CELL & GENE THERAPY INSIGHTS

REVIEW

Advances and challenges of successful cell therapies for liver disease

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End-stage liver disease is a common cause of morbidity and mortality. Currently, the gold standard treatment is orthotopic liver transplant; however, the mismatch between organ demand and supply, and the risks associated with organ transplantation has led to a search for alternative therapies. Cellular therapies could potentially present such an alternative to transplant or bridge to transplant. The earliest work has involved transplantation of allogeneic hepatocytes, with modest benefits shown. Hepatic progenitor cells have also been used in experimental settings, but cellular engraftment is challenging. Use of embryonic stem cells and pluripotent stem cells to form hepatocyte-like cells has also been investigated. Various bone marrow cells have shown therapeutic potential, likely via paracrine effects on liver repair and regeneration. This review summarizes the key advances in cellular therapies for liver disease, and discusses the challenges that need to be overcome before these therapies can be translated into clinical practice.

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Liver disease is a common cause of morbidity and mortality worldwide and causes include acute and chronic liver failure along with inherited metabolic diseases [1]. Currently the only effective treatment for advanced liver disease is orthotopic liver transplantation (OLT). However, demand for organs exceeds supply, with approximately 20% of patients dying whilst on the transplantation waiting list [2]. Furthermore, liver transplantation is associated with major surgical risks, with many patients being insufficiently fit for transplant due to co-morbidities and the debilitating effects of liver disease. Moreover, after transplantation, patients are required to take life-long immunosuppression, which has numerous side effects and can impact quality of life.

Auxiliary partial OLT has been used successfully to treat certain metabolic liver diseases and acute liver failure [3], suggesting that the functions of the liver can be performed without the whole organ. This has prompted research into novel cellular therapies for the treatment of liver disease.

Cellular therapies could potentially be used as an alternative to OLT, or as a bridge to transplant. They offer potential logistical and safety advantages over whole-organ transplant as summarized in **Box 1**: they are usually administered by

BOX 1

Summary of some of the key advantages of cellular therapies over orthotopic liver transplant (OLT).

- Less invasive
- May utilize organs unsuitable for OLT
- One donor can supply cells to multiple recipients
- The native liver can remain *in situ*
- Lower financial costs

intravascular catheters, so are less invasive than OLT, with lower immediate morbidity and mortality. For hepatocyte transplantation the same organ can provide cells to multiple recipients, thereby improving the ratio of donors to recipients on the waiting list. Currently, many donated livers are not accepted for OLT, yet these livers could be used to provide therapeutic cells. Another advantage is that cellular therapies do not necessitate the removal of the native liver, which in the setting of acute liver failure would provide the native liver with an opportunity to recover.

In selecting a cell therapy for a patient with liver disease, it is important to have an understanding of the underlying disease process, as the requirements may be very different. Inherited metabolic diseases are usually characterized by a single enzymatic defect within the hepatocyte, and therefore replacement with functional allogeneic hepatocytes would be therapeutically effective. Chronic liver failure is usually associated with liver cirrhosis, which is characterized by deposition of extra-cellular matrix proteins throughout the liver, alteration in the hepatic architecture and loss of hepatic parenchymal cells. This commonly results in impaired liver function, and the clinical need is restoration of synthetic function and reduction in liver fibrosis. Resolution of fibrosis would depend on either apoptosis of the myofibroblast cells which synthesize the fibrous tissue, or enzymatic breakdown of the fibrous tissue by matrix metallic proteinases (MMP) [4]. In acute hepatic inflammation, as seen in autoimmune liver disease, there is a need to dampen inflammation and thus cells with immunomodulatory properties, such as regulatory T or mesenchymal stromal cells, are required.

This review article provides an overview of the advances in cellular therapies for the treatment of liver disease, as well as highlighting some of the current challenges associated with their use.

OPTIONS FOR CELLULAR THERAPIES

Some cellular therapies in liver disease aim for homologous reconstitution of the native liver; however, the seeding of healthy hepatocytes may not always be necessary to treat liver disease. In rats it was shown that the use of cultured supernatant from cells and fragmented cells was beneficial in the setting of acute liver failure [5]. Therefore, other cell types that can influence cell generation and repair are additional candidates for cell therapies.

