Stem Cell Research (2013) 11, 1348–1364

Available online at www.sciencedirect.com

ScienceDirect

www.elsevier.com/locate/scr

REVIEW

Transplantation of mesenchymal stem cells for the treatment of liver diseases, is there enough evidence?

Raphael P.H. Meier[a], Yannick D. Müller[a,b], Philippe Morel[a], Carmen Gonelle-Gispert[a,1], Leo H. Bühler[a,*,1]

[a] *Surgical Research Unit, Transplantation and Visceral Surgery, University Hospital and Medical Faculty, Geneva, Switzerland*
[b] *Division of Clinical Immunology and Allergology, Department of Medical Specialties, University Hospital and Medical Faculty, Geneva, Switzerland*

Received 3 April 2013; received in revised form 18 August 2013; accepted 21 August 2013
Available online 29 August 2013

Abstract Mesenchymal stem cells or multipotent mesenchymal stromal cells (MSCs) have been extensively investigated in small animal models to treat both acute and chronic liver injuries. Mechanisms of action are not clearly elucidated but may include their ability to differentiate into hepatocyte-like cells, to reduce inflammation, and to enhance tissue repair at the site of injury. This approach is controversial and evidence in large animals is missing. Side effects of MSC infusion such as the contribution to a fibrotic process have been reported in experimental settings. Nevertheless, MSCs moved quickly from bench to bedside and over 280 clinical trials are registered, of which 28 focus on the treatment of liver diseases. If no severe side-effects were observed so far, long-term benefits remain uncertain. More preclinical data regarding mechanisms of action, long term safety and efficacy are warranted before initiating large scale clinical application. The proposal of this review is to visit the current state of knowledge regarding mechanisms behind the therapeutic effects of MSCs in the treatment of experimental liver diseases, to address questions about efficacy and risk, and to discuss recent clinical advances involving MSC-based therapies.

Contents

Abbreviations: CCl-4, carbon tetrachloride; GVHD, graft versus host disease; HGF, hepatocyte growth factor; ISCT, International Society of Cellular Therapy; INR, international normalized ratio; IFN-γ, interferon-γ; MSC, mesenchymal stem cell; MELD, Model for End-Stage Liver Disease; TGF-β, transforming growth factor-beta 1; TNF-α, tumor necrosis factor-alpha.

* Corresponding author at: Surgical Research Unit, Transplantation and Visceral Surgery, University Hospital of Geneva, 4 rue Gabrielle-Perret-Gentil, Geneva, Switzerland. Fax: +41 22 372 76 89.
E-mail address: leo.h.buhler@gmail.com (L.H. Bühler).
[1] Contributed equally.

1873-5061
http://dx.doi.org/10.1016/j.scr.2013.08.011

Introduction

Mesenchymal stem cells (MSCs), also called multipotent mesenchymal stromal cells, are adult progenitor cells originating from neural crest and mesoderm (Slukvin and Vodyanik, 2011; Vodyanik et al., 2010; Takashima et al., 2007). Originally, MSCs were found to reside in the stromal fraction of the bone marrow, wherein they contribute to nonhematopoietic stromal cell renewal including osteoblasts, adipocytes and chondrocytes (Pittenger et al., 1999; Friedenstein et al., 1976; Prockop, 1997). In addition to their mesenchymal differentiation capacities, MSCs may have a multidirectional differentiation potential and differentiate into cell types normally derived from endoderm or ectoderm such as hepatocytes (Hong et al., 2005; K.D. Lee et al., 2004; Snykers et al., 2009). Further, it has been shown that MSCs can be immunosuppressive (Bartholomew et al., 2002; Di Nicola et al., 2002; Glennie et al., 2005; Krampera et al., 2003; Le Blanc et al., 2003; Tse et al., 2003; Le Blanc and Ringden, 2005). Given these remarkable properties, their easy accessibility and strong in vitro expansion ability, MSCs were considered as an ideal cell source for autologous stem-cell-based replacement therapies. Horwitz et al. demonstrated their utility in the treatment of osteogenesis imperfecta in children by taking advantage of the bone microenvironment regeneration capacities of MSCs (Horwitz et al., 1999). Children had increased growth velocity and total body mineral content, and fewer fractures. MSC capacity to regulate the immune system has been investigated in diverse diseases; Le Blanc et al. showed that systemically injected MSCs were effective for the treatment of steroid resistant graft versus host disease (GVHD) in bone marrow transplanted patients (Le Blanc et al., 2004, 2008); of note, 55% of them had a complete response with MSC-based treatment. Other beneficial effects were observed in various clinical situations, such as renal transplantation (as an induction therapy) (Tan et al., 2012), multiple sclerosis (Karussis et al., 2010) or systemic lupus erythematosus (as an adjuvant treatment) (Sun et al., 2009).

Currently, MSCs are investigated with the perspective to treat both acute and chronic liver diseases. Some studies provided experimental and clinical evidences suggesting that transplantation of MSCs can sustain liver function in the situation of an acute or chronic liver injury (Peng et al., 2011; Li et al., 2012). However, the mechanisms of action underlying the anti-fibrotic effects are currently not well understood and long term safety and efficacy of such treatment remain to be determined. MSCs may carry a risk of fibrotic reaction (Baertschiger et al., 2009; Forbes et al., 2004; Li et al., 2009), malignant transformation (Casiraghi et al., 2013) and virus transmission (Sundin et al., 2006, 2008) that may not be outweighed by the clinical benefit. In this context, large animal studies are needed to provide enough valid data to allow large clinical trials to start.

The proposal of this review is to visit the current state of knowledge regarding mechanisms responsible for the therapeutic effects of MSCs in the treatment of experimental liver diseases, to address questions regarding efficacy and safety, and to discuss recent clinical advances involving clinical MSC-based therapies.

MSCs and their physiological function

In the late 1960s Friedenstein and colleagues described bone marrow stromal cells and referred to them as nonhematopoietic colony-forming-unit fibroblasts (Friedenstein et al., 1968, 1970). Later, these cells were found to renew themselves and differentiate in vitro in osteoblasts, chondrocytes and adipocytes (Pittenger et al., 1999; Friedenstein et al., 1974, 1976). Multipotency and self-renewal being the hallmarks of "stemness", MSCs were recognized as osteogenic (i.e. nonhematopoietic) "stem" cells of the bone marrow (Friedenstein et al., 1987), wherein they constitute a small subset accounting for 0.001 to 0.01% of the cells. However, their contribution to nonhematopoietic stromal cell renewal in vivo was demonstrated much later (Morikawa et al., 2009). The term "mesenchymal stem cells" was used first by Caplan (1991). Later on, these cells were found to reside in adipose tissue (Hauner et al., 1989; Zuk et al., 2002; R.H. Lee et al., 2004), peripheral blood (Zvaifler et al., 2000; Fernandez et al., 1997), umbilical cord blood (Erices et al., 2000), various fetal tissues (in't Anker et al., 2003; da Silva Meirelles et al., 2006), placenta (Fukuchi et al., 2004), pancreas (Baertschiger et al., 2008) and liver (Najimi et al., 2007). Thus, the term "mesenchymal stem cell" was used to qualify stromal cells isolated following different protocols and arising from diverse tissues. Facing the heterogeneity of what was called "MSC", the International Society of Cellular Therapy (ISCT) proposed a new terminology, calling

these cells "multipotent mesenchymal stromal cells (MSCs)" (Dominici et al., 2006). It was recommended to specify the tissue origin of MSCs, e.g. bone marrow-derived MSCs and adipose tissue-derived MSCs. The ISCT established criteria to define MSCs: (i) plastic-adherent cells under standard culture conditions, (ii) CD105, CD73 and CD90 positive, (iii) CD11b, CD14, CD34, CD45, CD79a and HLA-DR negative, and (iv) ex-vivo differentiation into osteocytes, adipocytes and chondrocytes should be demonstrated.

