Hepatic Regeneration in Cirrhosis

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End-stage liver disease is characterized by massive hepatocyte death resulting in clinical decompensation and organ failures. Clinical consequences in cirrhosis are the results of the loss of functional hepatocytes and excessive scarring. The only curative therapy in advanced cirrhosis is orthotropic liver transplantation, but the clinical demand outweighs the availability of acceptable donor organs. Moreover, this also necessitates lifelong immunosuppression and carries associated risks. The liver has a huge capability for regeneration. Self-replication of quiescent differentiated hepatocytes and cholangiocytes occurs in patients with acute liver injury. Due to limited hepatocyte self-renewal capacity in advanced cirrhosis, great interest has therefore been shown in characterizing the possible role of hepatic progenitor cells and bone marrow-derived stem cells to therapeutically aid this process. Transplantation of cells from various sources that can be properly differentiated into functional liver cells or use of growth factors for ex-vivo expansion of progenitor cells is needed at utmost priority. Multiple researches over the last two decades have aided researchers in refining proliferation, differentiation, and storage techniques and understand the functionality of these cells for use in clinical practice. However, these cell-based therapies are still experimental and have to be used in trial settings. (J CLIN EXP HEPATOL 2022;12:603–616)

L iver has remarkable regeneration capacity. Systemic inflammation, hepatocyte death, and fibrosis with decreased matrix remodeling are the hallmark of liver cirrhosis. Progression of these changes is associated with impaired liver regeneration and risk of dysfunction and failure of organ systems. Effective and practical alternate approaches to liver transplantation are needed. The challenges to regeneration in patients with liver cirrhosis are different from those with acute hepatic injury. In cirrhosis, there is the massive deposition of extracellular matrix (ECM) and tissue scarring, which results in cellular or functional loss of regenerative niche, architectural distortions, vascular reorganization, depletion of parenchymal cells, and persistent inflammatory response, this

would result in failure of engraftment and regeneration of transplanted cells. The discovery of molecular pathways in hepatic regeneration during the last two decades has opened up new vistas in the treatment of liver cirrhosis and given rise to new optimism. Cellular debris, to begin with, should be cleared and inflammation subsided. Hepatocyte progenitor cells (HPC) and bone marrow stem cells (BMSC) play an important role (Figure 1). In this paper, we review the mechanisms of liver regeneration and current therapeutic approaches for enhancing liver regenerative capacities in patients with liver cirrhosis.

CELLULAR COMPOSITION OF LIVER

The understanding of the mechanisms of liver injury and the cell types involved in hepatic regeneration requires a thorough knowledge of normal liver cellular composition and architecture. The liver lobule is the basic unit of the liver. It contains cords of hepatocytes and supporting cells, including liver sinusoidal endothelial cells (LSEC), Kupffer cells (KC), cholangiocytes, hepatic stellate cells (HSC), and many other immune cells.[1] The unique arrangement of hepatocytes and supporting cells in zones is essential for the wide range of functions performed by the liver. In addition, the liver also contains hepatic stems that are potentially capable of self-renewal.[2] Three main types of stem cells in liver are (i) Sox9^{+} cells in the portal area that express both hepatic and bile duct cells lineage markers and are referred to as hybrid cells.[3] These cells are primarily involved in hepatocyte regeneration after chronic liver injury. (ii) Portal area also contains hepatic progenitor cells (HPCs contain oval-shaped nuclei and sparse cytoplasm) that are capable of differentiating in hepatocytes

Three Arms of Hepatic Regeneration

Hepatocyte or cholangiocytes self replication	Activation , migration and differentiation of hepatic stem and progenitor cells	Activation , migration and engraftment of Bone marrow stem and progenitor cells

All theses three arms are normal in normal liver

All theses three arms are impaired to various extent in chronic liver injury and results aberrant regeneration

Hepatic Regeneration

BONE MARROW CELLS RESTORE NATIVE LIVER REGENERATING NICHE

BM Clear the damage, provide HSC,EPC,MSC and immune cells	Provide paracrine support for parenchymal and non parenchymal cell regeneration	Rescue of lost non-parenchymal cell mass and causes Remodelling of extracellular matrix

Both adult and progenitor cell mediated regeneration is compromised with disease severity
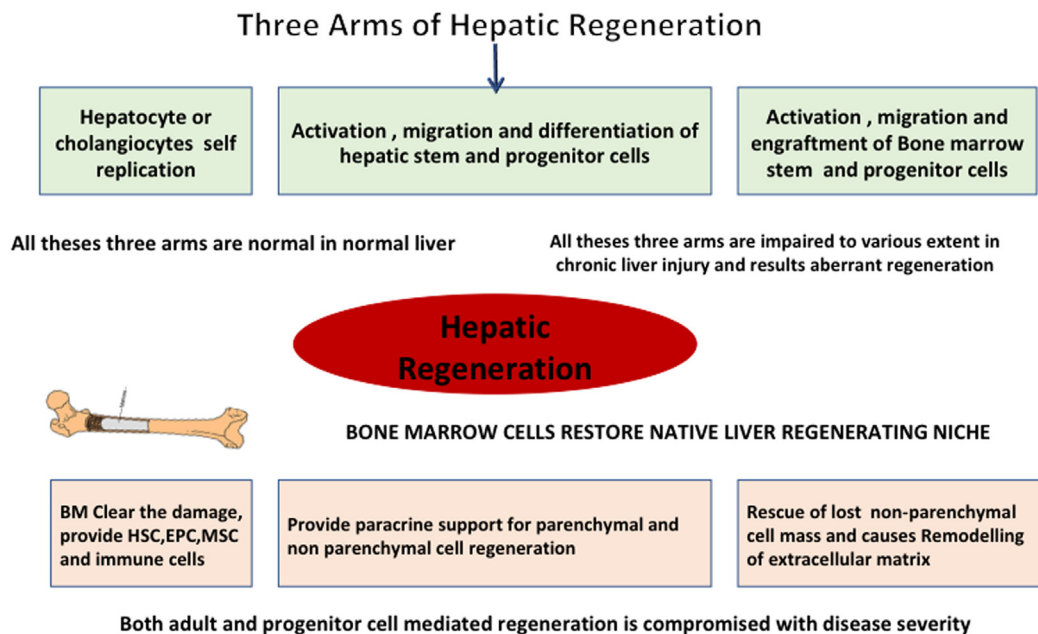
Figure 1 Three arms of hepatic regeneration in relation to chronic liver disease.

