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Concise Review: Bone Marrow Autotransplants for Liver Disease?

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ABSTRACT

There are increasing reports of using bone marrow-derived stem cells to treat advanced liver disease. We consider several critical issues that underlie this approach. For example, are there multipotent stem cell populations in human adult bone marrow? Can they develop into liver cells or supporting cell types? What are stromal stem/progenitor cells, and can they promote tissue repair without replacing hepatocytes? Does reversal of end-stage liver disease require new hepatocytes, a new liver microenvironment, both, neither or something else? Although many of these questions are unanswered, we consider the conceptual and experimental bases underlying these issues and critically analyze results of clinical trials of stem cell therapy of end-stage liver disease. STEM CELLS *2013;31:2313–2329*

Disclosure of potential conflicts of interest is found at the end of this article.

INTRODUCTION

Liver transplantation is the only effective therapy of end-stage liver disease. However, the vastly increasing prevalence of end-stage liver disease without a parallel increase in donor livers has precipitated a search for alternative therapies. Recently, there is considerable interest in using stem cells to repair or improve liver function in persons with end-stage liver disease. One possible source of stem cells is from the bone marrow and other hematopoietic tissues. We reviewed data of whether transplanting these cells, typically given as an autotransplant, can improve impaired liver function in persons with end-stage liver disease.

WHAT ARE STEM CELLS?

The term stem cell was first used by Haeckel, a German biologist, in a late 19th century to define the origin of the blood system in embryology [1, 2]. Since then many different cells have been defined as stem cells. Some believe only the fertilized egg is the ultimate stem cell because it is totipotent and able to give rise to the embryo and extra-embryonic structures needed to develop entire organism [3]. More commonly, a stem cell is defined as "A cell that can continuously produce unaltered daughters and also has the ability to produce daughter cells that have different, more restricted properties" [4]. Embryonic stem cells (ESCs) are pluripotent stem-cell lines derived from early embryos before formation of the tissue germ layers which has unlimited self-renewal capacity and are able to differentiate to all three embryonic layers. Somatic or adult stem cells also develop in the embryo and retain self-renewal capacity throughout life but can develop into some, but not all, cell lineages of the adult organism [5].

Induced pluripotent stem cells (iPSC) are a type of pluripotent stem cell artificially derived from a nonpluripotent cell—typically an adult somatic cell—by inducing expression of specific genes. iPSCs are similar to ESCs in many respects but the full extent of their relation to natural pluripotent stem cells is unknown [6].

The definition of stem cells also varies based on the field of study (regenerative medicine, aging, gene therapy, etc.), the organism being studied (*Drosophila sp*, mice, humans, etc.), persistence through life, and other variables. For example, in some organisms, such as *Planarians sp*., pluripotent cells are maintained throughout life whereas mouse and human ESCs, which are also pluripotent, are present only during embryonic development [7].

Another term sometimes confused with stem cell is progenitor cell. Progenitor cells are often defined as immature cells able to differentiate into specific cell types. Progenitor cells have less proliferative potential than stem cells. In developmental biology, progenitor cells are defined as "cells with proliferative capacity that may or may not be committed to a lineage choice but are not terminally differentiated" [4]. Sometimes precursor cells also are confused with stem cells. Precursor cells are usually, although not always, postmitotic, but have the capacity to assume one of several differentiated fates. Neither progenitor nor precursor cells are typically categorized as stem cells by most cell biologists [4, 8].

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There is considerable confusion and controversy, sometimes *bona fide*, of how these terms are used within a field and between fields [2–4]. The aforementioned definitions are a consensus and boundaries between cell types may change, blur or disappear as knowledge develops. The important point is misuse of these terms can result in confusion regarding which therapeutic effects are reasonably be expected from the manipulation and transplantation of these cells. As we will see, this imprecision becomes important when we consider the use of bone marrow or blood cells to treat end-stage liver disease.

"Hematopoietic Stem Cells" As Progenitor Cells

Most hematologists think human bone marrow and blood (under special circumstances) contains stem cells. For example, bone marrow and blood cell transplants are often referred to as *stem cell transplants*. However, hematopoietic cells lack critical features of ESCs. They are more accurately defined as a subset of progenitor cells derived from embryonic mesoderm. Biological features of hematopoietic stem cells include: (a) multipotency and asymmetrical cell divisions which can give rise to different cell types; (b) persistence in a quiescent state and a slow rate of self-renewal; (c) ability to remain in an undifferentiated state in specific microenvironment sites called stem cell niches; (d) ability to restore bone marrow function in lethally irradiated animals including rodents and sub-human primates; and (e) ability to differentiate into diverse hematopoietic lineages including red blood cells (RBCs), myeloid and lymphoid cells, and megakaryocytes [8, 9]. Here, we use the term "hematopoietic cells" instead of "hematopoietic stem cells" to minimize confusion between readers from diverse disciplines.

Bone Marrow–Derived Mesenchymal Stromal/Stem Cells

Mesenchymal stem cells are a rare population of mesenchymal cells with self-renewal and differentiation characteristics. Mesenchymal stem cells differ from mesenchymal stromal cells most of which lack stem cell features (mesenchymal stem cells and mesenchymal stromal cells have the same abbreviation [MSC] resulting in confusion and potentially incorrect conclusions). We restrict most of our discussion to mesenchymal stromal cells which may contain a small fraction of mesenchymal stem cells. Most studies of cells described as MSCs are actually mesenchymal stromal cells and refer to a plastic-adherent population of cells obtained after *in vitro* culture and may be different from analogous primary uncultured cells that are even more difficult to characterize [10]. The properties of MSCs in the context of tissue regeneration are largely related to the release of bioactive molecules rather than from inherent stem cell characteristics.

Mesenchymal stromal cells can be obtained from different tissues such as bone marrow, adipose tissue, placenta, umbilical cord blood, umbilical cord perivascular cells, umbilical cord Wharton jelly, dental pulp, skin, amniotic fluid, synovial membrane, and breast milk [10]. Although MSCs from these sources share some characteristics, significant differences are described [11]. For example, adipose tissue and skin exhibit differences in molecular phenotype and differentiation potential. Further studies are required to determine whether this nomenclature is appropriate [10].

Mesenchymal stromal cells are not easily defined, because they lack a unique marker or surface antigen. Current criteria are from 2005 and need revision. These criteria are based on three characteristics: plastic adherence, a cell surface immune type lacking hematopopietic determinants, and the ability to undergo differentiation *in vitro* to osteogenic, chondrogenic, and adipogenic lineages [10, 12]. Mesenchymal stem cells are more difficult to define. Only three studies report stem cells among human mesenchymal stromal cells. Their frequency varies but seems to be less than one per 1,000 [13–15]. This low frequency contrasts with studies in mice showing much higher frequencies.

Stromal vascular fraction (SVF) cells are typically obtained by collagenase digestion of adipose tissues although other sources are sometimes used. SVF cells are diverse and include B-cells and T-cells, endothelial cells, fibroblasts, macrophages, pericytes, preadipocytes, and others. Under appropriate conditions, cultures of SVF cells yield an adherent subpopulation termed adipose-derived stromal/stem cells (ASCs). These ASCs are relatively homogeneous with similar, but not identical, cell surface antigens to bone marrow-derived mesenchymal stromal/stem cells. However, the frequency of these cells in the SVF is substantially higher than in the bone marrow. Some studies suggest ASCs may be useful to correct structural disorders such as occurs in end-stage liver disease [16].

Modulatory effects of MSCs on the immune system are well-known. MSCs have effects on the adaptive and innate immune systems including suppressing T cells and dendritic cells, inhibiting B-cell activation and proliferation, inhibiting proliferation and cytotoxicity of natural killer cells, and promoting generation of regulatory T-cells via suppression of interleukin (IL)−10. MSCs mediate some of these processes by affecting the expression of inflammatory cytokines [10, 17]. Because of diverse definition of stromal or mesenchymal stem cell in discussed clinical trials we use term mesenchymal stromal/stem cells (MSCs) throughout.