In addition to their mesenchymal differentiation capacities, MSCs have a multidirectional differentiation potential including transdifferentiation into endothelial cells (Oswald et al., 2004), cardiocytes (Makino et al., 1999), neurocytes (Snykers et al., 2009; Weiss et al., 2006), insulin producing beta-cells (Xie et al., 2009) and hepatocyte-like cells (Hong et al., 2005; K.D. Lee et al., 2004; Snykers et al., 2009). However, their differentiation into these types of cells is still controversial. The major concern is the lack of reproducibility in the generation of well differentiated cells which might be explained by the use of different subpopulations of MSC. Currently, standardized protocol for isolation, expansion and characterization is not uniformly used by researchers. Although it has been suggested that such transdifferentiation, crossing lineage barrier, occurs in pathologic situations, to our best knowledge, no evidence clearly demonstrated that such a phenomenon can occur in a physiologic situation (Duncan et al., 2009). Hepatocytes seemed to be renewed from small hepatocyte-like progenitors (Gordon et al., 2000; Vig et al., 2006) originating from a periportal niche and migrating to the central vein (Fellous et al., 2009).

MSCs have been studied in much detail regarding their differentiation potential and immunomodulatory properties when transplanted as therapeutic agents, but their physiologic role in vivo remains largely undefined (Nombela-Arrieta et al., 2011). Their small number and the lack of specific markers made them difficult to identify in vivo until recently. Studies showed that they constitute a population positive for CD146 present mainly in the adventitia of microvessels (Morikawa et al., 2009; Sacchetti et al., 2007). Furthermore, nestin marker was reported to selectively label MSCs and allowed to confirm their role as osteochondral progenitors (Mendez-Ferrer et al., 2010). In the bone marrow MSCs provide soluble factors and extracellular matrix proteins that promote hematopoietic stem cell maintenance (Mendez-Ferrer et al., 2010), and are possibly issued from blood vessel pericyte (Hirschi and D'Amore, 1996; da Silva Meirelles et al., 2009; Odorfer et al., 2011). Despite the fact that MSCs possess both in vivo and in vitro immunomodulatory activities (Nauta and Fibbe, 2007), no evidence exists to date that these regulatory properties are an inherent role of MSC in its native localization in the tissues. However, a physiological role in immune regulation of MSCs in the bone marrow is plausible, as it is the site where the adaptive immune responses originate.

Mechanisms implicated in the therapeutic effects of MSCs

Hereafter, we will focus on the properties that may allow MSCs to treat liver diseases. First, according to some investigators, MSCs are able to differentiate into hepatocyte-like cells and thus to compensate for impaired liver function. Second, MSCs have immunomodulatory properties and secrete anti-inflammatory cytokine.

MSCs as hepatocyte-like cells

Using specific culture conditions, some studies showed that MSCs undergo a phenotypic change, express genes typically expressed in hepatocytes, fulfill some metabolic functions similar to hepatocyte, and thus postulated that MSCs can transdifferentiate into hepatocyte-like cells in vitro (K.D. Lee et al., 2004; Li et al., 2010; Ong et al., 2006; Banas et al., 2007; Piryaei et al., 2011; Aurich et al., 2007; Campard et al., 2008; Aurich et al., 2009).

First, it is crucial to define what characteristics are needed for a differentiated cell to be comparable to a primary hepatocyte. The minimal set of functions of a true hepatocyte includes (Hengstler et al., 2005) (i) metabolic function (detoxification of xenobiotics (i.e. diazepam, lidocaine) and endogenous substances (i.e. ammonia)); (ii) synthetic function (i.e. albumin, clotting factors, complement, lipids); and (iii) storage (glycogen and fat soluble vitamins). For example, a candidate hepatocyte-like cell would be almost indistinguishable from a primary hepatocyte if the following criteria are fulfilled: synthesis of albumin, urea, and fibrinogen; functional cytochromes P450 2A6, 1A2, 2C9, 2B6, 2C19, 2D6, 3A4, and 2C8; and functional uridine 5′-diphospho-glucuronosyltransferase, glutathione-S-transferase, and sulfotransferase (Hengstler et al., 2005). In addition to this, other markers such as α-fetoprotein, cytokeratin-18, or hepatocyte nuclear factor 4 are used to detect hepatocyte-like cells. However, taken individually, these hepatic lineage markers are not restricted to the liver; for example, cytokeratins are present in gastric cardia, skin (Korbling et al., 2002), and lung (Schlichenmaier et al., 2002); albumin and α-fetoprotein are detected in pancreas (Beerheide et al., 2002). Overall, RNA and protein analyses alone are not sufficient, and functionality must be tested. The systematic assessment of the complete phenotype is probably unrealistic, and it is commonly accepted that the demonstration of at least one metabolic function, one synthetic function, and one storage function provides sufficient evidence to define a differentiated cell as a hepatocyte-like cell. It is much more challenging to determine whether a cell is a true hepatocyte in vivo. Immunostaining for albumin, cytokeratin-18 or hepatocyte nuclear factor 4 is a recognized indicator of hepatocyte transdifferentiation but does not allow to asses functionality. Models allowing cells to completely replace deficient native hepatocytes can provide sufficient evidence of complete functionality (Lagasse et al., 2000), although cell fusion cannot be excluded. Other strategies include isolation of newly formed hepatocytes followed by ex-vivo phenotype assessment or detection of human proteins (e.g. albumin) in xenotransplantation models.