or cholangiocytes whenever the capability of hepatocytes to sufficiently self-renew is compromised in response to injury.[4] (iii) Axin2^{+} cells around the central vein endothelium.[5] Various study have shown the distinct cell population in liver with hepatic stem cell-like properties, but whether they are facultative or liver has liver stem cells still remains controversial.[6]

NORMAL LIVER TISSUE TURNOVER

The average life span of adult hepatocytes varies from 6 to 9 months. For explaining hepatocyte turnover, two competing hypotheses have been presented. As per the "streaming liver" hypothesis, young hepatocytes or cholangiocytes originate from hepatic stem/progenitor cells present in the portal zone.[7] Young hepatocytes then subsequently migrate and mature towards the central vein.[7] Based on some evidence against the streaming liver hypothesis, the "self-replicating model" was proposed.[8] This model suggests that the majority of liver tissue maintenance is accomplished through hepatocytes and cholangiocyte cell division. Currently, there is debate about the role of specialized HPC in proliferation and differentiation into the hepatocytes in hepatic lobules, or every hepatocyte has the capability of repopulating the liver according to the local microenvironment.[9] More recently, the pericentral diploid hepatocytes produced by endothelial cells are recognized, which have extensive proliferative capacity yielding mature hepatocytes in response to Wnt signals.[5] However, a follow-up study has found that Axin2+ hepatocytes contribute little to a normal hepatocyte turnover and are limited to pericentral

hepatocytes.[10] Wnt-responsive hepatocytes expressing Lgr4 or Lgr5 have shown limited cell division resulting in limited hepatocyte turnover during homeostasis.[11] Lin et al have recognized the specific hepatocytes with high Telomerase reverse transcriptase (Tert[high]). These cells are seen scattered throughout the liver lobule without zonal dominance and are clonally capable of repopulating the liver for around a year.[12] This study poses a different perspective that multiple populations of regenerating hepatocytes could maintain liver homeostasis without any distinct zonal dominance. However, there is still debate whether there is a differential proliferation of hepatocytes subsets during homeostasis. Some evidence suggests that while Major Facilitator Superfamily Domain containing 2A [Mfsd2a]— expressing periportal hepatocytes are markedly decrease in number during homeostasis,[13] whereas Lgr5+ expressing pericentral hepatocytes persist for long term.[11]

REGENERATION IN NORMAL LIVER

Liver regeneration is primarily accomplished through the self-replication of hepatocytes or cholangiocytes. The liver can quickly regenerate back to its former size after partial hepatic resection. The mechanism of hepatocyte regeneration varies depending on the degree of liver resection. Hepatocytes are hypertrophied when 30 percent of the liver is resected, and fast division of hepatocytes occurs to replace the hepatocyte mass when 50–70 percent of the liver is resected.[14] It is unclear what regulates the hepatocytes for hypertrophy or rapid proliferation depending on different degrees of liver injury. Other cell sources such as

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Foxl1^{+},[15] MIC1-1C3[+],[16] or CK19[+][17] cells have been shown to yield hepatocytes during a liver injury, but their exact role is still debatable. Some studies have reported transdifferentiation of CK19+ biliary epithelial cells into new hepatocytes and vice-versa.[15,17] Few studies have shown liver regeneration to be primarily carried out by hepatocytes as opposed to other cell types.[18–20] Hepatocyte damage in one zone causes a compensatory proliferative response in noninjured hepatocytes in other zones that attribute to the remarkable regenerating potential of hepatocytes in response to injury. In support of this, Pu et al had shown that when the pericentral hepatocytes are injured by CCl4, Mfsd2a+ periportal hepatocytes develop and extend in the liver.[13] Periportal hepatocytes expressing Sox9 and/or Hepatocyte Nuclear Factor 4 Alpha [Hnf4a] may multiply and replace hepatocytes when pericentral hepatocytes are chronically damaged.[13] Conversely, damage to periportal hepatocytes stimulates compensatory proliferation of pericentral and mid-lobular hepatocytes.[20]

Kupffer cells are the primary hepatic macrophages in the liver that detect damage. Hepatocytes remain in a quiescent state (G0 phase) under physiological conditions, and they respond to TGF generated by LSEC by inhibiting their proliferation. KC gets activated in response to liver injury, which causes HSC and LSEC to become activated as well. Activated KC also produces IL-6 and TNF, which drive G0 hepatocytes to undergo a G0/G1 transition, making them more susceptible to future mutagenic signals.[21,22] Angiopoietin 2-mediated TGF synthesis by LSEC decreases in the early stages, releasing the brake on primed hepatocytes. Further, activated HSC generates stromal derived factor 1 (SDF1), hepatocyte growth factor (HGF), Hedgehog (Hh), and Notch ligands.[23] Hh and Notch ligands aid in the proliferation of cholangiocytes. LSEC having SDF1 receptors CXCR4 and CXCR7 activated produce the hepatocyte mitogens HGF and Wnt2 through ID1. Primed hepatocytes begin to rapidly multiply in response to HGF and Wnt2. Moreover, they also produce growth factors such as PDGF, VEGF, FGF1, FGF2, and SCF that aid in the regeneration of nonparenchymal cells.[23] Hepatocytes proliferation is followed by KC and cholangiocytes proliferation. LSEC regains Ang2 expression later in the process.

