



# Clinical application of stem cell in patients with end-stage liver disease: progress and challenges

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**Abstract:** End-stage liver disease (ESLD) is life-threatening disease worldwide, and patients with ESLD should be referred to liver transplantation (LT). However, the use of LT is limited by the lacking liver source, high cost and organ rejection. Thus, other alternative options have been explored. Stem cell therapy may be a potential alternative for ESLD treatment. With the potential of self-renewal and differentiation, both hepatic and extrahepatic stem cells have attracted a lot of attention. Among them, multipotent stem cells are most widely studied owing to their characteristics. Multipotent stem cells mainly consist of two subpopulations: hematopoietic stem cells (HSCs) and mesenchymal stem cells (MSCs). Accumulating evidences have proved that either bone marrow (BM)-derived HSCs mobilized by granulocyte colony-stimulating factor or MSCs transplantation can improve the biochemical indicators of patients with ESLD. However, there are some challenges to be resolved before stem cells widely used in clinic, including the best stem cell source, the optimal route for stem cells transplantation, and the dose and frequency of stem cell injected. The purpose of this review is to discuss the potential of stem cell in liver diseases, particularly, the clinical progress and challenges of multipotent stem cells in the field of ESLD.

**Keywords:** End-stage liver disease (ESLD); stem cells; mesenchymal stem cells (MSCs); hematopoietic stem cells (HSCs)

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## Introduction

End-stage liver disease (ESLD), decompensated liver cirrhosis or liver failure, is the final stage of liver disease and is the major cause of mortality worldwide. Patients with ESLD should be referred to liver transplantation. However, serious problems, including shortage of donors, surgical complications, organ rejection and high cost, have restricted the use of liver transplantation (1). Other treatment strategies have been explored to mitigate the clinical challenge.

Stem cell therapy, an emerging field, has brought

substantial benefits to patients with various diseases and may also be a potential alternative for patients with ESLD. Considerable preclinical research and clinical trials have been performed. This article discusses the potential of stem cell in liver diseases. In particular, we focus on the clinical progress and challenges of multipotent stem cells in the field of ESLD.

## Hepatic stem cells

In response to mild acute liver injury and partial hepatectomy, the mature liver cells begin to proliferate to

reconstitute the liver mass, whereas in the setting of severe or chronic injury in which the proliferative capacity of hepatocytes and cholangiocytes is blocked, hepatic stem cells may contribute to liver regeneration (2).

Adult hepatic stem cells, mainly referred to hepatic progenitor cells (HPCs) in humans and oval cells in rodents, maintain quiescent in normal liver homeostatic condition. They are able to differentiate into hepatocyte and cholangiocyte lineages with stimulus from stem cell niche. Stem cell niche includes not only the site where stem cells are located but also the surrounding cells and the composition of microenvironment, all of which help to maintain the characteristics of stem cells by preserving their quiescent state in normal condition and regulating their proliferation and differentiation capacity after activation (3). The well-known niche for hepatic stem cells is the Canal of Hering. Hepatic stem cells can be activated with signaling factors and cytokines released by damaged hepatocytes, inflammatory cells and adjacent liver cells including Kupffer cells, hepatic stellate cells and liver sinusoidal endothelial cells (2,4).

However, the benefit of HPCs to chronic liver injury is controversial. Other than hepatocytes and cholangiocytes, HPCs may also differentiate into myofibroblasts and promote the development of fibrosis and even cirrhosis during chronic liver injury (5,6). The precise mechanism underlying hepatic differentiation of HPCs is still unclear, thus more efforts are needed to govern HPCs committed differentiation to hepatocytes and cholangiocytes both *in vivo* and *in vitro*.

### Pluripotent stem cells

Embryonic stem cells (ESCs) are pluripotent cells isolated from the inner mass of an embryo. They can differentiate into any cell type of three embryonic germ layers (7). Although human ESC-derived hepatocyte-like-cells (HLCs) have been reported to rescue liver function and promote liver repair in mice (8,9), the clinical application of ESCs and their differentiated progeny is still limited owing to the safe issue (tumorigenesis) and ethical concerns. Induced pluripotent stem cells (iPSCs) have pluripotent properties like ESCs. However, iPSCs may develop gene mutation during differentiation, which may even lead to teratoma formation (10).

