Review Article

Role of Granulocyte Colony-stimulating Factor Therapy in Cirrhosis, 'Inside Any Deep Asking Is the Answering'

Cyriac Abby Philips*[1], Philip Augustine[2], Rizwan Ahamed[2], Sasidharan Rajesh[3], Tom George[3], Gopakumar C. Valiathan[4] and Solomon K. John[4]

[1]*The Liver Unit and Monarch Liver Lab, Cochin Gastroenterology Group, Ernakulam Medical Centre, Kochi, Kerala, India;*
[2]*Department of Gastroenterology, Cochin Gastroenterology Group, Ernakulam Medical Centre, Kochi, Kerala, India;*
[3]*Interventional Radiology, Hepatobiliary Division, Cochin Gastroenterology Group, Ernakulam Medical Centre, Kochi, Kerala, India;*
[4]*Department of Hepatobiliary and Transplant Surgery, Cochin Gastroenterology Group, Ernakulam Medical Centre, Kochi, Kerala, India*

Abstract

Liver cirrhosis progresses through multiple clinical stages which culminate in either death or liver transplantation. Availability of organs, timely listing and prompt receipt of donor-livers pose difficulties in improving transplant-listed and transplant outcomes. In this regard, regenerative therapies, particularly with granulocyte colony-stimulating factor (GCSF), has become a lucrative option for improving transplant-free survival. However, the literature is confusing with regards to patient selection and real outcomes. In this exhaustive review, we describe the basics of liver fibrosis and cirrhosis through novel insights from a therapeutic point of view, discuss preclinical studies on GCSF in advanced liver disease to improve on clinical utility, shed light on the pertinent literature of GCSF in advanced cirrhosis, and provide astute inputs on growth factor therapy in decompensated cirrhosis.

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Abbreviations: ACLF, acute-on-chronic liver failure; C/EBP-α, CCAAT enhancer-binding protein-alpha; CTGF, connective tissue growth factor; CTP, Child-Turcotte-Pugh; ECM, extracellular matrix; EpCAM, epithelial cell adhesion molecule; FGF, fibroblast growth factor; GCSF, granulocyte-colony-stimulating-factor; GCSF-R, GCSF receptor; HBV, hepatitis B virus; HGF, hepatocyte growth factor; HSCs, hepatic stellate cells; IL, interleukin; JAK, Janus kinase; LSECs, liver sinusoidal endothelial cells; MAPK, mitogen-activated protein kinase; MCP, macrophage colony-stimulating factor; MELD, model for end-stage liver disease; MMPs, matrix metalloproteinases; MSCs, mesenchymal stem cells; NF-κB, nuclear factor kappa-light-chain-enhancer of activated B cells; PDGF, platelet-derived growth factor; PPAR, peroxisome proliferator-activated receptor; SMT, standard medical care; STAT, signal transducer and activator of transcription; TGF-β, transforming growth factor-beta; TIMPs, tissue inhibitors of MMPs; TNF-α, tumor necrosis factor-alpha; VEGF, vascular endothelial growth factor.

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***Correspondence to:** Cyriac Abby Philips, The Liver Unit and Monarch Liver Lab, Cochin Gastroenterology Group, Ernakulam Medical Centre, Automobile Road, Palarivattom, Kochi, 682025, Kerala, India. Tel: +91-484-2907000, Fax: +91-484-2907000, E-mail: abbyphilips@gmail.com

Introduction

Cirrhosis is the final common pathological pathway of ongoing liver injury that arises due to multiple etiologies of insults that vary geographically. Even though the etiologies differ, there are multiple drivers and progression factors, which culminate in common pathological characteristics that lead to cirrhosis. These include hepatic necrosis, degeneration, and replacement of hepatic parenchyma by scar tissue surrounding failed regeneration in the form of hepatic nodules that ultimately lead to portal hypertension and liver failure. Fibrosis is the most crucial precursor that drives the central pathological process in cirrhosis.

Currently, strategies in the treatment of cirrhosis are aimed at management of complications of cirrhosis and portal hypertension, and direct treatment strategies for cirrhosis, except treatment for known causes for cirrhosis progression (such as autoimmune hepatitis, and chronic viral hepatitis B and C), are lacking. Treatment of cirrhosis and restoration or replacement of functional regenerative potential can only happen once the molecular mechanisms that drive progression to cirrhosis are better understood. These molecular mechanisms, even though generally understood, lack clarity in the current literature.[1]

In the current review, we discuss pertinent mechanisms that lead to cirrhosis, and regenerative or restorative therapeutic strategies aimed at prevention and reversal of cirrhosis based on the currently known molecular mechanisms of cirrhosis. We also explore the current literature on clinical trials of regenerative strategies in cirrhosis and provide a critical appraisal of the same with particular emphasis on granulocyte-colony-stimulating-factor (GCSF)-based treatments.

Pathogenesis of cirrhosis and complexities associated with ideal therapeutic implications

Chronic liver injury results in progressive accumulation of extracellular matrix (ECM) with distortion of hepatic parenchymal architecture, an event that is spearheaded by myofibroblasts that form due to activation of hepatic stellate cells (HSCs) and multiple other cell types. This fibril-forming collagen deposition that replaces the low density, basement membrane-like interstitial matrix, along with the accumulation of other matrix proteins such as hyaluronan, elastin, fibronectin and proteoglycans, is central to fibrosis and its

progression to cirrhosis.[2] The ECM has the potential to secrete various cytokines, such as transforming growth factor-beta (TGF-β), platelet-derived growth factor (PDGF), hepatocyte growth factor (HGF), connective tissue growth factor (CTGF), tumor necrosis factor-alpha (TNF-α), fibroblast growth factor (FGF) and vascular endothelial growth factor (VEGF), that promote angiogenesis, another critical event that leads to exaggerated wound healing in the form of fibrous tissue formation. While this injury and ECM deposition is ongoing, remodeling of the ECM to preserve healthy hepatic parenchymal structure and function becomes critical in the maintenance of liver health.[3]

This balance is maintained by the matrix metalloproteinases (MMPs; specifically, MMP-1, -2, -8 and -13) and their inhibitors, the tissue inhibitors of MMPs (TIMPs). When the injury is chronic and the ECM deposition is overwhelming due to the persistence of HSC activation and abnormal neoangiogenesis, the activity of TIMPs takes the upper hand. This tipping of balance toward prolonged activity of TIMPs (mostly TIMP-1 and -2) results in anti-apoptotic effects on HSCs that further promoted fibrogenesis.[4]

Chronic liver inflammation leads to hepatocyte necrosis/apoptosis, paracrine stimulation, Kupffer cell activation, reactive oxidation and cytokine deliberation that activate HSCs that then transform into myofibroblasts with profibrogenic potential. HSC activation also occurs through lipid peroxide release, TNF-α and interferon-gamma production via the activation of nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB)-interferon regulatory factor 3 pathway and toll-like receptors (through lipopolysaccharide production by gut microbial dysbiosis).[5] Stimulated HSCs secrete macrophage colony-stimulating factor (MCP)-1, interleukin (IL)-6 and chemokines that act on their respective receptors, leading to macrophage activation, neutrophil infiltration, and chemotaxis, which activate mitochondrial oxidation in hepatocytes leading to their apoptosis; furthermore, these become a strong trigger for fibrogenesis, as phagocytosis of damaged hepatocytes by myofibroblasts enhances the fibrogenic activation through NADPH oxidase and the Janus kinase (JAK) signal transducer and activator of transcription (STAT) and phosphoinositide 3-kinase/Akt pathways.[6,7] The HSCs and myofibroblasts proliferate and lay down ECM, and further promote hepatic inflammation through enhanced TIMP expression, also under the influence of secreted angiotensin II [via mitogen-activated protein kinase (MAPK) signal transduction pathways], upregulated cannabinoid receptor 1, and circulating adipokine leptin [via (JAK)-signal transduction, leading to suppression of peroxisome proliferator-activated receptor γ (PPAR-γ)].[8]

