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Recent advances in the prevention and treatment of decompensated cirrhosis and acute-on-chronic liver failure (ACLF) and the role of biomarkers

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ABSTRACT

The progression of cirrhosis with clinically significant portal hypertension towards decompensated cirrhosis remains clinically challenging and the evolution towards acute-on-chronic liver failure (ACLF), with one or more extrahepatic organ failures, is associated with very high mortality. In the last decade, significant progress has been made in the understanding of the mechanisms leading to decompensation and ACLF. As portal hypertension advances, bacterial translocation across an impaired gut barrier culminates in endotoxaemia, systemic inflammation and cirrhosis-associated immune dysfunction (CAID). Gut-derived systemic inflammation and CAID have become the logical targets for innovative therapies that prevent hepatic decompensation episodes and the progression to ACLF.

Furthermore, classification of disease and biomarker discovery to personalise care have advanced in the field. This review discusses progress in biomarker discovery and personalisation of treatment in decompensated cirrhosis and ACLF.

WHAT IS ALREADY KNOWN ON THIS TOPIC

⇒ Decompensated cirhosis empases and spectrum of disorders, from stable decompensated cirrhosis to unstable decompensated cirrhosis, pre-acute-on-liver-failure (ACLF) to ACLF

⇒ Several key pathogenic mechanism has been implicated in the diagnosis, progression and treatment of such disease including inflammatory biomarkers and microbiome phenotypes.

WHAT THIS STUDY ADDS

⇒ There are established guidances by the regulatory agencies that can support the development of newer diagnostic/prognostic tools and therapies in the field of decompensated cirrhosis/ACLF

⇒ We provide several concepts for therapeutic research development that can help in the regulatory process.

HOW THIS STUDY MIGHT AFFECT RESEARCH, PRACTICE OR POLICY

⇒ Understanding the scope of each specific biomarker will tailor trials in ACLF to improve clincial care in these patients.

INTRODUCTION

Cirrhosis is a common chronic condition associated with high morbidity and mortality. The development of decompensation, that is, ascites, hepatic encephalopathy (HE) and variceal bleeding, increases the healthcare burden due to frequent hospital admissions and the yearly mortality rate from less than 1% to 10%–50%.[1] The prognosis of decompensated patients also depends on the time of development of the decompensation. While a slow development of ascites or jaundice represents a non-acute decompensation (NAD), the rapid development of those defines an acute decompensation (AD).[2] These clinical pathways are distinctly characterised by the velocity, severity and clinical urgency of the occurring event(s). Non-elective/emergency hospitalisation due to existing decompensation may represent a viable stratum to distinguish AD from NAD since it implies clinical relevance and urgency for medical intervention. While NAD features a slow and progressive development of complications, AD is characterised by a more severe and accelerated clinical course, leading to the development of its distinct subphenotypes and, frequently, progression towards acute-on-chronic liver failure (ACLF). The NAD pathway may also constitute a transitioning hub to a state of recompensated cirrhosis.[3] ACLF characterises the most aggressive form of AD and is determined by the development of hepatic and/or non-hepatic organ failures associated with a very high short-term mortality, as high as 20%–80%, within 28 days.[4]

The pathogenetic mechanisms underpinning the development of complications in cirrhosis are mainly related to the presence of clinically significant portal hypertension (CSPH) defined as hepatic-venous pressure gradient (HVPG) equal to or greater than 10 mm Hg.[5] Subsequently, the circulatory shifts related to portal hypertension may generate either underperfusion or congestion, resulting in shear stress-induced endothelial dysfunction of sensitive organs and impairing their regular function.[1] Further, systemic inflammation caused by the progression of hepatic inflammation, organ dysfunction or individual precipitating events constitutes a primary driver of disease progression in NAD and AD.[2] Systemic inflammation is mainly related to the development of AD and ACLF, with a continuous increase of systemic inflammation surrogates across AD and ACLF grades (number of organ failures).[4] However, it must be mentioned

that treatment of portal hypertension using transjugular intrahepatic portosystemic shunt (TIPS) may prevent further decompensation[6] and thereby NAD/AD and organ failures.

While the mechanisms driving the development of NAD/AD are shared, the stage of decompensation guides the standard of care, in particular due to the level and number of organ dysfunction (online supplemental table 1). This review aims to dissect the evidence on biomarker guidance of therapies and recent advances in the prevention and treatment of AD and ACLF but avoids overlap with recent reviews addressing definitions of aetiology, ACLF and post-transplant outcomes.

CURRENT RECOMMENDED THERAPIES

The PREDICT study included patients with AD at hospital admission. At the baseline visit, the medical history was recorded, in terms of previous decompensations, in order to map the trajectory of disease before admission.[7] Using data from more than 1200 patients, PREDICT showed that patients transiting from NAD to AD had a more benign prognosis than patients developing ACLF, who recorded a relatively short history of cirrhosis. PREDICT further identified three clusters of patients with different clinical trajectories.[7] However, the treatments of different complications should be adopted and stratified according to the course of decompensation. Although the treatments are shared, the situation and mode of application may differ between NAD and AD. For example, in AD presenting with overt HE, acute rifaximin administration would be of no help. Nevertheless, this is a standard secondary prophylaxis in NAD. Similarly, one aims to differentiate between the different types of ascites and their differential treatment.Online supplemental table 1 illustrates how the clinical situation and the course of disease may influence treatment regimens, as already currently recommended.[5 8–10]

While stratification according to the stage and the course of disease leads to personalisation of care, individualisation of care requires more than that. In order to predict and guide treatment, biomarkers must be measured in individual patients to guide initiation of the most appropriate therapy.

BIOMARKER-GUIDED TREATMENTS

In 2015, the Food and Drug Administration (FDA)—National Institutes of Health Joint Leadership Council harmonised the terms used in translational science and medical product development with a focus on study endpoints and biomarkers. As a result, the BEST (Biomarkers, EndpointS and other Tools) resource was published, which clarifies biomarker definitions, describes their hierarchical relationships, connections and dependencies among the terms it contains.[11] BEST recommends a comprehensive description of the biomarker and proposed to specify the biomarker according to its biological plausibility (ie, relevance to the ACLF condition) and its measurement method and units for consistency when reporting such biomarker evaluation. Using neutrophil gelatinase-associated lipocalin (NGAL) as an example, Ariza *et al* studied urine and plasma NGAL concentrations in 148 patients with ACLF and 716 without ACLF. The authors found that urine NGAL was an independent predictor of the development of ACLF and of 28-day transplant-free mortality and that hepatic *LCN2* gene expression was markedly upregulated in patients with ACLF.[12] In addition, urinary NGAL may differentiate between the aetiologies of acute kidney injury (AKI) in cirrhosis, as shown recently by Gambino *et al*,[13] thereby determining the utility of terlipressin in AKI.

The BEST framework can guide novel therapies in ACLF and understanding the taxonomy of each category can guide the design of future studies in the field (figure 1).

A diagnostic biomarker is used to detect or confirm the presence of a disease or condition of interest or to identify a subtype of disease. The utility of a diagnostic biomarker is determined by its ability to accurately diagnose the condition of interest against the reference standard as shown by sensitivity, specificity or area under the receiver operating characteristics curve (AUROC).[11] Phase angle (PhA) is a low-cost nutritional marker obtained from