Both HPCs and iPSC are considered as candidates for hepatocytes and hepatocyte-like cells generation. However, long-term culture of cells with traditional two

dimensional (2D) system *in vitro* may lead to the loss of stem cells characteristics and chromosome alterations in higher passages (11). Moreover, cells grown in 2D culture system cannot recapitulate *in vivo* cell polarization and cellular interaction. Recently, these gaps are partly filled by 3D culture technology, or term organoid culture techniques. Organoids retain a tissue structure and function similar to the original tissue *in vivo* under physiological and pathological state, paving a way for the study of tissue development, physiology and disease (11). Organoids can be generated from both tissue-resident stem/progenitor cells and pluripotent stem cells (12). Liver organoids allow long-term expansion of liver stem cells with the preservation of their stable chromosome and structural level over months, and these cells can differentiate into functional hepatocytes *in vitro* and upon transplantation *in vivo* (13), which has the advantage over iPSCs who have an unpredictable degree of differentiation and a higher risk for tumorigenesis. Up to now, most liver organoids studies are in preclinical stage.

### Multipotent stem cells

Multipotent stem cells, also known as adult stem cells, are characterized by a relatively limited differentiation potential and are able to generate specialized cell types of adult tissue (14). The two major populations of adult stem cells are hematopoietic stem cells (HSCs) and mesenchymal stem cells (MSCs). They may be mobilized and migrate to the injured liver, which form the basis of regenerative medicine for the treatment of specific diseases.

#### *Hematopoietic stem cells*

Human bone marrow (BM) and peripheral blood are easily available sources for HSCs isolation. HSCs are able to self-renew and differentiate to replenish short-lived mature blood cell types (15). HSCs also have the potential of plasticity, meaning that HSCs can generate non-hematopoietic cell types when exposed to appropriate stimuli, for example hepatocytes (16). Surface marker cluster of differentiation (CD) 34 is often used to identify HSCs in human beings and CD133 may represent a more stem cell-enriched subpopulation of the CD34+cells (7).

BM is the classical stem cell niche for HSC. A body of evidence has proved the importance of CXCR4/stromal-derived factor-1 (SDF-1) axis in the mobilization of HSCs from BM and in the homing of HSCs to damaged liver (17,18). Granulocyte colony-stimulating factor (G-CSF) has

**Table 1** Clinical application of HSCs or MNCs in patients with ESLD

Authors	Disease	Cell therapy	Source of MSCs	Dose of injection	Way of injection	No. patients	Outcome
Salama <i>et al.</i> (24)	End stage liver disease	Autologous CD34 <sup>+</sup> CD133 <sup>+</sup> stem cell	Bone marrow	0.5×10 <sup>8</sup> cells	Portal vein	90	Improvement of liver function
Gordon <i>et al.</i> (25)	Chronic liver failure	Autologous CD34 <sup>+</sup> stem cell	Peripheral blood	1×10 <sup>6</sup> to 2×10 <sup>8</sup>	Portal vein or hepatic artery	5	Improvement of ALB and bilirubin
Newsome <i>et al.</i> (28)	Liver cirrhosis	Autologous CD133 <sup>+</sup> stem cell	Peripheral blood	0.2×10 <sup>6</sup> /kg	Peripheral vein	26	No evidence of the improvement of liver function and MELD score
Bai <i>et al.</i> (29)	Decompensated cirrhosis	Autologous MNCs	Bone marrow	Unclear	Hepatic artery	32	Improvement of liver function and reduction of the incidence of serious complications
Terai <i>et al.</i> (30)	Liver cirrhosis	Autologous MNCs	Bone marrow	(5.20±0.63)×10 <sup>8</sup> cells	Peripheral vein	9	Improvement of ALB and Child-Pugh scores

HSCs, hematopoietic stem cells; ESLD, end-stage liver disease; ALB, albumin; MNCs, mononuclear cells; MELD, model for end-stage liver disease.

been proved to antagonize CXCR4 and SDF-1 interaction and mobilize HSC from BM to peripheral circulation (19,20). It is thought that the secretion of SDF increases during tissue injury, and these CXCR4 positive stem cells may response to the increased SDF-1 gradient by chemotaxis thereby migrating to damaged liver (21). Except for SDF-1, hepatocyte growth factor (HGF) is also upregulated in liver injury (22). HGF can facilitate the mobilization of stem cell via HGF/c-MET axis (23).