Other cells of the liver microenvironment regulating enhancement or reduction of fibrosis involve natural killer cells, T cells, monocytes, liver sinusoidal endothelial cells (LSECs), ductular cells, cholangiocytes, and portal fibroblasts.[9] The role of monocytes in inflammation and fibrosis is important regarding GCSF therapy. Fibrogenesis is promoted by the subset of proinflammatory monocytes (CD14 + and CD16+ in humans). Monocytes are a source of circulating fibrocytes, which differentiate into collagen-producing fibroblasts, which are in turn closely related to the bone marrow mesenchymal stem cells (MSCs).[10,11] The TGF-β secreted by myofibroblasts promotes hepatocyte apoptosis after activation. TGF-β1 activates Smad2, and Smad3 promote fibrogenesis. Reduction and clearance of activated HSCs are central to fibrosis regression.

In clinical and experimental fibrosis models, control or elimination of the etiological agent responsible for the chronic inflammation has been shown to promote fibrosis reversal, due to the complete disappearance of myofibroblasts. Even then, a small subset of myofibroblasts can escape apoptosis during liver fibrosis regression, acquiring a phenotype similar to but distinct from quiescent HSCs. These 'fugitives' then rapidly reactivate into myofibroblasts in response to repetition of fibrogenic stimuli and rapidly contribute to liver fibrosis.[12,13] Early liver fibrosis, which lacks ECM crosslinking and marked angiogenesis, has the best potential to revert to typical architecture, provided the chronic insult is adequately controlled. Hence, the initiation, progression and reversal of fibrosis is a highly complex process with multiple interactions at the cellular and molecular levels.[14,15]

Taken together, the pleomorphic action of GCSF through multiple molecular mechanisms in the liver microenvironment could increase fibrosis and liver disease progression apart from its beneficial effects on granulopoiesis. Hence, to promote liver regeneration or restoration, the therapeutic intervention(s) must target multiple pathways and not just one of the 'central' pathways. Progression of fibrosis to cirrhosis happens through multiple 'central' pathways — this is akin to a control headquarters (chronic injury) and multiple 'metro-rail-lines' (molecular mechanisms) and major associated stations (central pathways) in a large city, rather than a single central railway station in a town. This complicated aspect of liver fibrosis progression and the complexities associated with treatment of fibrosis/cirrhosis is demonstrated in Fig. 1.

Pathophysiology associated with GCSF in the context of chronic liver disease

GCSF is a 25 kDa secreted glycoprotein encoded by the CSF3 gene. The central physiological role played by GCSF is in the regulation of neutrophil production in health and particularly in emergency responses to infections and bone marrow aplasia. In healthy humans, the serum concentrations of GCSF are typically undetectable or detectable at deficient levels, which markedly increases in the presence of an infectious stimulus. Most of the tissues in the body secrete GCSF after stimulatory effects, such as induction of IL-1, lipopolysaccharide and TNF-α produced by the macrophages, endothelial cells, fibroblasts and related mesenchymal cells (Fig. 2).

IL-17 is a potent upstream extracellular regulator of tissue production of GCSF, especially in the bone marrow. Ligation of the extracellular domain of the GCSF receptor (GCSF-R) by GCSF results in cellular responses due to signals that arise from the cytoplasmic domain of the GCSF-R. The GCSF-R is expressed by neutrophils and its precursors, such as metamyelocytes, myelocytes, promyelocytes, myeloblasts, myeloid progenitor cells, and primitive hemopoietic stem cells. The GCSF-R signals through the JAK/STAT pathway and through Lyn phosphorylation that activates PI3-kinase/Akt pathways, which are pertinent to the progression of liver fibrosis.

GCSF also activates Ras-MAPK through activation of tyrosine kinases, Lyn and Hck. This is of utmost importance because the Ras/Raf/MEK/ERK signaling pathway has been implicated in the occurrence and development of hepatocellular carcinoma in cirrhosis. GCSF has been shown to stimulate tumor cell growth and migration *in vitro*, and to promote