IMPAIRED HEPATOCYTE REGENERATION IN CHRONIC LIVER INJURY AND CIRRHOSIS

Unlike after partial hepatectomy and acute liver injury where liver architecture is intact, in cirrhosis, there are marked changes in liver architecture with fibrosis. There is an increase in the fraction of senescent hepatocytes (with cell arrest at G1/S transition) and telomere shortening.[24] In the rodent model, chronic ethanol exposure with partial hepatectomy significantly impair hepatocyte replication.[25] Similarly, patients with alcoholic hepatitis showed a marked decrease in Ki67+ hepatocytes and an increase in HPC expansion,[26] which also correlate with treatment nonresponse.[27] AH patients also showed a significant reduction in cytokines and growth factors associated with liver regeneration[26] and had upregulation of cell cycle inhibition. Even in NAFLD, triglycerides in hepatocytes are linked to defects in liver volumetry, suggesting regeneration impairment.[28] Underlying cause of poor hepatocyte proliferation or replicative senescence of hepatocyte in cirrhosis is not clearly defined. Exacerbation of cytokine production,[29] deficiency in the EGFR pathway,[30] and oxidative stress[30–32] also contribute to poor hepatocyte proliferation in NAFLD-related cirrhosis.

In normal hepatocytes, mitochondrial oxidative phosphorylation is the primary source of energy. With a progressive decline in mitochondrial function in cirrhosis, there is a transformation in the energy source in cirrhotic hepatocytes from oxidative phosphorylation to glycolysis.[33] In advanced cirrhosis, downregulation of HNF4α (regulates the expression of glucokinase) leads to failure in maintaining glycolysis.[33]

HEPATIC REGENERATION IN CHRONIC LIVER DISEASE

Gut-derived endotoxins (Pathogen-associated molecular patterns, PAMPs) and DAMPs (Damage-associated molecular patterns) from direct hepatocyte injury activates KC to release IL 6 and TNF. But due to enhanced HSC mediated fibrosis that inhibits hepatocyte self-replication, the regenerative effects of TNF and IL-6 are disrupted in cirrhosis.[34] Unlike patients with acute liver injury, hepatocyte self-replication (increased hepatocyte Ki67 expression) is limited. In mild Chronic Injury, hybrid periportal hepatocytes (HybHP) proliferate to regenerate the liver. By contrast, in advanced cirrhosis, both hepatocyte and HybHP are senescent, and there is a ductular proliferation in an attempt to restore liver mass.

A typical ductular reaction appears in the periportal region and is made up of HPCs, inflammatory cells, endothelial cells, and mesenchymal cells.[35] HPCs are diverse, with cells having biliary or hepatoblast or stem cell markers.[36] In vitro, these cells differentiate into hepatocytes and biliary cells and form hepatocyte buds.[37] However, not all studies have confirmed the role of HPCs in regeneration in human liver cirrhosis.[38,39] Type and extent of liver injury decide the fate of HPCs. The differentiation into intermediate hepatocytes suggests that HPCs are committed to hepatocyte lineage. This is mediated by Wnt, Notch, and fibroblast growth factor pathways.[40,41] During cell death, activated macrophages promotes hepatocyte differentiation through the Wnt pathway and by inhibiting the Notch pathway.[40] Hedgehog ligands also recruit macrophages that modulate HPC differentiation into hepatocytes.[41] The tumor necrosis factor ligand superfamily member 12

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(TNFSF12 or TWEAK) and TNFRSF12A pathway stimulated by T cells and KC is also involved in the commitment of HPCs to hepatocyte lineage in cirrhosis[42,43] (Figure 2). Myofibroblasts mediated Notch ligand expression in response to chronic biliary injury induces the HPCs to differentiate into cholangiocytes. Hepatic nonparenchymal cells such as activated HSC might also actively participate in repopulating the liver.[44] Activation of HPCs is also linked with an excessive fibrogenic response in liver cirrhosis.[37,45,46] Studies in NASH patients have shown a positive correlation between fibrosis stage and ductular reaction.[47]

Spontaneous recruitment of BMSC in response to liver injury in cirrhosis is limited. Exogenous G-CSF supplementation promotes the recruitment of BMSC and MSCs in the diseased liver and potentiates the regenerative response. A repeated injury also perturbs the endothelial regenerative angiocrine support. BMSC stimulates LSECs for tube formation and angiogenesis.[49,50] G-CSF also mobilizes functional neutrophils to the liver. All these processes augment regeneration. On the other hand, by expressing monocyte chemoattractant protein 1 and platelet-derived growth factor, HPCs attract activated HSCs and promote fibrosis.

In alcohol-associated hepatitis, HPCs are not capable of producing mature hepatocytes, and expansion of HPC positively correlated with liver disease severity and short-term mortality.[51,52] These findings support the notion that HPCs have a lesser repopulating capability than hepatocytes. We have earlier demonstrated that despite very high HPC expansion in ACLF patients, they do not contribute to the patient's outcome, and only hepatocyte replication is associated with spontaneous recovery.[53] Recently, Hyun et al[54] have shown that Inflammatory cytokines generated by heavy ethanol ingestion inhibits epithelial splicing regulatory protein 2 (ESRP2) and thereby limit the transition of adult hepatocyte to progenitor-like cells. This suggests that increased HPC response in cirrhosis is not due to activation and conversion of hepatic stem cells to hepatocytes; rather, it is dedifferentiation of the remaining hepatocytes toward the progenitor cells in response to a change in the inflammatory environment of the liver that further aggravates the inflammation and fibrosis.