Development of Hematopoiesis in The Embryo

To understand the biology of hematopoietic cells, it is important to consider the ontogeny of hematopoiesis. In humans, the earliest hematopoietic cells in the embryo arise in the foregut mesoderm aorta/gonad/mesonephros (AGM) area during the first trimester of gestation. These cells migrate to the liver bud in the second trimester after which they rapidly proliferate [18]. Consequently, the second trimester fetal liver is fundamentally a hematopoietic rather than hepatic organ concerned predominately with RBC production. During the third trimester, hematopoietic cells migrate to the newly formed internal spaces within the bones (bone marrow cavity) which were previously solid. Simultaneously, the anatomical liver space becomes filled with hepatocytes and bile duct lining cells which migrate in from the ventral endoderm and with supporting cells such as fibroblasts and Kupffer cells which also arise from mesoderm. At birth and thereafter the liver functions as a metabolic, detoxifying, glycogen storage, and protein-synthesizing organ (reviewed in 8, 9, 19). The liver plays no role in adult hematopoiesis except under special circumstances when the bone marrow space is fibrotic, filled with neoplastic cells, micro-organisms, or granulomas such as in persons with myeloproliferative neoplasm-associated

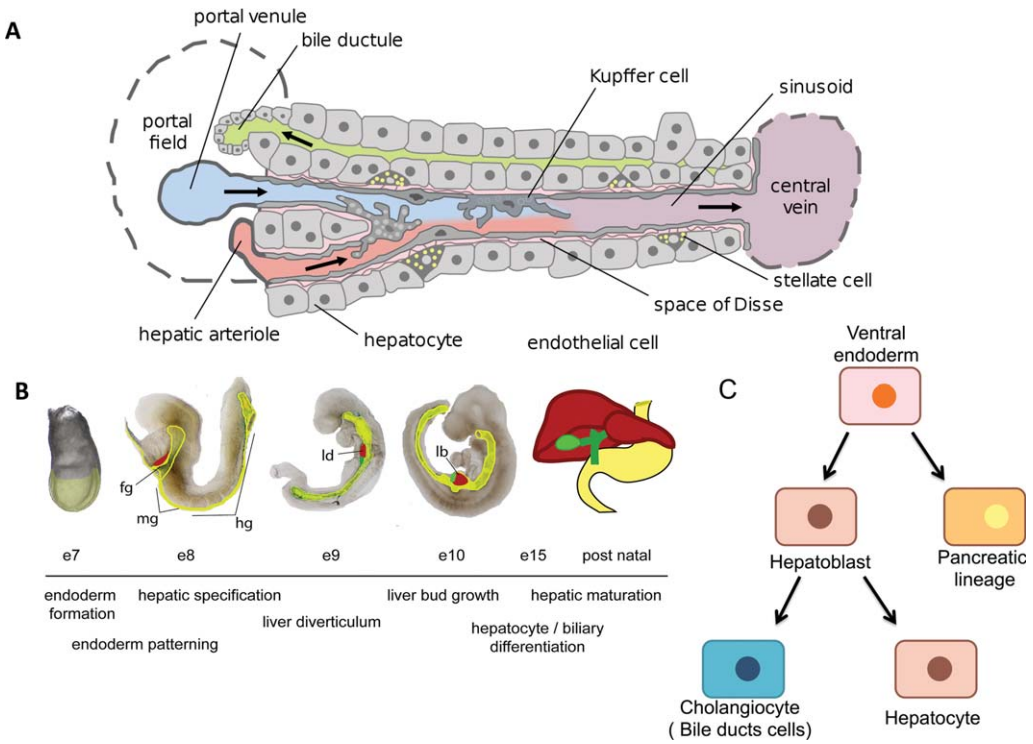

Figure 1. Liver architecture and development (**A):** Liver architecture and possible targets for cell therapy (Adapted from 23). **(B):** The schematic shows mouse embryos at different stages of development with the endoderm tissue highlighted in yellow, the liver in red and the gall bladder in green. The major developmental events are listed below. The endoderm germ layer is formed during gastrulation (e6.5-e7.5). Throughout gastrulation and early somite stages of development (e7-e8.5) the endoderm is patterned along the (A–P) axis into foregut (fg), midgut (mg), and hindgut (hg) progenitor domains. Morphogenesis forms foregut and hindgut pockets as the endodermal cup is transformed into a gut tube. By e8.5, hepatic fate specified in a portion of the ventral foregut endoderm adjacent to the heart. As the embryo grows the endoderm forms a gut tube and the liver domain moves to the midgut. The liver diverticulum (ld) forms by e9 and expands into an obvious liver bud (lb) by e10. The liver grows, and by e15 hepatoblasts are differentiating into hepatocyte and biliary cells. Final maturation of the liver is gradual and continues into the postnatal period (Adapted from 24). **(C):** Embryonic origin of the liver cells. Abbreviations: fg, foregut; hg, hindgut; mg, midgut; ld, liver diverticulum.

myelofibrosis or infections which result in bone marrow fibrosis. Some data suggest neoplastic hematopoietic cells, typically $CD34^{+}$, may selectively migrate to the liver and spleen but this is controversial.

Development of The Liver in The Embryo

The first molecular evidence for liver development occurs in a portion of the ventral endoderm adjacent to the developing heart. Liver progenitor cells expressing albumin, transthyretin, and α-fetoprotein localize in three regions of the endoderm and begin to differentiate at about 3 weeks of human gestation [19]. The hepatic endoderm forms by conjugation to become hepatocytes or bile duct epithelial cells [20]. At day 22, the liver diverticulum forms in the primitive gut by proliferation of hepatoblasts producing a pseudo-stratified epithelium-like tissue called the hepatic bud [21]. Columnar hepatoblasts go through an epithelial-mesenchymal transition while invading the septum transversum. Gradually, numbers of bipotent hepatoblasts decrease whereas numbers of mature cells increase [22]. Columnar hepatoblasts undergo an epithelial-mesenchymal transition while invading the septum transversum. Gradually, numbers of bipotent hepatoblasts decrease whereas numbers of mature cells increase (Fig. 1; 23, 24). (reviewed in 19-22).

Fetal Liver as a Hematopoietic Organ

Although fetal liver does not produce hematopoietic cells *per se* it is the main site of hematopoietic cells proliferation and differentiation. Hematopoietic cells development in fetal liver can be categorized in three phases: (a) seeding; (b) expansion; and (c) differentiation. Circulating hematopoietic cells from AGM and the placenta seed and colonize the fetal liver. Studies of mouse embryos indicate hematopoietic cells first appear in the fetal liver at E11.5 of embryonic development. During the expansion phase, hematopoietic cells undergo propagation and differentiation in liver tissue that reaches a maximum of approximately 1,000 hematopoietic cells by E15.5–16.5. Afterward, numbers of hematopoietic cells plateau and decline (Fig. 2; 25, 26).

Developing fetal liver tissue is a dynamic microenvironment where hematopoietic progenitor cells also develop. This may reflect the crucial role of fetal liver in providing a niche to produce differentiated blood cells. As the embryo develops, the hematopoietic profile of fetal liver changes. The early fetal liver is rich in erythroid and pro-erythroblasts colony-forming cells whereas myeloid and lymphoid progenitors accumulate in the later stages of fetal liver development in the second trimester [25].

Hematopoietic cells develop rapidly in fetal liver with high proliferation rates whereas hematopoietic cells in bone marrow are quiescent. Fetal stromal cells play a crucial role

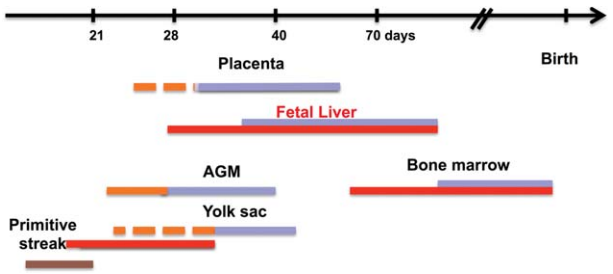

Figure 2. Sites of hematopoietic cells (HCs) in human embryos. The ages at which human hematopoietic sites are active. Brown bars, mesoderm; red bars, active hematopoietic differentiation; orange bars, HCs genesis; blue bars, presence of functional adult-type HCs. Broken orange bars for yolk sac and placenta indicate that de novo HCs genesis has not been experimentally proven (adapted and modified from 25). Abbreviation: AGM, aorta/gonad/mesonephros.

in the hematopoietic microenvironment of the developing liver. *in vitro* studies suggest fetal stromal cells support expansion of hematopoietic cells in co-cultures. Insulin-like growth factor-2 and angiopoietin-like proteins are believed to be the main molecular signals from the stroma [27, 28].