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Homologous reconstitution of liver cells

Hepatocyte transplantation

Due to limited organ availability, hepatocytes are usually sourced from whole livers considered unsuitable for OLT, which often means cell quality is poor. Currently, around 20–40% of donated organs are discarded [6]. Traditionally, cold static preservation of organs has been used, which is associated with ischemia–reperfusion injury; however, more recent methods of organ preservation, such as the use of enhanced machine perfusion devices, may allow greater utilization of marginal organs, and thus increase the number of transplantable liver organs as well as those that can be used for liver cell isolation [7,8].

Cells can be cryopreserved after isolation; however, repeated freeze–thaw cycles can compromise cells' metabolic function. Methods of reducing this damage are being investigated, such as the use of vitrification to prevent crystallization of cells, and encapsulation of hepatocytes prior to cryopreservation to provide mechanical protection. However, these protocols require validation and refinement [9–11].

Normal hepatocytes from allogeneic donors can be used to provide missing gene products in inherited metabolic diseases, as demonstrated in animals and humans with Criggler-Najjar syndrome, urea cycle disorders, and familial hypercholesterolemia [12–15]. Hepatocyte transplantation has also been undertaken in acute and chronic liver failure [16–19] where the goal is to restore parenchymal cell mass and synthetic function, although in most cases, the effects have been modest and of uncertain duration [20]. Although fetal hepatocytes have been shown to demonstrate anti-fibrotic properties in rats, ethical concerns have thus far restricted their use to animal experiments [21].

Until recently, it was difficult to measure and track transplanted hepatocytes. However, new non-invasive methods are being developed, such as MRI detection of hepatocytes labelled with iron oxide nanoparticles [22]. The challenge is to identify markers that are safe to patients, do not compromise function/engraftment of infused cells and are readily visualized.

Currently, one of the major limitations of hepatocyte transplantation is limited availability of good quality cells. In an attempt to overcome problems of cell shortages, research has included methods of immortalizing cells, the use of xenogeneic hepatocytes, and the use of autologous cells. Gene transfer from the simian virus 40 (SV40) to hepatocytes can immortalize them, although there are concerns about the transfer of malignancy to the host [23]. Use of xenogeneic hepatocytes from pigs has been explored, although immunogenic differences between pigs and humans can result in hyper-acute rejection due to xenoreactive antibodies against components of the porcine endothelium (such as the Galal-3Galp1–4G1cNAc oligosaccharide) [24]. Genetic knockout of these antigens can reduce the risk of immune rejection [25], although there still remains a risk of transferring zoonotic diseases, such as porcine endogenous retroviruses (PERV), to patients [26]. Use of autologous cells would reduce the problem of cell shortage and avoid the need for immunosuppression. In inherited

metabolic disease, this requires prior correction of the genetic defect. In humans with familial hypercholesterolemia, where the defective LDL receptor was genetically modified before re-implantation, there was a mild, albeit not clinically significant, reduction in serum cholesterol [19]. An alternative means of avoiding immunosuppression could be to genetically modify host or donor cells to prevent immune rejection, by for example introduction of immunomodulatory genes into donor hepatocytes before transplantation [27].

Another challenge is that there is difficulty in achieving long-term hepatocyte engraftment and survival in damaged liver, which means that repeated infusions may be required. Furthermore, after hepatocyte transplantation, it is difficult to achieve sufficient *in vivo* proliferation. Although hepatocytes have excellent regenerative capacity, physiological mechanisms ensure that hepatocyte numbers are constant, and thus hepatocytes usually require a stimulus to proliferate. In murine models of hereditary tyrosinemia and alpha-1-antitrypsin deficiency, transplanted hepatocytes have a survival advantage over the defective endogenous cells, enabling the liver to be repopulated with healthy cells [28,29]. However, in most other inherited liver diseases, such as Criggler-Najjar syndrome, the regenerative capacity of the native hepatocytes remains normal, and suppression of native cell proliferation is needed to promote proliferation of transplanted cells. In experiments, this is often achieved by irradiation of the liver or partial hepatectomy [30,31]. Although notably fetal hepatocytes can proliferate without any stimulus, this has not been a consistent finding [32,33].