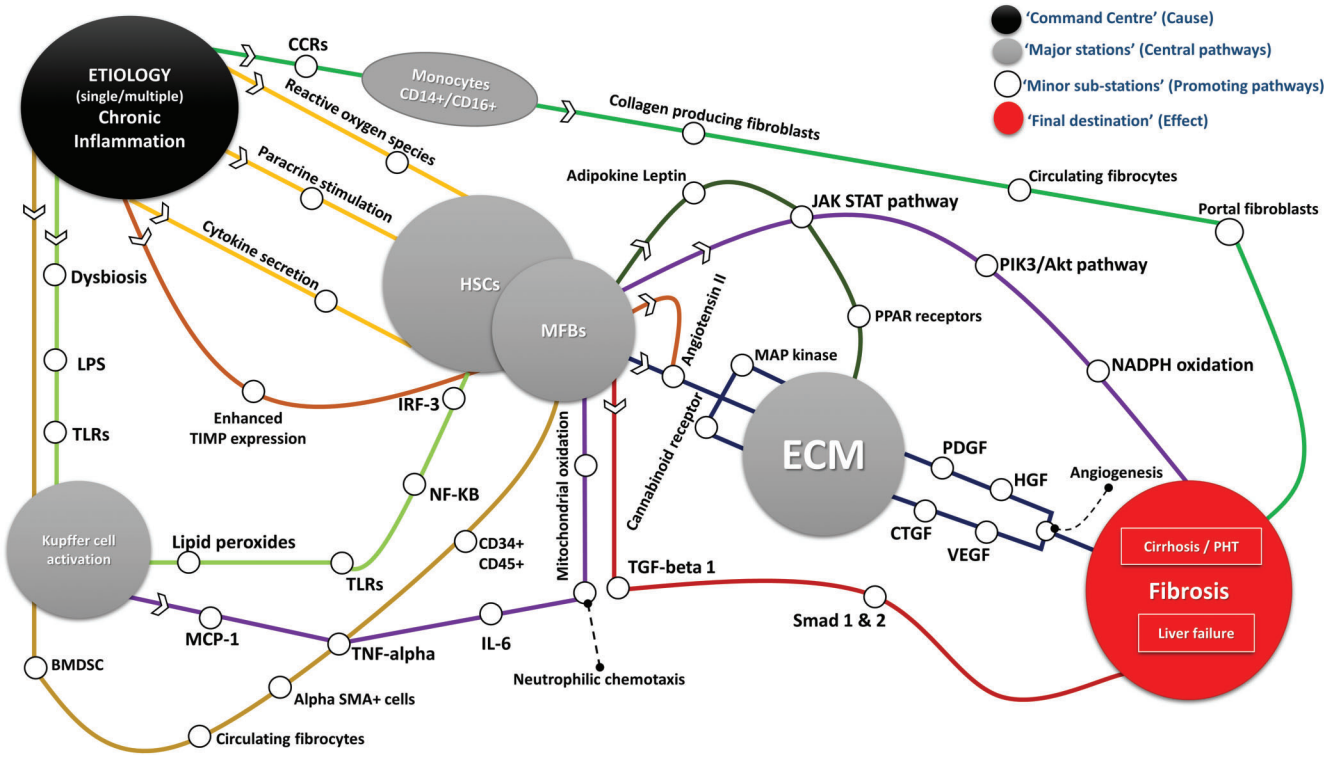

Fig. 1. The 'Metro-Rail Concept' to targeted therapy of the pathophysiology of cirrhosis. The development of cirrhosis follows well-coordinated steps that begin with the etiology, leading to sustained chronic inflammation (the control or command center; black bubble) that activates and promotes multiple pathways (rail-pathways, colored lines) that feature prominent mediators of inflammation and fibrosis (central stations, grey bubbles) and parallel assisting pathway intermediaries (secondary stations, small white bubbles) that ultimately lead to the destination (red bubble). Therapies that target only few of the pathway mediators do not tend to improve outcomes as expected; however, targeting the command center (etiology) along with controlling the central-stations (central inflammatory and fibrosis pathways) would impede progression to destination (cirrhosis).

Abbreviations: BMDSC, bone marrow-derived stem cells; CCRs, chemokine receptors; CD, cluster of differentiation; CTGF, connective tissue growth factor; ECM, extracellular matrix; HGF, hepatocyte growth factor; HSC, hepatic stellate cell; IL, interleukin; IRF, interferon regulatory factor; JAK/STAT, Janus kinase/signal transducers and activators of transcription; LPS, lipopolysaccharide; MAP, mitogen activated protein; MCP, monocyte chemoattractant protein; MFB, myofibroblasts; NADPH, reduced form of nicotinamide adenine dinucleotide phosphate; NF-kB, nuclear factor kappa B; PDGF, platelet derived growth factor; PHT, portal hypertension; PI3K/Akt, phosphoinositide-3-kinase-protein kinase B; PPAR, peroxisome proliferator-activated receptors; SMA, smooth muscle antibody; SMADs, homologues of the Drosophila protein, mothers against decapentaplegic (Mad) and the *Caenorhabditis elegans* protein Sma; TGF, transforming growth factor; TIMP, tissue inhibitors of matrix metalloproteinase; TLR, toll-like receptor; TNF, tumor necrosis factor; VEGF, vascular endothelial growth factor.

tumor progression *in vivo* by autocrine stimulation of tumor cells and paracrine activation of the tumorigenic stroma.[16,17]

Ordelheide *et al.*[18] demonstrated that GCSF promoted free fatty acid-induced insulin resistance in humans and that human adipocytes and myotubes treated with GCSF became insulin-resistant. Insulin resistance is a major driver of liver fibrogenesis and carcinogenesis. In cirrhosis patients with metabolic syndrome, obesity and insulin resistance, the use of GCSF could probably augment the disease process.[19] CCAAT enhancer-binding protein-α (C/EBP-α) regulates adipocyte differentiation and induces apoptosis in HSCs *in vivo* and *in vitro*. Tao *et al.*[20] showed that in the mouse liver fibrosis model, the upregulation of C/EBP-α decreased ECM deposition, including collagen and hydroxyproline content, and markers of liver damage were reduced significantly; immunohistochemistry showed an increase of apoptosis in HSCs, while hepatocytes were less affected.

On the other hand, C/EBPβ was found to be selectively upregulated in granulocytic-macrophage progenitors in the presence of GCSF. However, beneficial C/EBPα is not induced by GCSF in hematopoietic stem cells, and hence the utility of exogenous GSCF to decrease hepatocyte apoptosis cannot be possible through this mechanism of action.[21] Buck *et al.*[22] showed that, in response to liver injury, activation of ribosomal S6 kinase phosphorylation of C/EBPβ in activated HSCs is critical for the progression of liver fibrosis. Hence, in the presence of GCSF, molecular mechanisms of chronic liver injury that increases fibrosis are possibly upregulated to augment disease progression.

GCSF is also produced by a variety of nonhematopoietic cells, including fibroblasts and endothelial cells, and induces the proliferation and migration of endothelial cells, promotes angiogenesis, and upregulates inflammatory cell infiltration into tissues where GCSF-R is expressed.[23] Shojaei *et al.*[24] identified GCSF as a strong inducer of prokinectin-Bv8 expression, both *in vitro* and *in vivo*, the latter of which promotes neovascularization and tumoral progression in gastrointestinal malignancies. Hepatic angiogenesis is closely associated with the progression of fibrosis in chronic liver diseases and GCSF demonstrably induces endothelial activation

