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BONE MARROW-DERIVED STROMAL CELL THERAPY IN CIRRHOSIS: CLINICAL EVIDENCE, CELLULAR MECHANISMS AND IMPLICATIONS FOR THE TREATMENT OF HEPATOCELLULAR CARCINOMA

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Abstract

Hepatocellular carcinoma (HCC) is the 5th most common malignancy worldwide and accounts for nearly 10% of cancer deaths annually [1]. In patients with well-preserved liver function and limited disease, surgical resection offers the best chance of cure. For the 80% of HCC patients with underlying cirrhosis, however, liver transplantation remains the only available curative treatment, and then only in Child-Pugh (C-P) class A or B patients [1]. Even in transplant-eligible patients, the shortage of available donor livers severely limits the number of HCC patients that ultimately undergo transplantation [2]. Thus, for the majority of HCC patients, which present at advanced stage or with significant liver dysfunction, non-surgical interventions remain the only treatment option [1].

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Keywords

Cirrhosis; Bone Marrow-Derived Stromal Cell Therapy; Hepatocellular Carcinoma; Radiation Induced Liver Disease

RADIATION THERAPY (RT) FOR HCC

Traditionally, the role of RT in hepatic malignancy had been limited by the presumed low radiation tolerance of the liver, after early studies demonstrated whole liver radiation in excess of 30-35 Gy in conventional fractionation to be associated with a high risk of potentially lethal liver injury [3-6], termed as radiation-induced liver disease (RILD) later (reviewed in [7,8]). Technical advances in the delivery of RT, particularly 3D-conformal RT (3D-CRT), intensity modulated RT (IMRT), and stereotactic body radiotherapy (SBRT), with respiratory gating and image guidance, have facilitated the safe use of radiation dose escalation in unresectable liver cancers [9-11]. Pioneering studies at the University of Michigan demonstrated that image-guided 3-D-CRT planning using nonaxial beams and normal tissue complication probability (NTCP) [12] and principal component analysis [13] modeling enables investigators to escalate RT doses in patients with unresectable hepatobiliary cancers, after exclusion of radiologically “normal” liver from the treatment field [14,15]. Radiation doses were escalated to parts of the liver to levels as high as 90 Gy (median 58.5 Gy, range 28.5-90 Gy), in combination with concurrent chemotherapy, to obtain local tumor control in patients with unresectable hepatobiliary cancers [15,16]. On multivariate analyses, escalated RT dose was independently associated with improved progression-free and overall survival [15]. More recently, SBRT has shown great promise for the treatment of HCC with 1-2 year local control of 70-90% [17-21] and dose identified as a significant predictor of survival [17,21]. RT has also been combined with transarterial chemoembolisation (TACE) to improve tumor response and survival in unresectable HCC [22,23]. Despite advances in RT treatment planning, RILD remains a chief concern [24,25] and the volume of tumor limits the dose of RT that can be safely administered to the liver. Thus, the RT dose is reduced with an increase in the volume of liver tumors, resulting in the failure of RT to control the liver tumors.

RADIATION INDUCED LIVER DISEASE (RILD)

Classic RILD is characterized by the development of worsening hepatomegaly and ascites 1-2 months after RT, usually in non-cirrhotic patients. In contrast to decompensated liver cirrhosis, liver function testing in RILD is typified by disproportionately elevated alkaline phosphatase with only moderate transaminitis and minimal hyperbilirubinemia [26]. The pathological hallmark of classic RILD is described as a veno-occlusive disease (VOD), predominantly involving the small central and sublobular hepatic veins with relative sparing of the portal, arterial, and larger venous vasculature [3]. Morphologically, VOD is characterized by occlusion of the central vein lumen by erythrocytes trapped in a dense meshwork of reticulin and collagen fibers, with atrophy of centrilobular liver plates and loss of acinar zone 3 hepatocytes typically observed [27,28]. However, in patients with underlying viral hepatitis and cirrhosis, hepatic radiation injury is characterized by a general

decline in liver function, elevation of liver enzymes, hyperbilirubinemia and reactivation of HBV, a syndrome termed as “non-classic RILD” .

Sinusoidal endothelial injury has been traditionally postulated as the initiating lesion of VOD in RILD with deposition of fibrin and collagen along the hepatic sinusoidal endothelium being implicated as an early step in the cascade that eventually leads to veno-occlusion [3]. Recently, the term sinusoidal obstructive syndrome (SOS) has been proposed as a better description of the pathology of liver injury seen after the administration of chemotherapy with or without RT. In addition to endothelial cell damage, hepatic stellate cell activation is noted in patients with severe congestive changes of classic RILD. Hepatic stellate cells have multiple functions, including participation in the regeneration of hepatocytes, secretion of lipoproteins, growth factors, and cytokines that play a key role in modulating inflammation and fibrosis. In the nonclassic RILD syndromes, hepatocellular loss and dysfunction along with hepatic sinusoidal endothelial death and stellate cell activation have also been noted. This pathology could be secondary to a radiation-induced mitotic catastrophe of regenerating hepatocytes in cirrhotic livers and the reactivation of hepatitis B virus in patients with chronic viral hepatitis.

Of the cytokines involved in RILD, transforming growth factor- β (TGF- β) has been implicated in the subendothelial and hepatic fibrosis in RILD [29,30]. Anscher et al described the radiation dose dependence of the level of TGF- β in the irradiated rat liver (23). Proinflammatory cytokines IL-1-beta, IL-6, and tumor necrosis factor alpha, as well as, numerous chemokines have also been implicated to play a role in the early phase development of RILD [31-34]. Irradiation of rat liver in vivo induced early and extensive upregulation of IL-1-beta, IL-6, TNF-alpha, and CCL2, CXCL1, CXCL8, and CXCR2 chemokines. The elevated cytokines and chemokines can recruit inflammatory cells such as neutrophil granulocytes [31], influence liver cells gene expression [32-34] and alter liver metabolism [32-34].

The management of RILD is generally only supportive, with mortality rates exceeding 75% reported [24,25]. Advanced liver cirrhosis is recognized as perhaps the most important risk factor for the development of RILD, and is associated with particularly high risk of death from RILD [24,25,35]. Furthermore, hepatic irradiation can induce reactivation of hepatitis B virus (HBV) in HBV carriers, further complicating the clinical manifestations of RILD [36]. The role of systemic chemotherapy is similarly limited by abnormal liver function in cirrhosis [37]. Novel therapies that ameliorate hepatic dysfunction could potentially improve the tolerability of both RT and chemotherapy in patients with cirrhosis-related HCC, allowing for more aggressive and effective treatment approaches.