Co-culture studies also suggest that immature hepatic progenitor cells provide an appropriate microenvironment for hematopoiesis. This is not so of mature hepatocytes. This difference may explain the migration of hematopoietic cells to bone marrow in late gestation [29, 30].

Conversely, expression of oncostatin M by hematopoietic cells may enhance hepatocyte maturation [31]. These studies show the interaction between the blood and parenchymal compartments within the fetal liver which control the development of the liver and bone marrow.

Liver Regeneration

Normal hepatocyte turnover is slow. Bromodeoxyuridine labeling studies estimate the rate of liver cell turnover at about 1 in 20–40,000 at any time. Furthermore, the average lifespan of adult hepatocytes is 200–300 days. Streaming liver is the current model for normal hepatocyte maintenance. This model proposes that in a healthy liver, young hepatocytes originate in the portal zone and migrate toward the central vein. Other mechanisms are also suggested [32, 33].

The regenerative capacity of liver in response to partial resection or injury is well-studied. In rodents, partial hepatectomy is followed by mitosis in the residual hepatocytes restoring the hepatocyte mass to normal or near normal [34–36]. However, the resected lobe(s) never regenerates. (Prometheus [Prometheus is a Titan in Greek mythology. Because he gave the sacred fire to humans his liver was consumed daily by a vulture. However, it regrew overnight. Some think the myth indicates that Greek knew of the liver's remarkable self-renewal capacity.] would be surprised to learn this.) Recent data using enhanced yellow fluorescent protein indicate that almost all new hepatocytes derive from adult hepatocytes rather than from liver stem cells. These data also indicate that progenitor cells in the liver are not involved in normal liver homeostasis and regeneration after partial hepatectomy [37]. This situation differs fundamentally from the hierarchal structure of hematopoiesis discussed above where hematopoietic cells give rise to mature end cells equivalent to mature hepatocytes. This structural disparity may reflect the extraordinarily different demands for cell replication of the liver and bone marrow. As indicated, hepatocytes survive a long time compared with hematopoietic cells. For example, granulocytes survive only a few hours or days and it is estimated the bone marrow must produce several billion new cells daily to maintain normal levels of end cells in the blood. This is in striking contrast to the normal liver and requires an entirely different hierarchal and structural organization,

Studies in dogs and primates (including humans) also show a proportional regenerative response to the size of the resected liver. Several cytokines are important in the process of liver regeneration after partial hepatectomy including hepatocyte growth factor, IL-6, tumor necrosis factor-α, transforming growth factor-α, and epidermal growth factor (EGF).

In contrast to liver regeneration after partial hepatectomy, proliferation of hepatocytes is blunted or absent after severe and/or chronic liver injury caused by drugs, viruses, and toxins. Here, hepatic repopulation appears to occur via differentiation of progenitor cells (reviewed in 38). This distinction is of fundamental importance in considering therapy interventions for end-stage liver disease.

Prolonged liver trauma induces proliferation of a heterogeneous population of liver cells of small size (relative to normal hepatocytes), ovoid nuclei, and high nuclear: cytoplasmic ratio. These cells, termed oval cells, are thought to be hepatic progenitor cells. Oval cells are rare in normal adult liver, are primarily found in the peri-portal region, and are usually quiescent [39]. Data from studies in rodents indicate proliferation of oval cells between the hepatic cords in the liver parenchyma after exposure to toxins including 1,4-bis [*N*,*N*′-di(ethylene)-phosphamide] piperazine (DIPIN) [40] and a choline-deficient, ethionine-supplemented diet [41]. Activated oval cells are also found in humans with liver diseases such as hepatitis-C virus (HCV), hemochromatosis, and alcohol-induced liver disease [42]. Surprisingly, these hepatic progenitor cells that express hepatic and biliary lineage markers share common characteristics with hematopoietic cells supporting the notion of an extrahepatic origin of hepatic progenitor cells (oval cells) [43]. Some studies suggest oval cells originate from bone marrow progenitors [44]. However, most recent studies do not support this notion emphasizing the intrahepatic origin of oval cells [45, 46].

Can Bone Marrow–Derived Cells Help Liver Recovery in Animals?

Mobilization of hematopoietic cells into the blood may occur in response to liver injury. An increase in blood $CD34^+$ cells is reported in some persons with acute liver injuries such as paracetamol toxicity and alcoholic hepatitis [47, 48]. $CD34^+$ cell mobilizing sometimes occur after partial hepatectomy and small-for-size liver transplantation [49–51]. Stromal-derived factor-1 (SDF-1) and its receptor, CXC receptor four (CXCR4), may also be involved in this response [52] (reviewed in 48).

Some data suggest oval cells express granulocyte colony-stimulating factor (G-CSF) and G-CSF receptors after liver injury by 2-acetylaminofluorene (2-AAF) and partial hepatectomy in rat [53]. Giving G-CSF also significantly increased numbers of oval cells in this model. Additionally, G-CSF is a chemoattractant and mitogen for oval cells in vitro [53].

Li *et al*. mobilized $CD34^+$ human bone marrow cells with G-CSF, collected them, and infused them into liver injured mice. The infused human cells repopulated approximately 30% of the NOD/SCID mice liver tissue [54]. Zhang *et al*.

[55] reported giving G-CSF improved survival of rats with acute liver failure induced by D-galactosamine. Qujeq *et al*. [56] reported giving G-CSF improved recovery of rats with carbon tetrachloride-induced liver failure. Mizunaga *et al*. suggest G-CSF and IL-1β levels rose 2 weeks after bone marrow infusion in mice and humans with cirrhosis. However, it is not clear whether the bone marrow infusion, G-CSF, or other effect(s) improved liver function [57].

Meng *et al*. reported a stable increase in stem cell factor (SCF) and granulocyte-macrophage colony-stimulating factor (GM-CSF) levels for 1 week after 70% partial hepatectomy in mice. They suggested that this increase contributed to bile duct remodeling function and to deregulation of the activity of key signaling intermediates involved in cell expansion and multipotent differentiation [58]. However, as indicated, this effect was not seen in rats with carbon tetrachloride-induced liver injury [59]. In addition, Ogiso *et al*. [60] reported giving G-CSF suppressed liver cell proliferation by upregulating IL-1β after di-methyl-nitrosamine-induced liver injury in rats. These data suggest hormones involved in hematopoiesis, such as G-CSF and GM-CSF may influence liver recovery under some experimental conditions. Most of these data are from models of drug-induced liver injury and cannot be extended to all liver conditions including chronic virus-induced liver diseases.

Several studies report in vitro differentiation of bone marrow-derived cells into hepatocyte-like cells [61, 62]. Although these studies suggest plasticity of hematopoietic cells toward a hepatic lineage, more data are needed to determine whether a homogeneous population of functional hepatocytes can be developed from bone marrow cells.

Differentiation of bone marrow-derived cells into liver cells *in vivo* is also reported in rodents with liver injury [63–65]. Most studies attempted to address several issues: (a) the capacity of bone marrow-derived cells to engraft and repopulate the liver; (b) the role of liver injury and its severity in bone marrow engraftment; (c) which population of bone marrow cells, if any, is responsible for liver repopulation; and (d) how functional are bone marrow-derived liver cells.

Peterson *et al*. studied hepatic trans-differentiation after cross-sex or cross-strain bone marrow transplants into lethally radiated rats followed by giving 2-AAF to suppress hepatocyte proliferation and by giving carbon tetrachloride to induce liver injury. Donor-derived hepatic cells were found in host animals post-transplant [44]. The authors concluded bone marrow-derived cells were hepatocyte progenitors. Bone marrow-derived cells are also reported to repopulate the normal rodent liver. Theise *et al*. [63] transplanted bone marrow cells from male mice into irradiated female mice and found substantial numbers of donor-derived hepatocytes. Other studies in mice indicate that a single male hematopoietic cells transplanted into an irradiated female recipient can differentiate into several tissues including epithelial cells and cells in the gastrointestinal tract, bronchus, and skin [64].