The administration of G-CSF would significantly increase peripheral CD34<sup>+</sup> cell counts and improve the outcome of patients with ESLD. Salama *et al.* (24) separated CD34<sup>+</sup> and CD133<sup>+</sup> stem cells in patients with ESLD after G-CSF administration for five consecutive days. These patients were subsequently infused with CD34<sup>+</sup> and CD133<sup>+</sup> through portal vein, and their liver functions were greatly improved, suggesting that these autologous stem cells infusion may serve as a possible therapeutic protocol. Gordon *et al.* (25) obtained CD34<sup>+</sup> cells from peripheral blood after G-CSF mobilization and observed the improvement of serum bilirubin and albumin in patients with liver failure after these CD34<sup>+</sup> cells infusion, and no adverse effect related to the procedure were observed. The mechanism of G-CSF in protecting against liver injury may be multiple, including but not limiting to its potential of mobilizing and homing HSCs (26). G-CSF may also increase the level of HGF and induce the proliferation of HPCs (27).

Although the promising studies have been reported (*Table 1*), a multicenter, open-label, randomized, controlled phase 2 trial performed by Newsome and colleagues found that G-CSF or autologous HSC infusions did not improve the disease severity, fibrosis, or quality of life in patients with compensated cirrhosis, as no evidence of differences in the change of model for end-stage liver disease (MELD) scores over time between the treatment and control groups was observed (28). Moreover, serious adverse events, such as ascites and encephalopathy, were more frequent in patients receiving stem cell transplantation. The reason why this study observed the absence of any effect of HSCs on the fibrosis and liver function might be that the dose and viability of transfused HSCs (0.2×10<sup>6</sup>/kg) in this report was lower than that in other studies (31). Besides, patients enrolled in Newsome study are heterogeneous at baseline. Some patients with ascites, variceal bleeding and/or encephalopathy are classified to compensated cirrhosis. The different background of the enrolled patients may affect the results. It may be hasty to doubt the effect of HSCs in ESLD. Thus, rigorous randomized and controlled clinical trials are required to confirm the efficacy of stem cell therapy in patients with severe ESLD.

In adults, HSCs predominantly reside in marrow cavity of long bones. MSCs are another major stem cell type in BM. Some investigators infused a mixed cell population derived from BM into the liver and also found the promising results. Bai *et al.* (29) demonstrated that the

infusion of autologous BM mononuclear cells (MNCs) in patients with decompensated cirrhosis was safe and effective, significantly improving liver function and short-term quality of life. Terai *et al.* (30) treated 9 cirrhotic patients with autologous MNCs through peripheral vein. Significant improvements in serum albumin, total protein and Child-Pugh scores were observed after MNCs infusion. AFP and proliferating cell nuclear antigen (PCNA) expressions were markedly elevated in liver biopsy tissue. However, it was unclear which subpopulation in the MNCs imparts the maximum benefits owing to the infused unsorted BM-derived stem cell. Herein, most clinical trials observed short-term adverse effects after stem cell transplantation. Nevertheless, concerns regarding the medium- to long-term adverse effects after stem cell therapy including progressive liver fibrosis and hepatocellular carcinoma should also be noted, as it has reported that BM-derived cells may contribute to the development of liver fibrosis and hepatocellular carcinoma (7). Thus, more clinical trials are needed to monitor the adverse effects for a long enough time to confirm the safety of stem cell therapy.