HEPATOCTE TRANSPLANTATION (HT) FOR RILD AND METABOLIC LIVER DISEASES

HT has been proposed as an alternative to orthotopic liver transplantation for the treatment of metabolic and end-stage liver diseases [38]. Guha and colleagues examined the potential of HT in ameliorating RILD in F344 rats subjected to partial hepatectomy and whole liver RT. Intrasplenic or intraportal transplantation of adult primary hepatocytes, four days after

high-dose liver RT, ameliorated liver function and improved survival of these animals [39]. Importantly, the transplanted hepatocytes engrafted and extensively proliferated and selectively replaced the irradiated host hepatocytes to maintain normal physiological function of the heavily irradiated rat liver. While HT has been shown to be safe in humans, its applicability has remained limited by a number of critical barriers, including the limited availability of human hepatocytes and failure of donor hepatocytes to engraft and repopulate the host liver [40,41]. The studies of HT in RILD paved the way for investigators to develop a preparative regimen of liver irradiation for enhancing engraftment and selective repopulation of donor hepatocytes after HT [42-48]. Since then, HT after a preparative regimen of hepatic irradiation in combination with a hepatotropic growth stimuli, has been successfully used to ameliorate liver function in a variety of animal models of inherited metabolic diseases, such as, Crigler-Najjar syndrome [43,49] and primary hyperoxaluria [50,51]. Encouraged by these results, a clinical trial has been initiated in the University of Pittsburgh by Ira J. Fox for the treatment of phenylketonuria (NCT01465100) and other inherited liver-based metabolic diseases (NCT01345578). In contrast to metabolic liver diseases, HT has not been successful in the treatment of cirrhosis because the underlying portal hypertension poses a barrier to successful engraftment and repopulation of donor hepatocytes. As an alternative to HT, we review the potential role of bone marrow-derived stem cell (BMSC) therapy as a promising strategy to ameliorate liver function in cirrhosis and RILD.

BONE MARROW DERIVED STROMAL CELL (BMSC) THERAPY IN CIRRHOSIS

Three types of multipotent bone marrow (BM) derived cells have been shown to have significant therapeutic potential in liver cirrhosis: hematopoietic stem cells (HSCs), mostly myeloid progenitors, mesenchymal stem cells (MSCs), and endothelial progenitor cells (EPCs). HSCs are the predominant stem cell population within BM, express the cell surface antigen marker CD34, and are capable of both self-renewal and differentiation into the progenitor cells, or colony forming units, of the hematopoietic system [52,53]. MSCs are a rarer population of BMSCs capable of both self-renewal and differentiation into multipotent adult progenitor cells (MAPCs), which can give rise to hepatocytes as well as mesenchymal cell types including osteoblasts, adipocytes, chondrocytes, neurons, glial cells, and cardiomyocytes [54-65]. EPCs are immature endothelial cells found in both BM and the periphery, which can be mobilized to participate in neovascularization at sites of ischemic or damaged tissue, including myocardium, lung, hindlimb, and liver [66-74]. Due to their common expression of CD34, EPCs and HSCs are postulated to derive from a common hematopoietic-endothelial precursor, the “hemangioblast” [66,71,72,75-79]. Precise characterization of EPCs has proven challenging, complicated by evidence that EPCs can differentiate from a range of cell lineages and vary their surface marker expression by tissue of origin [66,75,80-86]. Nonetheless, circulating bone marrow derived CD34⁺ Flk-1⁺ CD133⁺ cells are widely thought to represent an “early” population of EPCs, which possess robust and clinically promising neoangiogenic capabilities [86-88]. The cell surface antigenic markers and commonly used isolation techniques for each cell population are detailed in Table 1.

PRECLINICAL STUDIES AND MECHANISMS OF BMSC REPAIR OF LIVER INJURY

Numerous pre-clinical studies have demonstrated the ability of HSCs, MSCs, and EPCs to improve hepatic function, reduce liver fibrosis, and contribute to hepatic regeneration in animal models of cirrhosis and liver injury [89-93]. Although confirmatory evidence in humans is currently lacking, a wide range of mechanisms by which each of these BMSC populations mediates amelioration of liver function have been proposed (Fig. 1 & Table 2)

Hematopoietic Stem Cells & Repair of Liver Injury – Transdifferentiation or Cell Fusion?

Hepatocytes derived from HSCs have been shown to contribute to regeneration of damaged liver, both *in vitro* and *in vivo* [94-97]. Mobilization of HSCs from bone marrow to areas of hepatic injury is a well-studied process mediated primarily by chemokine stromal cell-derived factor-1 (SDF-1) and CXCR4 receptor signaling, as well as HGF, interleukin-8 (IL-8) and matrix metalloproteinase-9 (MMP-9) [55,98]. The fate of HSCs once they reach the liver, and the subsequent mechanisms by which they exert their therapeutic effects, however, remain uncertain. Two competing theories have garnered considerable support in recent years: (a) generation of *de novo* hepatocytes by trans-differentiation, and (b) genetic reprogramming of resident hepatocytes by cell fusion [99].

Evidence in support of the cell fusion hypothesis comes from experiments on a mouse model of tyrosinemia-induced liver failure, in which lethally irradiated fumarylacetoacetate hydrolase deficient (*Fah*^{-/-}) mice who underwent transfusion of BM from *Fah*^{+/+} donor mice recovered normal liver function [100]. Examination of regenerative hepatic nodules 4-5 months after transfusion revealed low levels of donor wild type *Fah* alleles within host liver and concurrent expression of both donor and host genes within individual hepatocytes, suggesting fusion between hepatocytes and donor HSCs. In a similar study of BMT from female *Fah*^{+/+} mice into lethally irradiated *Fah*^{-/-} males, cytogenetic analysis of BM-derived hepatocytes isolated from host livers showed an abundance of (80,XXXXY) and (120, XXXXY) hepatocyte karyotypes, supporting cell fusion as a common event in the generation of *FAH*-expressing hepatocytes [100]. In contrast, hepatocytes lacking Y chromosomes were rarely detected, indicative of a rarity of transdifferentiation events. Further differentiated hematopoietic lineage cells have also been shown to be sufficient for cell fusion and the generation of metabolically functional hepatocytes. Transplantation of *Fah*^{+/+} myelomonocytic cells and BM-derived macrophages from lymphocyte depleted *Rag1*^{-/-} mice generated metabolically functional hepatocytes and cured *FAH* deficiency in non-irradiated recipient mice [101,102]. The ability of these more mature myeloid cell types to fuse with hepatocytes and restore hepatic function in the absence of host marrow engraftment by donor HSCs raises intriguing possibilities for these cells' potential in future regenerative liver therapies.

While these findings indeed appear to support cell fusion, rather than transdifferentiation, as a mechanism by which HSCs may ameliorate liver disease, the use of the tyrosinemia mouse model raises concerns about their broader applicability. Both *FAH*-deficient mice and humans with tyrosinemia have been reported to bear a number of cytogenetic abnormalities,

including aberrant karyokinesis and multinucleation [103,104]. Such reports raise the possibility that selective pressures in *Fah*^{-/-} recipient mice may promote cell fusion events that would otherwise be unlikely to occur, bringing into question the generalizability of these findings. Further evidence from animal models without inherent predispositions to cytogenetic instability may therefore be necessary to better establish the role of cell fusion in liver injury repair and hepatic regeneration after HSC transplantation.