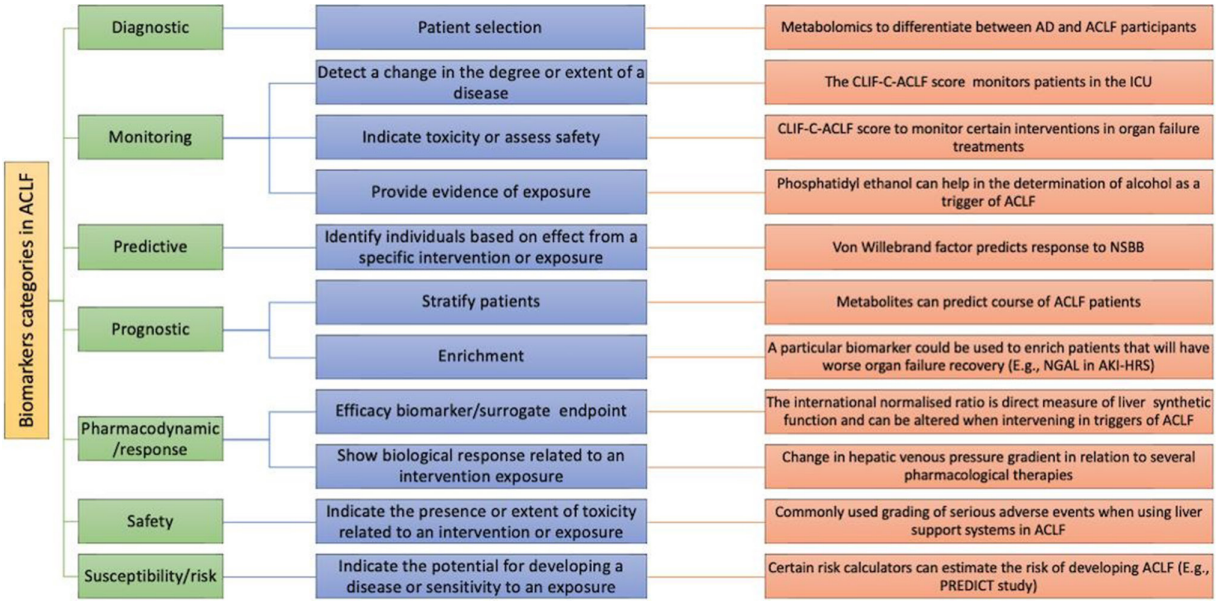

Figure 1 Biomarkers, endpoints and other tools biomarker classification proposed by the Food and Drug Administration.[11] We included some examples adapted to ACLF to demonstrate how each biomarker clarifies and describes their hierarchical relationships, connections and dependencies. ACLF, acute-on-chronic liver failure; AD, acute decompensation; AKI, acute kidney injury; CLIF, chronic liver failure; HRS, hepatorenal syndrome; NGAL, neutrophil gelatinase-associated lipocalin; PREDICT, predicting acute-on-chronic liver failure in cirrhosis.

point-of-care bioelectrical impedance analysis avoiding radiation exposure and validated against CT. In 163 patients spanning the spectrum of cirrhosis, Ruiz-Margáin *et al* showed that PhA had an AUROC of 0.70 (95% CI 0.61 to 0.79), and 5.6 was identified as the best cut-off value, yielding a sensitivity of 94% and a specificity of 63%.[14] More specifically, in ACLF, Pose *et al*, using untargeted metabolomics in 42 patients with decompensated cirrhosis and 25 participants from the LIVERHOPE-SAFETY trial (simvastatin+rifaximin vs placebo) identified functional pathways associated with ACLF and generated a metabolomicbased signature characteristic of ACLF.[15] The metabolic signature can change in response to treatment and serve as a diagnostic biomarker to distinguish ACLF from AD. Another example of a diagnostic biomarker was provided by Fernández *et al*, who used plasma renin concentration as a biomarker of circulatory dysfunction to assess the effect of antibiotics plus albumin vs antibiotics alone in a randomised clinical trial of 118 patients with cirrhosis.[16] The authors concluded that treatment with antibiotics plus albumin decreased circulatory dysfunction.

We use monitoring biomarkers to repeatedly assess disease progression or response to treatment. This type of biomarker is particularly important in the development and trials of new drugs or devices. A classic example is the monitoring of cholesterol levels in response to statins. In ACLF, such an example is the composite CLIF-C-ACLF score (CLIF-C-ACLF=10×(0.33×CLIF COFs+0.04×age+0.63×ln (white blood cell (WBC) count)–2)).[17] The score ranges from 0 to 100 and is used to monitor ACLF progression or response to treatment.[18] Ripoll *et al* designed a prospective, multicentre, open, 1:1-randomised, controlled parallel-group trial (LIVER-HERO) to compare the 12-month liver transplant-free survival in patients assigned to TIPS compared with the standard of care (terlipressin and albumin). While transplant-free survival is the primary endpoint, creatinine serves as a monitoring biomarker and the authors will assess reversal of hepatorenal syndreom (HRS)-AKI at 3–12 months or the response to the assigned treatment.[19]

When it is the purpose of the biomarker to show the biological response, either beneficial or harmful, after a particular treatment, we apply a response biomarker with two subtypes: the pharmacodynamic biomarker and the surrogate biomarker. The term 'surrogate' endpoint in ACLF requires further clarification. The FDA defines a 'surrogate endpoint biomarker' as a response biomarker that serves as an endpoint used in clinical trials as a substitute for a direct measure of how a patient feels, functions or survives. A surrogate endpoint does not measure clinical benefit per se but rather is expected to predict clinical benefit or harm based on epidemiological, therapeutic, pathophysiological or other scientific evidence. There are variations of such surrogate endpoints (Table of Surrogate Endpoints That Were the Basis of Drug Approval or Licensure | FDA): validated surrogate endpoint, reasonably likely surrogate endpoint and candidate surrogate endpoint, depending on the degree of scientific evidence supporting such association between the surrogate endpoint and the desired outcome.

Another very commonly used surrogate endpoint is HVPG, which has been shown to predict major liver outcomes (eg, variceal bleeding). A randomised placebo-controlled trial to assess whether nitric oxide (NO)-independent soluble guanylyl cyclase (sGC) activator BI 685509 has an effect on HVPG in patients with CSPH is currently running.[20] In the aforementioned study by Pose *et al*, the authors also identified plasma metabolites that were modified following treatment with simvastatin plus rifaximin in patients with decompensated cirrhosis.[15]

In ACLF, we usually encounter predictive and prognostic biomarkers and while they seem synonymous, they are not. A predictive biomarker in ACLF would identify patients who are more likely to have a favourable (or unfavourable response) to a particular treatment. For example, Jachs *et al* found in 159 patients with clinically stable decompensated cirrhosis that those with a decrease in von Willebrand factor ≥5% were deemed to be 'responders' to application of non-selective beta-blockers as determined by HVPG response.[21] As a result, when designing trials in ACLF, predictive biomarkers not only help to personalise therapies in the field but can also enrich sample size calculation when designing clinical trials. Du *et al* developed a predictive score so clinicians can identify the subgroups who may benefit from plasma exchange-centred artificial liver support system (ALSS) therapy in patients with hepatitis B-ACLF. In a cohort of 601 patients, they found that patients with a PALS score 6–9 could still benefit from ≥6 sessions of ALSS therapy compared with ≤2 sessions (63.6% vs 97.0%, p<0.05). The score is a composite of the presence of cirrhosis, total bilirubin, presence of infection, HE and INR.[22]

In contrast, a prognostic biomarker in ACLF would be used to identify the likelihood of a clinical event or progression in patients who already have ACLF. Weiss *et al* showed in two prospective multicentre large cohorts from (CANONIC (discovery, n=831) and PREDICT (validation, n=851)) that models including metabolites (CLIF-C MET)—here the prognostic composite biomarker—reflecting SI, mitochondrial dysfunction and sympathetic system activation were superior to prognostic short-term mortality using established clinical scores (eg, MELDNa).[23] Such prognostic biomarkers are also used in clinical trials as a method to identify patients who might have a higher risk of developing the event of interest, and, as such, enable enrichment of trials and reduce sample size (figure 1).

When developing new drugs or treatments, ACLF research may be interested in biomarkers that are measured before or after the patients are exposed to the treatment of interest: this is what the FDA calls a safety biomarker. Bilirubin and aminotransferases are the most common safety biomarkers used in drugs with potential hepatotoxicity. Agarwal *et al* recently reported on a randomised clinical trial in 32 patients undergoing a novel liver dialysis device (DIALIVE), and the primary outcome was at least one serious adverse event between days 1 and 10. Several biomarkers were used to monitor serious adverse events, including platelet count drops and severe hypotension, which can be applied as safety biomarkers.[24]

Finally, susceptibility/risk biomarkers indicate the potential of developing a disease (eg, ACLF) in an individual who does not currently have a clinically apparent disease. Trebicka *et al* identified a particular phenotype in the PREDICT study, namely pre-ACLF, in patients who later developed ACLF, in a cohort of 1071 patients without ACLF. The formula provided by this study shows how a composite biomarker can determine the susceptibility risk for the development of ACLF.[7]

In summary, these FDA biomarker classifications applied to ACLF can assist researchers in novel trial designs in the field of ACLF, facilitating and expediting future regulatory approval.

ONGOING STUDIES, NOVEL DRUGS AND OTHER APPLICATIONS

Over the last decade, we have largely focused on the treatment of two major mechanisms, portal hypertension and systemic inflammation, as well as the main precipitants of decompensation, such as infection, bacterial translocation and organ failure.