Thus, hepatocyte transplantation offers promise for the correction of specific disease processes such as urea cycle enzyme defects. However, cell availability and *in vivo* proliferation is a limiting factor. The challenges of engraftment in end-stage liver disease render it a more difficult target. In these cases, it may be just as important to target the surrounding environment, rather than just the hepatocytes.

Transplantation of hepatic progenitor cells

In response to mild liver injury, mature hepatocytes contribute to the majority of liver regeneration [34] whereas in severe liver disease this regenerative ability in endogenous hepatocytes is exhausted. In this setting, there is activation of endogenous liver stem cells, known as hepatic progenitor cells or oval cells, which are multipotent cells in the canals of Hering [35]. Oval cell proliferation may be induced experimentally through targeted gene deletion to suppress hepatocyte proliferation [36]. Oval cells can differentiate both into hepatocytes and cholangiocytes, and have been implicated in liver regeneration and repair [37].

Yovchev *et al* demonstrated that rat fetal hepatic progenitor cells, when transplanted into rats with thioacetamide-induced fibrosis/cirrhosis, repopulated the liver and exerted an antifibrotic effect [21]. Moreover, ablation of oval cells, identified using the marker fox1, was found to impair recovery from liver injury [38].

Unfortunately, hepatic progenitor cells are only available in limited numbers, and usually require significant regenerative stimulus to proliferate. There are also concerns

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that activation of oval cells may drive a fibrogenic response, through differentiation into cholangiocytes which secrete pro-fibrogenic factors acting on myofibroblasts and hepatic stellate cells [39]. Oval cells cannot currently be delivered as cell suspensions for use in clinical trials, and therefore have not been tested. Interestingly, use of G-CSF has been associated with clinical improvement in patients with chronic liver disease [40]. Oval cells express a G-CSF receptor, and levels of oval cell mobilization and proliferation have shown to increase following G-CSF infusion [41]. The underlying mechanism of G-CSF action may be stimulation of endogenous repair.

Transplantation of embryonic stem cells

Embryonic stem cells (ESCs) are pluripotent cells derived from the blastocyst, approximately 5 days following fertilization. However, their extraction requires destruction of the blastocyst, thus presenting ethical and legal concerns to their use, which limits their clinical application.

Using the appropriate culture medium, they can be maintained in an undifferentiated state and directed to differentiate into every cell type, including hepatocytes [42]. Takayama *et al* used two transcription factors, FOXA2 and HNF1α, to induce differentiation of human ESCs into hepatocyte-like cells, which were functionally similar to hepatocytes producing albumin and urea, taking up indocyanine green, and metabolizing drugs [43]. Transplantation of ESC-derived hepatocytes in a mouse model of acute CCl_4 injury increased host hepatocyte proliferation and revascularization, whilst reducing alanine aminotransferase levels [44]. ESCs thus potentially provide an infinitely expandable source of hepatocyte-like cells. As yet however, these cells are more similar to fetal hepatocytes than adult hepatocytes when differentiated *in vitro* and thus further refinements to differentiation protocols are needed.

Direct injection of undifferentiated ESCs causes the formation of teratomas containing hepatocyte-like cells, which creates an unacceptable cancer risk and thus restricts their use. No teratomas were found when ESCs were induced to differentiate into hepatocyte-like cells prior to transplantation, although further longer-term studies are required.

Transplantation of induced pluripotent stem cells

Induced pluripotent cells (iPSCs) were first generated in 2006 [45]. They are somatic cells that have undergone reprogramming to adopt a phenotype similar to embryonic stem cells. This reprogramming is achieved using transcription factors, such as Sox2, KIf4 and c-myc, and iPSCs can be generated from almost any tissue type. Human iPSCs can therefore be induced to differentiate into hepatocyte-like cells, in a process similar to differentiation of ESCs [45]. iPSCs avoid ethical issues associated with use of ESCs, and can potentially be used autologously, removing the need for immunosuppression.

iPSC-derived hepatocyte-like cells were able to engraft and proliferate after transplantation into fumarylacetoacetate hydrolase knockout mice ($FAH^{-/-}$) mice [46]. Notably iPSCs from patients with alpha-1-antitrypsin deficiency were

genetically modified to correct the defect, and were, after successful differentiation *in vitro* to hepatocyte-like cells, able to repopulate recipient murine liver with alpha-1-antitrypsin-positive cells [47].

