Mesenchymal Stem Cell Transplantation in Liver Diseases

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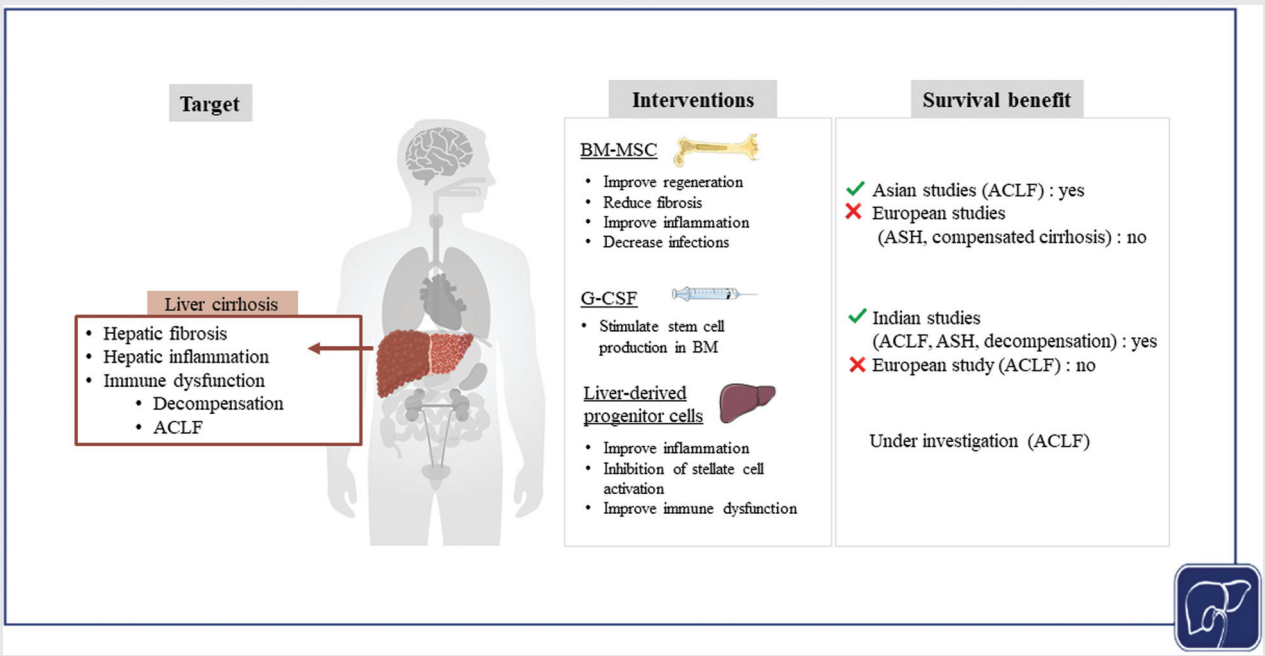
Graphical Abstract



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Abstract

Promising preclinical data suggested that bone marrow–derived mesenchymal stem cells (BM-MSC) can reduce hepatic fibrosis and stimulate liver regeneration. Preclinical studies moreover suggested that the immunomodulatory and anti-inflammatory functions of MSCs may reduce hepatic inflammation, improve liver function, and decrease infection incidences which are deemed especially important in the case of acute-on-chronic liver failure (ACLF). Studies in patients with decompensated cirrhosis demonstrated that injection of BM-MSC resulted in an improvement of biochemical tests and led to a survival benefit in ACLF. Most of these studies were performed in hepatitis B virus infected patients. However, two adequately powered studies performed in Europe could not confirm these data. A possible alternative to mobilize BM-MSC into the liver is the use of granulocyte colony-stimulating factor (G-CSF) which has proregenerative and immunomodulatory effects. In Indian studies, the use of G-CSF was associated with improvement of survival, although this finding could not be confirmed in European studies. Human allogeneic liver-derived progenitor cell therapy represents a potential treatment for ACLF, of which the main action is paracrine. These human liver–derived MSC can perform various functions, including the downregulation of proinflammatory responses. The clinical beneficial effect of these cells is further explored in patients with alcoholic cirrhosis and ACLF in Europe.