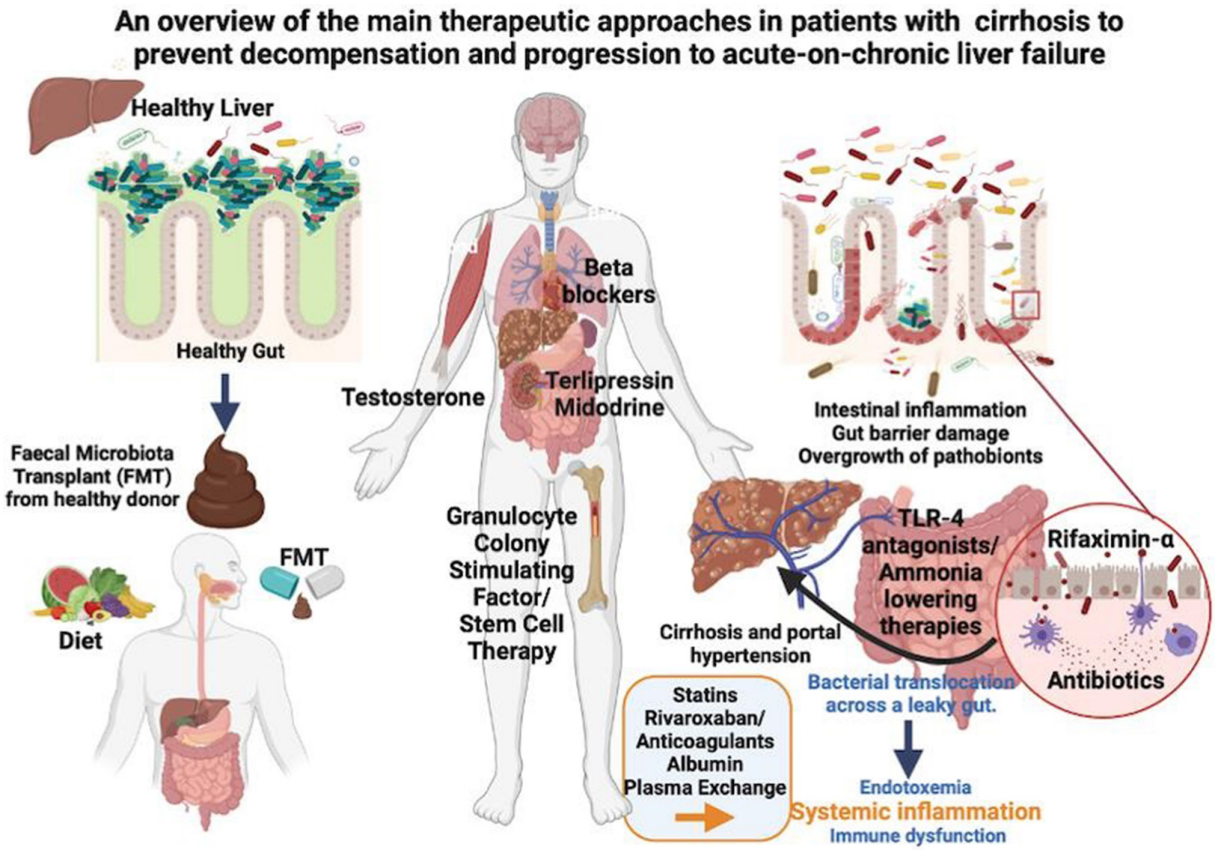

Figure 2 An overview of the main therapeutic approaches in patients with cirrhosis to prevent decompensation and progression to acute-on-chronic liver failure. Created with BioRender.com with publication licence. TLR-4, toll-like receptor 4.

Therapies in patients with cirrhosis and CSPH to prevent hepatic decompensation and progression to ACLF

The pathophysiology of CSPH in patients with cirrhosis is underpinned by profound peripheral arterial vasodilatation and circulatory dysfunction. Activation of neurohormonal systems drives renal sodium and water retention.[25] This fragile balance maintains organ function in a stable state, but as portal hypertension advances, bacterial translocation across an impaired gut barrier culminates in endotoxaemia, systemic inflammation and cirrhosis-associated immune dysfunction (CAID).[1 26] Gut barrier damage arises from endothelial damage, which is exacerbated by reduced gut microbial diversity with an increase in pathobiont species. These pathobionts, which frequently translocate from the mouth, drive gut inflammation, mucosal degradation and enhanced ammonia production.[27 28] Gut-derived systemic inflammation and CAID, therefore, become the logical targets for therapies that prevent hepatic decompensation episodes and the progression to ACLF, which is often precipitated by infection and sterile inflammation (figure 2).[29]

Therapeutic targets for patients with cirrhosis and CSPH have historically focused on counteracting peripheral arterial vasodilatation, that is, non-selective beta blockers (NSBB), which was shown to be a mainstay therapy that may have benefits beyond the prevention of variceal bleeding. Indeed, the PREDESCI study showed that in addition to reducing ascites, long-term administration of NSBB may decrease the occurrence of ascites.[30] In a meta-analysis with 352 patients with compensated cirrhosis, the risk of decompensation was lower with carvedilol than in controls, mainly due to a reduced risk of ascites; the risk of death was also lower with carvedilol.[31] A recent study analysing over 1000 patients showed that use of NSBBs reduced the rate of patients developing sepsis within 1 year to approximately half, while the dose did not play a major role.[32] The BOPPP trial (NCT05872698), which will complete recruitment in 2024, is a prospective multicentre randomised-controlled trial comparing carvedilol with placebo in 764 patients with cirrhosis and grade 1 oesophageal varices to determine whether carvedilol does indeed improve decompensation-free survival (figure 3A).

Anticoagulation has been studied as a tool to prevent not only portal vein thrombosis but also decompensation.[33] The IMPORTAL study, a meta-analysis using individual data of patients with cirrhosis and PVT showed that anticoagulation (independent of the drug) had a significant effect on survival of patients with recanalisation of portal vein thrombosis.[34] Moreover, rivaroxaban improved survival and decompensation in patients with cirrhosis with moderate liver dysfunction in a double-blind, placebo-controlled trial, particularly in patients with a maximal Child-Pugh-Turcotte score of 7.[35] Finally, aetiological cure prevents further decompensation and mortality in cirrhotic patients with ascites as the single first decompensating event.[36]

The most effective therapy to reduce portal pressure is TIPS. Early or pre-emptive TIPS is recommended in patients at high risk of rebleeding after haemostasis, despite the presence of HE at admission. A study enrolling more than 2000 patients clearly showed that the presence of HE has no influence on the development of post-TIPS encephalopathy.[37]

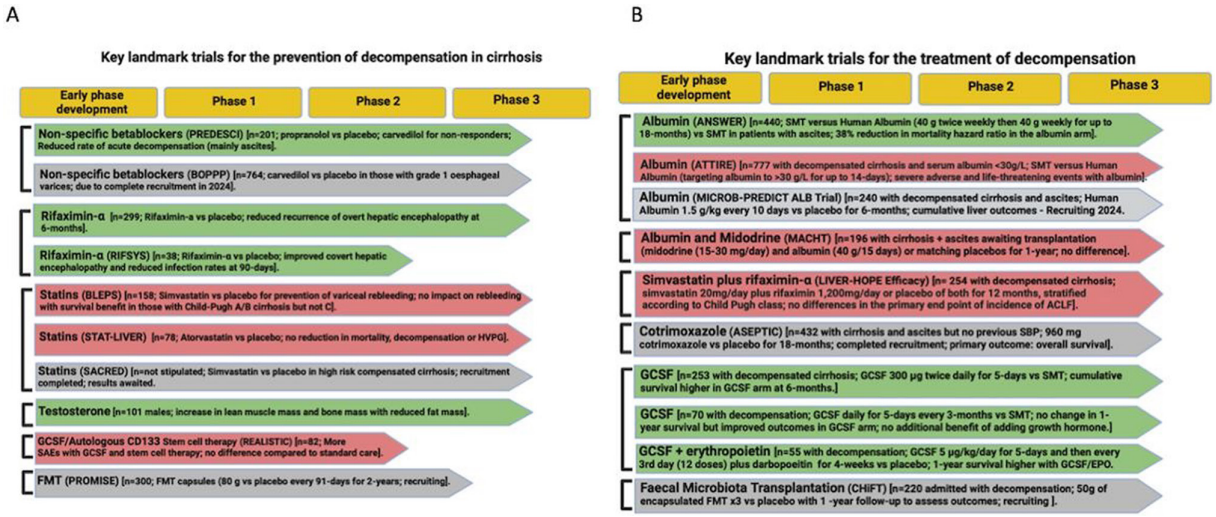

Figure 3 Key landmark clinical trials in cirrhosis in patients with compensated cirrhosis and clinically significant portal hypertension (A) and in patients with decompensated cirrhosis (B). (A) Illustrates recently published phase 2a/b and 3 clinical trials in patients with compensated cirrhosis and clinically significant portal hypertension with the outcome of improved decompensation-free survival. These include trials of non-specific beta blockers, rifaximin-α, statins, testosterone, granulocyte colony-stimulating factor (GCSF)/autologous CD133 stem cell therapy and faecal microbiota transplantation (FMT). The trials highlighted in green bars are positive, light red bars negative and in grey bars are still recruiting or await reporting. (B) Illustrates recently published phase 2a/b and 3 clinical trials in patients with decompensated cirrhosis with the main outcome of survival. These include trials of human albumin solution, midodrine, simvastatin, cotrimoxazole, GCSF and GCSF in combination with growth hormone/erythropoietin. HVPG, hepatic venous pressure gradient; SAEs, serious adverse events; SBP, spontaneous bacterial peritonitis; SMT, standard medical therapy. Created with BioRender.com with publication licence.