In vitro, the transdifferentiation of MSCs in hepatocyte-like cells was obtained when cells were cultured on Matrigel with a specific medium containing several growth factors e.g. fibroblast growth factor, epidermal growth factor, hepatocyte growth factor (HGF), insulin, and dexamethasone (Campard et al., 2008). Once differentiated, MSCs expressed cytoplasmic

markers of hepatic lineage including albumin, α-fetoprotein, cytokeratin-18, connexin-32, hepatocyte nuclear factor 4, hepatocyte-specific antigen, and dipeptidyl-peptidase IV or cell surface markers including Thy-1, c-Kit, and Flt-3 detected by RT-PCR or immunostaining (Hong et al., 2005; K.D. Lee et al., 2004; Aurich et al., 2007). Similar to hepatocytes, the differentiated MSCs stored glycogen, produced low quantities of urea, transported low-density lipoprotein (LDL), and expressed an inducible cytochrome P450 2B6 (assessed by RT-PCR and enzyme activity assay) (K.D. Lee et al., 2004). It is important to note that MSCs still expressed stem cell or mesenchymal markers such as CD90, alpha-smooth muscle actin, vimentin, and fibronectin suggesting that complete transdifferentiation was not achieved (Campard et al., 2008). Among the previously cited studies (K.D. Lee et al., 2004; Li et al., 2010; Ong et al., 2006; Piryaei et al., 2011; Aurich et al., 2007; Campard et al., 2008; Aurich et al., 2009), MSC-derived hepatocyte-like cells fulfill only a low functionality by percentage (as defined by Hengstler et al. (2005)) compared to professional hepatocytes, i.e. ranging from 8% to 23%. And finally, it is likely that only a small fraction of MSCs underwent into hepatocyte transdifferentiation (Lin et al., 2011).

MSCs from other sources, such as adipose tissue were also successfully used to demonstrate hepatocyte transdifferentiation. Seo et al. showed that human adipose tissue-derived MSCs differentiated into hepatocyte-like cells by the treatment of HGF, oncostatin M and dimethyl sulfoxide in vitro (Seo et al., 2005). MSC-derived hepatocyte-like cells expressed albumin and alpha-fetoprotein and showed LDL uptake and production of urea. Others demonstrated that umbilical cord blood-derived MSCs were also able to differentiate into hepatocyte-like cells in vitro (Hong et al., 2005). MSCs expressed typical hepatocyte markers and were reported to incorporate LDL. Altogether, these experiments suggest that certain MSC populations, either clonally expanded or expanded as heterogenic population, independently from tissue of origin, have the potential to transdifferentiate into hepatocyte-like cells. Inconsistencies between studies may be due to different isolation protocols and culture conditions which may be fundamental to select growth of subpopulation of MSCs which are more prone for genetic reprogramming (Phinney and Prockop, 2007).

In vivo, human MSCs were reported to differentiate into hepatocyte-like cells when transplanted during acute or chronic liver failure in rodents (Sato et al., 2005; Kuo et al., 2008). Human MSC xenografts were shown to rescue nonobese diabetic immunodeficient mice from carbon tetrachloride (CCl-4)-induced fulminant hepatic failure (Kuo et al., 2008); in this situation 4% of the liver was repopulated by MSCs of human origin. Further, labeled human adipose tissue-derived MSCs were retrieved in the liver of CCl-4-injured severe combined immunodeficiency mouse after intravenous injection and differentiated into hepatocyte-like cells (Seo et al., 2005). Sato et al. used a specific marker for human Y chromosome and observed that human MSCs can transdifferentiate in hepatocyte after xenotransplantation by intrahepatic injection to allyl alcohol-treated rats (Sato et al., 2005). Evidence of the ability of MSCs to transdifferentiate and integrate into liver tissue was also reported in larger animals. Chamberlain et al. injected human clonally derived MSCs by an intraperitoneal or intrahepatic route into preimmune fetal sheep in the absence of liver injury (Chamberlain et al., 2007). The intrahepatic injection resulted in the more efficient and widespread generation of hepatocytes 70 days after xenotransplantation (13% of the hepatocytes). These experiments suggested that hepatocyte-like cells issued from MSCs could replace damaged hepatocyte and thus provide a supportive effect in an acute or chronic liver injury situation. Although it is possible that the microenvironment of the injured liver allows appropriate conditioning for implantation and transdifferentiation, in vivo signals driving transdifferentiation into hepatocytes are yet not identified. A further issue not usually discussed is phenotype stability, even if MSCs engraft in the injured liver and form into hepatocyte-like cells, it is likely that chronic injury provocation (e.g. high levels of TGF-β) precludes those cells to stay epithelial-like. In this respect, Kisseleva et al. showed that cells recruited from the bone marrow to the fibrotic liver (induced by bile-duct ligation) finally become collagen-producing fibrocytes (Kisseleva et al., 2006). Moreover, Sato et al. showed that after a very small increase in the percentage of MSCs that transdifferentiated in hepatocyte-like cells in allyl alcohol-injured livers (i.e. 0.5% at day 28 after cell transplantation), this percentage drops to zero at day 58 (Sato et al., 2005). Finally, MSCs are not the only bone-marrow progenitor cell that can give rise to hepatocytes, hematopoietic stem cells were shown to significantly contribute to liver regeneration after acute liver injury (Lagasse et al., 2000) and multipotent adult progenitor cells can differentiate into hepatocyte-like cells (Schwartz et al., 2002). The contribution of hematopoietic stem cells, unlike MSCs, has been demonstrated to occur by cell fusion rather than by transdifferentiation (Wang et al., 2003).

Immunomodulation by MSCs

Liver injury caused by persistent inflammation is accompanied with T cell, B cell and monocyte infiltration of the liver (Kisseleva and Brenner, 2012). It has been demonstrated that immunosuppressive therapies can prevent the recurrence of some liver disease before and after liver transplantation (Dmitrewski et al., 1996; Manousou et al., 2010; Mohamadnejad et al., 2005). Moreover, decreased inflammation is likely to be beneficial to liver regeneration during acute hepatocellular insufficiency (Yang et al., 2007; Sgroi et al., 2011). In this respect, MSC immunomodulatory and immunosuppressive properties are potentially involved in the favorable effect of MSC transplantation in chronic and acute liver diseases. First, MSCs are nonimmunogenic cells. Indeed, MSCs have few MHC-I, and lack MHC-II as well as costimulatory molecules CD80, CD86 or CD40 on their surface, and thus fail to stimulate an allogeneic T-cell response (Jacobs et al., 2013). Second, MSCs are immunosuppressive; they suppress naive and memory T lymphocyte activation, proliferation and cytotoxicity (Uccelli et al., 2008). In vitro, solid evidence of MSC immunosuppressive effect was provided by Di Nicola et al. (Di Nicola et al., 2002). Autologous and allogeneic bone marrow-derived MSCs dose-dependently and contact-independently reduced CD4(+) T cell proliferation and CD8(+) T cell cytotoxicity CD8(+) stimulated by allogeneic cells, polyclonal activators (Di Nicola et al., 2002), or specific antigen (Krampera et al., 2003). The