BONE MARROW STEM CELL NICHE IN ADVANCED CIRRHOSIS

Intact BM is critical for hepatic regeneration in cirrhosis. HSCs increase in the early stages of cirrhosis and decrease with the severity of cirrhosis, regardless of cause.[55] The periarteriolar niche made up of Nestin MSCs, sympathetic nerves and related Schwann cells keeps HSCs in a state of quiescence. But in cirrhosis, Nestin MSCs are lost due to the degeneration of the niche.[55] Moreover, the growth
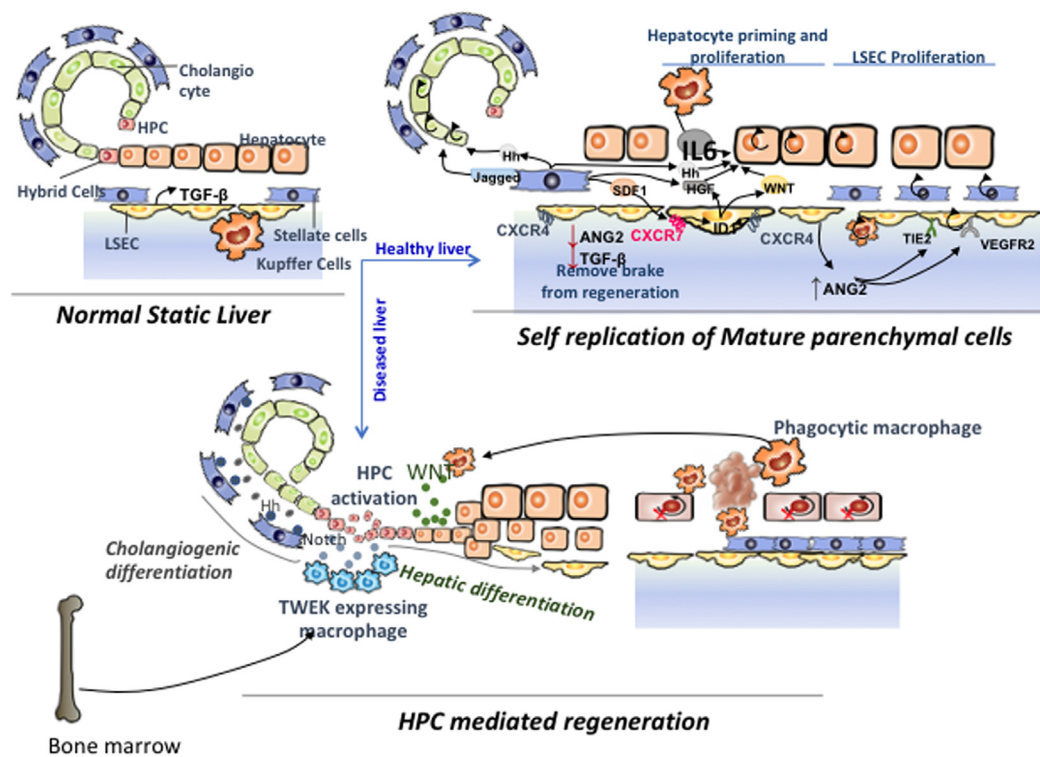

Figure 2 Cellular and molecular mechanisms of hepatic regeneration in chronic liver disease.

factors required for HSCs differentiation in the niche are also reduced. Bone marrow nerve injury is known to impair hematopoietic regeneration.[56] Alcohol-associated cirrhosis may have reduced bone marrow hematopoiesis secondary to bone marrow suppression secondary to prolonged and excess alcohol use.[57] Persistent accumulation of proinammatory cytokines (TNF-a, IFN-Y, and IL-1b) and oxidative stress lead to a decline in BM-HSC pool[55] (Figure 3).

THERAPY FOR HEPATIC REGENERATION IN CIRRHOSIS

In cirrhosis, massive deposition of extracellular matrix and tissue scarring and consequent cellular or functional loss of regenerative niche, architectural distortions, vascular reorganization, persistent inflammatory response, and depletion of parenchymal cells results in failure of engraftment and regeneration of transplanted cells. Multiple researches over the last two decades have enabled researchers to understand the functionality of different sources for use in clinical practice.

METHODS OF REGENERATIVE THERAPY (TABLE 1)

Hepatocyte Replacement

This can be achieved by two predominant methods, *one* to completely transplant liver in the setting of decompensated cirrhosis and *second* cell therapy with an aim to replace senescent hepatocytes with healthy hepatocytes or its progenitor as a definitive treatment or a bridge to liver transplantation.

Orthotropic Liver Transplantation (OLT)

Liver transplant is a well-established treatment modality in advanced cirrhosis, and with improved surgical technique and organ preservation methods over time, the rate of graft failure and mortality has substantially decreased. The main issues are the limited availability of suitable organs and the high procedure cost. With respect to OLT, cell therapy is still in the experimental stage.

Hepatocyte Cell Transplantation

Hepatocytes have unique proliferative and regenerative potential. Hepatocyte cell transplantation has been studied in UGT1 enzyme deficiency (Crigler-Najjar syndrome) and low-density lipoprotein receptor deficiency (familial hypercholesterolemia). However, studies in cirrhosis are limited to small case series with variable results.[58–62]

The use of hepatocyte transplantation in practice has limitations (1) *lack of suitable hepatocytes* due to organ shortages, poor cell survival, and difficulties in isolation, characterization, and failure of long-term cryopreservation of cells.[63] The cell numbers decrease after thawing, and the freezing process can cause loss of metabolic function and downregulation of adhesion proteins (integrin-b1 and E-cadherin).[64] Optimizing cryopreservation and thawing techniques and the use of apoptosis inhibitors[65] and N-acetylcysteine[66,67] can improve cell quality and viability. (2) Difficulties in *delivery or transfusion of the isolated hepatocytes* into the liver sinusoids. The cells can be injected either through the portal vein, peripheral vein, intrasplenic or intraperitoneal route. Multiple risks are involved in cirrhosis in view of coagulopathy, portal hypertension mediated shear forces causing transplanted
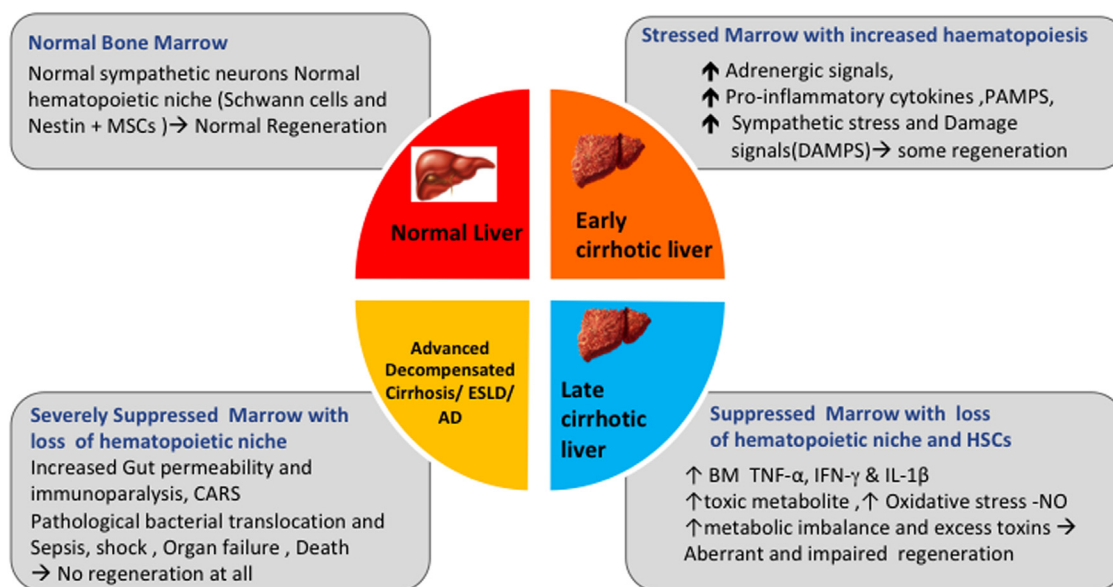