To this end, several studies in non-FAH deficient mice have provided strong evidence both in support of transdifferentiation and against cell fusion. Jang demonstrated the multipotent plasticity of HSCs isolated from C57Bl6/NCR mice utilizing trans-well membrane barrier co-culture of purified HSCs with liver damaged by CCl₄ or regenerating after partial hepatectomy [94]. Co-cultured HSCs lost their hematopoietic surface markers, underwent fetal hepatocyte differentiation pathway changes, including transient expression of alpha-fetoprotein, GATA4, HNF4, and HNF4 β , and ultimately expressed markers of mature hepatocytes, including albumin, CK18, fibrinogen, and transferrin. Genetic analysis of HSC-derived hepatocytes revealed no transfer of genetic material from injured liver cells, suggesting that paracrine signaling, rather than direct cell-to-cell contact, was responsible for mediating HSC conversion into hepatocyte-like cells. Subsequent transplantation of HSCs from male C57Bl6/NCR donors into lethally irradiated CCl₄-injured females resulted in rapid repopulation of recipient livers with XY and YXY hepatocyte-like cells within 2 days, suggesting HSC transdifferentiation rather than fusion as the source of these novel hepatocytes. Hepatic synthetic function was significantly improved at 2 days and nearly normalized by 7 days, demonstrating the ability of HSC-derived hepatocytes to support hepatic function.

In perhaps the most convincing argument against cell fusion, Harris utilized a sophisticated Cre/lox recombinase reporter system to show that fusion events occurred only exceedingly rarely in lethally irradiated female Cre-recombinase mice who received BMT from male Z/EG Cre-reporter strain mice [105]. While this experiment does not necessarily provide evidence for trans-differentiation, it does confirm the absence of HSC-hepatocyte fusion. Of note, in contrast to the fusion studies in *Fah*^{-/-} mice, the transplanted mice in this experiment did not suffer from either metabolic or induced liver disease, and thus did not have a hepatic regenerative stimulus present to potentially act upon transplanted donor HSCs. This important distinction in study design suggests the possibility that hepatocyte loss and resultant hepatic regenerative stimuli may play a role in inducing cell fusion events, although this effect was not seen in the study by Jang above [94].

Regardless of whether cell fusion or transdifferentiation actually take place, HSC do not likely constitute a significant source of hepatocytes in the injured liver [106,107]. Rather, HSC may contribute to the repair of liver injury and regeneration by providing macrophages that produce collagenases [108] and phagocytose dead parenchymal cells [109]. During chronic liver injury, macrophages phagocytose cellular debris from damaged hepatocytes and begin to release Wnt3a [110]. Wnt3a then stimulates the nuclear translocation of β -catenin in hepatic progenitor cells, which promotes their differentiation towards the hepatocellular lineage [110]. Additionally, macrophages have been shown to facilitate liver regeneration through the secretion of IL-6 after partial hepatectomy [111]. This emerging

data favors the role of macrophages as the predominant mechanism by which HSCs contribute to hepatocellular regeneration, with transdifferentiation and cell fusion considered as infrequent events that contribute minimally to generation of hepatocytes under either physiological or pathological conditions.

Vasculogenic Potential of HSCs

In addition to their ability to contribute to hepatocellular repair in liver injury, HSCs also appear capable of stimulating neoangiogenesis. Due to their close temporal and physical proximity in the developing embryo, endothelial and hematopoietic cells have long been postulated to share a common progenitor cell, termed the “hemangioblast” [112]. This bipotential progenitor cell has since been narrowed to a population of c-kit⁺, Sca-1⁺, lineage⁻ HSCs (“KSL cells”) by the demonstration that GFP⁺ KSL cells stably integrated into host endothelial surfaces for up to 8 months following transplantation of KSL cells from male green fluorescent protein transgenic (GFP⁺) mice into lethally irradiated C57Bl/6 female recipients [75,78,113]. GFP⁺ endothelial cells (ECs) integrated throughout hepatic sinusoids, central veins, arterioles, and portal veins, and were also found in abundance in cardiac, muscle, pulmonary, and intestinal endothelium. Transplantation of a single GFP⁺ KSL cell into irradiated recipients resulted in both (a) GFP⁺ myeloid and lymphoid hematopoiesis and (b) engraftment of GFP⁺ ECs into host endothelium that remained detectable 6-8 weeks later, confirming the bipotential differentiation capacity of KSL cells [78]. Notably, DNA and karyotype analysis of GFP⁺ ECs from recipient animals confirmed that the vast majority of these cells arose from trans-differentiation of donor HSCs, rather than from cell fusion events. Bailey further demonstrated that more mature common myeloid progenitor (CMP) and granulocyte/macrophage progenitor (GMP) cells are also capable of differentiation into vascular endothelium [114]. The pluripotency of these late myeloid progenitor cells, which can be isolated by routine CD34⁺ hemapheresis, raises intriguing possibilities for the potential of BMSC therapy of liver disease, in which endothelial cell dysfunction is a central mechanism in the pathogenesis of portal hypertension and its sequelae [115].

Angiogenic, Antifibrotic, and Hepatic Regenerative Effects of EPC

EPCs are the immature endothelial cells, which arise from bipotential hemangioblast, and have been shown to participate in angiogenesis and repair of damaged hepatic microvasculature in regenerating liver. EPCs are involved in neovascularization of damaged or ischemic tissues throughout the body, including myocardium, hindlimb, cornea, corpus luteum, endometrium, and skin [67,71,73,74,116-118]. Taniguchi demonstrated the capacity of human EPCs to play a similar role in CCl₄ mediated liver injury in nude mice [69]. As early as 2 days after CCl₄ injection, transplanted human EPCs incorporated both at foci of necrotic centrilobular hepatocytes and into hepatic sinusoids, reflecting their ability to integrate into diverse specialized endothelial niches within the liver [69]. EPC-derived blood vessels surrounding areas of hepatocyte proliferation were seen by day 14, suggesting the importance of vascular support by transplanted EPCs in the repair of acute liver injury [69]. Most importantly, EPC transplantation prevented death from liver failure after CCl₄ injection, improving 7-day survival from 28.6% in control mice to 85.7% in transplanted mice [69].