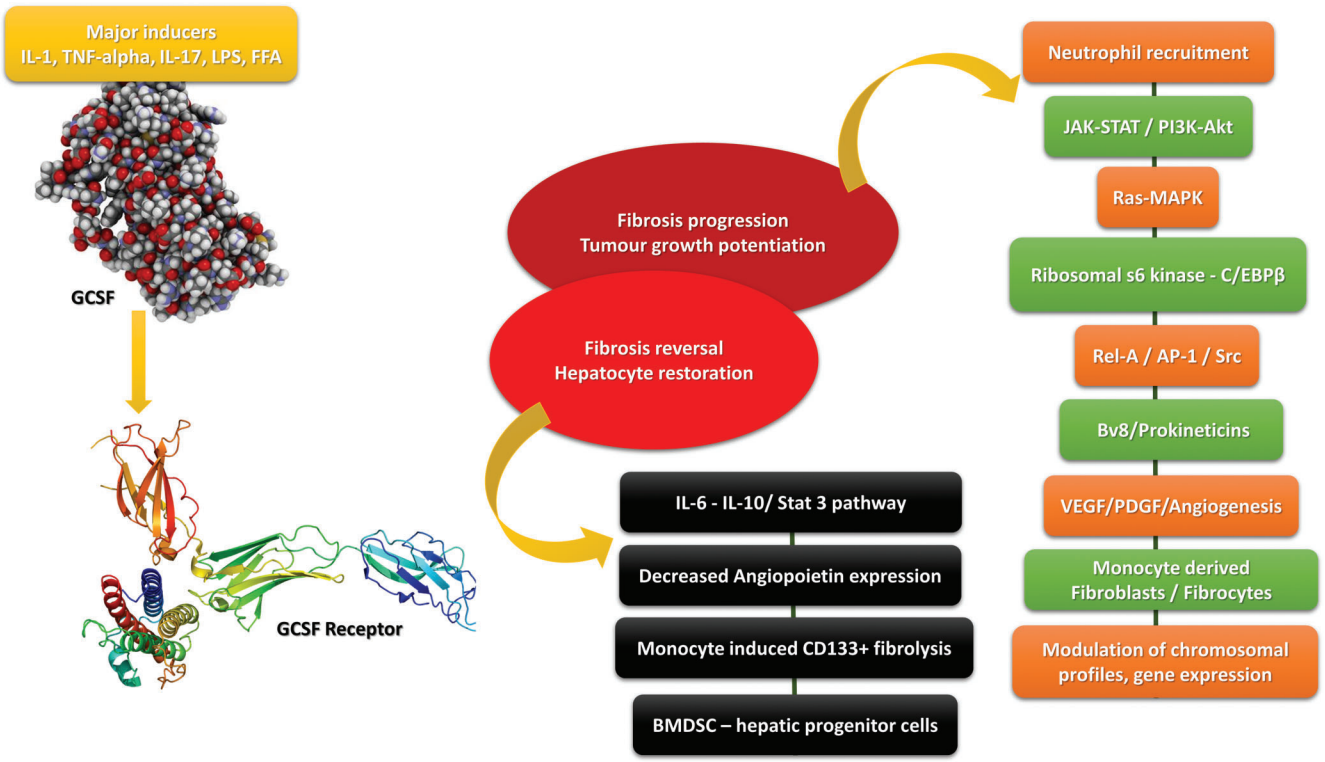

Fig. 2. GCSF-activated molecular pathways associated with fibrosis regression and progression. GCSF is a 'double-edged sword' and cannot be considered a true ally in the armamentarium against fibrosis and cirrhosis.

Abbreviation: GCSF, granulocyte colony-stimulating factor.

and upregulates downstream inflammatory pathways associated with angiogenesis and fibrosis progression.[25]

The belief that GCSF improves synthetic liver function and decreases fibrosis stems from the understanding that mobilization of CD34+ bone marrow-derived cells home into the liver microenvironment, transform into hepatic progenitor cells, and restore lost hepatocyte volume. Subsequently, possible reduction in expression in angiopoietin which leads to decreased neoangiogenesis ameliorates fibrosis. The enhancement of fibrolytic activity of CD133+ cells induced through GCSF on monocyte and bone marrow activation possibly through IL-10 mediated Stat3 regeneration pathway has been postulated. However, most of these processes remain clinical hypotheses, lacking proper 'fate of cell' tracer studies.[26–28]

Several studies have shown that GCSF promoted chromosomal changes in healthy persons associated with the modification of gene expression profile. Even though the long-term mutagenic implications in healthy persons with such changes are insignificant, in cirrhosis patients, in the presence of an inflammatory microenvironment, exogenous GCSF-associated genetic expression as well as chromosomal aberration are concerning and need further study.[29] Healthy persons, when injected with GCSF, develop marked neutrophilic response within 4 h, mobilize bone marrow-activated stem cells after 3 days, that peaks at the fifth day, which is associated with splenic enlargement over a week's time. Stroncek *et al.*[30] demonstrated that the spleen length increased by 20% or more in healthy subjects treated with GCSF at 10 mcg/kg/day for 5 days. In portal hypertension, splenic congestion and splenomegaly are attributed to portal congestion, elevated portal pressures and closely related to increased tissue hyperplasia and fibrosis. The increase in spleen size results in increased splenic blood flow, translating to increased portal hypertension, which may worsen with repeated GCSF use in patients with cirrhosis.[31]

In the study by Nakamura *et al.*,[32] hepatic arterial infusion of low, mid and high doses of autologous-derived CD34+ cells mobilized by GCSF showed mild improvement in serum albumin level, without significant sustained improvement in liver disease severity scores. The spleen size in cirrhosis patients did increase with GCSF but it was a transient phenomenon since the GCSF treatment was only for 5 days. Similarly, Gaia *et al.*[33] reported significant reversible spleen enlargement with stable serum liver enzyme levels after GCSF administration in patients with alcoholic cirrhosis, and Lorenzini *et al.*[34] demonstrated increased splenomegaly with stable levels during GCSF administration in patients with viral hepatitis. These studies concentrated on short-term use of GCSF, and longer use or multiple dosing regimen of GCSF as seen with recent studies could be associated with greater chances of splenic enlargement and elevation in portal pressures. All of these studies lacked the fate of hematopoietic cell investigation and, as such, conclusive evidence of liver cell restoration or regeneration could not be ascertained.

In humans, GCSF could either be beneficial or detrimental, depending on the disease in context. For example, perioperative GCSF use was found to reduce postoperative morbidity by

decreasing monocyte and lymphocyte activation. However, in patients with chemotherapy-related lung injury and fibrosis, GCSF worsened clinical outcomes by exacerbating lung injury. A similar detrimental outcome was noted in patients with rheumatoid arthritis.[35] The role of GCSF in cell recruitment in infected and healthy individuals is different from a potential role in producing tissue injury. These are yet to be studied in different stages of advanced liver cirrhosis. However, preclinical studies[36,37] that shed light on various pleomorphic actions of GCSF in patients with advanced liver disease, with regards to pro- and anti-inflammation, fibrosis reversal or promotion, and intermediate and long-term portal hypertensive outcomes, especially in decompensated cirrhosis, are currently lacking and mandatory before further trials on GCSF is undertaken.

Critical appraisal of GCSF in decompensated cirrhosis

One of the strategies to restore liver functionality is cell therapy, aimed at restoring or regenerating hepatocytes that maintain liver function, an aspect that is physiologically lacking in cirrhosis. Sources of hepatocytes include normal liver in which hepatocytes themselves proliferate to restore functionality, liver progenitor cells that differentiate and proliferate under specific circumstances, and blood-derived stem cells that infiltrate the liver, transform into hepatocytes and proliferate. The ideal source of liver cell function and mass restoration in a chronic liver disease state is currently poorly understood. Most clinical trials on GCSF have been conducted without adequate knowledge on the accurate beneficial pathway that promotes quantity restoration of existing healthy hepatocytes or regeneration or formation of new liver cells.

Beneficial control over molecular mechanisms involved in HSC activation, amelioration in production and enhanced degradation of ECM with mitigation in activated myofibroblasts form the components of ideal 'regenerative' therapy for liver cirrhosis. Such an ideal scenario is currently unavailable with cell therapy-based interventions in cirrhosis. Treatment or control of etiology responsible for chronic liver inflammation (i.e. abstinence from alcohol, weight loss in obese patients, and antiviral therapy for hepatitis B and C) is the best currently available intervention that can truly 'regenerate/restore' liver function in cirrhosis. However, in patients with advanced cirrhosis, control of etiology may not fully establish acceptable liver function, and such patients become ideal candidates for cell therapies aimed at liver regeneration in the absence of liver transplantation options. At present, the various cell-based therapeutic strategies studied in patients with cirrhosis include infusion of autologous hepatocytes, epithelial cell adhesion molecule (EpCAM) positive fetal liver stem cells, bone marrow-derived differentiated or undifferentiated MSCs, autologous GCSF mobilized cultured CD34+ bone marrow stem cells, peripheral blood mononuclear cells from GCSF mobilized peripheral blood, and direct use of GCSF to induce bone marrow-derived stem cells in peripheral circulation. Conclusive evidence for improving transplant-free survival or sustained improvement in liver disease severity scores have not been fully realized with these treatments[38] (Table 1).