Keywords

- liver cirrhosis
- ACLF
- alcoholic hepatitis
- human allogeneic liver progenitor cells

The use of mesenchymal stem cell (MSC) therapy may be an attractive approach to treat liver failure. Stem cells are multipotent and may differentiate to become functional hepatocytes that could replace hepatocyte mass, function, and ultimately reverse fibrosis. Alternatively, these cells may secrete growth factors and anti-inflammatory cytokines that can impact on regeneration and inflammation in the failing liver. The use of MSC therapy may be especially applicable and relative in acute-on-chronic liver failure (ACLF), a devastating clinical syndrome with a prevalence in hospitalized patients with cirrhosis with acute decompensation ranging from 24 to 34%. This syndrome is characterized by hepatocyte failure, systemic inflammation associated with various degrees of organ dysfunction, and high associated mortality.[1–3] All of these characteristics may potentially benefit from MSC therapy. In the absence of effective therapy able to reverse all components of decompensated cirrhosis and ACLF, liver transplantation remains the only definitive therapeutic option. However, in case of decompensated cirrhosis not every patient can receive a graft due to organ shortage and most patients with ACLF cannot be listed due to rapid disease progression, active alcoholism, uncontrolled infections, and multiple organ failure. MSC therapy may also be an attractive indication in cirrhosis complicated by severe alcoholic hepatitis (ASH) not responding to corticosteroids which is, in the western countries, a frequent trigger of ACLF.[4,5]

Bioartificial liver (BAL) support devices gained a lot of attention several decades ago as a treatment of liver failure. If they contain sufficient liver cell mass, they have the theoretical possibility to compensate both the failing synthetic function and impaired detoxification activity of the liver.[6,7] The origin of the cells used in the most important early clinical trials with BAL devices were primary porcine hepatocytes. The major concern with the use of xenogeneic liver cells was the risk associated with zoonotic infections that led to the implementation of a moratorium on xenotransplantation in Europe.[8] As an alternative for primary porcine hepatocytes, transformed malignant human liver cell lines have been used as a cell source. However, cell lines have an impaired and often highly limited spectrum of preserved liver functions and concerns exist regarding "malignant seeding," should these cells escape the bioreactor environment.[9] This led to the development of stem cell–derived hepatocytes.[10,11] Many obstacles, however, remained. Modeling of bioreactor requirements indicated that at least 20% of the native liver cells that corresponds to 200 g or 20×10^9 hepatocytes are required.[9] In this regard, in the Demetriou trial, 7 billion porcine hepatocytes within the hollow-fiber bioreactor were used, and in the Extracorporeal Cellular Therapy (ELAD) trial, four cartridges containing approximately 440 g of C3A cells.[12,13] Despites large investments made, both in the development of the sources of these cells and in the development of three-dimensional (3D) complex bioreactors, none of the trials with such devices observed a survival benefit in both acute liver failure (ALF) and ASH.[12–15] Regression analysis in the ELAD trial revealed that patients with older age, renal impairment, and severe coagulopathy contributed to the negative outcome observed in this study.[13]

Following the disappointing data generated by clinical trials utilizing BAL support devices to replace liver function showing no benefit, there use has been largely abandoned since the last disappointing trial in 2017.[13] Treatment of ACLF shifted toward the use of cell-free liver support devices utilizing albumin dialysis and high volume plasma exchange to improve the detoxification capacity of the liver. These cell-free based artificial liver support therapies remove albumin-bound toxins which cannot be extracted by conventional hemodialysis, with the expectation that this will improve the

clinical state of the patient.[16] Artificial liver support devices, such as the molecular adsorbent recirculating system (MARS) and fractionated plasma separation and adsorption (Prometheus), were evaluated in randomized controlled trials but again, failed to show improvement in survival of patients with ACLF.[17,18] A meta-analysis based on individual data from three randomized trials with MARS revealed that a survival benefit was seen only in patients receiving more than four treatment sessions.[19] However, the primary endpoint in these trials were not reached, so that these types of liver support are not incorporated into widespread clinical utilization anymore.[20] Only high-volume plasma exchange improved survival in patients with ALF.[21] This suggests that circulating factors, produced by the failing liver or immune cells, propagate disease progression and a randomized, controlled trial on survival in patients with ACLF, using this therapy is currently in progress.