The mechanism of HSCs in alleviating liver damage is yet not fully understood. BM-derived HSCs of host origin can migrate to the liver and differentiate into hepatocytes and cholangiocytes (32). However, some publications demonstrated that the new appearance of hepatocytes in animal liver was caused by the fusion of HSCs with resident hepatocytes rather by the trans-differentiation of HSCs to hepatocytes (33-35). Some authors proposed that epithelial cells in the lung, skin, and liver could develop from BM-derived cells through a mechanism other than cell fusion (36). Other explanations for HSCs in the improvement of liver function are the induction of angiogenesis by releasing vascular endothelial growth factor (VEGF) and the suppression of apoptosis by upregulating the Bcl-2 (2 B-cell leukemia/lymphoma 2) gene (37,38). In addition, HSCs may stimulate the oval cells in the liver and facilitate liver regeneration (39).

### MSCs

MSCs are defined as a heterogeneous subset of cells characterized with potency of self-renewing, giving rise to various cell lineages, homing to damaged sites, modulating the host immune system and paracrine actions, which make them numerically the most favored cell type under clinical trials at present (40). MSCs can be defined based on the propensity to adhere to the plastic surface of culture

vessels, the differentiation potential and the expression of some special surface biomarkers, positive for CD90, CD29, CD44 and CD105 and negative for CD45, CD34, CD117 and human leukocyte antigen (HLA)-DR (41).

MSCs trials are happening with high activity in the field of liver diseases. Accumulating evidences have validated therapeutic effects of MSCs in experimental animal models of liver injury (42,43), paving the way to the execution of clinical trials. Clinical trials of human MSCs are evolving rapidly with ambitious goals of improving the outcome of patients with ESLD.

MSCs are typically isolated from BM and the clinical application of these undifferentiated cells has attracted a lot of attentions (Table 2). Peng *et al.* (44) recruited 527 patients with liver failure caused by hepatitis B, among whom 53 received a single transplantation of autologous BM MSCs through hepatic artery. In transplantation group, short-term outcomes were favorable, with the significant improvement of ALB and TBIL levels, MELD scores and prothrombin time (PT) at 2-3 weeks after transplantation, whereas long-term benefits were limited. Lin *et al.* (45) transplanted allogeneic BM-derived MSCs to patients with hepatitis B virus (HBV)-related acute-on-chronic liver failure (ACLF), and found that serum total bilirubin levels and MELD scores were markedly improved in patients with MSCs infusion. No severe infusion-related side effects were reported. In the study of Kharaziha *et al.* (46), MSCs injection through either peripheral or the portal vein decreased the MELD scores, serum creatinine and bilirubin levels in patients with liver cirrhosis. And no adverse effects were reported. El-Ansary *et al.* (47) and Salama *et al.* (48) also proved the significant improvement in liver function by autologous BM-MSCs in liver cirrhosis induced by hepatitis C.

For clinical use, the isolation of MSC from BM is an invasive and painful procedure. Besides, the proportion of MSCs in BM is very low (0.001-0.01%), and their number and differentiation potential may decline with increasing age (41,51). Other adult tissues, such as umbilical cord blood (UC) and adipose tissue (AD), are alternative options for MSCs isolation. These MSCs isolated from different tissue share some common features, morphological and immunophenotypic properties.

The promising results of UC- MSCs have been observed in experimental animal models of liver cirrhosis and liver failure (52,53). For clinical trial, Li *et al.* (49) reported that MELD scores, PT and liver function were markedly improved in patients with HBV-related ACLF after UC-MSCs transplantation, and the inspiring results were also