Unfortunately, results of these and similar studies are inconsistent. Wagers *et al*. [66] performed single cell transplants of green fluorescent protein (GFP)-marked transgenic hematopoietic cells into sublethally irradiated normal mice. Hepatocytes were found only at very low frequency (1 in 70,000 cells) despite complete donor hematopoietic engraftment. Wang *et al*. reported transplants of hematopoietic cells corrected liver disease in a mouse model of tyrosinemia. They show bone-marrow-derived hepatocytes can repopulate the liver of mice by cell fusion [67]. In contrast, Jane *et al*. [68] reported infusion of bone marrow-derived hematopoietic cells into mice with liver injury convert into hepatocytes after infusion and improve liver function without cell fusion. These contradictory data come from studies in two mouse models of liver injury. Whether either is correct is unclear.

Kanazawa *et al*. generated a GFP^+/GFP^- parabiotic mice. They observed hematopoietic cross-engraftment without engraftment of nonhematopoietic tissues. They also used different liver injury models to evaluate hepatic regeneration after gender-mismatched bone marrow transplants with no detectable contribution of bone marrow-derived cells to liver cells [69]. Other studies report greater adult stem cell plasticity suggesting that moderate to severe liver injury enhances the level of hepatic differentiation of hematopoietic cells [70].

The effect of liver injury on differentiation of hematopoietic cells to liver cells remains unclear because of these contradictory data. Different bone marrow-derived cell populations might have different ability for transdifferentiation into liver cells. Most initial studies used unfractionated bone marrow cells making it difficult to address this question.

A series of studies using an in vivo mouse model (the GFP/CCl_4 model) reported GFP-positive bone marrow cells infused intravenously efficiently repopulated cirrhotic livers, reduced liver fibrosis, increased serum albumin levels, and significantly decreased survival rate [65, 71]. These studies also reported increased production of collagenases including matrix metalloproteinase (MMP)−9 after bone marrow cell infusion [71]. Another study reported increased expression of fibroblast growth factor (FGF) receptors after bone marrow cell infusion and enhanced repopulation of GFP-positive bone marrow cells with increased Liv-2 positive cells after giving FGF-2 [72]. These data suggest FGF-2 is an important liver growth factor. Some data suggest pretransplant splenectomy enhances repopulation of the cirrhotic liver and decreases fibrosis by increased expression of MMP-9 produced by donor bone marrow cells favoring the notion some bone marrow cells capable of repopulating the liver may be trapped in the spleen after intravenous injection [73].

Suh *et al*. [74] used in vitro co-culture of bone marrow cells with hepatic stellate cells in a mouse carbon tetrachloride-induced liver fibrosis model. They reported infusion of co-cultured bone marrow cells reduced liver fibrosis and enhanced hepatic expression of IL-10. They also reported two distinct bone marrow cell subpopulations [CD11b(+) Gr1(high) F4/80(−) and CD11b(+) Gr1(+) F4/80(+)] suppress expression of collagen and α-smooth muscle actin in hepatic stellate cells via IL-10. Consistently, human bone marrow cells express more IL-10 after co-culture with human hepatic stellate cells lines (LX-2 or hTERT). Serum IL-10 levels were significantly increased in persons with liver cirrhosis after a bone marrow autotransplant [74].

Although the presence and severity of liver injury may be important in regulating the extent of bone marrow cells plasticity and engraftment, there is marked disagreement between reports. Detailed analyses of these models show different subpopulations of bone marrow cells may have different levels of functional plasticity. Initial studies used unfractionated bone marrow cells. However, subsequent studies suggest $CD34^+$ bone marrow cells have higher levels of hepatic engraftment. Whether this is so is controversial [75].

Despite these extensive experimental data, much of it contradictory and/or controversial, the fundamental question of whether bone marrow-derived cells can differentiate into liver cells remains unresolved. Most recent data support the notion of a paracrine effect of transferred bone marrow cells on improved liver function rather than transdifferentiation of bone marrow cells into hepatocytes.

Bone Marrow-Derived Mesenchymal/Stromal Cells in Animal Models

Studies *in vitro* and *in vivo* report the ability of MSCs to improve hepatocyte function and proliferation by providing cytokines (such as hepatocyte growth factor [HGF], EGF, IL-6, SCF, and tumor necrosis factor alpha [TNF-α]), connexins, cell contact, and extracellular matrix (reviewed in 76) rather than by cell replacement. In addition, anti-inflammatory properties of MSCs provide promising therapeutic potential for liver diseases, specially in acute or acute on chronic hepatic conditions.

Macrophages with the F4/80 cell surface marker and MSCs are two subpopulations of bone marrow cells able to repopulate the liver of cirrhotic mice in the GFP/CCl_4 model [71]. In another report in a mouse model, overexpression of CXCR4, a specific receptor for SDF, enhanced mobilization and engraftment of MSCs into small-for-size liver grafts where these cells promoted early regeneration of the remnant liver probably by a paracrine mechanism [77].

Kuo *et al*. reported a high frequency of multi-potent bone marrow derived MSCs differentiating into functional hepatocyte-like cells under highly defined experimental conditions. The authors induced lethal liver failure in NOD/SCID mice using carbon tetrachloride followed by intrasplenic or i.v. transplants of MSC-derived hepatocytes and undifferentiated MCSs. Infused cells in both groups engrafted into the recipient livers and differentiated to functional hepatocytes which prevented death from liver failure. Interestingly, intravenous transplants were more effective than intrasplenic transplants [78]. MSCs were more resistant to reactive oxygen species in vitro and reduced oxidative stress in recipient mice. MSCs also accelerated repopulation of hepatocytes after liver damage. These data are consistent with paracrine effects of MSCs. In a similar route, Ali *et al*. [79] reported a nitric oxide donor (sodium nitroprusside) augments the ability of MSCs to repair carbon tetrachloride-induced liver fibrosis in mice.

Wang *et al*. also reported that paracrine signals from liver MSCs direct hepatic stem cells into specific adult cell fates. In this study, subpopulations of liver-derived MSCs including angioblasts, mature endothelia, hepatic stellate cell precursors, mature stellate cells (pericytes), and myofibroblasts were isolated. Later, each of these populations were co-cultured with human hepatic stem cells. Feeders of angioblasts yielded self-replication, stellate cell precursors caused lineage restriction to hepatoblasts, mature endothelia produced differentiation into hepatocytes, and mature stellate cells and/or myofibroblasts resulted in differentiation to cholangiocytes. Paracrine signals of each feeder were identified after analysis and feeders were replaced by adding them in media [80]. Similarly, a supportive role of bone marrow-derived MSCs on liver function is reported in large animals. Li *et al*. transplanted human bone marrow-derived MSCs into pigs with fulminant hepatic failure. All control animals died. In contrast, most animals in the intraportal injection cohort survived more than 6 months. In immune histochemical staining for human albumin, hepatocytes derived from human bone marrow-derived MSCs were widely distributed in the hepatic parenchyma 2–10 weeks after infusion and 30% of hepatocytes were of human origin. However, numbers of human cells decreased significantly at week 15 and few cells were found in regenerated liver lobules at week 20. These data suggest a possible "bridging" effect for transplants to support autologous liver recovery [81]. A recent report claimed development of fetal liver buds in co-cultures of human iPSC-derived hepatic endoderm cells with human umbilical vein endothelial cells and human MSCs suggesting supportive role of these cells in liver development [82].