Decompensated cirrhosis and of ACLF are characterized by immune dysfunction and at risk to develop multiorgan failure.[22,23] Clinical trials utilizing albumin dialysis and high-volume plasma exchange focusing on improvement of synthetic and detoxification activity of the liver, failed to demonstrated a survival benefit in ACLF. This strongly suggests that other approaches, aimed at targeting not only these functions but also modulating immune function would likely be required in the management of this complex disease entity.

Human Mesenchymal Stem Cells

Human MSCs (hMSCs) are multipotent stem cells capable of self-renewing and differentiation in vitro into different kinds of cell types. In vivo hMSCs are sources of trophic factors capable of modulating the immune system and inducing intrinsic stem cells to repair damaged tissues.[24] Currently, there are multiple clinical trials using hMSCs for therapeutic purposes in various clinical settings, such as traumatology, neurology, cardiology, and immunology. In all of these studies, similar cell isolation protocols have been used and no serious adverse effects have been reported.[25]

The best-studied stem cell is the hematopoietic stem cell (HSC) that can be harvested from different sources (bone marrow [BM], blood, and umbilical cord blood) in sufficient numbers for transplantation. HSCs have then also been used for cell-based therapies, especially in an allogeneic setting for more than a quarter of a century. Other adult stem cell populations that are being evaluated clinically are MSCs and mesenchymal adult progenitor cells (MAPCs) both derived from human postnatal tissues. Apart from being able to differentiate to multiple cell types in vitro and in vivo, MSCs have extensive immunomodulatory and immunological tolerance inducing characteristics. MSCs efficiently suppress an immune response by modulating T-cell activation and proliferation.[26] This immunomodulating effect of MSCs is being explored as adjuvants during allogenic transplantation to prevent graft versus host disease, during organ transplantation to prevent immune rejection and have being evaluated in the setting of autoimmune diseases.[27,28] In preclinical studies published between 2000 and 2010, it has been shown that these multipotent stem cells can produce innumerable growth factors and cytokines that may play a crucial role in tissue repair and regeneration by differentiating into several cell types and replacing injured tissue. However, mechanism of MSC epithelial differentiation still remains unclear and controversial with transdifferentiation or fusion events being evoked.[29] MSCs have homing potential to the site of injury are not recognized as "non-self" by immune cells. MSCs have the luxury of being tolerated by the host immune system due to low immunogenicity. MSCs that characteristically lack expression of MHC-II, CD40, CD80, and CD86 but express MHC-I present themselves as nonimmunogenic. Although the presence of MHC-I may activate T-cells, due to lack of costimulatory molecules, MSCs fail to elicit an immune response.[30]

In case of the liver disease, homing BM-derived MSCs (BM-MSCs) have been shown to transdifferentiate into hepatocyte-like cells in the local hepatic microenvironment. The beneficial effect of MSC is thought to be principally mediated by paracrine mechanisms. Promising preclinical data suggested that administration of BM-derived cells can reduce hepatic fibrosis and stimulate liver regeneration, thereby improving the synthetic function of the liver, although the mechanisms by which these effects are achieved are yet to be clearly elucidated.[31–37] Thus, although the exact mechanisms by which BM-MSC exert their effects remain uncertain, these studies have shown that it likely entails and dependent on engraftment into the liver and, differentiation in this microenvironment into hepatocyte-like cells. This observation was confirmed when it was shown that Y-chromosome positive hepatocyte-like cells could be found in the liver of a women who had received allogenic BM transplantation from male donors.[38,39]