Hepatocyte-like cells produced from iPSCs may also be used for creating patient-specific *in vitro* disease models, so called 'disease in a dish'. Furthermore they could be used for drug testing, for both toxicity and therapeutic assessment as for example has been tried in familial hypercholesterolemia where cells demonstrated an increase in LDL uptake in response to lovastatin [48]. They have also been used to model alpha-1-antitrypsin deficiency and hereditary tyrosinemia [49].

There are still differences in the gene expression and functionality of hepatocytes produced from iPSCs and primary hepatocytes [50]. The 'hepatocyte-like cells' generated from iPSCs are more similar to fetal hepatocytes than adult hepatocytes, for example because they express alpha fetoprotein, and demonstrate embryonic p450 activity [51]. Following transplantation, hepatocytes from iPSCs do not proliferate as effectively as primary hepatocytes [52]. Furthermore, there remain concerns about the long-term sequelae of genetic reprogramming, and the potential for malignant transformation of cells.

Transplantation of directly programmed cells

In experimental studies, fibroblasts can be directly programmed into hepatocytes with use of specific transcription factors, Hnf4alpha plus Foxa1 [50]. By 'bypassing' the pluripotent stage, there is a reduced risk of malignant transformation with these cells.

In a recent study, fibroblasts were programmed directly into a readily expandable pool of multipotent progenitor cells, which then differentiated into hepatocytes [53]. When transplanted into a model of $FAH^{-/-}$ mice, these cells demonstrated higher rates of repopulation compared to cells derived from iPSCs, although still lower than that of adult hepatocytes.

Transplantation of cells that modulate liver regeneration & repair

Experimental and clinical studies suggest that infusion of bone marrow cells can modulate liver fibrosis [54–56]. Some studies have found an improvement in fibrosis following infusion of unsorted bone marrow cells, whilst others found no effect, and in some, there was worsening of fibrosis [57–60]. Bone marrow contains a mixture of cells, including hematopoietic stem cells, mesenchymal stem cells (MSCs) and macrophages and using such mixed populations limits our understanding of their mechanisms of action. Therefore several studies have attempted to isolate the various bone marrow cell populations to study them individually.

Macrophages in liver disease

Macrophages are derived from monocytes, and can play a role in both progression and regression of fibrosis [61]. There are different subsets of monocytes and macrophages, which differ in their expression of adhesion molecules and chemokine receptors, for example, human monocytes can be defined by differential expression of CD14 and CD16, whereas the corresponding murine markers are $Ly6C^{hi}$ and $Ly6C^{lo}$.

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Ruhnke *et al* demonstrated that monocytes can differentiate into hepatocyte-like cells after treatment with macrophage colony stimulating factor/interleukin (IL)-3 and culture in hepatocyte medium [62]. The resulting cells expressed specific hepatocyte markers and demonstrate functional similarity to hepatocytes. Most evidence suggests that their main action is likely mediated through paracrine effects.

Thomas *et al* administered bone marrow-derived macrophages in CCl_4-induced murine liver fibrosis and demonstrated increased albumin production and reduced liver fibrosis with an associated upregulation of MMP enzymes [60]. Administered macrophages also promoted recruitment of host macrophages, thereby achieving an amplified response. Ramachandran *et al* found that different monocyte subsets were present at different timepoints in liver injury; soon after injury there was a predominance of $Ly6C^{hi}$ monocytes associated with inflammation, whilst at maximal scar resolution there were more $Ly6C^{lo}$ monocytes. These $Ly6C^{lo}$ monocytes were associated with increased expression of MMP, growth factors and phagocytosis-associated genes. Macrophage depletion using mice transgenic for the CD11b promoter diphtheria toxin receptor caused preferential depletion of $Ly6C^{lo}$ monocytes, and persistence of fibrosis in mice [63].