Gut-derived systemic inflammation and CAID as a target of therapy

As the gut drives endotoxaemia and low-grade systemic inflammation, manipulating the gut microbiome towards health will reduce bacterial translocation, the predisposition to spontaneous bacterial peritonitis (SBP) and also lower blood ammonia, which has recently been shown to predict hospitalisation, liver-related complications and mortality.[27] Therefore, dietary interventions with increased consumption of fruit, vegetables, fibre and fermented food substances, prebiotics (lactulose), probiotics and synbiotics should be evaluated.[38] There is robust data now to suggest that rifaximin-α not only reduces the recurrence of overt HE[39] but may also favourably manipulate the gut microbiome. A recent mechanistic randomised-controlled trial showed that rifaximin-α suppressed oralisation of the gut, reducing levels of mucin-degrading sialidase-rich species, *Streptococcus* spp, *Veillonella atypica* and *parvula*, *Akkermansia* and *Hungatella*. Rifaximin-α also promoted a TNF-α-enriched and interleukin-25-enriched intestinal microenvironment, augmenting antibacterial responses to invading pathobionts and promoting gut barrier repair. Interestingly, patients on rifaximin-α were less likely to develop infection.[28] Faecal microbiota transplant (FMT) is a well-established treatment, acting as a 'whole ecosystem' approach to restore the homeostatic balance of gut microbiota. An open-label phase I pilot study of 10 patients with cirrhosis treated with rectally instilled FMT restored gut-microbiota diversity and improved HE. However, there were limitations in attributing results to FMT alone as patients were treated concurrently with broad-spectrum antibiotics, whereas those allocated to standard care were not.[40] A further 10-patient phase I trial using FMT capsules was associated with improved duodenal mucosal microbial diversity, gut microbial diversity, antimicrobial peptide expression and reduced lipopolysaccharide-binding protein.[41] FMT, therefore, could represent a promising non-antimicrobial therapeutic strategy to improve an array of clinical outcomes in cirrhotic patients, ranging from the development of encephalopathy and infection to reducing AMR rates. Moreover, in the recent PROFIT trial, FMT decreased microbial-associated ammonia production and augmented ammonia excretion via anaerobic metabolism of L-aspartate to hippurate, providing proof of concept that FMT enhances ammonia metabolism, central in the pathogenesis of HE in cirrhosis.[42] Finally, testosterone therapy in men with low serum testosterone safely increases muscle mass, bone mass and haemoglobin.[43] Increased muscle mass may indirectly lower blood ammonia and improve outcomes. Therefore, testosterone treatment could merit a larger scale investigation.

The role of toll-like receptor 4 (TLR-4) as a mediator of gut-derived systemic inflammation has been highlighted (figure 2). It is upregulated in patients with cirrhosis and attenuated by rifaximin-α.[28] TAK-242 is a small molecule that selectively binds and inhibits TLR-4 and will be examined in a multicentre European phase 2 study (A-TANGO) (NCT04620148) in combination with granulocyte colony-stimulating factor (G-CSF) (which promotes hepatic regeneration) which commenced in 2023.

Statins are anti-inflammatory agents, blocking the action of eNOS downregulators, such as oxidised low-density lipoprotein, TNF-α and caveolin-1, with antifibrotic effects which may not only reduce portal hypertension but also have a favourable impact on decompensation-free survival. Inhibition of HMG-CoA reductase can result in increased nitric oxide bioavailability through inhibition of the Rho-ROCK pathway with the potential to treat endothelial dysfunction.[44] In a study of simvastatin versus placebo for prevention of variceal rebleeding, there was no impact on bleeding but, remarkably, a survival benefit in those with Child-Pugh-Turcotte A/B cirrhosis but not C.[45] However, in a small, recently published randomised-controlled trial in patients with cirrhosis and CSPH, atorvastatin, while safe, did not reduce HVPG, liver-related complications or mortality.[45] In the LIVER-HOPE study, simvastatin was examined at high (40 mg) and low

doses (20 mg) with rifaximin-α 1200 mg daily versus placebo. The higher dose of simvastatin led to more serious adverse events with no favourable impact on clinical outcomes.[46] Over 84 000 patients were analysed from the VOCAL database.[47] Of these, over 46 000 received no statin therapy, 22 000 received statins at initial presentation and a further 15 000 received statins only during the observation period. Statin exposure was correlated with the prevention of ACLF, and all patients had an indication for statin treatment outside of prevention of decompensation. In the efficacy study of LIVERHOPE, the combination of simvastatin with rifaximin did not prevent ACLF development, nor did it have a survival benefit in decompensated patients.[48] Therefore, statins in cirrhosis seem beneficial for the traditional indications, but not specifically in the prevention of complications of cirrhosis.[5]

Human albumin as a drug

Albumin, synthesised exclusively by the liver, is the most abundant plasma protein, and is a major contributor to plasma oncotic pressure. It also serves as a pleiotropic scavenger, reversibly binding to many toxic metabolites, inflammatory mediators and reactive oxygen species accompanying endotoxaemia and systemic inflammation.[49] It is well established and used in the prevention and treatment of circulatory dysfunction and renal failure associated with large-volume paracentesis and SBP and it has been incorporated in international guidelines. However, its benefit in the treatment of decompensated cirrhosis remains a matter of intense debate. In the ANSWER study performed on 442 patients with persistent ascites, 40 g of human albumin, given weekly for up to 18 months, led to a 38% reduction in the HR for mortality.[50] This was accompanied by a 50% reduction in the incidence of refractory ascites, a reduction in renal dysfunction and HE and a reduction in SBP and non-SBP infections. Real-world evidence on long-term albumin treatment in patients with decompensated cirrhosis in Italy[51] demonstrated that administration of albumin led to a considerable reduction in ascites complications and hospitalisation. Moreover, a recent double-blind, placebo-controlled trial in cognitively impaired outpatients showed that albumin significantly improved psychometric tests over 5 weeks.[52] Conversely, the MACHT trial in patients with decompensated cirrhosis awaiting liver transplantation did not show any benefit.[53] The ATTIRE trial with 777 patients with decompensated cirrhosis and serum albumin <30 g/L also showed no benefit, with a higher number of patients in the albumin arm suffering severe adverse and life-threatening events.[54] This could be attributed to rapid albumin infusion and/or targeting rather high plasma levels of albumin leading to fluid overload-derived complications and thus should be avoided.[55]

The PRECIOSA study (NCT03451292) is currently exploring in patients with decompensated cirrhosis, with or without ACLF, whether albumin at a dose of 1.5 g/kg (maximum 100 g/session) every 8–12 days over 1 year will offer a survival benefit. The MICROB-PREDICT albumin trial (NCT05056220) will validate predictive biomarkers of response to treatment with long-term albumin.[56]

Targeting hepatic regeneration as a therapy

The discovery of bone marrow participation in hepatic regeneration led to the use of G-CSF as an experimental therapy in patients with decompensated cirrhosis.[57] G-CSF induces haematopoietic stem cell mobilisation into the peripheral blood with neutrophil activation. This has been postulated to overcome the functional immunoparesis in patients with advanced cirrhosis and CAID. G-CSF stimulates the bone marrow to release 'immature' neutrophils which are programmed to secrete pro-inflammatory cytokines on stimulation and do not exhibit effector functions (ie, antimicrobial functions, such as ROS production, degranulation, NET formation) that characterise mature neutrophils.[58] However, while mobilisation of stem cells might be beneficial, the effect on monocytes and macrophages might be proinflammatory and could worsen CAID and predispose it to infection. Several studies (figure 3B) suggested that G-CSF daily for 5 days vs standard medical therapy offered a survival benefit,[59–62] but two recent large multicentre European studies in both compensated (REALISTIC)[63] (figure 3A) and decompensated cirrhosis (GRAFT)[64] have contradicted these findings. Therefore, caution is required and a recommendation on G-CSF is not possible at this point in time.