Figure 3 Bone marrow niche in relation to hepatic regeneration and severity of chronic liver disease.

Table 1 Summary of Clinical Trials on Cell Therapy in Patients With Liver Cirrhosis.

Authors and journal	N	Type of study	Indication	Type of Cell therapy	Dose and route	Outcome
Hepatocyte cell transplantation						
Mito M et al, Cell Transplantation.[126] 1993	10	Observational, Hepatocyte transplantation in man.	Liver cirrhosis	Hepatocytes	Intraportal	Upto 11 month survival in one patient
Skvorak et al.[127] Mol Ther. 2009	Mice study	Open level experimental	Maple Syrup Urine Disease	Hepatocytes	Direct into liver	
Kobayashi et al. Cell Transplant. 2000	Mice study	Experimental open level	Chronic Liver Failure	Hepatocytes	Spleen	
Trials of unsorted Bone Marrow–Derived Mononuclear Cell Transplant in Liver Disease						
Saito et al, Stem Cell Dev,[128] 2011	5:Treatment 5:Controls	RCT	Alcoholic Cirrhosis	BM-MNC	Single dose, peripheral vein	Improved CTP scores and INR, higher serum albumin, and total protein
Lyra et al, Eur Jou of Gastr Hepatol,[77] 2010	15: Treatment 15: Controls	RCT	Decompensated cirrhosis on waiting list for LT	BM-MNC	Single dose, hepatic artery	Improved serum albumin and CP score up to 90days
Spahr et al,[129] PLoS One 2013	28: Treatment 30:Controls	RCT	Decompensated cirrhosis, mean MELD score-19	BM-MNC + GC-CSF	Single dose, hepatic artery	No significant differences between study groups
Trials of Sorted Hematopoietic Stem Cell Transplant in Liver Disease						
Gordan et al,[81] Stem Cell 2006	5	Phase 1 open Uncontrolled trial	Decompensated cirrhosis (Ethanol-4, HCV-1)	CD34+	Single dose, portal vein or hepatic artery	Serum albumin and T Bil improved
Spahr L et al,[130] Hepatology. 2008	11: control 13: treated	RCT	Alcoholic cirrhosis	CD 34+	10 μg/kg/day Subcutaneous G-CSF for 5 days.	Effective CD34+ cells mobilization; increased Hepatocyte Growth Factors
Levicar et al,[82] Cell Prolif 2008	5	Uncontrolled trial	Cirrhosis	CD34+	Single dose, hepatic artery	Improved T Bil and CP up to 12 months, no short- and long-term side effects
Trials of G-CSF–Mobilized Hematopoietic Stem Cell Transplant in Patients with Liver Disease						
Han Y et al. Cyto-therapy,[131] 2008	20: control 20: treated	Phase 2 open RCT	Decompensated cirrhosis	PBMCs from G- CSF mobilized PB	Single dose, hepatic artery Vs. peripheral vein for 4 days for HSC mobilization	GC-SF plus PBMNC group had better liver test results up to 6 month follow up, no major adverse effects
Shasthry SM et al,[86] Hepatology. 2019	14:Treatment 14:Placebo	RCT	Steroid Non responsive Severe Alcoholic Hepatitis	G-CSF	Multiple doses Subcutaneous	Decrease in MELD, and Maddrey's discriminant function, Infections and decreased 90-day mortality in the G-CSF arm
Kedarisetty CK et al.[88] Gastro 2015	29:Treatment 26:Placebo	Double blinded RCT	Decompensated cirrhosis	G-CSF+ Darbopoietin α	Multiple doses, Subcutaneous 4 weeks	Improved CTP, MELD and survival at 12 month. Decreased sepsis

Table 1 (*Continued*)

Authors and journal	N	Type of study	Indication	Type of Cell therapy	Dose and route	Outcome
Newsome PN et al.[90] Lancet Gastroenterol Hepatol. 2018	27: standard care 26: G-CSF 28: G-CSF plus stem-cell infusion	Multicentre, open-label, randomized, controlled phase 2 trial	Compensated liver cirrhosis and MELD scores of 11–15·5	G-CSF alone or G-CSF plus stem-cell infusion	G-CSF (lenograstim) at 15 μg/kg bodyweight daily for 5 consecutive days.	No significant changes in MELD score More ascites and encephalopathy in G-CSF group.
Philips CA.[91] J Clin Exp Hepatol. 2019	56: GCSF, per-protocol analysis 24: Matched historical controls	Retrospective study	Decompensated cirrhosis	G-CSF (5 μg/kg daily 5 days and every 3rd day thereafter until day 26)	Multiple doses Peripheral vein	Compared to a matched HC group, patients receiving GCSF had higher mortality (75% vs 46%, $P = 0.04$) at one year.
De A.[89] Clin Gastroenterol Hepatol. 2020	50: standard care 50: G-CSF	Open-label trial	Decompensated cirrhosis	5 days of G-CSF every 3 months	Multiple doses Peripheral vein	GCSF- Significantly more CD34+ cells on day 6 than on day 0 ($P < 0.001$) Significant reductions in Child-Turcotte-Pugh and model for end-stage liver disease scores, increased ascites control, fewer infections and hospitalizations, lower liver stiffness measurements, and increased quality of life