Mononuclear cells in liver disease

Bone marrow mononuclear cells include MSCs, hematopoietic stem cells, endothelial progenitor cells and stromal cells. Six clinical studies have been conducted – of which three were randomized controlled trials – where a single dose of bone marrow mononuclear cells was administered to patients with chronic liver disease [64–69]. Five of the trials demonstrated improvement in liver indices, such as serum albumin, total protein and Child-Pugh score, although the largest trial, with 28 patients in the treatment arm, showed no significant difference [69]. However, they were all small unpowered trials, and possible explanations for discrepancies in their results include different routes of administration, different rates of relapse in patients with alcohol-induced liver disease and different doses of cells infused.

MSCs in liver disease

MSCs are multipotent stem cells that can differentiate into cells of mesodermal tissues, such as bone, muscle, cartilage, fat and neural tissue [70], and can be isolated from a range of tissue sources, including bone marrow, fat and umbilical cord. As umbilical cord MSCs are more abundant than bone marrow, they are proving to be a more regular source of MSCs.

Tanimoto *et al* demonstrated that infusion of 5×10^5 bone marrow-derived MSCs significantly improved fibrosis in the nonobese diabetic/severe combined immunodeficiency mouse exposed to CCl_4 injury [71]. Similar results were obtained in further murine studies, with repeated infusions being more beneficial than a single infusion [72,73].

A number of clinical trials have utilized MSC therapy in liver disease, including two pilot studies, five randomized controlled trials, and several non-randomized controlled trials [74–83]. In all but one, there were some beneficial effects of MSC infusion, including improvement

in model for end-stage liver disease (MELD) score, quality of life, and albumin level for example, although there was no significant change in patient survival. There were no significant side effects and the therapy was well tolerated. Histological evaluation was only performed in two studies, which demonstrated histological improvement following cell transplantation in patients with liver cirrhosis [81,82]. Different doses of cells and routes of administration used in the trials may partially explain the variability in results.

MSCs can differentiate into hepatocytes; however, it is more likely that their immunomodulatory and anti-fibrotic effects will be most relevant. Adult MSCs can be induced to differentiate into hepatocyte-like cells *in vitro* and *in vivo* under the influence of hormones, cytokines, growth factors and other cells [84–86]. Luk *et al* describe differentiation of rat MSCs into hepatocytes following co-culture with hepatocytes, even in the absence of additional growth factors [87]. Rabani *et al* infused MSCs in mice with CCl_4-induced liver injury [88] and demonstrated a subsequent reduction in liver fibrosis, even though relatively few of the cells were found to engraft in the liver. Therefore, paracrine mechanisms are more likely to be responsible for the beneficial effects.

MSCs have immunomodulatory properties, which can influence cells responsible for liver injury, and protect hepatocytes from cell death. For example, prostaglandin E2 made by MSCs reduces production of pro-inflammatory cytokines such as TNFα by dendritic cells, Th1 and Th2 cells, and increases production of anti-inflammatory cytokines such as IL-10 from dendritic cells [89,90]. Prostaglandin E2 also stimulates proliferation of the immunomodulatory T-reg cells, and reduces cytotoxicity of NK cells [91]. Through downregulation of co-stimulatory molecules, C80 and CD86, on antigen presenting cells, MSCs can render T-cells anergic [89]. Xagorari *et al* demonstrated that hepatocytes in MSC-conditioned medium were relatively protected from CCl_4-induced apoptosis, mediated through increased IL-6 production [92].

MSCs exert anti-fibrotic effects, potentially mediated in part by release of IL-10, which reduces collagen deposition and increases expression of MMPs to aid resolution of scar tissue [93,94]. Furthermore, MSCs induced hepatic stellate cell apoptosis in co-culture, through the release of hepatocyte growth factor (HGF) and nerve growth factor [95].

Due to their multiple actions, the utilization of MSCs has been proposed for a range of liver diseases including liver cirrhosis, liver failure and immune-mediated liver diseases, although there are concerns that MSCs may give undergo unwanted differentiation into myofibroblasts, which is more likely to occur if they are injected during the acute liver injury rather than the "resolution phase" [96]. Allogeneic MSC transplantation brings with it risks of immune rejection, and transfer of viruses such as cytomegalovirus and herpes simplex virus [97]. Allogeneic MSCs can also trigger an immune response, leading to rejection of the transplanted cells and hence poor engraftment.