Injection of Bone Marrow–Derived Mesenchymal Stem Cells

Autologous multipotent BM-MSCs are characterized by their surface expression $CD34^+$ and $CD133^+$. These cells are generally considered to be HSCs capable of differentiation into all hepatic cell types. These cells can be mobilized from the patient's BM by administration of granulocyte colony-stimulating factor (G-CSF) and isolated using leukapheresis. Further use of FACS sorting gating on CD45+ and CD133+ results in a purified fraction of these cells. Direct administration via the liver vasculature enhances homing of the cells into the liver; therefore direct injection in the hepatic artery had a greater efficacy than the administering these cells via a peripheral vein.[38,39] Several studies reported that infusion of stem cells in patients with liver disease was safe, except when administered directly into the portal vein. Multiple single-center trials with a short-term follow-up originating mostly from China in hepatitis B virus (HBV) infected patients suggested that stem cell therapy was safe in decompensated cirrhosis and could result in a transient biochemical improvement of liver function. The majority of these trials, however, either included a limited number of patients, lacked appropriate control groups, or were too

heterogeneous in their design and methods of stem cell therapy.[40–42] An overview of the most recent randomized trial outside Europe with BM-MSC for decompensated cirrhosis is given in ►**Table 1**.[43–46] These studies included different etiologies of liver disease and followed-up patients for 12 to 24 months. The most common route of administration was a single injection into the hepatic artery using an interventional radiology approach. Of the four studies, three reported an improvement in laboratory investigations and some clinical improvement. In one study, an improvement in fibrosis was observed. No serious adverse events occurred, however, in one uncontrolled study, investigating reinfusion of $CD133^+$ cells for end-stage liver disease, worsening liver function and creatinine levels in Child-Pugh C patients was seen. No study observed malignant transformation during the short window of follow-up.[40,47]

Bone marrow–derived MSC have also been used to treat patients with ACLF.[48] Similar, as in case of decompensated cirrhosis, the mechanism of action and the downstream effects of BM-MSCs in the treatment of ACLF is not known. It has been postulated that the immunomodulatory and anti-inflammatory effects of MSCs may improve hepatic inflammation and liver function and may also decrease the incidence of infections in ACLF. A summary of the two most recent randomized trials outside Europe is given in ►**Table 2**. The two studies from China evaluated the use of MSC's in ACLF related to HBV. In the first study, MSCs were obtained from umbilical cord blood and in the second study, allogenic MSC's were obtained from healthy donors.[49,50] The cells were administered at 4 weekly intervals via a peripheral vein. The follow-up ranged from 24 to 72 weeks. In these two studies, MSC therapy improved biochemical parameters of liver function and model for end-stage liver disease (MELD) scores and survival benefit was reported in the study that utilized umbilical cord–derived stem cells. Except for fever, no serious side effects were reported in these studies. Short-term efficacy was favorable but long-term outcomes were not markedly improved.[51] Overall, studies utilizing MSCs in the management of decompensated cirrhosis and ACLF are encouraging but remain difficult to interpret, given that no uniform cell source is used, neither are the administration and interval of subsequent infusions standardized. There is still a need for more adequately powered studies in the Western countries where the dominant cause of ACLF is alcohol, to differentiate the effect of etiology of liver disease, type and source of stem cells, and the duration and interval of MSCs on the outcome of ACLF. This should resolve the current discrepancies existing in published literature.[40]