Possible Mechanisms by Which Bone Marrow Cells Might Improve Liver Function

During fetal development and in some diseases, hematopoiesis occurs in the liver in humans. Several, not mutually exclusive ways whereby bone marrow and other hematopoietic cells might affect or influence hepatic function and regeneration are indicated below, (reviewed in references 48, 76, 83 Fig. 3).

a. *New hepatocytes*: Transdifferentiation of bone marrow cells to hepatocytes. Some early studies support the notion bone marrow cells can transdifferentiate into hepatocytes. However, most recent studies are inconsistent with this notion [69, 81].
b. *Replacement of oval cells*: Although some data we cite suggest oval cells can develop from bone marrow progenitors, most data support an intrahepatic origin [46, 84].
c. *Paracrine effects*: Autocrine signaling is a form of signaling in which a cell secretes a hormone or chemical messenger (ligand) which binds receptors on the same cell. This receptor–ligand interaction results in biological changes in the cell which, in turn, fosters more ligand release. Paracrine signaling is a form of signaling in which a cell produces a signal to induce changes in nearby cells altering their behavior [83, 85]. As mentioned above, bone marrow cells can support hepatocyte function and liver regeneration through paracrine effects, providing extracellular matrix and anti-inflammatory effects (reviewed in 76). Some data suggest liver injury mobilizes bone marrow cells by releasing growth factors, cytokines, and chemotactic factors such as SDF-1, HGF, G-CSF [52]. Also, degradation of extracellular matrix by metalloproteinase [52, 71] and maintaining redox homeostasis [86] may reduce liver fibrosis and injury. Release of cytokines and growth factors by bone marrow cells is suggested to activate hepatocyte progenitor cells and increase proliferation of mature hepatocytes [48, 83].
d. *Amplifying liver regeneration through liver sinusoidal endothelial cells and their paracrine effects (LSECs)*: LSECs are small cells that spread into a very thin layer lining the hepatic sinusoids (Fig. 1A). In healthy liver, hepatocytes and hepatic stellate cells maintain the phenotype of LSECs by releasing vascular endothelial growth factor (VEGF). After liver injury and partial hepatectomy, bone marrow progenitor cells of LSECs (BM-SPCs) are recruited into the liver that results proliferation of BM SPCs to more than twofold and mobilization of BM-SPCs to the circulation twofold to fourfold in rodents. Recruited BM-SPCs are rich source of HGF [87].
Hepatic VEGF is a central regulator of BM-SPC recruitment which is critical to liver regeneration and increases in response to many forms of liver injury including partial hepatectomy. Hepatic VEGF has been shown to regulate each step of BM-SPC recruitment to the liver, proliferation of BM-SPCs, mobilization of BM-SPCs to the circulation, engraftment of BM-SPCs in the liver, and differentiation of BM-SPCs to fenestrated LSECs lining the sinusoids (reviewed in 87). Whether these effects occur in humans is unknown.

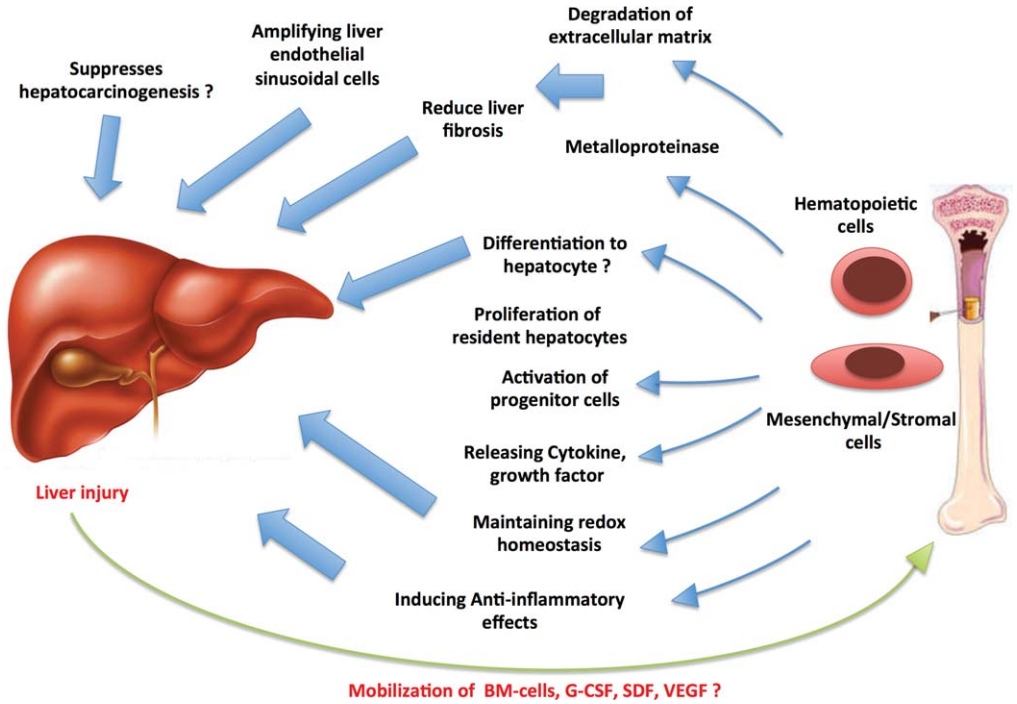

Figure 3. Possible mechanisms by which bone marrow cells might improve liver function. Abbreviations: BM, bone marrow; G-CSF, granulocyte colony-stimulating factor; SDF, stromal derived factor; VEGF, vascular endothelial growth factor.

e. *Immune modulation*: Modification of tissue macrophages (Kupffer cells) posttransplant is another possible bone marrow-liver interaction [88]. Anti-inflammatory effects might also promote improved liver function including: switching macrophages from M1 (classically activated macrophages: iNOS- or CD16/32-positive) to M2 (alternatively activated macrophages: arginase-1- or CD206-positive), the release of MSC-derived anti-inflammatory molecules (such as tumor necrosis factor-α [TNF-α] stimulated gene/protein 6 [TSG-6]) and reduced apoptosis of injured tissues [76].

Clinical Trials

Driven by the limited numbers of donor livers there are increasing trials of novels approaches to treat acute and chronic liver diseases. We focus on the ability of hematopoietic growth factors and of bone marrow-derived cells including hematopoietic cells and MSCs on improving advanced liver disease. Most trials are in early stages, include few subjects, and are designed to test safety rather than efficacy. Nevertheless, many authors comment on efficacy. These early trials involve complex issues of subject selection, use of hematopoietic growth factors (G- or GM-CSF) to collect blood or bone marrow cells, techniques of hematopoietic cell collection and processing, route of administration and response criteria (Tables 1–3; 89-123).

Trials of G-CSF

In several studies, subjects received G-CSF to mobilize hematopoietic cells into the blood for subsequent collection by apheresis. Although this strategy is effective in healthy individuals, there are few data on the types of bone marrow cells mobilized by G-CSF in persons with advanced liver disease many of whom have extensive portal hypertension and splenomegaly. Chronic liver failure itself probably does not stimulate mobilization of bone marrow cells into the blood. In a recent study, persons with chronic cirrhosis had similar numbers of blood $CD133^+/CD34^+$ cells as normals [121].

Safety and feasibility of giving G-CSF to persons with liver diseases (Table 1) was evaluated in several studies. For example, Gaia *et al*. studied the pattern of bone marrow cell mobilization in eight subjects with liver cirrhosis receiving G-CSF, 10 μg/kg per day, for 3 days. There were no controls. No important adverse events were seen save for a slight increase in spleen size. The authors reported clinical improvement which correlated with increased numbers of bone marrow-derived $CD34^+$ cells in the blood [89]. Campill and coworkers reported 24 subjects with acute or chronic liver failure who received G-CSF. They reported no adverse events and saw mobilization of $CD34^+$ cells into the blood [90]. Spahr and coworkers randomized 24 subjects with alcohol-induced fatty liver to receive G-CSF, 10 μg/kg per day, for 5 days or a placebo. G-CSF mobilized $CD34^+$ cells into the blood, increased serum HGF, and induced hepatocyte progenitor cells to proliferate within 7 days. However, liver function was not improved compared to controls [91].

Recently, Garg *et al*. reported a controlled, blinded (but not randomized) study in which 47 subjects with acute-on-chronic liver failure were randomized to receive G-CSF (5 μg/kg subcutaneously for 12 doses) or a placebo. Survival at 2 months was better in subjects receiving G-CSF. The authors also claimed that G-CSF therapy significantly reduced Child-Turcotte-Pugh score (CTP), model for end-stage liver disease (MELD), and sequential organ failure assessment score scores and decreased incidences of sepsis, hepato-renal syndrome, and hepatic encephalopathy compared with placebo controls [93].