depletion in transforming growth factor beta (TGF-β) 1 and HGF partially restored T cell proliferation (Di Nicola et al., 2002). However, the role of these two growth factors was not observed in other settings (Le Blanc et al., 2003; Tse et al., 2003). Aggarwal and Pittenger demonstrated that MSCs exert their immunosuppressive effects through the secretion of prostaglandin E2 that further promoted IL-10 secretion by dendritic cells (Aggarwal and Pittenger, 2005); this was confirmed by others (Rasmusson et al., 2005). Moreover, MSC caused an increase in regulatory T cells and a decrease in tumor necrosis factor alpha (TNF-α) (produced by dendritic cells), interferon-γ (IFN-γ) (produced by TH-1 cells), and IL-4 (produced by TH-2 cells) (Aggarwal and Pittenger, 2005). Another candidate mediator of MSC suppressive effects is indoleamine 2,3-dioxygenase. Meisel et al. showed that MSC expressed indoleamine 2,3-dioxygenase upon IFN-γ stimulation, leading to tryptophan depletion and thus inhibition of T cell proliferation (Meisel et al., 2004); this was further confirmed by others (Suva et al., 2008; Sheng et al., 2008). Another important factor involved in MSC-mediated immunomodulation is the human leukocyte antigen (HLA)-G5 protein. Selmani et al. demonstrated that MSCs produced soluble HLA-G5 that suppressed T-cell proliferation and increased regulatory T cell numbers (Selmani et al., 2008). MSC inhibition was also extended to B cell (Glennie et al., 2005; Corcione et al., 2006), dendritic cells (Nauta et al., 2006; Zhang et al., 2004; Jiang et al., 2005), and natural killer cells (Nauta and Fibbe, 2007; Sotiropoulou et al., 2006).

The suppressive capacities of MSCs were further confirmed in preclinical studies. A first report by Bartholomew et al. demonstrated that the injection of allogeneic bone marrow-derived MSCs prolonged third-party skin graft survival in baboon (Bartholomew et al., 2002). Other studies used models of immune-mediated diseases and confirmed with strong evidence that MSC can efficaciously modulate pathogenic B- and T-cell response (Zappia et al., 2005; Gerdoni et al., 2007; Augello et al., 2007; Urban et al., 2008).

In clinical studies, the in vivo immunosuppressive effect of transplanted MSCs has been successfully shown to treat acute steroid-resistant GVHD in hematopoietic stem cell-transplanted patients (Le Blanc et al., 2004, 2008). One to five doses containing 1.4×10^6 MSCs (HLA-matched or -mismatched) were infused in 55 patients, and a complete response or improvement was observed in 71% of them. The 2-year probability of survival was 35%, compared to 10% in historical series (Ringden and Nilsson, 1985; Deeg, 2007). Of note, the response to MSC treatment was not restricted to single organs: skin, gastrointestinal tract, and liver GVHD were significantly decreased (e.g. MSC decreases liver mononuclear cell infiltration and endothelialitis (Tobin et al., 2013)). Of note, no patients had acute or late side-effects (considering a median follow-up of 16 months). MSC effect has been attributed to the inhibition of donor T-cell reactivity to histocompatibility antigens of the recipient tissues. A possible drawback could be that infused MSCs might impair the therapeutic graft-versus-leukemia effect by recipient T-cell and increase the incidence of leukemia relapses (Ning et al., 2008).

Another clinical trial taking advantage of MSC immunomodulatory effects showed that autologous MSC infusion was effective as an induction therapy in kidney recipients (Tan et al., 2012). This therapy allowed reducing acute rejection rate, decreasing the risk of opportunistic infection, and increasing estimated renal function at 1-year confirming the immunosuppressive capacities of MSCs in human. These promising results open the path to the use of MSCs in liver transplanted patients (Popp et al., 2009).

Regardless of the definitive elucidation of the underlying mechanisms, these investigations suggest that MSCs have effective immunosuppressive properties that could theoretically participate in the reduction of leukocyte infiltration during acute and chronic liver injuries, and after liver transplantation.

Cytokines secreted by MSCs during liver injury

Chemokines and cytokines secreted by MSCs might be effective in reducing inflammation and hepatocyte apoptosis in both acute and chronic liver injuries. MSC-derived cytokines that were shown to improve liver injury are summarized in Fig. 1. In a rat model of D-galactosamine-induced acute liver injury, Parekkadan et al. showed that human bone marrow-derived MSC-conditioned medium injection, or an extracorporeal perfusion using a bioreactor containing MSCs, provided a significant survival benefit in the treated animal compared to controls (Parekkadan et al., 2007a). Although no specific mechanism of action has been identified, the authors suggested an effect on immune cell migration to the liver, and postulated that soluble factors may be implicated in the observed effects. Potential candidates included vascular endothelial growth factor (Zhen et al., 2010), HGF, insulin-like growth factor binding proteins (Leu et al., 2003), or IL-6. In a subsequent study using the same model of liver injury van Poll et al. observed that administration of MSC conditioned medium down-regulated blood levels of IL-1β, TNF-α and IL-6, increased levels of IL-10 (van Poll et al., 2008). This was accompanied with a lower lymphocyte infiltration in the liver, a reduced hepatocyte apoptosis and an increase in hepatocyte proliferation.

MSCs were shown to secrete epidermal growth factor that could promote hepatocyte proliferation and function during liver regeneration (Natarajan et al., 2007). MSCs were also shown to reduce the proliferation of stellate cells and collagen type I synthesis through the secretion of TNF-α (Parekkadan et al., 2007b), and to promote hepatic stellate cell apoptosis through the secretion of nerve growth factor (Lin et al., 2009). Higashiyama et al. suggested that MSCs mediate an antifibrotic effect through the expression of matrix metalloproteinase-9 that degrades the extracellular matrix (Higashiyama et al., 2007). There are probably other anti-inflammatory cytokines implicated, for example, some MSC subpopulations were shown to secrete IL-1 receptor antagonist and thus reduced fibrosis (Ortiz et al., 2007). However, MSCs did not only promote anti-inflammatory signals, they also secreted proinflammatory cytokines such as TGF-β 1 and 3, IL-6, monocyte chemoattractant protein-1, macrophage inflammatory protein-1α and β or monokine induced by IFN-γ (van Poll et al., 2008; Salazar et al., 2009; Boomsma and Geenen, 2012). Interestingly, it has been suggested that depending upon selective toll-like receptor activation, MSCs can polarize into two different phenotypes: the TLR4 activation promoting mostly proinflammatory