Table 1 Randomized trials with BM-MSC for decompensated cirrhosis

Study (year), Country	Etiology	Number of pts.	Number of cells (median)	Route of administration	Schedule	Follow-up (mo)	Side effects	Outcome	
								Biochemical test	Clinical
Lyra et al (2010), Brazil[43]	Mixed	15 vs. 15 controls	3×10^8	Hepatic artery	1x	12	No	Improvement in the first 90 days	Improvement of the Child Pugh score
Mohamadnejad et al (2013), Iran[44]	Mixed	15 vs. 12 controls	1.95×10^8	Peripheral vein	1x	12	No	No improvement	No survival benefit
Bai et al (2014), China[45]	HBV (mostly)	32 vs. 15 controls	NA	Hepatic artery	1x	24	No	Improvement during 3–12 months	Encephalopathy less SBP less
Suk et al (2016), South Korea[46]	Alcohol	18 vs. 19 vs. 18 controls	5×10^7	Hepatic artery	1x ($n = 18$ pts) 2x ($n = 19$ pts) (interval 1 month)	12	No	Improvement	Reduction in fibrosis after 6 months Improvement of the Child Pugh score

Abbreviations: BM-MSC, bone marrow–derived mesenchymal stem cells; HBV, hepatitis B virus; pts., patients; SBP, spontaneous bacterial peritonitis.

Mobilization of Bone Marrow–Derived Mesenchymal Stem Cells by Granulocyte Colony-Stimulating Factor

Granulocyte colony-stimulating factor (G-CSF) is a glycoprotein which stimulates the BM production of stem cells and release of BM-MSC's into the circulation. In addition G-CSF has a pro-regenerative capacity in other tissue sites.[52,53] In a proof-of-concept study, G-CSF was indeed able to increase circulating CD34+ cells and hepatocyte growth factor levels and the number of proliferating human progenitor cells and mature

Table 2 Randomized trials with BM-MSC for ACLF

Study (year), Country	Etiology	Number of pts.	Type of MSC	Number of cells (median)	Route of administration	Schedule	Follow-up (wk)	Side effects	Outcome
Shi et al (2012), China[49]	HBV	24 vs. 19 controls	Umbilical cord	0.5×10^6/kg body weight	Peripheral vein	3x/ 4-week interval	72	No	Survival benefit
Lin et al (2017), China[50]	HBV	56 vs. 54 controls	Bone marrow	$1.0–10 \times 10^5$	Peripheral vein	1/week, 4x	24	Fever	Severe infections: less survival benefit

Abbreviations: ACLF, acute-on-chronic liver failure; BM-MSC, bone-marrow-derived mesenchymal stem cells; HBV, hepatitis B virus; pts., patients.

Table 3 Randomized multicenter studies in Western countries of BM-MSC and G-CSF

Study (year), Country	Type of complication	Etiology	Number of pts.	Schedule	Route of administration	Follow-up (mo)	Serious side effects	Outcome
Spahr et al (2013), Switzerland[68]	Alcoholic hepatitis	Alcohol	28 vs. 30 controls	G-CSF 10 µg/kg 5 days andBM-MSC $0{,}47 \times 10^8$ /kg cells	G-CSF: subcutaneous BM-MSC: hepatic artery	3	No	MELD: no improvement Biopsy after 4 weeks: • improvement of steatosis • proliferation of progenitor cells: no
Newsome et al (2018), the United Kingdom[69]	Compensated cirrhosis	Mixed	26 (G-CSF) vs. 28 (G-CFS + stem cells) vs. 27 controls	G-CSF 15 µg/kg 5 days vs. G-CSF 15 µg/kg 5 days and CD 133 cells 0.2×10^6 cells/kg infusion 3x	G-CSF: subcutaneous Stem cells: peripheral vein	3	Complications of cirrhosis: 11 vs. 43 vs. 11% Mortality: $n = 0$ vs. 2 vs. 1	MELD: no improvement

Abbreviations: BM-MSC, bone-marrow-derived mesenchymal stem cells; G-CSF, granulocyte colony stimulating factor; MELD, model for end-stage liver disease; pts., patients.

hepatocytes, within 7 days after administration, in patients with alcoholic cirrhosis and ASH.[54] This finding was confirmed by several other studies investigating the use of G-CSF across the spectrum of liver disease including decompensated cirrhosis, ACLF, and in patients with ASH.