Novel targets and therapies in development of ACLF

Figure 4 summarises the novel targets and therapies in preclinical or early phase development of ACLF. Overall, therapies of interest largely target the downstream sequelae of toxic metabolites and systemic inflammation, including inflammasome pathways and serve as immune modulators or promote hepatic regeneration.[27]

The innate immune system detects pathogen-associated molecular patterns (PAMPs) via specific pattern recognition receptors (PRRs). An example is the binding of lipopolysaccharide and other gut microbial-derived metabolites to TLR-4, present on the surface of hepatic macrophages and Kupffer cells following bacterial translocation across the gut barrier into the portal vein.[26 65] This leads to the activation of nuclear factor-κB and interferon regulatory factor, inducing the release of proinflammatory cytokines.[66] This culminates in chronic low-grade systemic inflammation, which over time leads to loss of tolerance to antigen exposure and an augmented proinflammatory response.[26] Targeting gut-derived PAMPs, therefore, has potential in treating and preventing the development of ACLF and can be achieved by manipulation of the gut microbiome with rifaximin-α[28] and FMT[41] as discussed previously or via a non-antibiotic gut decontaminating product such as CARBALIVE. CARBALIVE, a novel engineered orally ingested macroporus carbon bead, binds toxins and cytokines with a molecular weight up to 70 kDa.[67] This has the potential to ameliorate systemic and gut inflammation. Also, large-volume plasma exchange may be beneficial in removing toxins in ACLF. The APACHE trial (NCT037002920) is a phase 3 multicentre, randomised-controlled, parallel-group, open-label study evaluating plasma exchange using 5% human serum albumin in 380 patients with ACLF.

Similarly, a phase 2 multicentre, randomised-controlled, open-label study evaluates the effects of the intraperitoneal, liposomal formulation VS-01 in patients with ACLF and ascites (NCT05900050). The intraperitoneally administered liposomes adsorb ammonia and other toxic metabolites. This is achieved using transmembrane pH-gradient liposomes containing citric acid. Once inside the peritoneum, uncharged ammonia diffuses out of the bloodstream into the peritoneal cavity and across the liposomal bilayer membrane, where it becomes trapped due to its positive charge. Other toxins, including urea, can be removed by the same mechanism.[68]

The products of necrotic cells are known as damage-associated molecular patterns. These also generate an inflammatory response mediated via PRRs, which physiologically stimulate tissue repair.[69] In the context of a patient with cirrhosis, it may

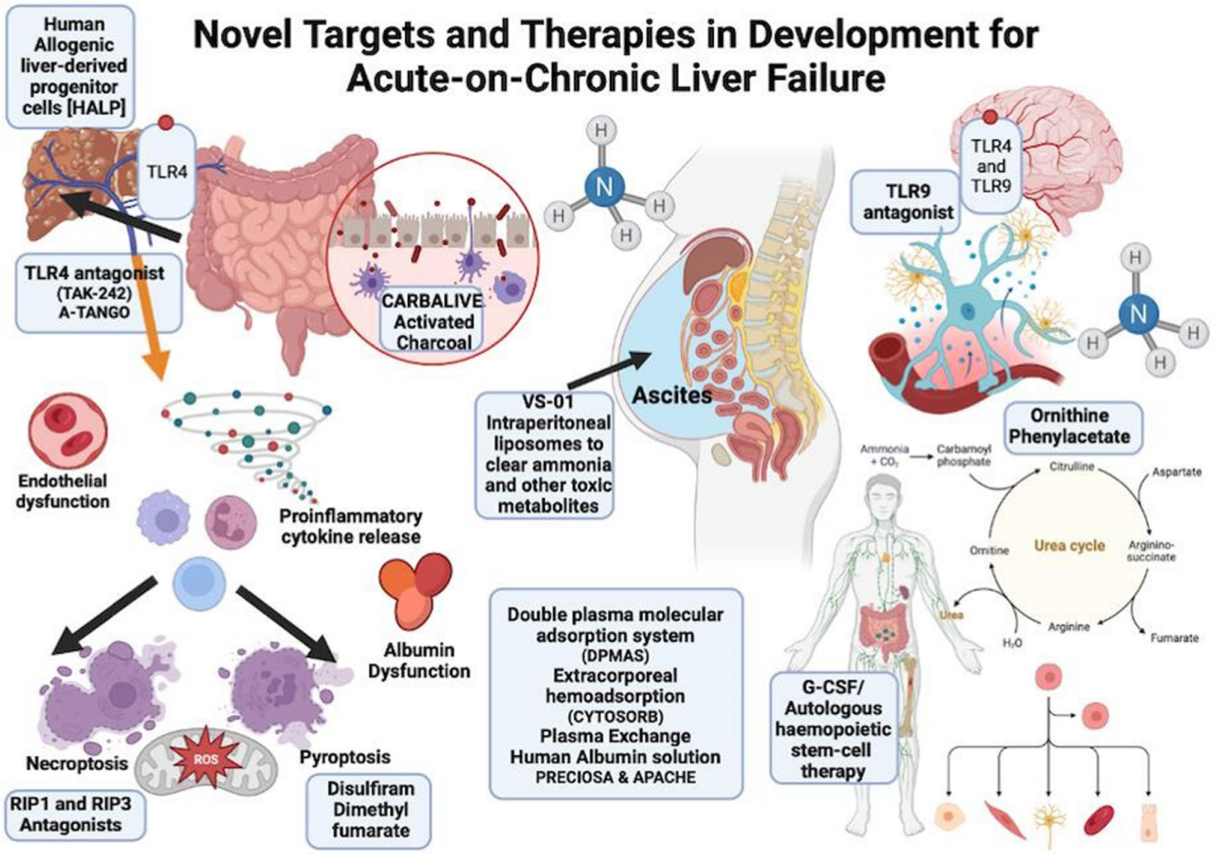

Figure 4 Novel targets and therapies for acute-on-chronic liver failure. The Figure illustrates novel targets and therapies that are in early development and/or phases 1 and 2 trials for acute-on-chronic liver failure. G-CSF, granulocyte colony-stimulating factor; RIPK, receptor interacting protein kinase; TLR, toll-like receptor. Created with BioRender.com with publication licence.

drive ACLF. Non-apoptotic cell death results in necroptosis and pyroptosis and is the predominant form of cell death in ACLF.[70] Necroptosis mediated by activation of the receptor-interacting protein kinases 1 and 3 forming necrosomes drives inflammation and organ dysfunction and may be associated with progression and severity of ACLF, especially in alcohol-related liver disease and infection.[71] Pyroptosis results from non-canonical inflammasome activation, which in turn is associated with ACLF development.[72] Currently, disulfiram is an effective inhibitor of non-canonical inflammasome and, thereby, pyroptosis.[73] Currently, a phase 2a trial of disulfiram in high-risk AD and ACLF is in progress.

Ammonia is also a key driver of the production of reactive oxygen species and systemic inflammation. Ammonia impairs neutrophil function by reducing chemotaxis and phagocytosis and increasing spontaneous oxidative burst[74]; this has been associated with 3-month and 1-year mortality in patients with cirrhosis.[75] In patients with ACLF, myostatin and hyperammonaemia are associated with higher mortality and prolonged ICU stays.[76 77] Ammonia also upregulates myostatin expression,[78] which contributes to sarcopenia and frailty by reducing muscle mass.[79] Ammonia-lowering strategies are thus key in ameliorating CAID and the increased risk of infection as a driver of hepatic decompensation and ACLF.[80] L-ornithine aspartate (LOLA), when added to lactulose and rifaximin in an RCT in patients with cirrhosis and severe HE, resulted in a lower 28-day mortality than with lactulose and rifaximin alone.[81] LOLA removes ammonia via two distinct mechanisms: by the synthesis of urea and by the synthesis of glutamine via the enzyme glutamine synthetase. L-ornithine phenylacetate lowers ammonia in patients with decompensated cirrhosis and has been shown to be safe.[82] A phase 2 study of L-ornithine phenylacetate in patients with cirrhosis and HE showed a shorter time to clinical improvement when compared with standard medical therapy (lactulose and rifaximin) with reduced intensive care stay.[83]