**Table 2** Clinical application of MSCs in patients with ESLD

Authors	Disease	Cell therapy	Source of MSCs	Dose of injection	Way of injection	No. patients	Outcome
Peng <i>et al.</i> (44)	HBV-related liver failure	Autologous MSCs	Bone marrow	Unclear	Hepatic artery	53	Improvement of ALB, TBIL, PT and MELD score
Lin <i>et al.</i> (45)	HBV-related ACLF	Allogeneic MSCs	Bone marrow	[1–10]×10 <sup>6</sup> /kg	Peripheral vein	56	Improvement of TBIL and MELD score and reduction of the incidence of severe infections
Kharaziha <i>et al.</i> (46)	Liver cirrhosis	Autologous MSCs	Bone marrow	[30–50]×10 <sup>6</sup> cells	Peripheral or the portal vein	8	Improvement of bilirubin, creatinine, INR and MELD score
El-Ansary <i>et al.</i> (47)	HCV-induced liver cirrhosis	Autologous MSCs	Bone marrow	1×10 <sup>6</sup> /kg	Peripheral vein	15	Improvement of ALB, bilirubin and MELD score
Salama <i>et al.</i> (48)	HCV-related ESLD	Autologous MSCs	Bone marrow	1×10 <sup>6</sup> /kg	Peripheral vein	20	Improvement of liver function and hepatic fibrosis
Li <i>et al.</i> (49)	HBV-related ACLF	Allogeneic MSCs	Umbilical cord	100×10 <sup>6</sup> cells	Hepatic artery	11	Improvement of liver function, PT and MELD score
Shi <i>et al.</i> (50)	HBV-related ACLF	Allogeneic MSCs	Umbilical cord	0.5×10 <sup>6</sup> /kg	Peripheral vein	24	Improvement of the survival rates, liver function, PT and MELD score

ALB, albumin; PT, prothrombin time; INR, international normalized ratio; MELD, model for end-stage liver disease; ACLF, acute-on-chronic liver failure; MSCs, mesenchymal stem cells; ESLD, end-stage liver disease.

reported in another study in which patients suffering ACLF treated with UC-MSCs (50). Although AD-MSCs transplantation improved serum biochemical parameters and slowed disease progression in experimental model of ACLF (54), no too many studies have been conducted in clinical trials.

Although MSCs have the capacity of differentiating into hepatocytes in acute and chronic liver injury, the mechanism of MSCs in liver injury might be primarily based on their immunomodulatory properties and paracrine action (55–57). With the potential of homing to the damaged sites and the pleiotropic properties of anti-apoptosis, anti-fibrosis, angiogenesis, anti-inflammation and growth factor production, MSCs contribute to the improvement of ESLD (58). In addition, MSCs might communicate with immune cells to regulate the inflammatory process.

### Challenges to be addressed

Although multipotent stem cells, particularly MSCs transplantation, might become a potentially efficacious tool to treat ESLD and large numbers of preclinical and clinical studies have been performed, there are still some vital challenges to be resolved before stem cells widely used in clinic.

### *Which are more safe, convenient and cost-effective, autologous or allogenic stem cells in the field of liver disease?*

Autologous stem cells are generally advantaged in terms of ethical consideration and risks of sensitization and cell rejection. Autologous HSCs are more common than allogenic HSCs in the field of liver disease, as allogenic HSCs may cause inflammatory neuropathy and graft versus-host disease (59). It is a little complicated when comes to MSCs. There is an argument on the pros and cons of autologous and allogenic MSCs transplantation. Autologous MSCs are safe and cause no transplant rejection. However, autologous MSCs derived from aged donors show increased number of senescent cells and low capacity of proliferation and differentiation (60). System disease may also affect the characteristic of autologous MSCs. Allogenic MSCs isolated from young and healthy donors are more effective and powerful than those from older and systemic sick individuals. Moreover, allogenic MSCs can be expanded and cryopreserved *in vitro*, which make them available off the shelf in emergency settings where stem cells are required immediately, whereas the extraction and large-scale expansion of autologous cells are expensive and time consuming (61).

However, the safety implication of allogenic MSCs in an

immunocompetent host needs reexamined. Previously, MSCs are usually considered to be immunoprivileged because of the absence or low expression of major histocompatibility complex (MHC) class II and costimulatory molecules (62), and allogenic MSCs may escape the recipient's immune surveillance. Nonetheless, several studies have documented that the transplantation of allogenic MSCs could elicit specific cellular and humoral immune responses, which may reduce the survival of allogenic MSCs *in vivo* (63,64). It is likely that compared with autologous MSCs, more allogenic MSCs are needed to ensure the comparable therapeutic effects. Therefore, allogenic MSCs are not fully immunoprivileged and their long-term benefits await more clinical validation.