Trials of G-CSF–Mobilized Hematopoietic Stem Cell Transplant in Patients with ACLF

Authors and journal	N	Type of study	Indication	Type of Cell therapy	Dose and route	Outcome
Garg et al.[118] Gastro 2012	23: Treatment 24: Placebo	Double blinded RCT	ACLF (APASL)	G-CSF	Multiple doses Peripheral vein	Improved MELD score, better patient survival Less sepsis, HRS and HE
Duan XZ[87] et al. WJG 2013	27: Treatment 28: Placebo	RCT	HBV related ACLF	G-CSF	Multiple doses Peripheral vein	Increased CD34 (+) cell mobilization, improved the liver function and survival rate.
Singh V[119] et al Am Jour Gastroenterol 2014	23: Treatment 23: Placebo	Open RCT	Severe Alcoholic hepatitis	G-CSF	Multiple doses Peripheral vein	Increased CD34 (+) cell mobilization, CTP, MELD, mDF score, and survival rate.
Engelmann C et al.[92] J Hepatol 2021	88: Treatment 88: SMT	Open-label, Plase 2 RCT	ACLF defined by EASL-CLIF criteria	G-CSF (5 μg/kg daily 5 days and every 3rd day thereafter until day 26)	Multiple doses Peripheral vein	No improvement in overall and transplant free 90 and 360 day survival. No prevention of infection.

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Table 1 (*Continued*)

Authors and journal	N	Type of study	Indication	Type of Cell therapy	Dose and route	Outcome
Trials of Mesenchymal Stem Cell Transplant in Liver Disease						
Peng et al,[97] Hepatology 2011	53: Treatment 105: Controls	Phase 2, open, RCT	HBV Related Cirrhosis-Decompensated	BM-MSC	Single dose infusion, hepatic artery	No mortality benefit. Decreased Bilirubin, improved INR and MELD score. No complications
Amin MA et al, Clinical Transplantation.[120] 2013	20	Open level, Uncontrolled trial, for safety	Post HCV child C liver cirrhosis	bone marrow derived mesenchymal stem cells	Intrasplenic injection	Decreased Bilirubin, AST, ALT, PT; improved Albumin, and INR
Mohamadnejad et al.[96] Liv Int 2013	15:Treatment 12: Placebo	RCT	Decompensated cirrhosis MELD >15	BM-MSC	Single dose, peripheral vein	No differences between the groups
Liang J et al, International Journal of Rheumatic Diseases. 2017	26	Open level, uncontrolled	Cirrhosis related to Autoimmune liver diseases	Allogeneic MSCs	Peripheral vein	improved MELD and liver function, without any side effect
El-Ansary et al.[121] Stem cell rev 2012	15:Treatment, and 10:Controls	Phase 2, open, Uncontrolled trial	HCV-related cirrhosis and MELD score >12	BM-MSC	Single dose, Peripheral vein	Decreased Bilirubin, improved INR, albumin, and MELD score
Trials of Mesenchymal Stem Cell Transplant in Patients with ACLF						
Shi M et al. Stem Cells Transl Med,[122] 2012	24:Treatment 19: Placebo	open-labeled and controlled	HBV related ACLF	UC-MSC	three times at 4-week intervals, Peripheral vein	Increase 90 day survival, reduced the MELD scores; increased serum albumin, and platelet counts
Li YH et al. Stem Cell Rev Rep,[123] 2016	11:PE + MSC 34:Only PE	Prospective study, open-labeled	HBV related ACLF	UC-MSC	Single doses, Peripheral vein	Improves the hepatic function and survival
Lin BL et al[124] Hepatology 2017	56:Treatment 54:Placebo	open-label, RCT	HBV related ACLF	Allogeneic BM-MSC	weekly for 4 weeks, Peripheral vein	Improved survival and liver function tests, Decrease incidence of Sepsis and multiorgan failure
Macrophage therapy						
Thomas JA et al,[104] Hepatology. 2011	Experiential mice study	Mice study	Liver Cirrhosis	Macrophages	Single dose treatment	Macrophage therapy for murine liver fibrosis recruits host effector cells improving fibrosis, regeneration, and function.
Bird TG et al, Proc Natl Acad Sci U S A.[125] 2013	Experimental mice study	Mice study	Liver cirrhosis	Macrophage	Single dose treatment, intravascular	Bone marrow injection stimulates hepatic ductular reactions in the absence of injury via macrophage-mediated TWEAK signaling.

cell destruction, and inadvertent arterial injections into the hepatic or splenic artery leading to embolic process.[68] (3) *Poor engraftment of transplanted cells* in the liver.

Hepatocyte Progenitor Cells (HPC)

HPCs have a high proliferative ability to differentiate into mature hepatocytes and cholangiocytes. Lgr5 in mice recognizes cells that have an HPC trait. These Lgr5+ cells may be developed into high-clonogenic-capacity organoids.[69] EpCAM+/NCAM+ progenitor cells in the fetal liver can expand and differentiate into hepatocytes.[70] Study of human fetal HPC transplant into patients with cirrhosis has shown clinical improvement.[71] In cirrhosis, intrasplenic injection of fetal hepatocytes has shown to improve MELD score[72]. In the future, well-designed and adequately powered studies to demonstrate safety and efficacy while overcoming technical issues are needed.