Several studies, mostly from India, found that G-CSF improved liver function and survival of patients with decompensated cirrhosis, ACLF, or ASH.[55–61] Only one of these studies from India, which used matched historical control group, reported a reduced survival.[62] The additional effect of erythropoietin and growth hormone on promoting liver regeneration was investigated in addition to MSC's; however, none improved the positive effect of G-CSF in these trials.[58,63,64]

In Europe, one adequately powered multicenter trial was performed in Germany to investigate the effect of G-CSF in patients with ACLF, In this study, 176 patients with ACLF (the European Association for the Study of Chronic Liver Faliure [EASL-CLIF] criteria) were randomized to receive G-CSF (5 µg/kg daily for the first 5 days and every third day, and thereafter for 26 days) plus standard medical therapy (SMT; $n = 88$) or SMT alone. Transplant-free and overall survival at 360 days did not differ between the 2 arms (hazard ratio [HR] = 0.998, 95% confidence interval [CI]: 0.697–1.430, $p = 0.992$; and HR = 1.058, 95% CI: 0.727–1.548, $p = 0.768$, respectively). G-CSF did not improve liver function scores, the occurrence of infections, or survival in subgroups of patients without infections, with alcohol-related ACLF or with ACLF defined by the the Asian Pacific Association of the Stuty of the Liver (APASL) criteria. In total, seven drug-related serious adverse reactions occurred in the G-CSF group.[65] A recently meta-analysis, aimed to resolve some aspects regarding the discrepancy in outcome in Asian versus European studies, assessing the effects of G-CSF in alcoholic hepatitis. Data of the effect of G-CSF on the 90-day mortality rate and risk of infection from patients with ASH were analyzed. There was a high heterogeneity between the Indian ($n = 5$) and European ($n = 2$) studies, with a very pronounced positive effect on survival in the Indian studies but no efficacy in European studies. In fact, the overall number of patients studied was relatively small and only three clinical centers were involved in India in generating the data. However, it is important to mention that meta-analyses using individual participant data are more likely to generate clinically relevant results than the type of meta-analyses performed in this study.[66] Therefore, the efficacy of G-CSF therapy for ACLF is still unclear.[67]

Randomized Studies in Western Countries of Bone Marrow–Derived Mesenchymal Stem Cells and Granulocyte Colony-Stimulating Factor

In western countries, two trials were performed (►**Table 3**). In one trial with adequately powered randomized controlled study from Switzerland, the whole BM mononuclear cells in combination with G-CSF were infused into 28 patients with alcoholic cirrhosis in whom the majority had evidence of ASH.[68] In this study, following G-CSF injection, mononuclear cells were isolated following Ficoll density separation with no further enrichment of CD34+ by FACS sorting, resulting in a mixed-cell population. The authors could confirm that administration of autologous BM mononuclear cell transplantation via the hepatic artery was a safe procedure, but that this therapy did not result in an increase in the hepatic progenitor cell compartment, nor did it improve liver function. However, ASH is a different clinicopathological entity to that seen in patients with compensated liver cirrhosis in which there is mostly minimal inflammation and more extensive fibrosis. An important difference in this study, compared with other studies, may have impacted the results that was the use of an infusion with mononuclear cells which contained a mixed-cell population. In another study from Europe performed in the United Kingdom, patients with compensated cirrhosis were included to study the effects of these therapies on less advanced cirrhosis.[69] The presumption was that stem cell therapy, in patients with decompensated cirrhosis, may be less likely to reverse fibrosis and improve liver function, than would be the case in compensated cirrhosis and may have accounted for the negative outcomes observed in some studies assessing a decompensated population. The patients in this study had a low MELD score of 11.0 to 15.5. G-CSF was administered at a dose of 15 µg/kg per day for 5 days to mobilize haemopoietic stem cells. This was a considerably higher dose than what was used in the other trials and was considered the optimum dose for effective stem-cell mobilization without inducing significant adverse effects. Flow cytometry sorting was then used to enrich the CD133+ CD45+ population before injection. However, G-CSF with or without hemopoietic stem-cell infusion did not improve liver dysfunction (MELD) or fibrosis (as measured by serum enhanced liver fibrosis [ELF] score and elastography) and serious adverse events were more frequent in G-CSF and stem-cell infusion group (43%) versus the G-CSF only group (11%) versus the standard care group (12%). This was mostly due to a higher rate of ascites development. The study included different etiologies of cirrhosis but no difference in response to the interventions was seen between the different subgroups.