Hematopoietic stem cells

HSCs are multipotent cells, characterized by the expression of cell-surface markers CD34 and CD133, and which have the ability to differentiate into all cells of hematopoietic lineage.

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A number of clinical studies have utilized purified HSCs. Autologous infusions of HSCs were given to patients undergoing portal vein embolisation prior to liver resection for metastatic liver cancer where immediate resection was not possible due to insufficient residual liver volume. HSC infusion was associated with a more rapid increase in liver volume, thus enabling surgery to be performed earlier [98,99].

In another early clinical study, G-CSF-mobilized peripheral blood was collected from nine patients with liver cirrhosis. $CD34^+$ cells were selected, and reinfused into the hepatic artery, and 2 months later serum albumin increased with a reduction in bilirubin [100]. In further studies, some groups obtained peripheral blood to select HSCs whilst others obtained them by direct bone marrow aspirate. The dose and route of injection also varied, with some studies administering HSCs via a peripheral vein, and others via the portal vein or hepatic artery [101–108]. These studies suggest that HSC administration is safe and feasible, with some improvement in liver blood tests and Child-Pugh score. Follow-up studies suggest that the beneficial effects are mainly up to 1 year post infusion [103]. In a non-randomized study of 50 patients with chronic liver cirrhosis secondary to hepatitis C, Salama *et al* found that patients receiving stem cell therapy reported greater improvement in quality of life [109]. However, most clinical trials using HSCs have been small, uncontrolled studies, with short follow up and larger controlled trials will be needed in order to draw robust conclusions.

HSCs can engraft into tissues and participate in tissue regeneration [110–113], although the mechanisms of action remain unclear. There are three main theories:

The first is that HSCs differentiate into functional hepatocytes. When lethally irradiated female mice received whole bone marrow transplants from males, a subgroup of these cells, which were $CD34^+lin^{neg}$, were found to engraft in the liver and up to 2.2% of the hepatocytes contained the Y chromosome [45] indicating a contribution from donor cells. However reports of transdifferentiation of bone marrow cells are variable [114,115], with some evidence suggesting that purified HSCs are more likely to transdifferentiate than other bone marrow cells. In a mouse model of hereditary tyrosinemia, injection of purified HSCs was associated with around 10% seeding in the liver tissue in clusters around the diseased areas. However, with non-HSC bone marrow cells, there was no significant engraftment in this study [110]. Jang *et al* injected HSCs into mice with acute CCl_4 liver injury and observed an increased number of hepatocytes. Through analysis of chromosomes, and protein expression, they concluded that it was transdifferentiation and not fusion that was responsible [116]. They found that the degree of conversion of HSCs into hepatocytes was related to the degree of injury, and within 7 days normal liver function was restored.

A second theory is that there is fusion of HSCs and hepatocytes, as demonstrated in the setting of hereditary tyrosinemia, using the $FAH^{-/-}$ mouse. When $FAH^{-/-}$ mice were transplanted with $FAH^{+/+}$ bone marrow cells they developed nodules containing genes from both the donor and the host [117,118],

which then had a survival advantage, and thus could repopulate the liver. However, in other models, cell fusion is a rare event, and if it occurs the chimeric cells only engraft in bone marrow for a short time [116,119,120]. This is likely because there is no sustained selection pressure or survival advantage. Thus cell fusion is unlikely to explain the effects of HSCs.

The third theory, which has greater support, is that stem cells exert a paracrine effect to promote regeneration and repair, although these exact mechanisms remain undefined. HGF is upregulated during liver injury and in liver failure, and is important in proliferation and development of HSCs [121]. It may be that HSCs secrete cytokines that stimulate endogenous hepatocyte proliferation, such as IL-6, TNF-α and HGF [122,123]. When Sakaida *et al* infused bone marrow into mice with CCl_4-induced liver injury, they demonstrated a significant reduction in liver fibrosis, associated with an increase in hepatic MMP expression [57]. Some studies suggest that donor bone marrow cells are the source of this MMP, or it may be that the HSCs help to recruit other cells which can release MMP, such as macrophages [57,60,124,125].