Cell therapy is an alternative approach for the treatment of regenerative failure in ACLF. Mesenchymal cells can be isolated from bone marrow, other adult tissues, for example, adipose or embryonic sources, for example, umbilical cord. As the availability of donor tissues often limits this approach, interest has moved towards using induced pluripotent stem cells with or without G-CSF. Human allogenic liver-derived progenitor cells are currently under investigation in ACLF in a phase 2b randomised placebo-controlled, double-blind multicentre study (NCT04229901). In patients with alcohol-related hepatitis, a phase 2 study showed that human allogenic liver-derived progenitor cells were safe and improved markers of systemic inflammation and liver injury over a 3-month period without a survival benefit.[84]

CHALLENGES AND OPPORTUNITIES

Clinical stratification as an example of prognostication

Patient heterogeneity poses significant challenges for managing individuals and designing clinical trials, especially in complex diseases. Existing classifications rely on outcome-predicting scores, potentially missing crucial

elements contributing to heterogeneity and impacting prognostic insights. To address patient heterogeneity at hospital admission, the Decision project developed a tool called ClustALL, a computational pipeline addressing diverse clinical data challenges, such as mixed types, missing values and collinearity. ClustALL enables unsupervised identification of robust patient stratifications,[85] which was applied to the PREDICT study.[86] ClustALL revealed several distinct stratifications at hospital admission, including markers of impaired liver function, organ dysfunction count and precipitating events, of which one stratification determined three patient clusters characterised by typical clinical features, but also exposing prognostic value. These findings were validated in the independent ACLARA-study.[87] Therefore, ClustALL may guide future clinical trial design by stratification of patient populations for a specific treatment.[85]

Practical aspects and applicability of biomarkers

The use of novel biomarkers for drug and trial development in ACLF implies a long road ahead (online supplemental figure 1). The applicability depends on regulations set by regulatory agencies, for example, FDA, shaping the research towards future therapy and/or biomarker regulatory approval (see https://www.fda.gov/drugs/biomarker-qualification-program/list-qualified-biomarkers) To date, only one biomarker has been approved by the Center for Drug Evaluation and Research, and the Center for Biologics Evaluation and Research Biomarker Qualification Programme database. Using ACLF, cirrhosis and end-stage liver disease as keywords, the magnitude of the complex shear modulus |G*|, a parameter corresponding to tissue 'stiffness' as measured by magnetic resonance elastography, is the single biomarker that met the FDA regulations (https://force-dsc.my.site.com/ddt/s/). The FDA approved the context of its use as 'a diagnostic biomarker to prescreen patients with clinical risk factors for chronic liver disease for enrolment in clinical trials to identify those at high risk of having histopathologic findings of significant fibrosis (≥F2), advanced fibrosis (≥F3) or cirrhosis (F4) on liver biopsy'. There is clearly an unmet need in biomarker development in ACLF. In addition, one may be probably more familiar with drug-induced liver injury (DILI) as a stopping rule and from the 2009 report where a combination of AST/ALT and bilirubin set forth the standard of DILI. Nonetheless, we think this will likely change in the future following the use of proteomic profiling to identify such candidates as recently shown for DILI.[88] We propose using the evidentiary framework and guidance drafted by the FDA (online supplemental figure 2). The needs assessment for a novel biomarker in ACLF includes the limitations of the current biomarkers and how this proposed biomarker will promote drug/therapy development in areas of ACLF where there is an unmet medical need or how such novel biomarker can address a particular aspect of ACLF pathophysiology more efficiently or effectively. The context of use (online supplemental figure 2) includes two components: (a) the biomarker category—previously described, either ACLF-related or treatment-related and (b) the biomarker's proposed use in drug development (purpose of use in drug development, the stage of drug development, patient population or model system and/or the therapeutic mechanism of action for which the biomarker is intended to have value). Third, benefits and risks assessment are important in biomarker development, for example, what is the added value to drug development or what are the anticipated consequences if the biomarker is unsuitable for its proposed use.

Finally, to determine the evidence to support the use of biomarkers in novel therapy development in ACLF, investigators need to provide not only the biological rationale for the use of the biomarker but also evidence supporting the relationship between the biomarker and the condition or clinical outcome of interest and the analytical performance (online supplemental figure 3). The latter is particularly important because a novel biomarker needs to provide enough data on reliability/agreement[89] before providing biomarker measurement cut-offs. Additionally, it will be important to provide performance characteristics of the existing measurement methods, the biological variability of the biomarker in different settings (eg, compensated cirrhosis vs unstable decompensated cirrhosis, pre-ACLF and ACLF), and the minimum magnitude of the biomarker change expected to affect clinical decisions. Altogether, we suppose the study is designed to study the diagnostic accuracy of a particular biomarker. We recommend considering the sample size to ensure adequate power to detect the clinically important difference, control for multiple comparisons, ensure that investigators collect and address key threats in the internal (biases/confounding) as well external validity and minimise missing data.[90 91]

CONCLUSIONS

Novel concepts dealing with different courses of cirrhosis as well as pathogenetic mechanisms have enhanced our understanding and given rise to many approaches for diagnosis and personalised approaches to treatment. Yet, prevention and treatment of ACLF remains a huge challenge for several reasons. Timing of prevention of ACLF may be different with different approaches. The dynamics of systemic inflammation and the influence of portal pressure may determine the choice of rationale and the specific treatment or treatment combination. Biomarker development to guide decisions is underway and needs to be robustly tested and prospectively validated in the clinic. Repurposed drugs and other approaches from critical care medicine may be appropriate at different stages of the disease, as highlighted above. Despite controversial and sometimes disappointing results, significant research has influenced the field. Many novel studies are underway and will change the future landscape. Personalised care and certification of biomarkers are crucial in this complex disease.