Possible Reason for the Conflicting Data Obtained in Clinical Trials

It is generally accepted that the proportion of BM-derived hepatocytes repopulating a diseased liver is limited. Therefore the number of MSC's infused may have been insufficient in these studies to ensure adequate engraftment and regeneration. Of note, no clinical study has been able to explore the level and efficacy of engraftment into the diseased parenchyma, partly due to the lack of sensitivity of cell tracing techniques that are available. Also studies differ with regard to administration of these cells via the hepatic artery or peripheral veins which may impact on the ability of these cells to be successfully engrafted into the liver parenchyma. Some have also postulated that the profibrogenic potential of BM-MSC's may counteract the otherwise beneficial effects that these cells may have. In animal models, for instance, mixed BM infusions have been reported to contribute to liver fibrosis, whereas purified hepatic stem cells reduced fibrosis.[36,40,70] Also, it is potentially possible that the response to stem cell therapy may be influenced by the etiology of liver disease and that the response to the proliferation stimulus may differ between HBV and alcoholic liver disease. In

addition, the effect of any intervention is more difficult to assess in patients with alcoholic liver disease due to the unpredicted rate of relapse which may mask any potential therapeutic benefit.

Human Allogeneic Liver–Derived Progenitor Cells

As discussed before, one of the most relevant beneficial properties of MSCs is related to their potent immunomodulatory actions and are therefore especially attractive to treat ACLF. Cirrhosis is not only characterized by a synthetic and detoxification deficit but also by cirrhosis associated immune dysfunction. Decompensated cirrhosis, especially, represents an immunological paradox, patients exhibit a hyperinflammatory state at the clinical and molecular level, but this coexists with profound cellular immunoparesis and increased susceptibility to bacterial infection. The severity of this immune dysfunction is dynamic and progressive and parallels the severity of cirrhosis. During cirrhosis progression, damage- and pathogen-associated molecular patterns activate immune cells and promote development of systemic inflammatory responses which may involve different tissues and compartments simultaneously. Triggers of inflammation in decompensated liver cirrhosis and ACLF are mainly derived from intestinal bacteria and bacterial products that breach the epithelial barrier. In patients with compensated disease or clinical decompensation with no organ failure, there is an exaggerated immune activation but the effector response against bacterial infections is not markedly compromised. In contrast, in patients with ACLF, there are high-grade inflammation and intense immune paralysis that critically increase the risk of infections and results in further multiorgan failure and death.[22,23,71–73] Since the disappointing results of BM-MSC therapy in the West, new strategies are currently being explored.