TRANSLATION INSIGHT

Cellular therapies offer exciting novel treatment options for advanced liver disease, and over the last two decades there have been many advances in this field. A variety of cell therapies have shown therapeutic potential, and many are in clinical trials (Figure 1).

Differentiated hepatocytes can be transplanted, into the liver or in an extra-hepatic location, and hold promise for the treatment of inherited metabolic diseases, and short-term correction of liver failure. However, shortage of organ donors, poor engraftment and the need for immunosuppression limits their use at present. Another option is to use pluripotent stem cells, including ESCs or iPSCs, although there are concerns about their potential to become phenotypically unstable and form tumors. Future work could further investigate mechanisms of preventing this, for example, 'suicide genes' that can be incorporated into cells, which become activated on consuming a particular drug. The use of induced pluripotent cells offers remarkable potential for *in vitro* modelling of human diseases to understand pathogenesis and response to treatments.

Alternative approaches using bone marrow-derived cells, including mononuclear cells/macrophages, MSCs and HSCs, which are promising due to their putative properties in promoting regeneration and repair. Specifically, they may be of particular use in reversing the distorted liver architecture in chronic liver disease, and several small trials have shown that this approach is safe and feasible. Larger trials with standardized treatment protocols will be required to draw robust conclusions.

As cellular therapies for liver disease are still a relatively new concept, much remains to be determined regarding optimizing cellular transplantation protocols. For example, there is a need to determine:

1. Best methods for cell isolation

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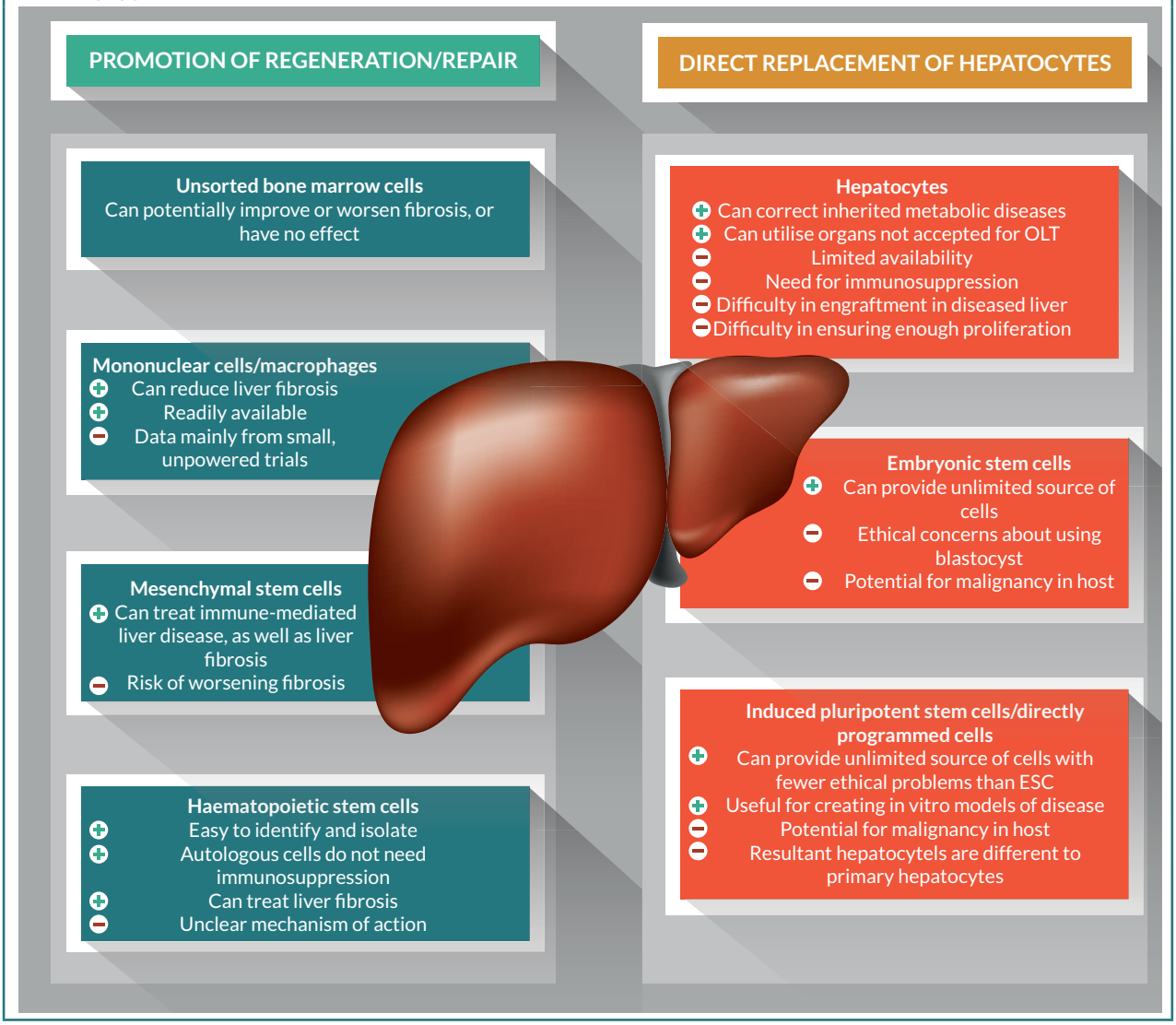
2. Optimal number of cells/ infusions required for transplant – further work should determine the number of cells per infusion and how many infusions are necessary for optimal effect. Research is in progress to find methods of delaying cell clearance, for example using encapsulation of hepatocytes in alginate beads to prevent exchange of antibodies and immune cells [126].

