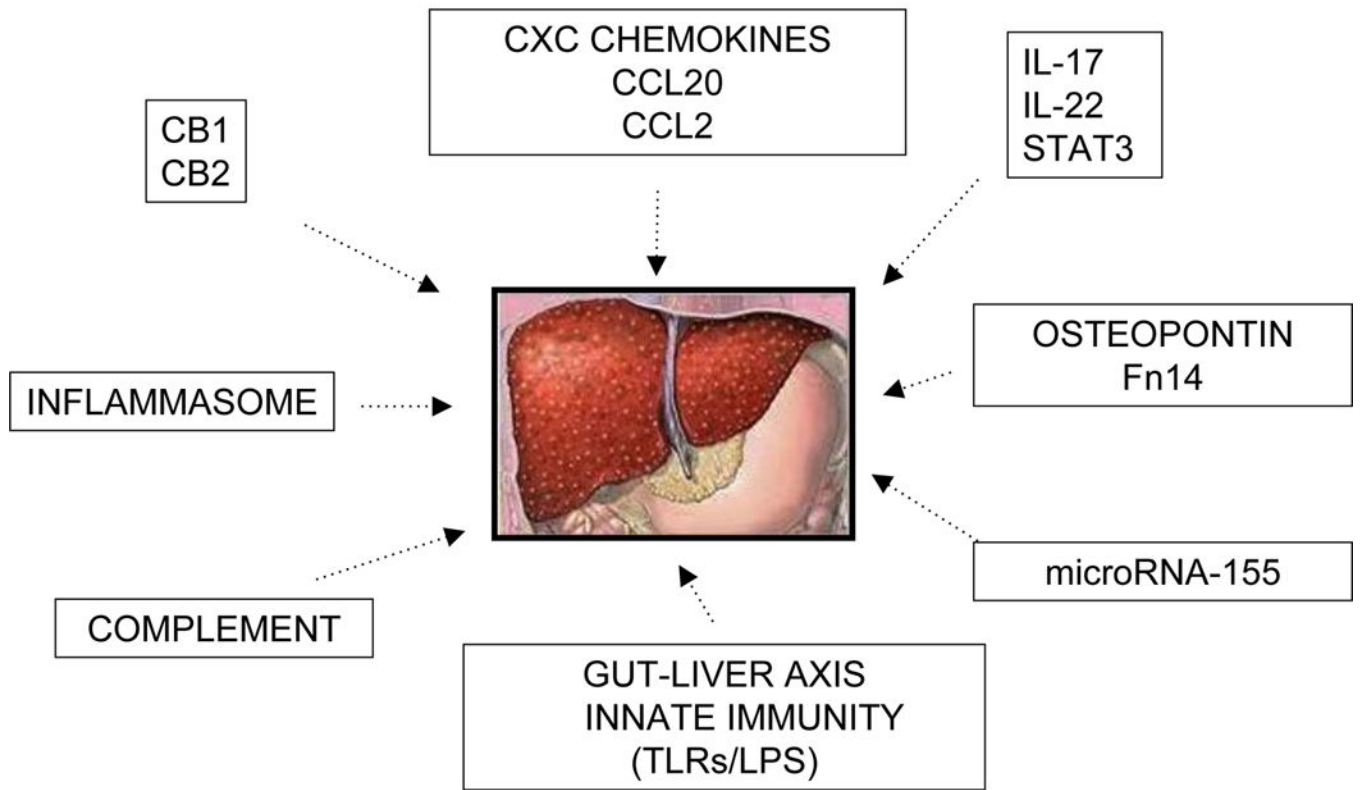
Figure 4.
Potential molecular and cellular targets for therapy for AH identified in translational studies including human samples.

Table 1

Current therapeutic interventions for management of alcoholic hepatitis.

Intervention Class	Examples, Indications and Limitations
Nutritional support	Address protein calorie malnutrition (High calorie meals and supplements), and vitamin deficiency (Vitamin A, Vitamin D, Thiamine, Folate) through supplementation.
Alcohol Abstinence	Most important component of therapy, often a multidisciplinary team recommended for successful management.
Corticosteroids	Prednisolone: first line therapy for severe AH (indicated with DF > 32 or HE) AASLD/EASL recommendations: 40mg/day for 4 weeks Assess response after a week: Lille score> 0.45, discontinue therapy Rule out infection and GI bleeding before initiating therapy
Phosphodiesterase inhibitor:	Pentoxifylline: inhibits TNF Second line agent. Not an effective rescue therapy agent.
Anti-TNF agents	Infliximab/Etanercept Large studies showed increased adverse effects: Infections, mortality Currently not recommended for AH
N-acetylcysteine	Replenishes Glutathione in damaged hepatocytes and prevents cell death. Used in Acute Hepatitis from other causes. Studies on Prednisolone and NAC combination therapy have shown reduction in 1-month mortality rate, HRS incidence, and Infection incidence, but no effect on improving 6-month mortality.

DF: Maddrey's discriminant function, HE: Hepatic encephalopathy, TNF: Tumor necrosis factor, HRS: Hepatorenal Syndrome

Table 2

Potential novel therapeutic targets for intervention.