Regenerative Niche Correction

Stem cells can be totipotent, pluripotent, multipotent, or unipotent. Stem cells are ideal for liver regeneration in cirrhosis due to their ability to divide, proliferate and differentiate into other cell types. Stem cells also provide a favorable milieu for hepatocytes cell growth. Main methodologies for stem cell-based therapies include (1) direct injection of cells, (2) in vitro differentiation to hepatocyte-like cells, and then transplantation, or (3) *Ex vivo* mobilization of stem cells into the regenerative niche.

Bone Marrow Stem Cell Therapy

Bone marrow (BM) is a common source of three different pluripotent cell types; hematopoietic stem cells (HSCs), mesenchymal stromal cells (MSCs), and endothelial progenitor cells (EPCs). In clinical trials, autologous BMSC transplantation had shown improved quality of life without complications.[73] BMSC role in liver regeneration has been studied in a number of studies in recent years. In comparison to hepatocytes, HSCs and MSCs can be collected from the BM of living donors, lowering the chance of graft rejection.[74] In recent years, the role of BMSC in liver regeneration has been explored in various trials. BM also contains macrophages that produce matrix metalloprotease (MMP) that are antifibrotic.

Unsorted BM-derived mononuclear cell (BM-MNC) transplant: Several trials and small studies have shown that autologous BM-MNCs transplantation is both safe and effective.[75,76] A pilot study by Lyra et al showed that infusion of autologous BM-MNC through hepatic artery causes liver function improvement in patients with cirrhosis.[77] A recent meta-analysis of 15 studies has shown the effectiveness of autologous BMSC therapy for liver improvement and coagulation in patients with liver cirrhosis. The therapeutic effect was generated at 2–4 weeks after transplantation. The effect lasted for 24 weeks but no more than 48 weeks. The greatest benefit to patients was observed with a 4×10^8 autologous BMSC transplant via the hepatic artery.[78]

Sorted hemopoietic stem cell transplant: CD34 is a cellular marker of HSCs. CD34+ HSC promotes repopulation of cells by fusion with hepatocytes forming hybrid cells, which helps in liver regeneration. In mouse models, purified BMSC infusion has shown improved regeneration in cirrhosis with a reduction in liver fibrosis.[79] Small case series have shown improvement in liver functions with HSC therapy, whereas larger randomized controlled trials showed mixed benefits.[80] A study by Gordon et al has shown improvement in serum bilirubin and albumin after CD 34+ cells injection via a portal vein or hepatic artery and with no complications.[81] Levicar et al in a similar study showed that the effect lasts for about 12 months.[82]

G-CSF mobilized HSC therapy: G-CSF stimulates the BM to release neutrophils and CD34+ HSC into the circulation. CD133+ cells are a subset of CD34+ cells that can differentiate more easily.[83] G-CSF acts as a chemoattractant and a mitogen for oval cells in vitro. G-CSF therapy results in a significant increase in oval cell reaction and liver repair.[84] However, G-CSF may sometimes activate molecular pathways that can be associated with fibrosis progression.[83]

G-CSF is mainly studied for treatment for severe alcoholic hepatitis (SAH) and ACLF. A recent meta-analysis by Marot et al showed that as compared to controls, G-CSF treatment is related with a 70% reduction in mortality after 3 months in SAH patients but shows a beneficial role only in Asian studies.[85] Shasthry et al used GCSF therapy in steroid nonresponsive SAH patients (12 doses of 300 mcg GCSF over 28 days) vs. placebo. There was no mortality benefit at day 28, whereas at day 90, there was a significant reduction in MELD, infection rates, and lower mortality with GCSF therapy.[86] A randomized study in hepatitis B-related ACLF patients have shown that G-CSF therapy improved liver function and survival.[87] In a randomized trial by Kedarisetty CK and colleagues, combination of G-CSF and darbopoietinα in decompensated cirrhosis was associated with survival benefit with decreased sepsis and reduction in liver severity scores as compared to placebo.[88] In another randomized trial by Arke De et al, administration of multiple cycles of G-CSF increases the numbers of hematopoietic stem cells and survival of patients with decompensated cirrhosis.[89]

Not all studies on G-CSF in cirrhosis have shown promising results. In a multicentre, open-label, randomized, controlled trial by Newsome and colleagues in patients with compensated liver cirrhosis and MELD scores of $11 \cdot 0$–$15 \cdot 5$[91]. Treatment with subcutaneous G-CSF (lenograstim) 15 μg/kg for 5 days, or treatment with G-CSF for 5 days followed by leukapheresis and intravenous infusion of three doses of CD133-positive hemopoietic stem cells

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(0·2 × 10⁶ cells per kg per infusion) did not improve liver dysfunction or fibrosis and was associated with increased frequency of adverse events such as ascites. In fact, survival was shorter than what was expected in the natural history of the disease after G-CSF use in patients with decompensated cirrhosis.[91] The study by Engelmann et al (GRAFT study) failed to show a significant beneficial effect of G-CSF in treating patients with acute-on-chronic liver failure.[92] The use of G-CSF neither improved 3- and 12-month transplant-free survival nor lead to improvement in MELD score or reduction in the incidence of new infections. This was independent of the nature of the precipitating event, the severity of ACLF, or the type of organ failure.

Mesenchymal stem cell therapy: MSCs are multipotent fibroblast-like cells that are mostly generated from the bone marrow but can also be obtained from the umbilical cord and adipose tissue. Phase I/II studies on MSCs based therapies in liver cirrhosis had shown promising results. MSCs reduce inflammation, fibrosis and increase liver regeneration response better and rapidly than HSCs.[93,94] MSCs increase MMP expression and phagocytosis, promoting the regenerative process.[95]

An RCT of peripheral administration of autologous MSCs showed minimal benefit in cirrhosis.[96] The subsequent RCT in cirrhotics patients using HSC given via portal vein followed by peripheral BM-MSCs infusion 1 week later showed improvement in liver functions.[97] In another study, MSCs given through the hepatic artery (two infusions of 50 million BM-MSCs) in alcoholic cirrhosis showed improvements in CTP score without any benefit in MELD score.[98] At present, large human studies on MSC are hindered by ethical and safety concerns, lack of molecular data, and immunological mismatch.