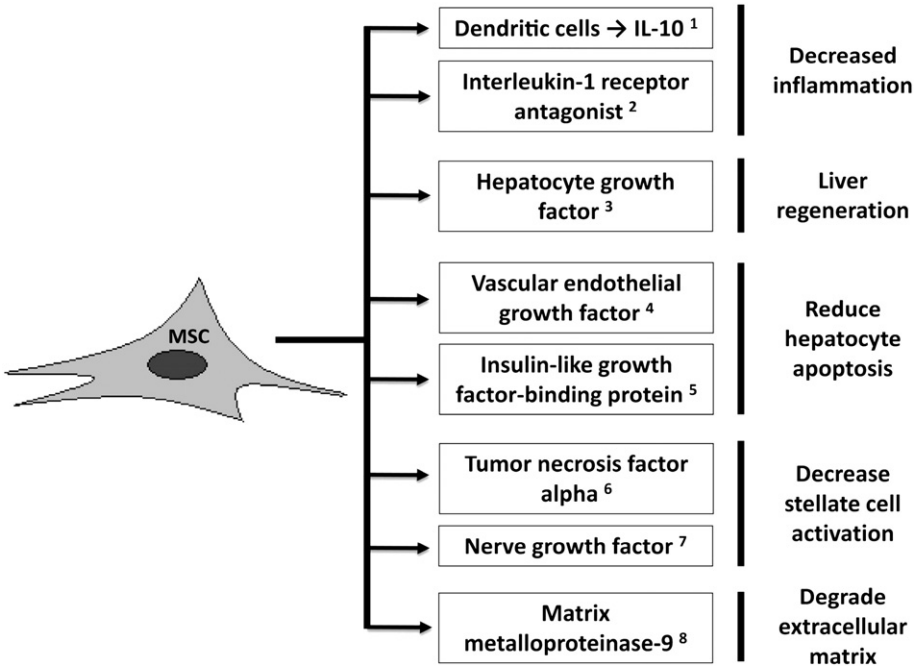

Figure 1 MSC-derived cytokines that potentially protect the liver during injury. 1. Aggarwal and Pittenger Blood 2005, Rasmusson et al. Exp Cell Res 2005. 2. Ortiz et al. Proc Natl Acad Sci USA 2007. 3. Di Nicola et al. Blood 2002, Parekkadan et al. PLoS One 2007. 4. Parekkadan et al. PLoS One 2007, Zhen et al. Cytotherapy 2010. 5. Parekkadan et al. PLoS One 2007. 6. Parekkadan et al. Biochem Biophys Res Commun 2007. 7. Lin et al. Life Sci 2009. 8. Higashiyama et al. Hepatology 2007.

signals, and TLR3 stimulation promoting mostly immunosuppressive ones (Waterman et al., 2010). In summary, MSC cytokine secretion is likely to reduce inflammation and to promote liver regeneration during acute and chronic liver injuries. However, exact mechanisms are unknown and MSC cytokine secretion could be proinflammatory in some circumstances.

MSC transplantation in animal models of liver injury

Hereafter, we will discuss the main findings regarding MSC transplantation in animal models of acute and chronic liver injuries.

Acute liver injury

Numerous studies tried to demonstrate the therapeutic potential of MSCs in the treatment of acute liver failure. A few studies showed beneficial effects. Using a model of carbon tetrachloride induced acute liver failure in immunodeficient mice Kuo et al. showed that intrasplenically or intravenously transplanted human MSCs engrafted into recipient liver, differentiated into functional hepatocytes, and rescued liver failure (Kuo et al., 2008). Similarly, intravenously injected human MSCs derived from adipose tissue also improved liver functions (i.e. decreased levels of ammonia, uric acid, alanine transaminase, and aspartate transaminase) in nude mice with CCl-4-caused liver injury (Banas et al., 2008). Soluble factors possibly responsible for this effect were numerous and included: IL-1R-alpha, IL-6, IL-8, granulocyte colony-stimulating factor, granulocyte macrophage colony-stimulating factor (GM-CSF), monocyte chemotactic protein-1, nerve growth factor, and HGF. More recently, in a rat model of hepatic ischemia–reperfusion injury, it has been observed that rat MSCs inhibited hepatocellular apoptosis and stimulated regeneration suggesting a favorable bystander effect of MSCs during acute liver injury (Kanazawa et al., 2011). More recently, rat bone marrow-derived MSC infusion was shown to reduce aspartate transaminase, alanine transaminase and alkaline phosphatase in rats with acute CCl-4 liver injury (L. Zhao et al., 2012). These studies have established the proof of principle in small animals, but the elucidation of mechanisms involved in the observed effect remained elusive. Studies on large animal models are sparse and to our best knowledge a few studies were performed on pigs. A validation in a large animal model is requested by many national regulatory agencies before starting clinical application, as for other innovative and invasive therapies such as xenotransplantation. Allotransplantation of bone marrow-derived MSCs significantly enhanced liver hyperplasia after selective portal vein ligation in pigs (Liska et al., 2009). This result was attributed to bystander effects of the transplanted MSCs as only few cells were detected in recipient livers two weeks after the infusion. Moreover, transaminases remained at high levels after MSC injection, indicating that the transplanted MSCs were not sustaining liver function. This might be due to the small number of cells that were reported to engraft in the liver tissue. Li et al. transplanted human bone marrow-derived MSCs in pigs with fulminant liver failure induced with D-galactosamine, and receiving no immunosuppression (Li et al., 2012). Thirty million human MSCs were infused intraportally or through peripheral veins. Thirteen of the 15 animals with intraportally

injected MSCs achieved long-term survival (>6 months), whereas no animal injected with MSCs in peripheral veins or sham injected survived. Up to 30% of the hepatocytes were bone marrow-derived MSC-derived during the early weeks following the procedure. Cao et al. transplanted human placenta-derived MSCs in miniature pigs with D-galactosamine-induced acute liver failure (Cao et al., 2012). MSC-treated animals had improved transaminases and bilirubin levels in the serum. Transplantation of human MSCs via the portal vein reduced liver inflammation, promoted liver regeneration and improved survival, whereas transjugular MSC injection did not. Human albumin and cytokeratin 18 positive cells were detected in recipient's liver 5 months after transplantation. Of note, only transplanted animals were immunosuppressed; this probably precludes a strict comparison with nontransplanted animals. Altogether, these experiments have shown positive effects in the large animal model, but again, no underlying mechanism was so far identified.

Some other studies on mice and rats testing MSC transplantation reported either no effect or effects which could potentially be harmful. After intrahepatic injection of human bone marrow-derived MSC transplantation into a mouse model of acute liver injury induced by 2/3 hepatectomy, MSCs expressed alpha smooth muscle actin and merged with collagen deposition suggesting that MSCs are able to adopt a fibrogenic phenotype (Baertschiger et al., 2009). These results were confirmed by other reports wherein MSCs adopted a myofibroblast-like shape after transplantation in a model of acute liver injury (di Bonzo et al., 2008). This fibrogenic potential may be related to the high expansion of MSCs; it is possible that some clones are prone to differentiate into fibroblast-like cells and thus contribute to the fibrotic process of the injured liver. Overall, in the situation of acute liver injury, MSC transplantation might lead to unwanted effects and contribute to the fibrotic reaction.