BM-derived EPCs contribute significantly to liver regeneration by replacing LSECs and providing HGF for hepatocyte proliferation [119]. After partial hepatectomy (PH) in rats, EPCs proliferate in the BM, followed by mobilization to the circulation, engraftment in the liver and differentiation to fenestrated LSECs [119]. Within three days after PH, 25% of LSECs were BM derived and these were the highest secretors of HGF. Each of these steps is regulated by VEGF, which appears to play a major role in hepatic regeneration. Hepatic VEGF increases in response to many forms of liver injury [69,120-122]. Beaudry and colleagues found that systemic administration of VEGF accelerated hepatic regeneration after PH [70]. Using mice with GFP⁺ reconstituted BM, they showed that vascular endothelium within areas of regenerating liver contained over 46% CD31⁺GFP⁺ ECs in VEGF-treated mice, compared with only 12.7% in saline-treated mice and 3.8% in mice who underwent sham-surgery, supporting the role of VEGF in recruiting EPCs from BM to regenerating liver. Interestingly, VEGF did not increase EPC migration to lung, demonstrating the preferential effect of VEGF on neovascularization of regenerating organs. In addition to its role in EPC recruitment, VEGF also stimulates hepatocyte proliferation by inducing LSEC to secrete HGF [123]. Thus, EPCs might promote liver regeneration and amelioration of fibrosis through secretion of paracrine factors [69,92]. Nakamura studied the effects of EPC transplantation on CCl₄ and thioacetamide (TAA) induced liver cirrhosis [92]. In this study, rats received either 9 weeks of TAA or 10 weeks of CCl₄, with EPCs infused either once or weekly starting in week 6 with continued administration of TAA or CCl₄. Repeated EPC treatments significantly reduced both histological degrees of liver fibrosis and mRNA levels of fibrogenic proteins, including α 2-(I)-procollagen, fibronectin, transforming growth factor- β (TGF β), and α -smooth muscle actin (α -SMA), a marker of activated hepatic stellate cells. EPC transplantation increased MMP-2, -9, and -13 activity and decreased expression of tissue inhibitor of metalloproteinase-1 (TIMP-1), suggesting that EPCs were able to reverse the hepatic fibrogenic state by promoting collagenolysis and remodeling of the stromal microenvironment. EPC transplantation further stimulated hepatocyte proliferation and induced the expression of several hepatotrophic growth factors, including HGF, EGF, and VEGF, consistent with prior reports [70,124-127]. Most importantly, EPC transfusion produced dramatic clinical improvements in CCl₄-treated rats, improving rodent survival as well as serum bilirubin, albumin, and total protein levels.

Rafii and colleagues demonstrated that PH activated Inhibitor of DNA binding 1 (Id1)-dependent gene expression of tissue-specific angiocrine factors, such as Wnt2 and HGF and induced VEGF/VEGFR2 signaling in LSECs. [121] Furthermore, Id1^{-/-} mice displayed impaired liver regeneration after PH, suggesting an impaired mobilization and recruitment of BM EPCs [120,121]. These findings illustrate the dynamic interplay between regenerating liver and BM EPCs that is mediated by VEGF and HGF, and suggest a potential therapeutic role for VEGF and HGF to augment hepatic neovascularization and regeneration in liver cirrhosis. Further investigations showed that divergent LSEC-derived angiocrine signals stimulate CXCR7-mediated liver regeneration after acute injury while switching to CXCR4-mediated hepatic fibrosis during chronic liver injury [128]. Thus, selective activation of CXCR7 in LSEC with concomitant inhibition of CXCR4 might be a therapeutic strategy for abrogating fibrosis in cirrhotic patients. Taken together, the above studies suggest a promising therapeutic role for EPC therapy in the treatment of liver

cirrhosis, mediated through a range of mechanisms including neovascularization of damaged and regenerating liver, paracrine stimulation of hepatocyte proliferation, remodeling of extracellular matrix, and inhibition of stellate cell activation and fibrogenesis.

Therapeutic Potential of Mesenchymal Stem Cells in Liver Fibrosis

Transplantation of MSCs has also shown preclinical efficacy in ameliorating liver fibrosis, similar to the effects of MSCs in animal models of lung, heart, and kidney fibrosis [90,91,129-133]. A recent clinical trial of MSC transplantation in decompensated liver failure has additionally demonstrated the safety of and suggested therapeutic potential for MSC transplantation in patients with advanced liver cirrhosis [134]. A number of strategies through which MSCs may promote their effects in liver cirrhosis have been proposed. Perhaps the most intriguing of these is the capacity of MSCs to differentiate into functional hepatocytes [56,57,135-137]. MSCs are the source of multipotent adult progenitor cells (MAPCs), which can be induced to differentiate into a range of cell types, including hepatocytes [56,57,136,138,139]. Following transplantation into rodent models of CCl₄- and dimethylnitrosamine (DMN) liver injury, MSCs have been shown to not only engraft and differentiate into functional hepatocytes, but also inhibit proinflammatory and fibrogenic cytokine activity, promote collagen degradation, stimulate hepatocellular proliferation, improve clinical measures of liver function, and increase rodent survival [90,91,129,138-141]. Only low numbers of MSC-derived hepatocytes, however, have been identified in host liver after transplantation, suggesting that mechanisms other than hepatocyte repopulation are chiefly responsible for the therapeutic effects of MSC therapy [91,138-140]. The demonstration that infusion of MSC conditioned media (CM) decreased serum markers of hepatocellular death and improved animal survival in rats with D-galactosamine-induced fulminant hepatic failure suggests that paracrine signaling by MSC secretory products is one such mechanism [142]. Treatment with MSC-CM additionally increased hepatocyte proliferation, reduced hepatocyte apoptosis, and increased expression of several anti-inflammatory and anti-fibrotic cytokines compared with saline-treated controls. MSCs are known to produce a number of soluble factors with potential hepatotrophic effects, including HGF, VEGF, basic fibroblast growth factor, placental growth factor, monocyte chemoattractant protein-1, stem cell factor-1, SDF-1, Flt-3 ligand, G-CSF, GM-CSF, and numerous interleukins [143,144]. How each of these signaling molecules individually contributes to hepatic regeneration, however, remains to be further elucidated.

Modulation of Hepatic Stellate Cells and Matrix Metalloproteinase Activity by BMSCs

In addition to providing angiogenic support and stimulating hepatocyte proliferation, modulation of MMP/TIMP activity and induction of hepatic stellate cell apoptosis are important mechanisms by which BMSCs may mediate their anti-fibrotic effects in liver cirrhosis. Stellate cells are resident liver cells that in quiescence store vitamin A, but are activated by hepatic injury by proliferating into contractile and fibrogenic myofibroblasts, identified histologically by α -smooth muscle actin (α -SMA⁺) immunostaining [145]. Activated stellate cells produce a number of effectors of liver fibrosis and cirrhosis, including collagen type I, TGF- β 1, and TIMPs [145] [145]. Under normal physiological conditions, collagen turnover in the hepatic extracellular matrix (ECM) is regulated by a

delicate balance between TIMP and MMP activity. In response to either liver injury or inflammation, however, TIMP expression by activated stellate cells inhibits MMP fibrolysis and ECM remodeling, leading to excessive collagen accumulation and fibrous scarring that eventually leads to cirrhosis [146]. This fibrogenic cascade is further perpetuated by autocrine/paracrine signaling by stellate cell derived TIMPs, which inhibit MMP-9 mediated stellate cell apoptosis [147,148].