Although these reports using G-CSF in persons with advanced liver disease are promising they do not address

Table 1. Clinical trials using mobilizing bone marrow cells with G-CSF with or without cell infusion

Reference	Patient group	Number of patients	Baseline measures	Source of stem cell	Type of bone marrow cells	Site of injection	Number of infused cells	Outcome time
(a) Only G-CSF								
Gaia *et al*., 2006 [89]	Severe liver cirrhosis	Study: 8, control: 40 healthy cell donors	CTP	N/A	G-CSF (5 μg/kg bid for 3 days)	N/A	N/A	G-CSF mobilization of BM occurs in cirrhotic patients even in the presence of splenomegaly
Di Campli *et al*., 2007 [90]	Acute on chronic liver failure	24	N/A	N/A	G-CSF (5 or 15 μg/kg S.C. for 6 days)	N/A	N/A	G-CSF mobilized CD34+ cells, in patients with acute on chronic liver failure
Spahr *et al*., 2008 [91]	Alcoholic steatohepatitis	Study: 13, control: 11	CTP-liver biopsy	N/A	G-CSF (10 μg/kg/da S.C. for 5 days)	N/A	N/A	G-CSF mobilized CD34+ cells, increase HGF, and induces hepatic progenitor cells to proliferate within 7 days
Lorenzini *et al*., 2008 [92]	HCV, alcoholic liver cirrhosis	18	MELD/CTP	N/A	G-CSF (optimum 15 μg/kg/da S.C. for 5 days)	N/A	10×10^6	G-CSF mobilized CD34+ cells, no significant clinical improvement in 1 month
Garg *et al*., 2012 [93]	Acute on chronic liver failure	Study: 22, control: 23	MELD/CTP/SOFA	N/A	G-CSF (5 μg/kg SC, 12 doses)	N/A	N/A	Improvement of liver function and MELD score
(b) G-CSF with infusion of mobilized blood cells								
Gordon *et al*., 2006 [94]	HBV or HCV, alcoholic cirrhosis, PSC	5	CTP	Peripheral blood, G-CSF mobilized cells	Autologous, $CD34^+$	Portal vein in three cases hepatic artery in two cases	10^6-10^8	Improvement in albumin or bilirubin in three of five patients after 60 days
Yannaki *et al*., 2006 [95]	Alcoholic cirrhosis	2	CTP, MELD	Peripheral blood post-G-CSF	BM-$CD34^+$	Peripheral vein	$2–4 \times 10^6$ per kg	Improvement in the CTP and MELD scores after 30 months
Gasbarrini *et al*., 2007 [96]	Drug-induced acute liver failure	1	Liver function tests and clotting tests	Peripheral blood, post-G-CSF	BM-$CD34^+$	Portal vein	5×10^6	Improvement in clotting (PT), AST and ALT up to 30 days, patient dies of sepsis at day 60
Pai *et al*., 2008 [97]	Alcoholic liver cirrhosis	9	CTP	Peripheral blood	Post-G-CSF, $CD34^+$ cells expanded in vitro	Hepatic artery	2.3×10^8	Improvement in bilirubin and liver enzymes after 12 weeks

Table 1. *Continued*

Reference	Patient group	Number of patients	Baseline measures	Source of stem cell	Type of bone marrow cells	Site of injection	Number of infused cells	Outcome time
Khan *et al*., 2008 [98]	HBV, HCV	4	CTP/MELD	Peripheral blood	Autologous, post-G-CSF, $CD34^+$ cells	Hepatic artery	0.1×10^8	Improvement in liver function after 1 month
Han *et al*., 2008 [99]	HBV	G-CSF only: 20 G-CSF + cell transplant: 20	CTP	Peripheral blood	Peripheral blood monocyte cell	N/A	??	Significantly improved liver function was observed in cell transplanted group after 6 months
Levicar *et al*., 2008 [100]	HCV, alcoholic cirrhosis, PSC	5	Liver function tests + CTP	Leukapheresis	BM-$CD34^+$ cells after G-CSF mobilization	Portal vein or hepatic artery	1×10^6 and 2×10^8	Initial improvement in four patients, beneficial effect seemed to last for around 12 months
Salama *et al*., 2010 [101]	HCV:36 AIH: 12	48	Bilirubin ALT, INR albumin/ MELD	Peripheral blood post-G-CSF	Partially differentiated BM-$CD34^+$	Hepatic artery or portal vein	1 billion	Improvement of liver function test specially in autoimmune group. 20.8% of patients died during 1 year follow-up

Abbreviations: AIH, autoimmune hepatitis; ALT, Alanine aminotransferase; BM, bone marrow; CTP, Child-Turcotte-Pugh score; G-CSF, granulocyte colony-stimulating factor; HGF, hepatocyte growth factor; HBV, hepatitis B virus; HCV, hepatitis C virus; INR, International normalized ratio; MELD, model for end-stage liver disease; PSC, primary sclerosing cholangitis; SOFA, sequential organ failure assessment score.

Table 2. Clinical trials using bone marrow-derived hematopoietic cells for liver disease*

Reference	Patient group	Number of patients	Baseline measures	Source of stem cell	Type of bone marrow cells	Site of injection	Number of infused cells	Outcome/time
Am Esch *et al.*, 2005 [102]	Patients with liver cancer, no cirrhosis	Study: 3, control: 3	Liver volumetry	Iliac crest	BM-CD133$^+$ after PVE	Portal vein	2.4–12.3 $\times$ 10^6	2.5-fold volume increase in left lobe in study group on CT volumetry 150 days
Terai *et al.*, 2006 [103]	Liver cirrhosis patients	9	CTP and albumin	Iliac crest	Concentrated BM-mononuclear cell	Peripheral vein	5.20 $\times$ 10^9	Improvement CTP and albumin after 24 weeks
Fürst *et al.*, 2007 [104]	Patients with liver cancer, no cirrhosis	Study: 6, control: 7	Liver volumetry	Iliac crest	BM-CD133$^+$ after PVE	Portal vein	8.8–12.3 $\times$ 10^6	Increased hepatic regeneration compared with control up to 5 weeks
Lyra *et al.*, 2007 [105]	Alcoholic, HCV, cholestatic, and cryptogenic cirrhosis	10	CTP	Iliac crest	Mononuclear-enriched BM-cells	Hepatic artery	100 millions	Improvement in CTP bilirubin, and albumin after 4 months
Mohamadnejad *et al.*, 2007 [106]	Decompensated cirrhosis	4	MELD	Iliac crest	BM-CD34$^+$ cells	Hepatic artery	3–10 $\times$ 10^6	Two patients showed minor improvement in albumin, one had nephropathy and hepatorenal syndrome, trial was terminated after 12 months
Lyra *et al.*, 2010 [107]	HBV, HCV, alcoholic, and cryptogenic cirrhosis	Study: 15, control: 15	CTP/MELD	Iliac crest	BM-Mononuclear-enriched cells	Hepatic artery	N/A	Improvement in liver function after 1 year
Kim *et al.*, 2010 [108]	HBV	10	CTP/MELD	Iliac crest	BM-cells	Peripheral vein	10^8 cells/kg	Improvement in albumin, quality of life, and the CTP after 1 year
Couto *et al.*, 2011 [109]	HCV, alcoholic and nonalcoholic cirrhosis	8	MELD/(99m)Tc scan, scintigraphy	Iliac crest	Autologous BM-MNCs	Hepatic artery	2.0–15.0 $\times$ 10^8 cells	One case of dissection of the hepatic artery and one case of Tako-tsubo syndrome occurred.
Nikeghbalian *et al.*, 2011 [110]	AIH, cryptogenic cirrhosis, hemochromatosis	6	MELD	Iliac crest	Bone marrow-derived CD133$^+$ or BM-MNCs	Portal vein	CD133$^+$: 6.4 $\pm$ 3.2 $\times$ 10^6, MNC: 13 $\times$ 10^8	Improvement of liver function test in both group after 24 months
Ismail *et al.*, 2010 [111]	Liver cirrhosis with HCC	Study: 10, control: 10	Liver function tests + CTP + liver volumetry	N/A	BM-MNCs liver resection	N/A	N/A	Improved surgical outcome 12 weeks postoperative in comparison to placebo
Saito *et al.*, 2011 [112]	Alcoholic liver cirrhosis	Study: 5, control: 5	Liver function type IV collagen 7S domain, Indium-111-scintigraphy	Iliac crest	BM-MNCs	Peripheral vein	8–7.3 $\times$ 10^9	Improvement of liver function test in three patients