3. Best route of delivery – hepatic or splenic artery infusion is associated with a risk of embolic complications and cell damage from shear stress [127]. Hepatic artery infusion has been associated with artery dissection, Tako-Tsubu syndrome and one cause of radiocontrast nephropathy causing fatal hepatorenal syndrome [101,128]. Portal vein injection is associated with a rise in portal pressures, which can limit the numbers of cells administered at any given time.

4. Methods of improving engraftment – cell transplantation initiates an inflammatory response, causing release of molecules to make the endothelial lining more

FIGURE 1

Summary of some of the key advantages and disadvantages of various cellular therapies for treatment of liver disease.



permeable. The transplanted cells must then adhere to and migrate through the sinusoidal endothelium into the liver sinusoids. Subsequent integration into the liver parenchyma depends on physical joining of the transplanted and native hepatocytes, mediated by MMP enzymes [129]. Only 10–20% of transplanted hepatocytes actually integrate into the tissue. This could be due to damage from the inflammatory response, or a failure to adhere to sinusoidal endothelium for example. Researchers have investigated ways of improving engraftment. For example, donor cells may be modified to include extracellular matrix components to aid adhesion to the liver sinusoidal endothelial cells [130]. Pre-treatment of rats with a TNF-α antagonist, etanercept, dampened the inflammatory response to hepatocyte transplantation, prolonging cell survival [131]. Agents such as doxorubicin can be given to disrupt the hepatic endothelial barrier permitting easier passage of transplanted cells.

5. Methods for tracking cells – genetically labelled probes that are often used in animal studies are not safe for use in humans. Short-term cell tracking, for example to assess cell distribution, can be achieved using indium-111 and 99m technetium. However, further work is needed to find the best way of assessing the fate of cells over a longer period.

6. The optimal immunosuppression regime – allogeneic cell transplantation produces an immune response which can lead to rapid cell clearance. Further work should look into the best choice of immunosuppressive agents to prevent this happening. Ideally, cellular therapies should remove the need for immunosuppression all together.

CONCLUSION

Cellular therapies have the potential to transform the future management of acute and chronic liver diseases. Although experimental work and small clinical trials suggest therapeutic benefit, the underlying mechanisms are uncertain. Further work needs to establish practical aspects of how the cell therapy should be delivered, and ideally to remove the need for immunosuppression. In the use of stem cells, the theoretical risk of malignancy needs to be researched and minimized further. A greater understanding of the underlying mechanisms of various cell therapies is crucial to tailoring the cell therapy for specific disease processes.

Once the above issues are addressed and tested in large randomized, controlled trials with long-follow up periods, cellular therapies have the potential to revolutionize the management of advanced liver disease.

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