Spahr *et al.*[39] showed that GCSF mobilized CD34+ cells, increased HGF, and induced proliferation of hepatic progenitor cells within 7 days of administration. HGF has been found to ameliorate liver fibrosis and prevent fulminant hepatic failure in elegant animal studies. About 5 to 10 ng/mL of HGF is required for growth promotion of adult rat hepatocytes in primary culture, and the ideal blood levels required for human hepatocyte growth promotion in human studies have not been confirmed. Exogenously administered HGF in animal models of acute liver failure has shown beneficial effects in improving survival, and the role of HGF in clinical practice is to predict prognosis in patients with severe liver failure. HGF has a very short half-life (~ 5 m), and the quality of HGF induction with GCSF use and its continued benefits remain unknown.[40]

In another study, Spahr and colleagues[41] randomized 58 patients with alcoholic hepatitis and underlying cirrhosis with mean model for end-stage liver disease (MELD) score of 19, early after hospital admission, to standard medical therapy (SMT) or combined with GCSF injections and autologous hepatic arterial infusion of bone marrow-derived mononuclear cells. At the end of 90 days follow-up, two and four patients died in the experimental and SMT groups, respectively. Adverse events were not significant between groups, and on follow-up liver biopsy from the baseline, improvement in steatosis was notable but proliferating hepatocyte progenitor cells decreased in both groups. A weak regenerative stimulation and resistance to the promotion of regeneration in decompensated alcoholic cirrhosis could have led to the poor responses seen with regenerative therapy in this study.

Han and colleagues[42] administered GCSF at 5 to 10 mcg/kg/day for 4 days to patients with decompensated hepatitis B-related liver cirrhosis and compared them to those receiving GCSF mobilized peripheral blood mononuclear cells, collected by leukapheresis followed by infusion through the hepatic artery. In the GCSF group, one patient died from variceal bleeding combined with hepatic coma at 3 weeks after GCSF, and two patients from repeated variceal bleeding at 5 months after GCSF. Among the surviving patients, only two required no albumin supplementation during the follow-up, while ten required continued albumin supplementation 4 weeks after GCSF therapy due to repeated ascites removal and new-onset hepatorenal syndrome in one patient. The differences in serum albumin and Child-Turcotte-Pugh (CTP) scores between the two groups were significant. This was indicative of worsening clinical outcomes with GCSF in patients with decompensated liver cirrhosis.

Xing and colleagues[43] studied the effects of GCSF in patients with hepatitis B virus (HBV)-related cirrhosis. They found that CD34+ cells were already higher at baseline in cirrhosis patients compared to healthy controls and that GCSF use dramatically increased circulating numbers of CD34+ bone marrow-derived stem cells. However, such increments in levels of the CD34+ stem cell population in the systemic circulation did not translate to clinical improvements in the treated patients compared to the control group.

Gaia *et al.*[44] studied the effects of multiple courses of GCSF (3-day GCSF course, 5 mcg/kg every 12 h; administered at 3-month intervals for a total of four courses) in patients with decompensated cirrhosis. CD34+ bone marrow-derived stem cells were found to increase in patients receiving GCSF during the first cycle, without peak level maintenance during subsequent cycles. Four patients died of progressive liver failure during the treatment period, in whom peak levels of CD34+ cells were comparable to those who completed treatment. The CTP score improved without sustenance, while the MELD score did not show significant changes from baseline but rather increase beyond 9 months after GCSF use. The authors also studied the fate of induced CD34+ stem cells

Table 1. Clinical trials of granulocyte-colony stimulating factor (GCSF) therapy in patients with cirrhosis, outcomes, and critique

Author / Country / Study type / Year	Follow up / Outcome / Salient features	Comments
Spahr *et al.*[39]/ Switzerland / randomized controlled trial (RCT) / 2008 GCSF vs. standard of care	· 7 days follow up · Clinical outcomes not discussed · Drug safety demonstrated · Changes in CD34+ cells demonstrated · Cytokines and aminopyrine breath tests similar between treated and control groups	· Initial study showing safety of GCSF and mobilization of bone marrow-derived stem cells in patients with advanced liver disease · Included patients with cirrhosis and alcoholic hepatitis
Han *et al.*[42]/ China / RCT / 2008 GCSF vs. autologous peripheral blood monocyte cell (PBMC) transplantation	· 6 months follow up · Both groups showed improvement in serum albumin and prothrombin time · Liver tests did not show significant improvement between groups · Improved Child-Turcotte-Pugh (CTP) score in the PBMC group	· GCSF did not improve liver function or liver disease severity · Autologous PBMC transplantation was a superior modality of treatment in advanced liver disease compared to GCSF
Spahr *et al.*[41]/ Switzerland / RCT / 2013 GCSF vs. standard of care	· 3 months follow up · Clinical outcomes similar between treated and control groups · Primary end point of 3-point decrease in model for end-stage liver disease (MELD) score same with GCSF and standard medical care · Histologically, only steatosis improved	· GCSF and stem cell infusions did not result in expansion of the hepatic progenitor cell compartment within the liver microenvironment · No improvement in liver function · Authors concluded insufficient regenerative stimulation or resistance to liver regenerative drive in patients with decompensated alcoholic cirrhosis with exogenous therapy
Xing *et al.*[43]/ China / RCT / 2013 GCSF vs. standard of care	· In hepatitis B virus (HBV)-related cirrhosis · Proportion of CD34+ cells increased after GCSF · Matrix metalloproteinase level significantly high before and after GCSF · Short-term disease severity not affected by GCSF use	· No significant differences in total bilirubin, albumin and prothrombin time between the treated and control groups · No significant differences were observed in the cure and improvement rates between the two groups
Gaia *et al.*[44]/ Italy / non randomized, control study/ 2013 Multiple courses of GCSF in decompensated cirrhosis	· 12 months follow up · 3 day GCSF course (5 mcg/kg every 12 h), administered at 3-month intervals for a total of four courses · Feasibility and safety explored · Telomere length was monitored to rule out early cell aging caused by GCSF	· GCSF could be safely administrated up to four times over a 1-year period in decompensated cirrhotic patients · CD34+ cells increase unsustained peak levels in subsequent cycles · Four patients died of progressive liver failure (CD34+ cells comparable to those who survived) · CTP score improved without maintenance, MELD score had no significant changes but worsened beyond 9 months · CD184 (repair of liver injury) reduction and loss of C-met (increases fibrosis) noted

(*continued*)

Table 1. (*continued*)