Table 2. *Continued*

Reference	Patient group	Number of patients	Baseline measures	Source of stem cell	Type of bone marrow cells	Site of injection	Number of infused cells	Outcome/time
Chernykh *et al*., 2007 [113]	HBV, HCV, HDV, alcoholic hepatitis, AIH, cryptogenic cirrhosis	47 patients	Liver function tests + CTP	Iliac crest	BM-CD34$^+$ cells	N/A	78×10^7	Improvement of liver function test after 12 months
am Esch *et al*., 2012 [114]	Patients with liver mass	a. PVE and CD133$^+$: 11, b. PVE; 11, c. controls: 18	Liver function tests and liver volumetry	Iliac crest	BM-CD133$^+$ cell after PVE	Portal vein	N/A	Higher hepatic growth in stem cell transplanted group and better survival after 28 months

Abbreviations: AIH, autoimmune hepatitis; BM, bone marrow; CTP, Child-Turcotte-Pugh score; HBV, Hepatits virus; HCV, hepatitis C virus; HDV, hepatitis D virus; HCC, hepatocellular carcinoma; MELD, Model for End-Stage Liver Disease; MNC, mononuclear cells; PVE, Portal vein embolization; TACE: transarterial chemoembolization

long-term benefits of this approach. Most studies had no controls, placebo or otherwise, and were not blinded or randomized. Multiple etiologies of liver failure were included and it is unclear whether improvements in liver function tests, if any, are related to a direct effect of G-CSF on liver cells, increased numbers of bone marrow-derived cells in the blood, both, or neither. One should be cautious giving G-CSF to persons with liver cirrhosis and splenomegaly because of the risk of spleen rupture.

Trials of G-CSF and Infusion of Bone Marrow Cells Mobilized into the Blood

Several trials evaluated outcomes of giving G-CSF to persons with advanced liver disease, collecting blood cells presumably containing cells released from the bone marrow and reinfusing these cells into the subject, a form of autotransplant. For example, Gordon *et al*. collected CD34$^+$ cells from the blood after giving G-CSF to five subjects with virus-related or alcoholic cirrhosis. They cultured these cells in minimal essential medium (MEM) supplemented with fetal bovine serum and cytokines and give the cells to the subjects via a hepatic artery. The authors reported decreased levels of serum bilirubin and increased levels of albumin in some subjects. There are, of course, several issues with this study including few subjects, lack of controls, no blinding, and a short observation interval (2 months) [94]. The use of bovine serum albumen in the cultures is a concern because of the possible induction of serum sickness.

Pai *et al*. studied nine subjects with alcoholic liver cirrhosis. They gave G-CSF and collected bone marrow-derived blood cells by leukapheresis. CD34$^+$ cells were isolated and expanded in vitro in MEM supplemented with human serum and cytokines for 7 days and infused into the subjects via the hepatic artery. The authors report short-term (90-day) improvement of serum albumin, CTP score, and ascites in most subjects. Again, there were neither controls nor blinding [97].

Lorenzini *et al*. reported giving G-CSF followed by collection of bone marrow-derived mobilized blood cells was safe in 18 subjects with liver cirrhosis. Oddly, collected cells were not reinfused. No improvement in liver function [92]. Salama *et al*. studied 48 subjects 36 of whom had chronic end-stage hepatitis C-virus-induced liver disease and 12 of whom had end stage autoimmune liver disease. Subjects received G-CSF and their blood cells were collected. CD34$^+$ cells were isolated, expanded, and partially differentiated in vitro toward hepatocytes. The cells were then injected into the subjects via the hepatic artery or portal vein. The authors reported 20% improvement in survival at a year compared to historical controls based on MELD scores [101]. Problems with the use of historical controls and other biases preclude accepting these conclusions without validation in a randomized study.

A few studies compared safety and efficacy of giving G-CSF only versus giving G-CSF followed by blood cell collection and an autotransplant. Han *et al*. gave G-CSF to 40 subjects with Hepatitis B virus (HBV)-induced decompensated liver cirrhosis. Some subjects also received collected blood cells; others did not. The study was not randomized, blinded, or placebo-controlled. Infusing blood cells after G-CSF therapy was claimed to be associated with a better outcome including improved serum albumin levels and CTP scores at 6 months compared with subjects receiving only G-CSF [99].

Recently, Spahr *et al*. reported a randomized, controlled open-label trial of 58 subjects with decompensated alcoholic

Table 3. clinical trials using bone marrow mesenchymal stromal/stem cells for liver diseases

Reference	Patient group	Number of patient	Baseline measures	Source of stem cell	Type of bone marrow cells	Site of injection	Number of infused cells	Outcome time
El-Ansary *et al*., 2012 [115]	Chronic hepatitis C virus	I-MSCs group: 15 Ia & Ib (undifferentiated and differentiated), control: 10	MELD, liver function test, CTP	Iliac bone	BM-derived undifferentiated and differentiated MSCs	Portal vein	1 million MSCs/kg	Undifferentiated or differentiated MSC showed partial improvement after 6 months
Liao *et al*., 2012 [116]	Cirrhosis + portal hypertension caused by HBV	Control: 6, study: 6	Liver function tests	Iliac crest	$CD117^+$ cells and $CD184^+$ cells after in vitro expansion for 7 days	Hepatic artery during splenectomy	8.24×10^7	Improved hepatic function after 24 hours in study group
Peng *et al*., 2011 [117]	Liver failure caused by hepatitis B	Control: 422, study: 105	MELD, liver function test	N/A	Autologous BM-MSCs	Hepatic artery	N/A	Short-term efficacy was favorable, but long-term outcomes were not markedly improved
Kharaziha *et al*., 2009 [118]	Hepatitis B or C, alcoholic and cryptogenic	8	MELD	Iliac crest	Autologous BM-MSCs	Peripheral or portal vein	30–50 million	Significant improvement in MELD Score after 24 weeks
Mohamadnejad *et al*., 2007 [119]	Decompensated cirrhosis	4	MELD	Iliac crest	Autologous BM-MSCs	Peripheral vein	31.37×10^6	MELD score improvement in 2 patients after 6 months
Amer *et al*., 2011 [120]	HCV cirrhosis	Study: 20, control: 20	CTP, MELD, fatigue scale	Iliac crest	Partial differentiated BM-MSCs	Intrasplenic and portal vein?	2×10^8	Safety and short-term efficacy was reported

Abbreviations: BM-MSC, bone marrow mesenchymal stem/stromal cells; CTP, Child-Turcotte-Pugh score; HBV, Hepatitis B virus; HCV, Hepatitis C virus; MELD, model for end-stage liver disease.

cirrhosis comparing conventional standard medical therapy to similar therapy combined with G-CSF and collection and infusion of blood cells into the hepatic artery. Marginal improvement was claimed for both cohorts [122].

Bone Marrow Hematopoietic Cells

Results of treating persons with advanced liver disease by collecting and infusing unselected autologous bone marrow mononuclear cells are reported in several studies. Terai *et al*. studied nine subjects with liver cirrhosis and reported no viable hepatocellular carcinoma on diagnostic imaging. Bone marrow cells were infused via the portal vein. The authors claim improved serum albumin levels, total protein levels, and CTP score at 6 months [103]. The study was small, uncontrolled and not blinded. In a similar uncontrolled study, Kim *et al*. reported increased serum albumin levels, CTP score, liver volume, and accumulation of ascites in 10 subjects with HBV-related decompensated liver cirrhosis. They reported gradual activation of the hepatic progenitor cell from liver biopsies which peaked after 3 months suggesting the possibility of hematopoietic cells activation as the underlying mechanism of clinical improvement. There was no significant change in grade or stage of liver fibrosis or stellate cell activation [108]. A follow-up study compared autologous bone marrow infusion via a peripheral vein in five subjects with alcoholic liver cirrhosis and five subjects with similar condition as controls. There is no indication the investigators were blinded. The authors report short-term improved liver function tests. They also measured type IV collagen 7S domain levels in blood as a surrogate of fibrosis and claimed improvement. Indium-111-chloride bone marrow imaging showed bone marrow activation in some subjects [112].