A recent pooled analysis, including 39 studies, have concluded that MSC-based therapy is relatively safe and improves liver function during the first 6 months after administration.[99] A single injection administration via the hepatic artery and MSCs derived from bone marrow are optimal in terms of improving liver function. However, the long-term efficacy of MSC therapy remains unknown.

Endothelial progenitor cell (EPC) therapy: EPCs could repair endothelial injury of hepatic sinusoids, reduce fibrosis and stimulate liver regeneration.[100] EPC also has immunomodulatory effects for better homing and expansion to injured organs. EPCs were shown to be antifibrotic and capable of inducing liver regeneration in rat models of liver fibrosis by Nakamura et al.[101] Kaur and colleagues showed increased levels of EPC in cirrhosis, and these cells stimulated angiogenesis in vitro.[50]

Macrophage Therapy

Monocyte-derived macrophages have a dual role. They recruit immune cells to the injury site and activate HSC, which promotes liver fibrosis.[102] They also initiate progenitor-mediated liver regeneration and hepatocytes differentiation. Despite significant chemotactic and paracrine actions, repeated injected macrophages are required as they last in the liver for a brief time. In cirrhosis, macrophages trigger the ductular response via Tweak/FN14 signaling.[103] In liver disease, BM-derived macrophages are given via venous or intrasplenic injections. Thomas et al showed reduced liver fibrosis after 4 weeks intraportal infusion of BM-derived macrophage on murine model.[104]

Embryonal Stem Cell (ESC) Therapy

Differentiation of cultured ESC toward hepatocyte-like cells involves the administration of several growth factors and cytokines in a sequential manner (fibroblast growth factor 2/4, bone morphogenetic protein 2/4, and activin A).[105] As hepatocyte isolation is difficult, Asialoglycoprotein receptor ASGPR1 (hepatocyte-specific cell surface marker) expression-based sorting is used to yield hepatocytes.[106] Despite the promising studies, there are ample ethical issues for the use of human ESCs in practice.[107]

iPSC Therapy (Induced Pluripotent Stem Cells (iPSCs))

iPSCs are induced pluripotent cells that are reprogrammed from adult cells using pluripotency factors (Activin A and Wnt3a) and maturation factors (hepatocyte growth factor and oncostatin-M) to form hepatocyte-like cells.[108] Unlike ESC, they do not require embryonic material, and since they are autologous, they need no immunosuppression. Nowadays, for the production of functionally efficient iPSCs, excisable viral transfection, microRNA transfection, and mRNA transfection techniques are being used.[109,110]

Liver Support Devices and Their Role in Improving Liver Regeneration

Extracorporeal liver support devices are primarily aimed at detoxification of the liver and thereby promote the microenvironment to facilitate regeneration.[111] They can be classified as (1) artificial liver support systems e.g. molecular adsorbent recirculating system (MARS), single-pass albumin dialysis (SPAD), and fractionated plasma separation and adsorption system (FPSA, Prometheus). (2) bioartificial liver support system e.g. Extracorporeal liver assist device (ELAS), Bioartificial liver assist system (BLSS), and Radial flow bioreactor (RFB). (3) Hybrid system e.g. Hybrid artificial liver assist system (TECA) and Molecular extracorporeal liver support system (MELS).[111,112]

Liver assist devices might be useful in a subgroup of patients with cirrhosis presenting with acute decompensation and ACLF, either as a bridge to transplant or regeneration.[113,114] Albumin dialysis helps by improvement in the cardiovascular system (by increasing systemic vascular resistance index and mean arterial pressure), cerebral function (by decreasing hepatic encephalopathy and

intracranial pressure), renal function (by increase in urine output and decrease in creatinine), liver function (increase in indocyanine green plasma disappearance rate, and improves others parameter) and improves the quality of life (by decreasing pruritus).[115] Extremely high costs, complexity, and shortage of suitable large prospective trials have curtailed the routine use of such systems to date.

LIMITATIONS OF CELL-BASED THERAPIES

It is unknown how a short course of therapy can have mortality benefit at three months. The homing of CD34 cells, particularly to the liver, after a peripheral mobilization, is unexplained. Some studies have documented the adverse effects related to treatment, such as new-onset ascites and portal vein thrombosis after hepatic artery infusion.[116] None of the proposed regenerative therapies have long-term effects. The effect wanes over time.[117] It is difficult to identify leucocytosis due to immune paresis in ACLF when G-CSF is used.

FUTURE PERSPECTIVE

Regenerating capacity of the normal liver is well known and this had revolutionized the concept of living donor liver transplant, where donor liver and recipient liver both get regenerated, but the same requires expertise, motivation of the donor, and other legalities. In the presence of organ shortage, high cost, and the need for life-long immunosuppression after liver transplant, various newer approaches of regenerative medicine can be helpful in the regeneration of the native liver. In patients with cirrhosis, the variations in intracellular matrix composition, paracrine effects from nonparenchymal mesenchymal cells, cytokines, and growth factors produced by inflammatory cells and bone marrow-derived stem and progenitor cells are the key elements involved in the supporting role in regeneration. Several studies conducted and still ongoing efforts are in place, which can provide mechanisms and processes to reverse the fibrosis or cirrhosis process, but all in experimental phases. G-CSF therapy seems to be the more efficient in certain group of patients with liver diseases, particularly those without organ failure and bacterial infection, but it is still a double-edged sword and intact BM is critical for hepatic regeneration in cirrhosis. Hope in positivity is the key, with constant efforts going in place despite the conflicting results.

AUTHOR CONTRIBUTION

AJ – Writing manuscript, critical analysis; RKJ – Compilation of data; AK – Writing manuscript.

AUTHOR DISCLOSURES

None.

CREDIT AUTHORSHIP CONTRIBUTION STATEMENT

Ankur Jindal: Conceptualization, Methodology, Writing – original draft, editing; **Rakesh K. Jagdish:** Writing – original draft, Figures, Tables; **Anupam Kumar:** Writing – original draft, Figure.

CONFLICTS OF INTEREST

The authors have none to declare.

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