Human allogeneic liver–derived progenitor cells (HALPC's) are liver-derived MSC like cells and are classified as the "Advanced Therapy Medicinal Product (ATMP)" by the European Medicine Agency which is the official designation of cell and gene therapy products and may represent an alternative to BM-MSC treatment for ACLF. These cells are obtained after primary culture of the parenchymal fraction of healthy human liver tissue and expanded by culture under good manufactory practice before use.[74] The cells are administered intravenously through a peripheral vein into the circulation from where they migrate to the liver to perform various functions including the downregulation of proinflammatory responses, as well as inhibition of hepatic stellate cell activation, reduction of collagen production that may possibly lead to a reduction in fibrosis.[75–78] These cells were called human liver–derived progenitor cells.[74] The term "progenitor" comes from original studies in which it was shown that these cells had the capacity to differentiate into hepatocyte-like cells in vitro when exposed to a specific cocktail of differentiation factors mimicking embryonic liver development.[74] They were on the contrary not able to differentiate into adipocytes, chondrocytes, or osteocytes which differentiates them from BM- or adipose tissue–derived MSC's and that was the reason for the terminology "liver progenitor." Hepatic progenitor cells are bipotential stem cells that reside in human liver and are able to differentiate toward the hepatocytic or cholangiocytic lineages. Hepatic progenitor cells are activated in the case of severe cell loss or when the replication of liver parenchymal cells is impaired, resulting in their proliferation and differentiation toward the cell type which is most affected (hepatocytes or cholangiocytes).[79–82] MSCs are called "stem cells" because it was originally intended that these cells will differentiate into regenerating tissue cells. The change of the name of MSCs to "medicinal signaling cells" more accurately reflect the fact that these cells home-in on sites of injury and inflammation where they secrete bioactive factors that are immunomodulatory and regenerative. However, it is in fact the tissue-specific mature cells and/or resident stem cells of the patient that are involved in constructing the new tissue further stimulating by the bioactive factors secreted by the exogenously administrated MSCs.[83] Immunosuppression is therefore not required, as these cells will not persist nor engraft. They rather act as a cargo to reach the liver and inflamed sites, where they release relevant cytokines to control inflammation.[75] In addition, one of the key advantages of these cells, beside their liver homing, is that they do not express human leucocyte antigen (HLA) class II, in contrast to BM or adipocyte cells.[84] The immune characteristics of these cells are currently under further investigation. The safety profile of these cell signaling technology has been extensively investigated in preclinical studies and the cells have been safely administered to children with inborn errors of metabolism.[84,85]

The clinical potential of the cells as a treatment for alcoholic cirrhosis, complicated by acute decompensation or ACLF, was recently explored in a feasibility phase 2a study (not placebo controlled). This indication was chosen since there is no effective treatment for ACLF, and alcohol is the most important trigger in western countries.[86] Given that ACLF is characterized by immune dysfunction this study explored whether cell signaling technology could bridge the patient past the acute phase.[86] The explorative study revealed that intravenous infusion of low doses of HALPCs is safe (1.0×10^6 cells/kg BW) and may have the potential as a treatment of ACLF.[87] A randomized placebo controlled multicenter trial has been initiated with overall survival as the primary endpoint (*ClinicalTrials.gov* identifier: NCT04229901). Other indications of this technology which are currently explored are chronic liver disease or complications of liver disease which are associated with extensive liver inflammation and fibrosis such as ASH or NASH.

Conclusion

Several decades of extensive research has been undertaken to offer patients with cirrhosis and liver failure, an alternative to liver transplantation. To date, this has not materialized in an acceptable effective therapy to achieve this goal. After the finding that BAL support and albumin dialysis devices were not able to improve survival, MSCs therapy became an attractive approach. Indeed promising preclinical data with

these cells have suggested that injections of BM-derived cells could reduce hepatic fibrosis and stimulate liver regeneration, thereby improving the synthetic function of the liver. Several clinical studies performed outside western countries seemed to support this notion and showed improvement of survival after direct infusion of BM-MSC's or following G-CSF administration in patients with decompensated cirrhosis and ACLF. However, larger adequately powered randomized studies from Europe could not replicate these findings. Recently, the focus has been changed. Instead of only aiming to induce regeneration and inhibit fibrogenesis, new work is focusing on modulating immune functions by the use of HALPCs. This is currently being explored as a potential treatment for the immune dysfunction in patients with ACLF.

In the future, long-term clinical efficacy and safety studies will be required to determine the place, if any, of these therapies in the management of decompensated cirrhosis. Studies will have to clarify whether extraction protocols of the cells, dose, duration, route, and cell type impact on the outcome of these therapies. This information will be crucial and will either lead to these therapies being accepted into clinical practice or dismissed as yet another promising tool that could not be translated in clinical medicine

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Conflict of Interest
None declared.

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