Author / Country / Study type / Year	Follow up / Outcome / Salient features	Comments
Kedarisetty *et al.*[47]/ India / RCT / 2015 GCSF + darbepoetin vs. placebo in decompensated cirrhosis	· 12 month follow up · GCSF (5 mcg/kg/d) for 5 days and then every third day (12 total doses) + subcutaneous darbepoetin-α (40 mcg/week) for 4 weeks · Liver disease severity scores, sepsis events and pre- and post-treatment liver biopsies assessed. Survival at 12 months higher in the GCSF + darbepoetin group (68.6% vs. 26.9%) · CTP scores were reduced by 48.6% in the GCSF group vs. 39.1% in the control group · MELD scores reduced by 40.4% after GCSF use · Need for large-volume paracentesis was significantly reduced and lower proportion of patients developed septic shock after GCSF use · On liver biopsy pre- and post-GCSF, increase in the proportion of CD34+ cells and CD133+ cells noted	· GCSF-only arm not studied · Role of darbepoetin in regeneration and amelioration of sepsis events not studied · Pre- and post-treatment liver biopsy done in only 5 patients in the treatment group and 2 patients in the control group. Under-powered conclusion regarding augmentation of hepatic regeneration
Prajapati *et al.*[56]/ India / RCT / 2017 GCSF in decompensated cirrhosis	· 6 months follow up · GCSF at 300 mcg subcutaneous twice daily for 5 days plus standard medical therapy (SMT) or SMT alone · In the GCSF group, 17 patients died and 9 were lost to follow-up · In the control group, 30 patients died and 11 were lost to follow-up · Survival with GCSF was higher (79 vs. 68%) · In the GCSF group, 66% of patients showed improvement or stability in the CTP score at 6 months, while in the control group it was 51%	· Acute-on-chronic liver failure patients also included · MELD progression not discussed · Specific extrahepatic and liver-related events between groups not discussed
Verma *et al.*[59]/ India / RCT / 2018 Multiple courses of GCSF with or without growth hormone in decompensated cirrhosis	· 12 months follow up · Growth hormone (1U subcutaneous per day) with GCSF (5 mcg/kg) subcutaneously every 12 hours for 5 days, then every 3 months for 3 days till 12 months · GCSF-only arm · Standard medical care-only arm · The primary outcome was transplant-free survival at 1 year · Survival significantly higher in GCSF-treated patients · CD34+ cells increased at day 6 · Significant decrease in clinical scores, improvement in nutrition, better control of ascites, lesser infection episodes · Striking decrease in liver stiffness after GCSF treatment	· Increase in CD34+ cells at day 6 was expected, does not translate to improved liver regeneration · Long-term sustenance in CD34+ cell levels and linked clinical events not studied · More than expected liver stiffness improvement not explained with GCSF use; antifibrotic effects of GCSF not studied and remain unexplained · Improved liver stiffness measurements not substantiated with liver histology assessment · Very low MELD score patients and those not requiring liver transplant listing also included in the study

(*continued*)

Table 1. (*continued*)

Author / Country / Study type / Year	Follow up / Outcome / Salient features	Comments
Newsome *et al.*[61]/ United Kingdom / RCT / 2018 Safety and efficacy of GCSF and haemopoietic stem cell infusions in patients with compensated cirrhosis	· 3 months follow up · Inclusion MELD scores of 11 to 15 · Subcutaneous GCSF (lenograstim) 15 mcg/kg for 5 days, or treatment with GCSF for 5 days followed by leukapheresis and intravenous infusion of three doses of CD133+ hematopoietic stem cells ($0{\cdot}2 \times 10^6$ cells per kg per infusion) · Co-primary outcomes included improvement in severity of liver disease (change in MELD) at 3 months and the trend of change in MELD score over time · No improvement in liver dysfunction or markers of liver fibrosis occurred after the administration of GCSF or GCSF + stem-cell infusions · GCSF / GCSF + stem infusions worsened liver function and increased patient morbidity and mortality	· The first multicenter, open-label, randomized, controlled phase 2 trial on GCSF in cirrhosis · Very rigorous high-quality trial · Sufficiently powered · Challenges findings of other similar studies · New onset ascites, sepsis and hepatic encephalopathy requiring multiple hospital admissions after use of GCSF/stem cell infusions compared to placebo · Adverse events were greater in the treatment groups compared to controls
Anand *et al.*[55]/ India / RCT / 2019 GCSF / GCSF + erythropoietin in decompensated cirrhosis	· 12 months follow up · GCSF given at a dose of 5 mcg/kg subcutaneous at days 1, 2, 3, 4, 5 and then every third day till day 60 (total 22 doses) · Erythropoietin given subcutaneously at dose of 500 IU/kg twice a week for 2 months (total 16 doses) · Follow-up until end of 12 months · Combination revealed significant improvement in CTP and MELD scores compared to GCSF alone · Reduction in mortality better with combination (16.6% vs. 36.7%) · The combination treatment showed decreased acute kidney injury, encephalopathy and refilling of ascites incidence compared to monotherapy · Response poor in grade 3 ascites and better in Child B cirrhosis with MELD <16	· Lower MELD and lower CTP score cirrhosis patients had better survival; this could be true even without treatment intervention · Response to treatment in patients with higher grades of liver disease severity was poor · Role of erythropoietin alone not assessed · Need for regenerative therapy in lower MELD scores debatable
Philips *et al.*[62]/ India / Real-world experience / 2019 GCSF in decompensated cirrhosis needing liver transplantation in the intermediate term	· 12 months follow up · GCSF 10 mcg/kg per day for 5 days, followed by 5 mcg/kg/day once every third day for total 12 doses · Per protocol analysis (n = 56) and intention to treat analysis (n = 100) · 16%, 43% and 75% patients died at 3, 6, and 12 months respectively · Sepsis most common cause of death, in 53% patients · 9% developed hepatocellular carcinoma at the end of follow-up · Patients receiving GCSF had higher mortality at end of 12 months compared to controls (75% vs. 46%)	· Non-randomized, historical controls · Included all patients who required short- and intermediate-term transplant-free survival · Large number of patients, clarity in follow-up, and definition and identification of events · Provided novel data on CTP (>11) and MELD (>20) cut-off at which GCSF use needs to be avoided

expressing immature CD133 and CD117 markers (considered to home into the liver microenvironment, promoting liver regeneration) and found no significant differences between pretreatment and post-treatment levels of the same. Interestingly, the authors found that CD184 and C-Met expression decreased significantly after treatment with GCSF in cirrhosis patients. CD184 is involved in repairing liver injury upon triggering MSCs to migrate, transdifferentiate, and fuse with hepatocytes, while the loss of c-Met was found to accelerate the development of liver fibrosis through deregulation of multiple molecular pathways. These findings demonstrate worsening of liver fibrosis in patients with advanced liver disease receiving GCSF.[45,46]

Kedarisetty *et al.*,[47] utilized GCSF and darbepoetin in patients with decompensated cirrhosis for comparison to placebo. The cumulative probability of survival at 12-months was 68.6% in the GCSF + darbepoetin group and 26.9% in the placebo-treated patients. Sepsis events were lower in the GCSF + darbepoetin group compared to placebo-treated patients. It has also been demonstrated that erythropoietin therapy reduced hypotension related to sepsis and promoted cardioprotective effects in animal models of sepsis, with a reduction in acute kidney injury events during endotoxemia.[48]

In a study by Preheim and colleagues,[49] GCSF was administered to control and cirrhotic rats before and after induction of pneumococcal pneumonia. The authors elegantly demonstrated that GCSF administered before infection did not protect cirrhotic or control rats but did so after infection and significantly reduced mortality in control but not cirrhotic rats. In human trials on GCSF in cirrhotics, the findings are quite the opposite (i.e. GCSF prevented sepsis events).[50] The mechanism of action of GCSF in amelioration or prevention of sepsis in advanced cirrhosis is not yet elucidated. However, in the study by Fiuza *et al.*,[51] GCSF was found to improve and increase recruitment of neutrophils to sites of infection and to enhance neutrophil transendothelial migration in cirrhotic patients in the absence of neutrophil adhesion, which could be one of the reasons underlying the beneficial action of GCSF in sepsis.