Increased MMP-9 collagenolytic activity has been observed to accompany histological improvement in models of both spontaneous and post-BMSC transplantation recovery from liver fibrosis [146,149-152]. Higashiyama *et al.* examined areas of fibrous hepatic septa during spontaneous recovery from CCl₄-induced liver fibrosis in mice with GFP⁺ hematopoietically reconstituted bone marrow, and found that half of the MMP-9 expressing cells in recovering areas also co-expressed GFP, suggesting their origin from BM [153]. Treatment of mice with G-CSF further increased the number of GFP⁺ MMP-9⁺ cells at sites of fibrosis and accelerated the resolution of fibrosis, supporting the key role of bone marrow derived cells and MMP-9 activity in the remodeling of fibrotic liver [153]. Similarly, studies of MSC and EPC transplantation in rodent models of DMN and CCl₄ liver injury have shown that resolution of fibrosis is accompanied by inhibition of TIMP-1 activity, enhanced MMP-9 activity, and improved animal survival [91,129] [92,149-152]. Decreased α -SMA⁺ and TGF- β 1 expression has also been observed in several studies, suggesting suppression of stellate cell activity during the reversal of fibrosis [92,129,149,150] Indeed, experiments by Parekkadan *et al.* of indirect co-culture of MSCs and activated stellate cells demonstrated that MSCs not only inhibit activated stellate cells, but actually induce their apoptosis [154]. In these experiments, both stellate cell proliferation and collagen synthesis were synergistically inhibited by MSC release of TNF- α and IL-10, and stellate cell apoptosis was shown to be dependent on HGF released by MSCs. This demonstration is consistent with prior reports that HGF accelerates hepatic regeneration and decreases liver fibrosis by promoting apoptosis of activated stellate cells [127,154,155]. BMSCs, therefore, appear to reverse hepatic fibrosis through a combination of mechanisms: (1) direct remodeling of fibrous ECM by MMP expression and (2) suppression of the pro-fibrogenic hepatic microenvironment by the induction of activated stellate cell apoptosis.

CLINICAL STUDIES OF BMSC TRANSPLANTATION FOR LIVER DISEASE IN HUMANS

Demonstrations of the capacity of MSCs, HSCs, and EPCs to ameliorate liver injury have raised considerable interest in the use of autologous BMSC transplantation as a potential therapy for liver cirrhosis [58,90,92,97,156,157]. A number of clinical trials have investigated autologous BMSC transfusion in patients with chronic liver disease (Table 3). Salama and colleagues randomized 140 C-P class B and C patients to either portal venous infusion of CD34⁺ CD133⁺ HSCs harvested from G-CSF mobilized iliac crest BM aspirate or usual supportive care [158]. At 6 months follow-up, patients in the treatment group showed significant improvements in overall survival, C-P class, performance status, ascites, HCV titers, and liver enzyme and synthetic function, with no adverse events, compared with untreated controls. Several smaller single arm phase I studies and retrospective analyses

have demonstrated similar improvements in hepatic function in patients with both mild and severe liver cirrhosis [134,159-169]. These studies also demonstrated the safety and feasibility of a number of BMSC isolation and infusion techniques, including iliac crest BM aspiration and peripheral blood leukapheresis, G-CSF administration for BMSC mobilization, and cell infusion via hepatic artery, portal or peripheral venous route.

BMSC infusion has also been used in combination with partial hepatectomy, portal venous embolization (PVE), and transarterial chemoembolization (TACE) to augment hepatic regeneration in patients with hepatic malignancies (Table 4) [170-172]. Ismail demonstrated that intrasplenic injection of BMMCs prior to partial hepatectomy for HCC improved liver function and prevented postoperative complications compared with patients randomized to routine pre-operative care [172]. Furst and am Esch showed that adding portal vein infusion of CD133⁺ HSCs to selective PVE prior to extended right hepatectomy in patients with right hepatic lobe tumors both increased future liver remnant volume and reduced time to surgical resection [170,171]. Combining hepatic artery infusion of BMMCs with TACE in patients with large HCC has produced similarly promising preliminary results [172].

CAN STROMAL CELL THERAPY SALVAGE LIVER FUNCTION IN PATIENTS WITH UNRESECTABLE HEPATOCELLULAR CARCINOMA?

Liver dysfunction in HCC with underlying cirrhosis poses a serious challenge for clinicians, significantly limiting the tolerability of current therapies. BMSC therapy has already shown potential as a means to improve cirrhotic liver function after surgical resection of HCC and to augment liver regeneration after PVE, albeit in small trials and case series [170-172]. Given the high risk of RILD and subsequent mortality associated with liver cirrhosis, BMSC therapy may prove to be of similar benefit for patients with HCC who are being considered for RT. One group has already demonstrated that hepatocyte transplantation can ameliorate the histopathological manifestations of radiation injury and improve survival in a rat model of RILD [39]. Clinical studies broadly demonstrate the safety and efficacy of BMSC infusion in patients with liver cirrhosis and hepatic malignancies. However, they generally suffer from a lack of prospective patient enrollment, randomized assignment of treatment groups, rigorous statistical analysis, and adequate patient numbers. Given the multifaceted role of BMSC transplantation in liver fibrosis in preclinical models and the encouraging results of clinical trials in liver cirrhosis and HCC, well-designed clinical studies are warranted to assess the therapeutic potential of autologous BMSC transplantation in HCC patients with underlying liver cirrhosis.

BMSCs stimulate hepatic regeneration in liver injury and cirrhosis through a number of cellular mechanisms, including (a) generation of *de novo* hepatocytes through transdifferentiation and/or cell fusion (b) paracrine stimulation of endothelial differentiation and vasculogenesis, (c) secretion of hepatotrophic growth factors and (d) antifibrogenic modulation of the stromal micro-environment by MMP, TIMP and stellate cell inhibition (see Fig. 1 & Table 2). This diverse range of mechanisms suggests a number of potential therapeutic roles for BMSC transplantation in patients with HCC (Fig 2). First, BMSC therapy may bolster the radiation and chemotherapy tolerance of cirrhotic liver, improving

our ability to safely and aggressively treat unresectable hepatic neoplasms. Second, BMSC therapy, either alone, or in combination with PVE, could stimulate hepatic regeneration to augment hepatic reserve, enabling safer resection of large tumors in poor surgical candidates. Third, BMSC transplantation may salvage liver function in patients experiencing hepatic toxicity (i.e. RILD) or decompensated liver failure after cancer therapy. Finally, BMSCs may serve as vectors for the delivery of tumoricidal agents, such as immunomodulatory cytokines, antiangiogenic agents, and suicide genes, to cirrhotic nodules bearing HCC. Despite the promise, caution must be exercised in its application due to the risk of deleterious tumor promoting effects upon recruitment of HSC, EPC and MSC in tumors, thereby, promoting tumor angiogenesis, immunosuppression and growth. Since we are proposing to bolster liver function before delivery of hepatic RT, we expect improvement of the therapeutic ratio upon amelioration of liver function in cirrhosis and control of the primary tumor by definitive doses of RT.