#### *Which is the optimal route for stem cells transplantation?*

The common routes for stem cell transplantation include peripheral vein, portal vein and hepatic artery. Peripheral vein is the most common and may be also the most safe and accessible route for transplantation (47,48,65,66). Nevertheless, infused stem cells may be trapped in the lung following peripheral intravenous injection (67-69). In the study of Eggenhofer *et al.* (69), these pulmonary trapped stem cell were short-term survival and disappeared from the lung, and the MSCs observed in the liver were MSC debris or phagocytosed MSC rather than living MSC. In summary, intravenously infused MSCs lead to a reduction in the number of viable stem cells homing to the target organ, which may inhibit stem cell harvest and decrease the treatment efficacy of stem cells. Although there were no significant differences in the therapeutic effects among the three transplanted routes in animal models and in some patients (46,70,71), the literatures always consisted of small cohort group not adequately characterized and might exist bias. Hence, investigators have explored and tried other routes for cell transplantation.

Theoretically, direct administration of stem cells into the vessels supplying the liver (the portal vein and hepatic artery) may be more effective. Although published reports have proved the safety and efficacy of stem cells injected through portal vein or hepatic artery (46,71-73), the invasive approaches may carry substantial risks: hemorrhage and thrombosis. The potential of portal hypertensive may increase if stem cells are delivered directly to the liver. Other adverse side effects, such as radiocontrast nephropathy and hepatorenal syndrome, although rare, were also observed in decompensated cirrhosis after infusion of HSCs through

hepatic artery (74). What described above has raised the significant concerns about the methodology used to deliver the stem cells.

#### *What is the optimal dose of stem cells for single injection? And what is the schedule of injection?*

The impact associated with the aforesaid pulmonary trapping may be mitigated if sufficient cells are infused (68). The dose of cell infusion affects the treatment effect and larger number of MSCs administration may induce more beneficial effects. However, as shown in *Tables 1* and *2*, there was obvious heterogeneity among studies regarding the dose of stem cell transplantation, which greatly limits the comparison of treatment effects across studies.

In addition, the therapeutic benefit of stem cells transplantation is transient. MSCs administration improved liver functions and decreased MELD scores in 3 patients on month six post-transplantation. Among these 3 patients, the MELD score of 1 patient came back to pre-transplantation status on month 12 post-transplantation (75). Repeated cell infusions seemed to induce more lasting clinical efficacy and improvement in liver function than single infusion (65). It is likely that the duration of therapeutic effects is limited after stem cell single injection. Therefore, the frequency of cell transplants to guarantee the sustained therapeutic effects in patients is still an open question and a matter of intensive investigation. Further studies may explore the standardized cell dose per injection and the life span of the injected cells, determining the frequency of stem cell infusion to guarantee the long-term therapeutic benefits in patients.

Except for these challenges above, other technical issues, for example the cryopreserved methods *in vitro*, are being explored. Stem cell should be cryopreserved without compromising their phenotypic characteristics, their proliferation and differentiation potential. During cryopreservation, the cooling rate, whether too fast or too slow, may cause cell injury. Cryoprotectants which are used to avoid minimize the freezing damage, can cause cell injury as well. Different storage period and temperature also have a great effect on the function of cryopreserved stem cells (41). At present, strategies are being explored to refine the cryopreservation procedure and to minimize the cryoprotectants toxicity and freezing-induced damage.

## **Conclusions**

ESLD is a severe clinical disease with high morbidity and

mortality. Stem cell therapies are promising agents and may act as a novel alternative for patients still waiting for liver transplantation. But stem cell transplantation is still in the early stage of development. And there still some challenges need to be addressed. Future clinical trials should be planned with standardized protocols to define the nature of stem cell, the transplantation procedure and even the evaluation system regarding efficacy. Optimism has been high for clinical application of stem cell therapy in the future.

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