Multiple studies have shed light on the detrimental effects of GCSF in sepsis. GCSF has immunosuppressive effects on monocytes, macrophages, dendritic cells, and T lymphocytes when exogenously administered, and high levels of GCSF have been found to negatively regulate IL-17 production, thereby worsening sepsis.[52] Studies have also shown that high level of GCSF at baseline was associated with poor outcome in sepsis. Stephens *et al.*[53] demonstrated worsening of liver dysfunction with elevations in troponin levels patients with sepsis treated with GCSF. Segal *et al.*[54] reported that the novel existence of a hepatobiliary hybrid progenitor population anatomically restricted to the ductal plate of fetal liver, with a transcriptional profile distinct from that of fetal hepatocytes, mature hepatocytes and mature biliary epithelial cells, in human fetal liver using single-cell RNA sequencing. This opens up newer horizons on regenerative therapies for advanced liver disease.

Strong conclusions cannot be made from the data in the Kedarisetty *et al.*[47] study on survival benefits with GCSF due to the absence of a GCSF-only treatment arm. Similar findings were echoed in the study by Anand *et al.*[55] The authors showed that addition of erythropoietin to GCSF led to better regenerative response than GCSF monotherapy in patients with low MELD score (<16). The need for regenerative cell therapy in patients with low MELD scores and relatively lower mortality rates in the short- and intermediate-term remain debatable in the absence of quality long-term effects with such experimental treatments.

Prajapati *et al.*[56] conducted the largest randomized controlled study of GCSF therapy, using 253 decompensated cirrhosis patients. The authors showed that the cumulative survival was significantly higher in GCSF-treated patients compared to controls (79 vs. 68%) and that significantly more patients in the GCSF group had an improvement in CTP scores at 180 days. Even though those authors stated that the inclusion of only decompensated cirrhosis as one of the strengths of their study, the table detailing patient characteristics shows the inclusion of acute-on-chronic liver failure (ACLF) patients also. ACLF patients have completely different disease mechanisms, progression, clinical outcome and possibly, distinct response to GCSF therapy in comparison to patients with decompensated cirrhosis, and hence the study by Prajapati and colleagues[56] does not fully realize the potential of GCSF use in decompensated cirrhosis. The authors do not discuss MELD progression in their study among GCSF-treated patients even though baseline values for the same are mentioned and the study is restricted to an intermediate follow up period.

Chavez-Tapia *et al.*[57] conducted a systematic review and metanalysis on GCSF use in patients with ACLF. The authors included all randomized clinical trials comparing the use of any regimen of GCSF against placebo or no intervention; ultimately, two trials involving 102 patients were included. A significant reduction in short-term overall mortality was observed in patients receiving GCSF compared to controls. Nonetheless, higher mortality secondary to gastrointestinal bleeding was noted in the GCSF-treated patients. The authors concluded that the GCSF-treated ACLF patients had significantly reduced short-term mortality with limited evidence. Another metanalysis by Yang *et al.*[50] included five studies with mostly 3 months follow-up in patients with ACLF. Those authors concluded that GCSF treatment in patients with advanced liver failure significantly improved liver function, reduced the incidence of sepsis, and prolonged short-term survival. Thus, the short-term survival with GCSF use in ACLF has been clearly defined. Long-term benefits, transplant-free survival, and GCSF use in different etiologies, in patients with sepsis and ACLF remain unknown and a matter for future study.

In patients with ACLF, in contrast to those with decompensated cirrhosis, immune dysregulation and higher sepsis events have been shown to predict poor outcomes. In studies on GCSF in ACLF, possible explanations for improved outcomes include a reduction in sepsis by amelioration of immune dysfunction observed in ACLF, through an increase in fraction of circulating and intrahepatic myeloid and plasmacytoid dendritic cells. GCSF has also been shown to improve survival in patients with ACLF due to reactivation of chronic hepatitis B as well as alcoholic hepatitis. However, these studies were mostly from single centers.

Robust data on the utility of GCSF in ACLF for specific etiologies is lacking, the precise mechanism of action promoting clinical benefit remains to be defined, and the dose and duration in specific groups of ACLF classified as per severity compared to decompensated cirrhosis is still warranted. Larger multicenter double-blind randomized trials in homogeneous patient groups for validating the role and potential mechanisms of action of GCSF in ACLF is the next step.[58] In this regard, the results of a large multicenter, open,

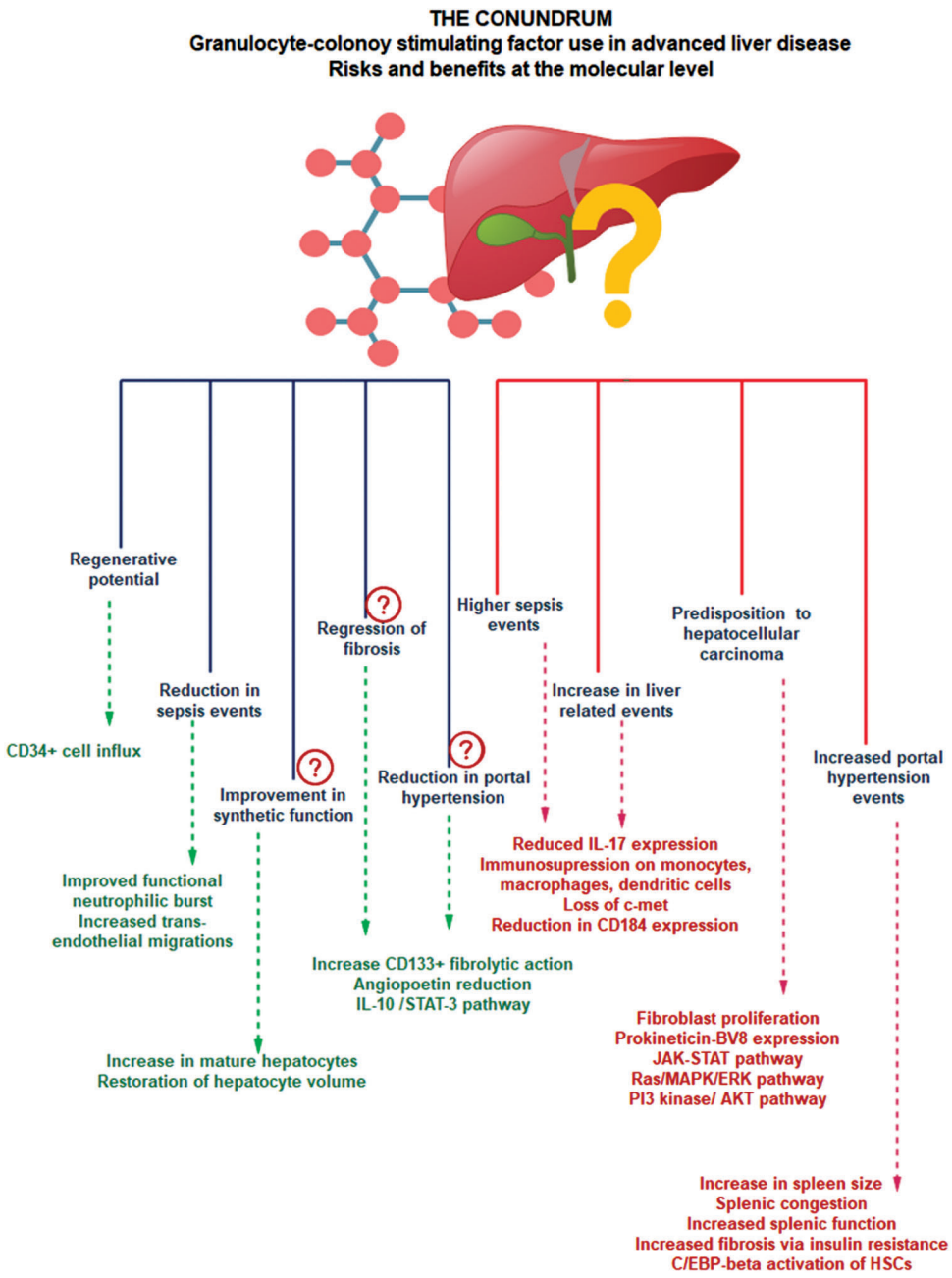

Fig. 3. The conundrum associated with GCSF and advanced liver disease in animal and human studies. Striking a balance is an unmet need to improve outcomes, which is currently still a matter of bench work before bedside use can be considered.