Chronic liver injury

MSCs have also been tested to reduce fibro-inflammatory reactions, and several studies reported that MSCs inhibit the progression of liver fibrosis. Fang et al. injected bone marrow-derived MSCs from male BALB/c mice intravenously into female BALB/c mice with CCl-4-induced liver fibrosis and observed reduced hydroxyproline levels in the serum, and fewer histological signs of hepatic necrosis when compared to controls (Fang et al., 2004). In this study MSCs engrafted into host's liver at low frequency suggesting a bystander effect. Similarly, others showed that rat bone marrow-derived MSC transplantation decreased collagen expression and liver hydroxyproline content (Abdel Aziz et al., 2007) and restored albumin production in rats with chronic CCl-4 liver injury (Oyagi et al., 2006). Human umbilical cord-derived MSCs (Tsai et al., 2009; Yan et al., 2009) and human placenta-derived MSCs (Lee et al., 2010) also mediated anti-fibrotic effects. More recently in vitro pre-differentiated hepatocyte-like cells were successfully used to treat experimental liver fibrosis (Piryaei et al., 2011; Mohsin et al., 2011). In these studies the authors reported that MSCs predifferentiated into hepatocyte-like cells were more efficient for preventing liver fibrosis. On the contrary, Tsai et al. observed favorable effects in the absence of MSC transdifferentiation (Tsai et al., 2009). Human umbilical cord-derived MSCs were directly injected into fibrotic livers of rats wherein MSCs engrafted and exerted their effect without transdifferentiation into hepatocytes. As suggested above, MSCs could exert their antifibrotic effects through secretion of matrix metalloproteinases (Higashiyama et al., 2007; Chang et al., 2009; Hardjo et al., 2009). These enzymes are normally upregulated during liver fibrosis in response to collagen accumulation, an increase in their activity could allow to a more efficient degradation of extracellular matrix. Zhao et al. observed an increase in the level of anti-inflammatory cytokines such as IL-10 and decreased levels of inflammatory cytokines such as IL-1β, IL-6, TNF-α and TGF-β in mice treated with intravenous infusion of MSCs (W. Zhao et al., 2012). Thus, MSCs may specifically and nonspecifically reduce local inflammation, providing a protection against chronic liver injury. In summary, protective effects of MSC transplantation on liver fibrosis have been shown in rodents, but again, the supposed mechanisms of action are not fully understood.

Several studies failed to observe significant beneficial effects of MSCs in chronic liver injury model. Some studies even indicated that MSCs might contribute to the fibrotic tissue. In a rat hepatic injury model with prolonged CCl-4 or allyl alcohol treatment, Popp et al. showed that MSCs injected into the portal vein or directly into the liver engrafted less efficiently compared to injected hepatocytes, and did not contribute to liver regeneration (Popp et al., 2007). Carvalho et al. observed unchanged transaminase levels, albumin levels, and liver fibrosis area between rats transplanted with rat bone marrow-derived MSCs and controls (Carvalho et al., 2008). Similar negative results were reported with human cord blood-derived MSCs injected through tail vein of rats with thioacetamide-induced liver fibrosis (Kim et al., 2011). MSCs engrafted into the liver but did not acquire mature hepatic phenotype. No differences were observed in biochemical markers or in the extent of liver fibrosis. di Bonzo et al. using intravenously transplanted human bone marrow-derived MSCs showed their potential to engraft into normal and CCl-4-injured liver parenchyma, however, according to morphologic examination, detection of albumin, alpha-fetoprotein, and cytokeratin 18, there was a very low number of transdifferentiated MSCs into hepatocyte-like cells (less than 0.3% of the cells) (di Bonzo et al., 2008). The detection of MSCs expressing alpha smooth muscle actin further lead di Bonzo et al. to point out a possible fibrogenic potential of MSCs. Another concern is the fact that in the absence of transplantation, native bone marrow-derived MSCs can migrate and engraft into damaged liver (Li et al., 2009). This phenomenon was shown to be dependent upon sphingosine 1-phosphate gradient and sphingosine 1-phosphate receptor type 3. In humans, the hypothesis that bone marrow-derived cells could contribute to liver fibrosis was confirmed in patients with liver fibrosis following sex mismatched transplantation. Analysis of livers from female donors transplanted in male patients, or livers of female patient who received a male bone marrow transplant showed that 7 to 22% of the liver fibrosis-related myofibroblasts contained the Y chromosome (Forbes et al., 2004), suggesting a bone marrow origin of myofibroblasts in

the liver (Dalakas et al., 2010). Further studies in irradiated mice transplanted with sex mismatched bone marrow demonstrated that in the cirrhotic liver, the contribution of bone marrow to parenchymal regeneration was minor (0.6% of hepatocytes), and by contrast, the bone marrow contributed to 68% of the hepatic stellate cell pool and 70% of myofibroblast populations (Russo et al., 2006). These studies demonstrate that MSCs might have negative effects, and suggest that there might be various MSC subpopulations with opposite effects.

MSCs are isolated and cultured under varying conditions which probably profoundly affects their plasticity and function. The different conditions during which MSCs are transplanted are likely to be the cause of conflicting results regarding engraftment rate and protective effects on liver fibrosis.

Clinical trials using MSCs for the treatment of liver diseases

Despite controversial results in experimental animal models, numerous clinical studies have been initiated to investigate the therapeutic potential of MSCs. A search on http://www.clinicaltrials.gov (March 2013) with the terms "mesenchymal stem cells" listed 282 trials (Table 1). Sixty-two were completed (22%), 146 were recruiting (52%), 28 were not yet recruiting (10%), and 46 were at undetermined stage (16%). These trials included 166 phase 1 (59%), 71 phase 2 (25%), and 11 phase 3 trials (4%); 34 were undetermined (12%). The number of trials per year increased almost exponentially from 2004 to 2012 (Fig. 2). Investigators used or are using culture-expanded allogeneic or autologous MSCs, originating either from their own cell isolation center or from a commercial origin (e.g., Prochymal®). Among these 282 trials, 28 concern the use of MSCs in the treatment of liver diseases (22 in cirrhotic patients and 6 in patients with acute liver failure).

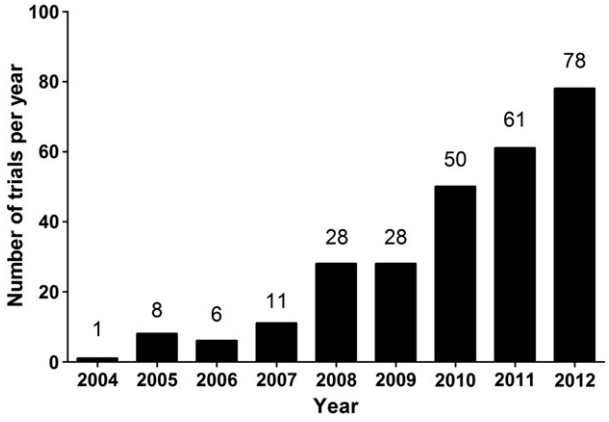

Figure 2 The number of trials per year registered on www.clinicaltrials.gov transplanting mesenchymal stem cells in patients from 2004 to 2012.
A search on www.clinicaltrials.gov (March 2013) with the term "mesenchymal stem cells" listed 271 trials from 2004 to 2012 (i.e. not including 2013).