Pathologic Process	Potential Therapeutic Targets	Mechanism of Action
Steatosis Decrease in fatty acid oxidation	CYP2E1 and PPAR-α	Ethanol upregulates CYP2E1 production resulting in hepatic oxidative stress, which in turn prevents up-regulation of PPAR-α, a nuclear hormone receptor, that regulates transcription of many genes involved in free fatty acid transport and oxidation.
Steatosis Increase in fatty acid uptake	SREBP-1c	Acetaldehyde upregulates SREBP-1c, a master transcription factor that upregulates the expression of genes encoding lipogenic enzymes, resulting in increased fatty acid synthesis.
	CB1, CB2	Endocannabimoids are also responsible for up-regulating SREBP-1c leading to increased fatty acid synthesis, but also mediate alcoholic liver injury through signaling through either of two cannabinoid receptors, CB1 and CB2. Signaling through CB1 worsens alcoholic liver injury while signaling through the CB2 confers a protective role in the development of steatosis.
	SIRT1	SIRT1, a NAD(+)-dependent class III protein deacetylase, also inhibits SREBP-1c activation decreasing fatty acid synthesis. SIRT1 agonists could be potentially be used to curtail the effects of ethanol on steatosis.
	Adiponectin, AMPK	AMPK, a serine-threonine kinase, inactivates the rate-limiting enzyme ACC in fatty acid synthesis. Inactivation of ACC leads to a reduction in the levels of malonyl Co-A, a precursor in fatty acid synthesis and an inhibitor of the rate-limiting enzyme CPT1, in fatty acid oxidation, resulting in less fatty acid synthesis and increased oxidation. While ethanol inhibits this pathway, adiponectin can confer protection by modulating this pathway.
Steatosis Autophagy	mTOR	Chronic alcohol consumption inhibits autophagy, leading to fat accumulation and. Inhibition of the mTOR signaling pathway inhibits progression to steatosis.
Steatohepatitis Bacterial translocation and the inflammatory cascade	LPS, TLR	Ethanol increases gut permeability resulting in translocation of bacterial products, such as LPS, into portal circulation, resulting in activation of Kupffer cells through TLR4 leading to production and release of pro-inflammatory cytokines. TLR antagonists have been proposed as potential therapeutic agents.
	IL-6, IL-10, STAT3	Kupffer cells secrete anti-inflammatory cytokines, IL-6 and IL-10, which bind STAT3 to curb inflammation and slow progression of liver injury.
Steatohepatitis Neutrophillic infiltration	IL-1, osteopontin, CXCL4, CXCL5, CXCL6	IL-1, osteopontin, CXCL4, CXCL5, and CXCL6 are chemokines that attract and activate macrophages.
Fibrosis Promotion of fibrosis	ERK1, ERK2, phosphinositide 3 kinase-Akt and JNK	ROS production stimulates collagen production though stimulation of pro-fibrogenic signaling pathways in HSCs including ERK1, ERK2, phosphinositide 3 kinase-Akt and JNK.
Fibrosis Inhibition of collagen degradation	metalloproteinases	ROS produced with heavy alcohol consumotion propagate collagen accumulation by preventing collagen degradation, through direct inhibition of metalloproteinases which degrade collagen.
Apoptosis and massive cell death	Caspase	Caspase inhibitors are known to inhibit apoptosis.
Innate immune response Neutrophil recruitment	IL-17, IL-8, CXCL1, ostepontin	IL-17 induces neutrophil recruitment by stimulating HSCs to secrete IL-8 and CXCL1. The modification of these chemokines may mediate neutrophil infiltration and attenuate alcoholic hepatitis. Osteopontin, an extracellular matrix protein, also contributes to neutrophillic recruitment, and is markedly upregulated in alcoholic hepatitis, and is an attractive target in considering new therapeutic agents.
Adaptive immune response T cells	Circulating T cells	Patients with alcoholic hepatitis have been found to have circulating T cells with antibodies to free-radical adducts
Intestinal dysbiosis	LCFA	Genomic and metabolomic analyses of intestinal bacteria revealed low levels of lactobacilli and reduced production of LCFA in those with

Pathologic Process	Potential Therapeutic Targets	Mechanism of Action
		alcoholic liver injury. Supplementation with LCFA restored eubiosis, intestinal barrier function, and reduced liver injury in mice.
Hepatic regeneration		The mechanism remains to be elucidated, but severe alcoholic hepatitis is marked by a failure of liver progenitor cells to progress past massive proliferation to maturation into mature hepatocytes. Agents to promote hepatic regeneration are being explored.

CYP2E1, cytochrome P450 2E1, PPAR-α: proliferator-activated receptor-α, SREBP-1c: sterol regulatory element-binding protein-1c, CB1: cannabinoid receptor 1, CB2: cannabinoid receptor 2, SIRT1: sirtuin 1, AMPK: AMP-activated protein kinase, mTOR: mammalian target of rapamycin, LPS: lipoplysaccharide, TLR: toll-like receptor, IL-6: interleukin-6, IL-10: interleukin-10, IL-1: interleukin-1, IL-17: interleukin-17, IL-8: interleukin-8, LCFA: long-chain fatty acids