In summary, BMSC holds great promise for the treatment of liver cirrhosis and HCC. Clinical trials are needed to evaluate whether peripheral blood HSC mobilization with a combination of G-CSF and a CXCR4 inhibitor, such as, plerixafor can enable successful recruitment of BMSC in the cirrhotic liver to modulate fibrosis and promote liver regeneration and amelioration of liver function. Alternatively, patients with unresectable unifocal HCC and Child-Pugh class B or C liver cirrhosis could receive either supportive care or transplantation of autologous BMSC, harvested from peripheral blood or BM, as an adjunct therapy, prior to definitive liver RT. In addition, selective targeting of CXCR7 on LSEC or inhibition of stellate cells could potentially be useful as an anti-fibrogenic therapy in these patients. Clinical endpoints should include appropriate liver function tests, time to liver function deterioration post-RT, indocyanine green (ICG) clearance and overall survival. ICG clearance assay has been utilized as an early predictor of liver dysfunction after both hepatic resection and liver RT [173-176]. Detailed cell surface marker characterization and fate mapping of transfused BMSCs, notably missing from the current studies, should also be performed to help identify mechanisms responsible for the therapeutic effects of BMSCs. Such studies would establish the utility of BMSC transplantation in improving the safety and efficacy of hepatic RT in HCC patients whose poor liver function would otherwise limit the feasibility of curative treatment options.

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Fig. 1.

Fig. 2.

Table 1

Cell Surface Markers of BMSC Populations.

Cell Population	Surface Antigen Expression Profile	Isolation Technique
Hematopoietic Stem Cells [47,59,86,126]	CD34 ⁺ c-Kit ⁺ Sca1 ⁺ Thy1 ⁺ CD105 ⁺ CD38 ⁻ Lin ⁻	– CD34 ⁺ magnetic bead sorting (MACS) of peripheral blood – Separation of bone marrow mononuclear cell (BMMNC) fraction from density gradient centrifuged iliac crest marrow aspirate
Mesenchymal Stem Cells [59,104,105]	SH2 ⁺ SH3 ⁺ CD13 ⁺ CD29 ⁺ CD44 ⁺ CD71 ⁺ CD90 ⁺ CD105 ⁺ CD106 ⁺ CD120a ⁺ CD124 ⁺ c-Met ⁺ CD14 ⁻ CD34 ⁻ CD45 ⁻	– Plastic adherent, fibroblast-like cells isolated from the 1.073 g/ml density interface of Percoll-centrifuged BM
Endothelial Progenitor Cells [7,18,30,31,47,77,102,113,137]	CD34 ⁺ Flk-1 (VEGFR-2) ⁺ CD133 ⁺ c-kit ⁺ CD31 ⁺ Tie-2 ⁺ FGFR-1 ⁺ Sca1 ⁺ CD38 ⁺ CD146 ⁺ CXCR4 ⁺ VE-cadherin ⁺ E-selectin ⁺ vWF ⁺ Precise characterization controversial	– CD34 ⁺ Flk-1 ⁺ CD133 ⁺ magnetic bead sorting (MACS) of peripheral blood

TABLE 2

Mechanisms of BMSC Therapy in Liver Disease

MECHANISM	CELL TYPE	MODEL SYSTEM	FINDINGS	REFERENCE
Transdifferentiation	HSCs	CCl ₄ (i.p.) – C57Bl6/NCR mice & an <i>in vitro</i> HSC/liver co-culture system	Transplanted HSC-derived cells in recipient liver found to express characteristic hepatocyte markers (albumin and E-cadherin), with no karyotypic evidence of fusion between donor male HSCs and host female liver cells. HSC tx improved recipient mouse ALT, PT, and fibrinogen levels by 2 d after Tx. Highly purified HSCs in isolated trans-well co-culture with CCl ₄ or acetaminophen injured liver sequentially expressed transcription factors and protein markers of hepatocyte differentiation, without evidence of horizontal gene transfer	Jang <i>et al.</i> [55]
		FAH ^{-/-} mouse model of tyrosinemia type I	Transplantation of HSCs engrafted in FAH ^{-/-} mouse livers and formed regenerative nodules of HSC derived hepatocytes, which expressed unique donor cell markers without evidence of fusion with host hepatocytes	Lagasse <i>et al.</i> [73]
	MSCs	AA (i.p.) – Sprague –Dewey rats	Human albumin (hAlb) detected in both liver and serum of rats receiving intrahepatic human MSC xenograft, but not in CD34 ⁺ MSC or non-MSC/CD34 ⁺ xenografts No colocalization of human and rat chromosomes detected on FISH; all human Y chromosomes found in nuclei of hAlb ⁺ cells Extremely low frequency of transdifferentiation (<0.06%)	Sato <i>et al.</i> [121]
		Pip/Rag2 ^{-/-} immunodeficient mice – Partial hepatectomy	MSCs differentiated in hepatocyte growth media <i>ex vivo</i> carried out glycogen synthesis, urea synthesis, and PCK-1 hepatocyte specific promoter activation <i>in vitro</i> MSC-derived hepatocytes injected intrasplenically into partially hepatectomized mice engrafted peritoneally and synthesized human albumin while carrying out other hepatocyte specific functions Lack of co-localization of human Alu sequences with mouse satellite DNA ruled out cell fusion as a common event	Aurich <i>et al.</i> [10]
Fusion	HSCs	CCl ₄ (i.p.) both prior to & after MSC tx – Fisher 344 rats	MSCs primed <i>ex-vivo</i> with HGF differentiated into albumin producing hepatocyte like cells <i>in vitro</i> HGF primed MSC injected via tail vein into CCl ₄ treated rats engrafted in injured liver, reduced serum AST & ALT levels, restored albumin to pre-injury levels, and suppressed development of liver fibrosis	Oyagi <i>et al.</i> [96]
		FAH ^{-/-} mouse model of tyrosinemia type I	Repopulating hepatocytes derived from transplanted HSCs in FAH ^{-/-} mouse livers were genotypically	Wang <i>et al.</i> [139]