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REFERENCES

1 Arroyo V, Angeli P, Moreau R, *et al*. The systemic inflammation hypothesis: towards a new paradigm of acute decompensation and multiorgan failure in cirrhosis. *J Hepatol* 2021;74:670–85.
2 D'Amico G, Bernardi M, Angeli P. Towards a new definition of decompensated cirrhosis. *J Hepatol* 2022;76:202–7.
3 Schulz MS, Angeli P, Trebicka J. Acute and non-acute decompensation of liver cirrhosis (47/130). *Liver Int* 2024.
4 Arroyo V, Moreau R, Jalan R. Acute-on-chronic liver failure. *N Engl J Med* 2020;382:2137–45.
5 de Franchis R, Bosch J, Garcia-Tsao G, *et al*. Renewing consensus in portal hypertension. *J Hepatol* 2022;76:959–74.
6 Larrue H, D'Amico G, Olivas P, *et al*. TIPS prevents further decompensation and improves survival in patients with cirrhosis and portal hypertension in an individual patient data meta-analysis. *J Hepatol* 2023;79:692–703.
7 Trebicka J, Fernandez J, Papp M, *et al*. The PREDICT study uncovers three clinical courses of acutely decompensated cirrhosis that have distinct pathophysiology. *J Hepatol* 2020;73:842–54.
8 Biggins SW, Angeli P, Garcia-Tsao G, *et al*. Diagnosis, evaluation, and management of ascites, spontaneous bacterial peritonitis and hepatorenal syndrome: 2021 practice guidance by the American association for the study of liver diseases. *Hepatology* 2021;74:1014–48.
9 European Association for the Study of the Liver. Electronic address: easloffice@easloffice.eu, European Association for the Study of the Liver. EASL clinical practice guidelines for the management of patients with decompensated cirrhosis. *J Hepatol* 2018;69:1207.
10 European Association for the Study of the Liver, Electronic address eee, European Association for the Study of the L. EASL clinical practice guidelines on acute-on-chronic liver failure. *J Hepatol* 2023;79:461–91.
11 FDA: BEST resource. In: *BEST (Biomarkers, EndpointS, and other Tools) Resource*. Silver Spring (MD) Bethesda (MD), 2016.
12 Ariza X, Graupera I, Coll M, *et al*. Neutrophil gelatinase-associated lipocalin is a biomarker of acute-on-chronic liver failure and prognosis in cirrhosis. *J Hepatol* 2016;65:57–65.
13 Gambino C, Piano S, Stenico M, *et al*. Diagnostic and prognostic performance of urinary neutrophil gelatinase-associated lipocalin in patients with cirrhosis and acute kidney injury. *Hepatology* 2023;77:1630–8.
14 Ruiz-Margáin A, Xie JJ, Román-Calleja BM, *et al*. Phase angle from bioelectrical impedance for the assessment of sarcopenia in cirrhosis with or without ascites. *Clin Gastroenterol Hepatol* 2021;19:1941–9.
15 Pose E, Solà E, Lozano JJ, *et al*. Treatment with simvastatin and rifaximin restores the plasma metabolomic profile in patients with decompensated cirrhosis. *Hepatol Commun* 2022;6:1100–12.
16 Fernández J, Clària J, Amorós A, *et al*. Effects of albumin treatment on systemic and portal hemodynamics and systemic inflammation in patients with decompensated cirrhosis. *Gastroenterology* 2019;157:149–62.
17 Jalan R, Saliba F, Pavesi M, *et al*. Development and validation of a prognostic score to predict mortality in patients with acute-on-chronic liver failure. *J Hepatol* 2014;61:1038–47.
18 Gustot T, Fernandez J, Garcia E, *et al*. Clinical course of acute-on-chronic liver failure syndrome and effects on prognosis. *Hepatology* 2015;62:243–52.
19 Ripoll C, Platzer S, Franken P, *et al*. Liver-HERO: hepatorenal syndrome-acute kidney injury (hrs-aki) treatment with transjugular intrahepatic portosystemic shunt in patients with cirrhosis-a randomized controlled trial. *Trials* 2023;24:258.
20 Reiberger T, Berzigotti A, Trebicka J, *et al*. The rationale and study design of two phase II trials examining the effects of BI 685,509, a soluble Guanylyl cyclase activator, on clinically significant portal hypertension in patients with compensated cirrhosis. *Trials* 2023;24:293.
21 Jachs M, Hartl L, Simbrunner B, *et al*. Decreasing von Willebrand factor levels upon nonselective beta blocker therapy indicate a decreased risk of further Decompensation, acute-on-chronic liver failure, and death. *Clin Gastroenterol Hepatol* 2022;20:1362–73.
22 Du L, Ma Y, Zhou S, *et al*. A Prognostic score for patients with acute-on-chronic liver failure treated with plasma exchange-centered artificial liver support system. *Sci Rep* 2021;11:1469.
23 Weiss E, de la Peña-Ramirez C, Aguilar F, *et al*. Sympathetic nervous activation, mitochondrial dysfunction and outcome in acutely decompensated cirrhosis: the metabolomic prognostic models (CLIF-C MET). *Gut* 2023;72:1581–91.
24 Agarwal B, Cañizares RB, Saliba F, *et al*. Randomized, controlled clinical trial of the DIALIVE liver dialysis device versus standard of care in patients with acute-On-chronic liver failure. *J Hepatol* 2023;79:79–92.
25 Schrier RW, Arroyo V, Bernardi M, *et al*. Peripheral arterial vasodilation hypothesis: a proposal for the initiation of renal sodium and water retention in cirrhosis. *Hepatology* 1988;8:1151–7.
26 Albillos A, Martin-Mateos R, Van der Merwe S, *et al*. Cirrhosis-associated immune dysfunction. *Nat Rev Gastroenterol Hepatol* 2022;19:112–34.
27 Trebicka J, Bork P, Krag A, *et al*. Utilizing the gut microbiome in decompensated cirrhosis and acute-on-chronic liver failure. *Nat Rev Gastroenterol Hepatol* 2021;18:167–80.
28 Patel VC, Lee S, McPhail MJW, *et al*. Rifaximin-alpha reduces gut-derived inflammation and mucin degradation in cirrhosis and encephalopathy: RIFSYS randomised controlled trial. *J Hepatol* 2022;76:332–42.
29 Clària J, Stauber RE, Coenraad MJ, *et al*. Systemic inflammation in decompensated cirrhosis: characterization and role in acute-on-chronic liver failure. *Hepatology* 2016;64:1249–64.
30 Villanueva C, Albillos A, Genescà J, *et al*. Beta blockers to prevent decompensation of cirrhosis in patients with clinically significant portal hypertension (PREDESCI): a randomised, double-blind, placebo-controlled, multicentre trial. *Lancet* 2019;393:1597–608.
31 Villanueva C, Torres F, Sarin SK, *et al*. Carvedilol reduces the risk of decompensation and mortality in patients with compensated cirrhosis in a competing-risk meta-analysis. *J Hepatol* 2022;77:1014–25.
32 Jensen MD, Watson H, Vilstrup H, *et al*. Non-selective beta-blockers and risk of sepsis in patients with cirrhosis and ascites: results from a large observational study. *Clin Epidemiol* 2023;15:775–83.
33 Villa E, Cammà C, Marietta M, *et al*. Enoxaparin prevents portal vein thrombosis and liver decompensation in patients with advanced cirrhosis. *Gastroenterology* 2012;143:1253–60.
34 Guerrero A, Campo LD, Piscaglia F, *et al*. Anticoagulation improves survival in patients with cirrhosis and portal vein thrombosis: the IMPORTAL competing-risk meta-analysis. *J Hepatol* 2023;79:69–78.
35 Sanchez AP, Turon F, Martinez J, *et al*. Rivaroxaban improves survival and decompensation in cirrhotic patients with moderate liver dysfunction. double blind, placebo-controlled trial. *J Hepatol* 2023;78:S2–3.
36 Tonon M, Balcar L, Semmler G, *et al*. Etiological cure prevents further decompensation and mortality in patients with cirrhosis with ascites as the single first Decompensating event. *Hepatology* 2023;78:1149–58.
37 Rudler M, Hernández-Gea V, Procopet BD, *et al*. Hepatic encephalopathy is not a contraindication to pre-emptive TIPS in high-risk patients with cirrhosis with variceal bleeding. *Gut* 2023;72:749–58.
38 Hatton G, Shawcross DL. Is treating the gut microbiome the key to achieving better outcomes in cirrhosis *Expert Rev Gastroenterol Hepatol* 2019;13:1–2.
39 Bass NM, Mullen KD, Sanyal A, *et al*. Rifaximin treatment in hepatic encephalopathy. *N Engl J Med* 2010;362:1071–81.
40 Bajaj JS, Kassam Z, Fagan A, *et al*. Fecal microbiota transplant from a rational stool donor improves hepatic encephalopathy: a randomized clinical trial. *Hepatology* 2017;66:1727–38.
41 Bajaj JS, Salzman NH, Acharya C, *et al*. Fecal microbial transplant capsules are safe in hepatic encephalopathy: a phase 1, randomized, placebo-controlled trial. *Hepatology* 2019;70:1690–703.
42 Shawcross D, Edwards LA, Woodhouse C, *et al*. Faecal microbiota transplant restores gut barrier function and augments ammonia metabolism in patients with advanced cirrhosis: a randomised single-blind placebo-controlled trial. *Res Square* [Preprint] 2023.