We found seven published clinical trials studying the effects of MSC transplantation in patients with liver cirrhosis (listed in Table 2). A pilot phase 1 study published in 2007 included four patients with decompensated liver cirrhosis (Mohamadnejad et al., 2007). Cells were derived from iliac crest puncture and expanded in culture during 73 days with 2.5 passages on average. Thirty-two million autologous MSCs were administrated through a single injection into the cubital vein of the arm. No severe side-effects were observed until the end of the follow-up at 12 months after the transplantation. Three out of four patients improved their Model for End-Stage Liver Disease (MELD) scores at 6 months after transplantation and two of them continued to improve up to 12 months. In another pilot phase 1 study, 30 to 50 million autologous iliac crest-derived MSCs were

Table 1 Investigations using MSC transplantation in humans.
A search on www.clinicaltrials.gov (March 2013) with the term "mesenchymal stem cells" listed 282 trials; a search on www.pubmed.com (March 2013) with the terms "mesenchymal stem cells" [title] or "mesenchymal stem cell" [title] with filters activated for Clinical Trial, Humans, with an additional search with the terms "mesenchymal stem cells" [title] and "trial" [title] (without filter) listed 81 studies.

Category	Number of ongoing trials (n = 282)	Published studies (n = 81)
Orthopedic diseases	64	5
Cardiovascular diseases	36	9
Neurological diseases	53	17
GVHD	25	8
Liver diseases	28	7
Diabetes	18	3
Hematological disorders	9	10
Crohn disease/ulcerative colitis	10	3
Pulmonary diseases	8	–
Dermatological diseases	9	2
Allograft rejection	7	1
Lupus	4	4
Others	11	12

Investigations using MSC transplantation in humans.

injected in the peripheral or portal vein of eight patients with end-stage liver disease (Kharaziha et al., 2009). The treatment was well tolerated and the average MELD score among patients improved from 18 to 11 after 24 weeks. In a phase 2 study, Amer et al. randomized 40 patients with end-stage liver failure due to chronic hepatitis C into two groups of 20 patients: the first group received autologous bone marrow-derived MSCs previously transdifferentiated in hepatocyte-like cells in vitro, the second group received standard supportive treatment (Amer et al., 2011). The patients receiving MSCs had significant improvement in Child–Pugh and MELD scores appearing after 2 weeks and maintained for 6 months when compared to 20 patients who received traditional supportive treatment. Scores measured by fatigue scale and performance status were also improved. Patients receiving MSCs through intrahepatic route had pronounced stronger improvement of MELD and fatigue scores compared to MSCs infused through intrasplenic route. In a phase 2 trial, Peng et al. transplanted autologous MSCs from iliac bone aspirates in 53 patients with liver failure caused by hepatitis B virus infection (Peng et al., 2011). Levels of albumin, bilirubin, prothrombin time and MELD score were improved at two to three weeks after MSC transplantation, compared with 105 control-patients. Long-term follow-up revealed similar results between groups in terms of hepatocellular carcinoma incidence and mortality. In another phase 2 trial, El-Ansary et al. transplanted autologous iliac bone-derived MSCs in patients with hepatitis C-related cirrhosis and MELD score >12 (El-Ansary et al., 2012). Fifteen patients received 1 million MSCs per kg-weight intravenously and were compared to 10 patients with conventional supportive treatment. Follow-up of MSC-transplanted patients at 3 and 6 months showed a significant improvement in serum albumin, prothrombin, and total bilirubin levels, resulting in an improved MELD score. In this study, there was no difference in curative potential between MSCs and MSC-derived hepatocyte-like cells. More recently, in phase 2 trial Zhang et al. randomized (2:1) 46 patients with chronic hepatitis B receiving either three injections with 0.5 million/kg allogeneic umbilical cord-derived MSCs (n = 31) or saline solution (n = 15) (Zhang et al., 2012). Patients receiving MSC infusion had improved MELD score, ascites and fibrosis marker levels up to 48 weeks after the treatment. The major concerns of this study are the lack of evidence that the patients have been given appropriate conventional anti-viral therapy and the absence of explicit data about the course of illness before inclusion, the virus genotype, or the length of abstinence from alcohol. A common problem in these studies was the lack of data regarding the MSC's fate once injected into the body. Gholamrezanezhad et al. addressed this issue and injected ^{111}In-oxine-labeled human bone marrow-derived MSCs in a peripheral vein of cirrhotic patients (Gholamrezanezhad et al., 2011). They observed an initial accumulation of MSCs in the lungs with a peak 20 min after injection, followed by a decrease of the lung signal and a gradual increase in the liver and spleen signal that was detected up to 10 days after injection (^{111}In half-life is 67 h), indicating that MSCs do not only entrap in the lungs, but also migrate to the liver following peripheral intravenous injection. Terai et al. transplanted an average of 70 million/kg unsorted bone marrow mononuclear cells from bone marrow aspirates (including other stem cell types as hematopoietic stem cells, MSCs, multipotent adult progenitor cells (Jiang et al., 2002)) in cirrhotic patients (Terai et al., 2006). This therapy allowed an improvement in serum albumin levels, Child–Pugh scores, alpha-fetoprotein levels and liver proliferating cell nuclear antigen up to 24 weeks after transplantation. Similar observations reported improvements with the infusion of unsorted bone marrow cells in cirrhotic patients (Amin et al., 2006; Lyra et al., 2007, 2010; Kim et al., 2010) and no improvement in patients with alcoholic steatohepatitis (Spahr et al., 2013). The pilot studies mentioned above suggested that autologous MSC infusion allows mild biological improvements in patients with liver diseases, but clear and significant clinical benefit was not yet reported. To our knowledge, none of these studies provided histologic evidence of improvement with MSC treatment. Of note, intraportal infusion seemed to be more efficient than peripheral route (Amer et al., 2011), and differentiation toward hepatocytes prior to infusion seemed not to increase MSC curative potential (El-Ansary et al., 2012). Overall, evidences provided by most of these clinical studies are quite poor. The cells are often poorly characterized; and improvements are claimed where there are insufficiently powered experimental/control groups, or lack of randomization to make this claim.

Finally, the mechanisms allowing MSCs to improve clinical parameters in liver disease are largely unknown. For example, it is not known whether MSC transplantation allows a significant reduction of liver fibrosis and whether these biological and clinical improvements have a significant impact on outcomes such as the patient's need for transplantation and survival. Furthermore, the long-term risk of developing malignancies should be evaluated.

Potential risks of MSC therapies

Tumorigenic potential of MSCs

Some concerns have been raised regarding MSC susceptibility to undergo malignant transformation or to promote pre-existing tumor growth. In order to obtain a sufficient number of MSCs prior to transplant, cells undergo extensive in vitro expansion, which increases the risk for genetic mutations, eventually leading to malignant transformation. After several passages (>3) murine MSCs accumulate chromosomal aberrations and spontaneously transform into malignant cells (e.g. sarcoma) in vitro and in vivo (Zhou et al., 2006; Miura et al., 2006; Li et al., 2007; Aguilar et al., 2007; Tolar et al., 2007). In contrast, after a few number of passages, human MSCs become senescent without any signs of immortalization both in vitro (Miura et al., 2006; Bernardo et al., 2007) and in vivo (Kim et al., 2009). Rubio et al. suggested that MSC could form tumors (Rubio et al., 2005). But it turned out that this was due to a cross-contamination with a cell line used in their laboratory (Garcia et al., 2010). Human MSCs seem to be at lower risk of malignant transformation. However, at very high passages (>170) MSCs may display telomeric deletions (Dahl et al., 2008); thus, analysis of chromosomal integrity prior to MSC transplantation could improve the safety of the procedure. Further, microsatellite instability, down-regulated genes involved in DNA

Table 2 Phase 1–2 studies using MSC transplantation to treat chronic liver diseases.
A search on www.pubmed.com (March 2013) with the terms "mesenchymal stem cells" [title/abstract] and "patients" [title/abstract] AND ("cirrhosis" [title/abstract] or "end-stage liver" [title/abstract]) with filters activated for Clinical Trial, Humans. Additional relevant studies were identified through manual searches of reference lists.