MECHANISM	CELL TYPE	MODEL SYSTEM	FINDINGS	REFERENCE
			heterozygous for both donor HSC and host liver alleles Female donor HSC derived hepatocytes in male recipient livers found to be cytogenetically 80,XXXXY and 120,XXXXYY	
		FAH ^{-/-} mouse model	FAH ^{+/-} β -gal ⁺ CD45 ⁺ single side population myelomonocytes rescued FAH ^{-/-} mice from liver failure Myelomonocytes fuse directly with host hepatocytes	Camargo <i>et al.</i> [19]
		CCl ₄ (i.p.) – P3D2F1 mice	HSCs from EGFP ⁺ male origin chimeric bone marrow infiltrated injured liver after G-CSF administration and formed Alb ⁺ glycogen storing EGFP ⁺ hepatocytes 74% of HSC derived hepatocytes co-localized hepatic cytokeratins, EGFP and Y-chromosome, demonstrating <i>in vivo</i> cell fusion between endogenous male hepatocytes and female donor EGFP ⁺ HSCs	Quintana-Bustamante <i>et al.</i> [110]
Inhibition of collagen deposition	MSCs	CCl ₄ (s.c.) – white Albino rats	MSC tx assoc with $\downarrow$ hydroxyproline content & $\downarrow$ collagen expression	Abdel Aziz <i>et al.</i> [1]
	EPCs	Phenobarbital (p.o.)/CCl ₄ (i.p.) and TAA (i.p.) both prior to and after tx - Wistar rats	EPC tx $\downarrow$ fibrosis in both Phenobarbital/CCl ₄ and TAA treated rats, and $\downarrow$ α 2-(I)-procollagen and fibronectin content in CCl ₄ treated rats	Nakamura <i>et al.</i> [91]
Paracrine Effects of BMSC Derived Growth Factors	EPCs	Phenobarbital (p.o.)/CCl ₄ (i.p.) and TAA (i.p.) both prior to and after tx - Wistar rats	EPC tx assoc with $\uparrow$ HGF, TGF- α , EGF, & VEGF expression and hepatic regeneration in recipient livers	Nakamura <i>et al.</i> [91]
		CCl ₄ (i.p.) – Balb/c nude mice	EPC tx assoc with $\uparrow$ HGF, TGF- α , EGF, & VEGF expression and hepatic regeneration in recipient livers	Taniguchi <i>et al.</i> [129]
	MSCs	<i>In vitro</i> co-culture of MSCs with CCl ₄ -injured hepatocytes	MSCs found to secrete HGF in co-culture with CCl ₄ injured hepatocytes	Oyagi <i>et al.</i> [96]
		D-galactosamine (i.p.)- Sprague Dewey Rats	Treatment with MSC-conditioned media reduced hepatocyte apoptosis, increased hepatocyte proliferation, and decreased mortality in rats with fulminant hepatic failure	Van Poll <i>et al.</i> (111)
Modulation of MMP expression and activity	EPCs	Phenobarbital (p.o.)/CCl ₄ (i.p.) and TAA (i.p.) both prior to and after tx - Wistar rats	EPC tx assoc with $\uparrow$ MMP-2, -9, and -13 expression & activity, and $\downarrow$ TIMP-1 expression in recipient livers. EPCs demonstrated to produce active forms of MMP-2, -9, and -13 <i>in vitro</i> .	Nakamura <i>et al.</i> [91]
	Whole marrow BMSCs	CCl ₄ (s.c.) – C57B16 mice	BMSCs derived from reconstituted EGFP ⁺ bone marrow migrate to cirrhotic liver and express MMP-9 during regression from liver fibrosis MMP-9 expression by EGFP ⁺ cells $\uparrow$ by G-CSF and HGF	Higashiyama <i>et al.</i> [45]

MECHANISM	CELL TYPE	MODEL SYSTEM	FINDINGS	REFERENCE
Neovascularization and Vascular Support	EPCs	CCl ₄ (i.p.) – Balb/c nude mice	Transplanted human EPCs incorporated into damaged central veins and hepatic sinusoids by 2 days post-tx. Hepatocyte proliferation abundant in areas of EPC derived blood vessels Survival dramatically increased in animals receiving EPC tx vs. saline	Taniguchi <i>et al.</i> [129]
		Partial hepatectomy – C57Bl/6 mice with reconstituted GFP ⁺ bone marrow	BM-derived EPCs accounted for over 46% of endothelium in regenerated liver after PH + VEGF. VEGF administration → enhanced hepatic regeneration after PH & increased efficiency of BM-derived EPC incorporation into regenerating liver	Beaudry <i>et al.</i> [14]
		Cell culture of MSCs isolated from human adult BM	Human adult MSCs and MAPCs were able to differentiate into CD31 ⁺ Flk-1 ⁺ vWF ⁺ endothelial cells <i>in vitro</i>	Jiang <i>et al.</i> (24); Oswald <i>et al.</i> (113)
	MSCs	Subcutaneous implantation of cultured MSCs (CD34 ⁺ CD31 ⁻ CD45 ⁻ Flk1/VEGFR-2 ⁺ Tie2 ⁺) from LacZ ⁺ C57Bl/6 mice resuspended in matrigel	Blood vessels which developed in matrigel plugs post-MSC tx <i>in vivo</i> contained intimal layer LacZ ⁺ CD31 ⁺ endothelial cells and subendothelial LacZ ⁺ vascular smooth muscle cells LacZ ⁺ MSCs contributed to only ~0.9% of new blood vessels, demonstrating that the vast majority of neovasculature developing post-MSC tx is of host origin Treatment of MSCs with anti-VEGF neutralizing antibody prior to tx prevented formation of LacZ ⁺ CD31 ⁺ endothelium and suppressed host-derived angiogenesis, demonstrating the dependence of MSC-mediated neovascularization on autocrine & paracrine VEGF-signaling	Al Khaldi <i>et al.</i> (47)
		Chronic Hind Limb Ischemia in Lewis rats	Intramuscular injection of LacZ ⁺ MSCs into ischemic limb 3 wks after femoral artery ligation improved arterial flow, vascular density, and arteriolar density compared with control LacZ ⁺ MSCs found to differentiate into endothelium, vascular smooth muscle, skeletal muscle, and adipocytes identified in ischemic limb MSC tx found to reconstitute femoral artery by formation of pelvic and abdominal collateral vessels	Al Khaldi <i>et al.</i> (114)
Inhibition of Hepatic Stellate Cell Activation, TGF- β Production, and Fibrogenesis	HGF alone	DMN (i.p.) – Sprague Dawley rats	HGF administration → inhibition of myofibroblast proliferation and enhancement of myofibroblast apoptosis	Kim <i>et al.</i> [67]
	EPCs	Phenobarbital (p.o.)/CCl ₄ (i.p.) and TAA (i.p.) both prior to and after tx - Wistar rats	EPC tx assoc with ↓ α -SMA ⁺ and TGF- β ⁺ cells in recipient livers	Nakamura <i>et al.</i> [91]
	MSCs	Human MSC and activated hepatic stellate cell co-culture	MSCs → IL-10 & TNF- α → inhibition of hepatic stellate cell activation, proliferation, and fibrogenesis MSCs → HGF → myofibroblast apoptosis	Parekandan <i>et al.</i> [99]

AA – allyl alcohol; Alb – a bumin; a-SMA – alpha smooth muscle actin; BM – bone marrow; BMSC – bone marrow-derived stromal cell; CCL4 – carbon tetrachloride; DDC – 3,5-dioxyacarbonyl-1,4-dihydrocollidine; EGF – epidermal growth factor; EGFP – enhanced green fluorescent protein; EPC – endothelial progenitor cell; FAH – fumarylacetoacetate hydrolase; FISH – Fluorescence in situ hybridization; G-CSG – granulocyte colony stimulating factor; h – human; HGF –hepatic growth factor; HSC – hematopoietic stem cell; i.p. – intraperitoneal injection; IL – interleukin; MMP – matrix metalloproteinase; MSC – mesenchymal stem cell; PH – partial hepatectomy; TAA – thioacetamide; TGF – transforming growth factor; TIMP – tissue inhibitor of metalloproteinase; TNF – tumor necrosis factor; Tx – transplantation; VEGF – vascular endothelial growth factor