In a controlled but neither blinded nor randomized study by Lyra *et al*., 30 subjects on a liver transplant waiting list received either a portal artery infusion of autologous bone marrow mononuclear cells ($n = 15$) of not ($n = 15$). Albumin levels and CTP scores improved in the treatment arm at 90 days follow-up [107].

In a recent study after two other similar trials from this group, am-Esch *et al*. evaluated safety and efficacy of infusion of $CD133^+$ bone marrow cells immediately before portal vein embolization and extended right hepatectomy in 11 subjects with liver cancer. The authors reported faster liver regeneration in persons receiving bone marrow cells compared to controls. This is a retrospective, nonblinded, nonrandomized study [102, 104, 114]. Consequently, validation of the authors' conclusions is needed.

Bone Marrow-Derived Mesenchymal/ Stromal Cells

Amer *et al*. infused partially differentiated bone marrow-derived MSCs into 20 subjects with HCV-induced cirrhosis via the intrasplenic or portal vein. The authors compared outcomes with that of 20 subjects receiving conventional therapy [120]. They claim recipients of MSCs by either route had improved Child and MELD scores and less fatigue compared to controls. However, this study was neither randomized nor blinded [120].

El-Ansary *et al*. [115] reported intravenous infusion of bone marrow-derived MSCs resulted in partial improvement of liver function tests in 15 subjects with HCV-induced cirrhosis compared to a placebo control group ($n = 10$). There were no controls. Peng *et al*. studied five subjects with HBV-related liver cirrhosis who received MSCs composed of $CD34^-CD44^+$ and $CD44^+CD45^-$ cells infused into the hepatic artery. One hundred and five subjects matched for age, gender, and liver biochemical indexes were controls. The authors claimed improvement of liver function tests and MELD scores within 2–3 weeks post-transplant compared with controls. By 4 years survival and incidence of hepatocellular carcinoma were similar [117]. This study has several limitations; it was neither blinded nor randomized. HBV viral load, genotype, and E antigen state for subjects and controls were not matched. The MSC population was poorly defined and subjects receiving anti-virus therapy were excluded [123].

Current Clinical Trials

There is considerable demand for new therapies for acute and chronic liver diseases. The magnitude of this unmet medical need is enormous and current treatments are inadequate. The potential regenerative capacity of bone marrow-derived cells has led to early phase clinical trials. Although results are encouraging, a critical analysis of efficacy based on these studies is premature. Short-term follow-up of the phase-1 studies suggest bone-marrow derived hematopoietic cells and MSCs safe. These preliminary data suggest benefit is short-lived and most likely to occur in persons with acute liver disease.

There is considerable diversity in clinical studies in subject selection, use of G- and GM-CSF, methods of hematopoietic cell collection and processing, route of administration, and response criteria. Which cells (bone marrow *vs*. blood, hematopoietic *vs*. stromal, etc.) are best, if any, is unresolved. Most studies included persons with liver failure from diverse etiologies including HBV, HCV, autoimmune hepatitis, alcoholic and cryptogenic cirrhosis. This diversity further adversely impacts our ability to determine efficacy. Focusing on one or only a few types of liver damage is recommended in future studies.

The studies we discuss used variety techniques for collecting bone marrow-derived cells and for processing them pre-transplant. In vitro expansion of mononuclear cells was used in some but not other studies. Some studies used cells the authors claimed were partially differentiated towards becoming hepatocytes. This requires culturing cells for several weeks in media containing animal-derived serum which can result immune reactions in the recipient and the risk of oncogenic transformation in vitro.

Diverse numbers of hematopoietic cells were transplanted in these studies from one million to several billion. No correlation between numbers of cells transplanted and response is reported but this is obviously not carefully studied. Nor is whether there is a benefit to culturing or trying to differentiate cells toward the hepatic lineage.

Different routes of injection were used in these trials. In most, hematopoietic cells were infused into the portal vein. However, this approach may cause transient portal hypertension and limit engraftment if there is hepato-fugal flow. Other studies infused cells into the hepatic artery or a peripheral vein. In an animal study in acute fulminant hepatitis only intraportal infusion of bone marrow-derived cells increased survival [81]. Consequently which route, if any, is best, also remains unknown.

Efficacy of bone marrow transplants in correcting liver failure was evaluated using liver function tests including bilirubin, albumin, and international normalized ratio and by

evaluation of ascites and CTP and MELD scores. Occasionally, liver biopsy pretransplant and/or post-transplant or measuring liver volume was used. All of these measures are unvalidated surrogates of survival in this setting. Survival must be the bottom line for definitive studies but no reliable survival data were reported in the studies we reviewed.

Transient or persisting improved liver function tests, bilirubin, and albumin, quality of life, and clinical variables such as ascites are frequently reported in these studies. However, most studies are too small to evaluate survival. Few studies advocate improved survival in short term or in acute conditions (Tables 1–3).

Some minor complications are reported including mild pain and discomfort at the site of cell infusion, transient thrombocytopenia after leukapheresis and low-grade fever. Use of G-CSF causes transient fevers and bone pain in some subjects. The main concern is portal hypertension which is uncommon [101]. Bone marrow infusions are not always safe; life-threatening complications may occur. Mohamadinejad *et al*. reported a death in a subject from radio-contrast-induced nephropathy and hepato-renal syndrome after infusion of concentrated $CD34^+$ cells from 200 mL of bone marrow through a hepatic artery in a subject with decompensated liver cirrhosis [106]. Salama *et al*. also reported 3 of 48 subjects had serious complications likely from the procedure requiring hospitalization; one died. Complication may be less with hepatic artery infusion of bone marrow cells but this is unproved [101].

Future and Challenges

Recent studies of molecular genetics and new animal models indicate complex cellular and molecular interactions between hematopoietic cells and the liver. Nevertheless, important theoretical and practical questions need to be addressed before advancing to large clinical trials. For instance, despite plasticity of hematopoietic cells toward the hepatic lineage *in vitro*, few data, most of it controversial, support the notion of bone marrow-derived cells can develop into oval liver cells. Furthermore, it is uncertain hematopoietic cells can differentiate into hepatocytes in vivo at a clinically useful and stable level. Mechanism(s) of action of hematopoietic cells within the human liver are elusive.

The goal for transplants of bone marrow-derived cells in persons with advanced liver disease is to enhance regenerative capacity of the liver and/or promote degradation of fibrous matrix typical of liver failure. Apparently, transplants of bone marrow-derived cells can provide an environment promoting liver regeneration by transiently supplying growth factors. However, repeated infusions might be needed for success. Most data suggest anti-inflammatory and paracrine mechanisms under liver improvement, especially after infusions of MSCs. Hematopoietic cell transplants seem to have better outcomes than giving only G-CSF.

Hematopoietic cell transplants might not succeed if the appropriate extracellular matrix of the liver is compromised or destroyed. Although the long-term replication potential of transplanted hematopoietic cells is unclear; the potential for neoplastic transformation must be considered in any analysis of benefit risk ratio. This is especially important given the increased risk of liver cancer in persons with liver failure, especially in the context of chronic HBV infection.

If in vitro expansion and differentiation of hematopoietic cells toward hepatocytes is to be used, xenobiotic-free and feeder layer free growth and differentiation conditions must be established which are effective, reproducible, robust, and relatively inexpensive. This may include the development and use of small molecules and synthetic biocompatibles.

Data from clinical trials are encouraging but inconclusive. Transplants are not without risk and should be done only in the context of clinical trials where safety and efficacy are studied. Randomized, controlled, double-blind clinical trial designs should be performed with focus on specific etiologies of liver failure specially in acute conditions. Serial infusions might be considered based on clinical outcomes.

A comprehensive understanding of bone marrow cell physiology in animal models of liver disease is essential to improve likelihood of success of future clinical trials in persons with end-stage liver diseases

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