43 Sinclair M, Grossmann M, Hoermann R, *et al*. Testosterone therapy increases muscle mass in men with cirrhosis and low testosterone: a randomised controlled trial. *J Hepatol* 2016;65:906–13.
44 Ramírez G, Briceño J, Rojas A. Statins and portal hypertension: a new pharmacological challenge. *Curr Vasc Pharmacol* 2012;10:767–72.
45 Abraldes JG, Villanueva C, Aracil C, *et al*. Addition of simvastatin to standard therapy for the prevention of variceal rebleeding does not reduce rebleeding but increases survival in patients with cirrhosis. *Gastroenterology* 2016;150:1160–70.
46 Pose E, Napoleone L, Amin A, *et al*. Safety of two different doses of simvastatin plus rifaximin in decompensated cirrhosis (LIVERHOPE-SAFETY): a randomised, double-blind, placebo-controlled, phase 2 trial. *Lancet Gastroenterol Hepatol* 2020;5:31–41.
47 Mahmud N, Chapin S, Goldberg DS, *et al*. Statin exposure is associated with reduced development of acute-on-chronic liver failure in a veterans affairs cohort. *J Hepatol* 2022;76:1100–8.
48 Pose E, Jiménez C, Zaccherini G, *et al*. Simvastatin plus rifaximin to prevent ACLF in patients with decompensated cirrhosis. A randomised, double-blind, placebo-controlled, phase-3 trial: the liverhope efficacy trial. *J Hepatol* 2023;78:S10–1.
49 Bernardi M, Angeli P, Claria J, *et al*. Albumin in decompensated cirrhosis: new concepts and perspectives. *Gut* 2020;69:1127–38.
50 Caraceni P, Riggio O, Angeli P, *et al*. Long-term albumin administration in decompensated cirrhosis (ANSWER): an open-label randomised trial. *Lancet* 2018;391:2417–29.
51 Laleman W, Trebicka J, Zaccherini G, *et al*. Real-world treatment of decompensated liver cirrhosis in Italy: a propensity score-matched analysis of long-term versus acute albumin therapy. *J Hepatol* 2023;78:S238.
52 Fagan A, Gavis EA, Gallagher ML, *et al*. A double-blind randomized placebo-controlled trial of albumin in outpatients with hepatic encephalopathy: HEAL study. *J Hepatol* 2023;78:312–21.
53 Solà E, Solé C, Simón-Talero M, *et al*. Midodrine and albumin for prevention of complications in patients with cirrhosis awaiting liver transplantation. A randomized placebo-controlled trial. *J Hepatol* 2018;69:1250–9.
54 China L, Freemantle N, Forrest E, *et al*. A randomized trial of albumin infusions in hospitalized patients with cirrhosis. *N Engl J Med* 2021;384:808–17.
55 Trebicka J, Garcia-Tsao G. Controversies regarding albumin therapy in cirrhosis. *Hepatology* August 7, 2023.
56 Torp N, Israelsen M, Coenraad M, *et al*. Personalised human albumin in patients with cirrhosis and ascites: design and rationale for the ALB-TRIAL - a randomised clinical biomarker validation trial. *BMJ Open* 2024;14:e079309.
57 Garg V, Garg H, Khan A, *et al*. Granulocyte colony-stimulating factor mobilizes Cd34(+) cells and improves survival of patients with acute-on-chronic liver failure. *Gastroenterology* 2012;142:505–12.
58 Montaldo E, Lusito E, Bianchessi V, *et al*. Cellular and transcriptional dynamics of human neutrophils at steady state and upon stress. *Nat Immunol* 2022;23:1470–83.
59 Kedarisetty CK, Anand L, Bhardwaj A, *et al*. Combination of granulocyte colony-stimulating factor and erythropoietin improves outcomes of patients with decompensated cirrhosis. *Gastroenterology* 2015;148:1362–70.
60 Verma N, Kaur A, Sharma R, *et al*. Outcomes after multiple courses of granulocyte colony-stimulating factor and growth hormone in decompensated cirrhosis: A randomized trial. *Hepatology* 2018;68:1559–73.
61 De A, Kumari S, Singh A, *et al*. Multiple cycles of granulocyte colony-stimulating factor increase survival times of patients with decompensated cirrhosis in a randomized trial. *Clin Gastroenterol Hepatol* 2021;19:375–83.
62 Venkitaraman A, De A, Verma N, *et al*. Multiple cycles of granulocyte colony-stimulating factor in decompensated cirrhosis: a double-blind RCT. *Hepatol Int* 2022;16:1127–36.
63 Newsome PN, Fox R, King AL, *et al*. Granulocyte colony-stimulating factor and autologous Cd133-positive stem-cell therapy in liver cirrhosis (REALISTIC): an open-label, randomised, controlled phase 2 trial. *Lancet Gastroenterol Hepatol* 2018;3:25–36.
64 Engelmann C, Herber A, Franke A, *et al*. Granulocyte-colony stimulating factor (G-CSF) to treat acute-on-chronic liver failure: a multicenter randomized trial (GRAFT study). *J Hepatol* 2021;75:1346–54.
65 Tranah TH, Kronsten VT, Shawcross DL. Implications and management of cirrhosis-associated immune dysfunction before and after liver transplantation. *Liver Transpl* 2022;28:700–16.
66 Gandoura S, Weiss E, Rautou P-E, *et al*. Gene- and exon-expression profiling reveals an extensive LPS-induced response in immune cells in patients with cirrhosis. *J Hepatol* 2013;58:936–48.
67 Caraceni P, Abraldes JG, Ginès P, *et al*. The search for disease-modifying agents in decompensated cirrhosis: from drug repurposing to drug discovery. *J Hepatol* 2021;75 Suppl 1:S118–34.
68 Uschner FE, Schulz M, Tyc O, *et al*. Safety and preliminary efficacy and pharmacokinetics of intraperitoneal VS-01 infusions in patients with decompensated liver cirrhosis: a first-in-human, open-label, phase 1B clinical trial. *Hepatology* 2021;74:1–156.
69 Iwasaki A, Medzhitov R. Control of adaptive immunity by the innate immune system. *Nat Immunol* 2015;16:343–53.
70 Macdonald S, Andreola F, Bachtiger P, *et al*. Cell death markers in patients with cirrhosis and acute decompensation. *Hepatology* 2018;67:989–1002.
71 Kondo T, Macdonald S, Engelmann C, *et al*. The role of Ripk1 mediated cell death in acute on chronic liver failure. *Cell Death Dis* 2021;13:5.
72 Monteiro S, Grandt J, Uschner FE, *et al*. Differential inflammasome activation predisposes to acute-on-chronic liver failure in human and experimental cirrhosis with and without previous decompensation. *Gut* 2021;70:379–87.
73 Hu JJ, Liu X, Xia S, *et al*. FDA-approved disulfiram inhibits Pyroptosis by blocking Gasdermin D pore formation. *Nat Immunol* 2020;21:736–45.
74 Shawcross DL, Wright GAK, Stadlbauer V, *et al*. Ammonia impairs neutrophil phagocytic function in liver disease. *Hepatology* 2008;48:1202–12.
75 Taylor NJ, Manakkat Vijay GK, Abeles RD, *et al*. The severity of circulating neutrophil dysfunction in patients with cirrhosis is associated with 90-day and 1-year mortality. *Aliment Pharmacol Ther* 2014;40:705–15.
76 Ruiz-Margáin A, Pohlmann A, Lanzerath S, *et al*. Myostatin is associated with the presence and development of acute-on-chronic liver failure. *JHEP Rep* 2023;5:100761.
77 Sawhney R, Holland-Fischer P, Rosselli M, *et al*. Role of ammonia, inflammation, and cerebral oxygenation in brain dysfunction of acute-on-chronic liver failure patients. *Liver Transpl* 2016;22:732–42.
78 Qiu J, Thapaliya S, Runkana A, *et al*. Hyperammonemia in cirrhosis induces transcriptional regulation of myostatin by an NF-kappaB-mediated mechanism. *Proc Natl Acad Sci U S A* 2013;110:18162–7.
79 Kumar A, Davuluri G, Silva RNE, *et al*. Ammonia lowering reverses Sarcopenia of cirrhosis by restoring skeletal muscle Proteostasis. *Hepatology* 2017;65:2045–58.
80 Tranah TH, Ballester M-P, Carbonell-Asins JA, *et al*. Plasma ammonia levels predict hospitalisation with liver-related complications and mortality in clinically stable outpatients with cirrhosis. *J Hepatol* 2022;77:1554–63.
81 Jain A, Sharma BC, Mahajan B, *et al*. L-Ornithine L-aspartate in acute treatment of severe hepatic encephalopathy: a double-blind randomized controlled trial. *Hepatology* 2022;75:1194–203.
82 Ventura-Cots M, Arranz JA, Simón-Talero M, *et al*. Safety of ornithine phenylacetate in cirrhotic decompensated patients: an open-label, dose-escalating, single-cohort study. *J Clin Gastroenterol* 2013;47:881–7.
83 Ventura-Cots M, Concepción M, Arranz JA, *et al*. Impact of ornithine phenylacetate (OCR-002) in lowering plasma ammonia after upper gastrointestinal bleeding in cirrhotic patients. *Therap Adv Gastroenterol* 2016;9:823–35.
84 Nevens F, Gustot T, Laterre P-F, *et al*. A phase II study of human allogeneic liver-derived progenitor cell therapy for acute-on-chronic liver failure and acute decompensation. *JHEP Reports* 2021;3:100291.
85 Palomino-Echeverria S, Huergou E, Ortega-Legarre A, *et al*. Clustall: a robust clustering strategy for stratification of patients with acutely decompensated cirrhosis. medRxiv [Preprint] 2023.
86 Trebicka J, Fernandez J, Papp M, *et al*. PREDICT identifies precipitating events associated with the clinical course of acutely decompensated cirrhosis. *J Hepatol* 2021;74:1097–108.
87 Farias AQ, Curto Vilalta A, Momoyo Zitelli P, *et al*. Genetic ancestry, race, and severity of acutely decompensated cirrhosis in Latin America. *Gastroenterology* 2023;165:696–716.
88 Ravindra KC, Vaidya VS, Wang Z, *et al*. Tandem mass tag-based quantitative proteomic profiling identifies candidate serum biomarkers of drug-induced liver injury in humans. *Nat Commun* 2023;14:1215.
89 Hernaez R. Reliability and agreement studies: a guide for clinical investigators. *Gut* 2015;64:1018–27.
90 Cook JA, Julious SA, Sones W, *et al*. DELTA(2) guidance on choosing the target difference and undertaking and reporting the sample size calculation for a randomised controlled trial. *Trials* 2018;19:606.
91 Little RJ, D'Agostino R, Cohen ML, *et al*. The prevention and treatment of missing data in clinical trials. *N Engl J Med* 2012;367:1355–60.