Table 3

Clinical Studies of Autologous BMSC Therapy in Liver Cirrhosis

Cell Type	N	Patient Characteristics	BMSC Source	BMSC Characteristics	Treatment	Follow-up	Outcomes	Reference
HSCs	48	- HCV (n=36) or autoimmune (n=12) related ESLD - C-P Class B-C - WHO PS 3 - MELD score > 15	Peripheral blood (G-CSF mobilized)	CD34 ⁺	PVI or HAI	48 wks	- OS 79% at 1 yr - ↓ Ascites (73% pts) - ↓ Bilirubin - ↑ Albumin - ↓ ALT - ↓ INR - 3 serious infusion related events (6%)	Salama et al. [120]
HSCs	5	- C-P Class A-B - WHO PS 0-1	Peripheral blood (G-CSF mobilized)	Plastic adherent CD34 ⁺ CD38 ^{low} CD33 ^{low} HLA-DR ^{low}	PVI or HAI	12 – 18 mo	- ↓ Bilirubin (4 pts) - ↓ Albumin (5 pts) - ↓ Ascites (1 pt) - No serious side effects	Levicar et al. [78]; Gordon et al. [38]
HSCs	9	- C-P Class B - WHO PS 0-1	Peripheral blood (G-CSF mobilized)	CD34 ⁺	HAI	3 mo	- ↓ C-P Score (7 pts) - ↓ Ascites (5 pts) - ↓ Bilirubin - ↑ Albumin - ↓ ALT/AST	Pai et al. [97]
HSCs	2	- C-P Class B-C - MELD score 14-23	Peripheral blood (G-CSF mobilized)	CD34 ⁺	Peripheral vein infusions (1-3 x)	30 – 34 mo	- C-P Score - ↓ MELD score - ↓ PS (2→0 in 1 pt) - No future cirrhosis related hospitalizations	Yannaki et al. [148]
HSCs	140	- C-P Class B-C - WHO PS 2	Iliac crest (G-CSF mobilized)	CD34 ⁺ CD133 ⁺ (MACS isolated)	Randomized to: (1) PVI (n=90) vs. (2) Supportive care (n=50)	6 mo	- OS 90% vs. 52% at 6 mo - ↓ C-P class in 53% vs. 0% - ↓ PS in 58% vs. 0% - ↓ Ascites in 65% vs. 0% (disappeared in 27%) - Improved LFTs: 54.5% vs. 0% - ↓ HCV titer: 59.1% vs. 0% - No infusion related serious events	Salama et al. [119]
HSCs	4	- HBV or HCV cirrhosis - C-P Class B-C - MELD score 18	Iliac crest (G-CSF mobilized)	CD34 (MACS isolated)	HAI	6 – 12 mo	- ↓ C-P Score - ↓ MELD Score (3 pts) - ↓ Bilirubin - ↓ ALT - ↑ Albumin - ↓ Serum HA	Khan et al. [64]
BMMCs	10	- HBV cirrhosis - C-P Class B	Iliac crest	CD45 ⁺	Peripheral vein infusion	12 mo	- ↑ Albumin - ↑ MRI Liver volume (8 pts) - ↓ Ascites - ↓ C-P Score - ↑ activation of the hepatic progenitor cell compartment (peak at 3 month)	Kim et al. [66]

Cell Type	N	Patient Characteristics	BMSC Source	BMSC Characteristics	Treatment	Follow-up	Outcomes	Reference
BMMCs	9	- C-P Class B-C	Iliac crest	CD45 ⁺	Peripheral vein infusion	6 mo	<ul style="list-style-type: none"> - ↑ PS at 6 months - ↑ QOL at 6 months - No serious adverse events - ↑ Albumin - ↓ C-P Score - ↓ Pro-collagen III peptide - ↑ Serum HGF - ↑ AFP & PCNA expression (liver regeneration markers) - ↓ Ascites (5 pts) 	Terai <i>et al.</i> [130]
BMMCs	30	- C-P Class A-C - Liver transplant wait-listed	Iliac crest	Ficoll-hypaque Gradient Enriched Mononuclear BM Fraction	Randomized to: (1) BMMC-HAI vs. (2) Observation	12 mo	<ul style="list-style-type: none"> - ↓ C-P and MELD scores in treatment group vs. controls - ↓ Bilirubin, ↑ albumin, ↑ INR in treatment group vs. controls 	Lyra <i>et al.</i> [83]
MSCs	4	- MELD score 16	Iliac crest	CD13 ⁺ CD31 ⁺ CD44 ⁺ CD90 ⁺ CD105 ⁺ CD166 ⁺ CD34 ⁻ CD45 ⁻	Peripheral vein	12 mo	<ul style="list-style-type: none"> - ↓ Bilirubin - ↑ Albumin - ↓ MELD Score - ↓ Peripheral edema - ↑ QOL (SF-36) 	Mohamadnejad <i>et al.</i> [88]

AFP – A pha-fetoprotein; ALT – Alanine transaminase; AST – Aspartate transaminase; BM – Bone marrow; C-P- Child-Pugh; ESLD – End stage liver disease; G-CSF – Granulocyte colony stimulating factor; HAI – Hepatic artery infusion; HGF – Hepatic growth factor; HSCs – Hematopoietic stem cells; HA – Hyaluronic acid; LFTs- Liver function tests; BMMCs – Mononuclear cells; MSCs – Mesenchymal stem cells; OS – Overall survival; PCNA – Proliferating cell nuclear antigen; PT – Prothrombin time; PS – Performance score; PVI – Portal vein infusion; QOL – Quality of life

TABLE 4

Clinical Studies of Autologous BMSC Transplantation in Hepatic Malignancies

Cell Type	N	Patient Characteristics	BMSC Source	BMSC Characteristics	Treatment	Follow-up	Outcomes	Reference
BMMCs	20	– Single right lobe HCC – C-P Class B or C	Iliac crest	Unsorted Mononuclear BM Fraction	Randomized to: (1) intrasplenic injection of BMMCs (n=10) vs. (2) no treatment (n=10) 3 wks prior to right lobe Hepatectomy	12 wks	– Improved LFTs (bilirubin, PT, albumin, bilirubin) – In treatment group vs. controls – ↑ Peri-op complications in treatment vs. control (i.e. bleeding, infection, liver failure, ascites)	Ismail et al.[51]
BMMCs	4	– Single HCC 6 cm – C-P Class B HCV-related cirrhosis	Iliac crest	Unsorted Mononuclear BM Fraction	Lipiodol TACE of HCC involved lobe + BMMC-HAI into non-tumor involved liver lobe	3 mo	– ↓ C-P score – ↓ Ascites & edema – ↑ Size of non-TACE treated lobe – ↑ Albumin – ↓ AST, ALT, bilirubin	Ismail et al.[51]
HSCs	13	– Primary (n=7) or metastatic (n=6) hepatic tumors	Iliac crest	CD133 ⁺ (MACS isolated)	(1) Selective PVE + HSC PVI (n=6) vs. (2) PVE alone (n=7); followed by extended right hepatectomy (non-randomized)	N/A	– Greater increase in future liver remnant volume in group 1 – Shorter time to surgery in group 1 (27 vs. 45 days)	Furst et al.[37]

ALT – Alanine transaminase; AST – Aspartate transaminase; BM – Bone marrow; BMMCs – Mononuclear cells; C-P- Child-Pugh; HSCs- Hematopoietic stem cells; LFTs- Liver function tests; N/A – not available; PVE – Portal vein embolization; PVI – Portal vein infusion; TACE – Transarterial chemoembolization.