Abbreviation: GCSF, granulocyte colony-stimulating factor.

randomized, and controlled trial in patients with ACLF (The Graft Trial) to evaluate efficacy and safety of subcutaneously administered GCSF is much awaited (ClinicalTrials.gov Identifier: NCT02669680).

Verma and colleagues[59] studied clinical outcomes in decompensated cirrhosis with multiple courses of GCSF with/without growth hormone compared to SMT. Very low MELD score patients who were exempt from liver transplantation listing were also included in the study. The mean survival and cumulative probability of transplant-free survival were higher in GCSF-treated patients associated with a surprisingly striking decrease in liver stiffness measurements. Explanations for such robust fibrosis reversal and improvement in synthetic liver function was not supported by strong research data in the discussion. Reasons for GCSF related to 'liver regeneration' as discussed by the Verma *et al.*[59] remain hypothetical.

The type of bone marrow-derived stem cell utilized has been shown to affect outcomes in cell therapy for liver diseases; for example, puritan mesenchymal cells, derived monocyte or macrophage fractions were shown to have

better efficacy in amelioration of chronic liver injury in preclinical studies. With GCSF use and an associated general increase in bone marrow-derived stem cells, the much needed 'fate of cell' tracer studies to correctly identify the probable beneficial pathway associated with this therapy remain to be performed.[60]

Newsome and colleagues[61] assessed the safety and efficacy of GCSF and CD133+ hemopoietic stem-cell infusions in patients with compensated liver cirrhosis with MELD scores ranging from 11 to 15.5 and found that liver dysfunction or fibrosis did not improve when compared to patients receiving standard care, and furthermore patients receiving the treatment had a higher incidence of adverse liver-related events. This study cautioned on the use of growth factor therapy in patients with compensated cirrhosis and moderately high MELD scores.

Recently, Philips and colleagues[62] published real-world experience of GCSF in a large group of patients with cirrhosis and active decompensations with higher MELD scores. Cirrhosis patients with active ascites, jaundice, or both completed GCSF treatment (10 mcg/kg/day for 5 days, followed by 5 mcg/kg/day once every third day for total 12 doses). A matched historical control group was used for comparing outcomes. Among them, 16%, 43% and 75% of patients died at 3, 6 and 12 months respectively, after GCSF treatment. Sepsis was the most frequent cause of death (in 53% of patients), followed by progressive liver failure (in 33%). Notably, a higher number of patients compared to the historical control group developed hepatocellular carcinoma at the end of 12 months. Acute variceal bleeds, overt hepatic encephalopathy, intensive care unit admissions, and liver disease severity scores were higher after GCSF use at 12 months. A CTP score of >11 and MELD-sodium score of >20 predicted worse outcomes at all time points and 12 months with GCSF use, respectively. The modified intention to treat analysis demonstrated poor overall survival at 6 months with GCSF therapy compared to the historical controls (48% vs. 75%, $p = 0.04$). The authors concluded that survival in decomposition was shorter than what was expected in the natural history of the disease after GCSF use in patients with advanced cirrhosis.

In the study by Kedarisetty *et al.*,[47] a lower proportion of patients developed septic shock during the follow-up period compared with controls, and by the end of 1 month after treatment the mean level of α-fetoprotein was significantly higher in the growth factor group (6.6 ± 3.6 ng/mL) than in the controls (4.7 ± 2.7 ng/mL). The latter was considered to be associated with hepatic regeneration. However, this was contrary to findings described by Philips *et al.*[62] and the occurrence of sepsis as well as liver cancer was found to be higher with GCSF use. Seehofer *et al.*[63] demonstrated, in an animal model of chronic liver disease, that hepatic regeneration was slightly inhibited in the GCSF group. A study on the effect of GCSF in liver fibrosis found that it significantly decreased the survival rate of mice.[64]

Conclusions

The way forward

Liver fibrosis and its progression, and the ultimatum of associated portal hypertension and liver failure, is a highly complex disease mechanism. Furthermore, the mechanisms that define human liver regeneration remain inadequately characterized. Much of essential basic science work on GCSF in liver fibrosis has not provided in-depth knowledge regarding its actions in amelioration of chronic liver injury and fibrosis. Even though clinical trials, mostly from the Indian subcontinent, have shown improved outcomes with GCSF use, rigorously designed high-quality trials and real-world evidence have shown the contrary (Fig. 3). Hence, in questioning the depth of available data, the answers regarding the utility of GCSF in treating patients with cirrhosis remain unclear.

To clearly understand the role of GCSF in liver cirrhosis, a systematic approach to the problem is warranted. First, one must try to define and delineate the pathways affected through use of exogenous GCSF in chronic liver disease animal models and in humans. It needs to be clear, with regards to GCSF therapy, if we are attempting to reduce portal hypertension or improve liver failure. With such an attempt, the deleterious effects of GCSF could also be studied. Second, dose-finding studies to clarify outcomes in chronic liver disease models need to be performed. Third, the roles of GCSF as an anti-inflammatory, antifibrotic or liver cell restoration therapy need to be clearly defined, and as such the dosing that promotes such specific activity needs to be identified through thorough quality bench work. Fourth, short-, intermediate- and long-term use of exogenously administered GCSF (at specific doses in the finite period or multiple courses over more extended periods) and its effect on fibrogenesis and carcinogenesis has to be demonstrated. All of these need thorough fate-of-cell tracer studies to identify the true potential and action of GCSF in the liver microenvironment.

Ultimately, once the ideal dose, duration, patient population and window of opportunity based on liver disease severity has been defined, large multicenter trials following this homogenous dosing regimen, with well-defined inclusion criteria and patient to follow up methodology, remain the unmet need. Until then, early and timely liver transplantation remains the most beneficial treatment for patients with decompensated cirrhosis.

Conflict of interest

Dr. Cyriac Abby Philips received advisory fees and research grant support from Cipla®, Mylan® and Samarth Life-Sciences®. The other authors have no conflict of interests related to this publication.

Author contributions

Designed the study and wrote the initial manuscript (CAP, PA, SR), conducted critical revisions to the manuscript (RA, TG, GCV, SKJ), made illustrations and obtained metadata for the manuscript (CAP, RA, SR, TG). All authors approved the final version of the manuscript.

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