Investigators	Study design	Liver disease	Source of MSCs	MSC derived in hepatocyte-like cells in vitro	Route of delivery	Number of patients treated
Mohamadnejad et al. Arch Iran Med 2007	Phase 1, open, safety	Decompensated liver cirrhosis (Cryptogenic, n = 3, autoimmune, n = 1)	Autologous, iliac crest	No	Cubital vein of the arm	4
Kharaziha et al. Eur J Gastroenterol Hepatol 2009	Phase 1, open, safety	Liver cirrhosis (cryptogenic, n = 2, hepatitis C, n = 1, hepatitis B, n = 3, alcoholic, n = 1)	Autologous, iliac crest	No	Peripheral or portal vein	8
Amer et al. Eur J Gastroenterol Hepatol 2011	Phase 2, open, safety, randomized	Child C due to chronic hepatitis C	Autologous, iliac crest	Yes	Intrasplenic (n = 10) or Intrahepatic (portal) (n = 10).	20
Peng et al. Hepatology 2011	Phase 2, open, safety	Chronic liver failure caused by hepatitis B virus	Autologous, iliac crest	No	Proper hepatic artery	53
El-Ansary et al. Stem cell reviews 2011	Phase 2, open, safety	Hepatitis C-related cirrhosis and MELD score >12	Autologous, iliac crest	Both	Peripheral vein	15
Gholamrezanezhad et al. Nucl Med Biol 2011	Phase 1, open, safety	Decompensated liver cirrhosis (cryptogenic, n = 3, hemochromatosis, n = 1)	Autologous, iliac crest	No	Peripheral vein	4
Zhang et al. J Gastroen Hepatol 2012	Phase 2, open, safety, randomized (2:1)	Chronic hepatitis B patients (n = 46)	Allogeneic, umbilical cord-derived	No	Peripheral vein	31

Table 2 (*continued*)

Investigators	Number of control patients	Dose	Early or late side effect	Main outcome measures	Main results	Comments
Mohamadnejad et al. Arch Iran Med 2007	0	32 mio	None	MELD score, serum creatinine	MELD score and serum creatinine improved at 6 and 12 months	
Kharaziha et al. Eur J Gastroenterol Hepatol 2009	0	30–50 mio	None	MELD score	MELD score improved from 18 to 11 after 24 weeks	
Amer et al. Eur J Gastroenterol Hepatol 2011	20	20 mio	Transient shivering in 3 patients	Child, MELD, fatigue scale, performance status	Child score and MELD score improved after 2 weeks and maintained for 6 months. Better score in fatigue scale and performance status	Authors found a better curative potential with MSCs infused through the intrahepatic route
Peng et al. Hepatology 2011	105	10 mio	None	MELD score, serum albumin	MELD score was improved at two to three weeks after MSC transplantation	Long-term follow-up revealed similar results between groups in terms of hepatocellular carcinoma incidence and mortality
El-Ansary et al. Stem cell reviews 2011	10	~70 mio (1 mio/kg)	Not reported	MELD score, serum albumin	MELD score and albumin were improved at 3 and 6 months after MSC transplantation	Authors compared MSCs to MSC-derived hepatocyte-like cells and found no difference in curative potential
Gholamrezanezhad et al. Nucl Med Biol 2011	0	270–400 mio	None	MSC engraftment, MELD score	MSC engrafted mainly in liver and spleen No significant improvement in liver function was noted after a 1 month period	The study was designed to trace MSC once injected
Zhang et al. J Gastroen Hepatol 2012	15	~35 mio (0.5 mio/kg), 3 injections (4 week intervals)	Fever (37–38 °C) 2–6 h after MSC transfusion	MELD Na score, ascites, liver fibrosis markers	MELD score, ascites, serum laminin, hyaluronic acid, procollagen type III, and type IV collagen were improved up to 48 weeks after MSC transplantation	MELD Na scores were simultaneously decreased in the control and MSC treatment groups

repair, and heteroplasmic point mutations had been observed in human MSCs (Oliveira et al., 2011). Moreover, the risk of tumorigenicity due to MSCs might be increased in patients receiving immunosuppression after liver transplantation with a previous MSC infusion.

A further potential risk of MSC transplantation is to promote the growth of masked pre-existing tumors or pre-cancer lesions, especially in immunosuppressed patients. In animal studies, co-transplanting MSCs with cancer cells promotes the growth of the neoformed tumors and their metastatic potency (Karnoub et al., 2007; Ramasamy et al., 2007; Bian et al., 2010; Kucerova et al., 2010). The mechanisms include the secretion of growth factors (i.e. TGF-β, HGF, and epidermal growth factor) which promote tissue growth and neo-vascularization (Zhu et al., 2006). This is not the case with every tumor, Cousin et al. highlighted long-lasting inhibition of tumor growth on aggressive pancreatic cancer cells implanted in vivo (Cousin et al., 2009). MSCs also showed effects on preestablished tumor growth in animal models either promoting (Hung et al., 2005; Beckermann et al., 2008; Lin et al., 2010; Sun et al., 2005) or inhibiting tumor growth (Khakoo et al., 2006; Dasari et al., 2010a,b; Secchiero et al., 2010). However, all studies reporting that MSCs promote tumor growth either used immortalized or modified MSCs (Casiraghi et al., 2013) and may not reflect properties of primary MSCs. Safety results of a pool of 700 subjects who received autologous or third party MSCs showed that none of them had major side effects, nor included the development of hematopoietic or solid tumors (Casiraghi et al., 2013). However, in most clinical trials the follow-up was relatively short (1 month to 6.8 years), and the occurrence of a tumor may take longer to appear. However, a correlation between cotransplantation of MSCs together with hematopoietic stem cells and higher incidence of relapses in patients with hematologic malignancy has been observed (Ning et al., 2008). Therefore, MSC expansion should be limited to keep tumorigenic risk low. Further, autologous or allogeneic MSC transplantation should not be considered if the donor is bearing a genetic disease associated with a tumorigenic risk (European-Union-Group-on-Ethics, 2004), and patients with known preexisting tumor should not undergo MSC therapy. Although evidence coming from pilot studies using MSCs is reassuring, risks need to be evaluated on a long-term basis.

Viral transmission by MSCs

In contrast to MSC autotransplantation, MSC allotransplantation (i.e. in the treatment of GVHD after hematopoietic stem cell transplantation (Le Blanc et al., 2008) or chronic liver diseases (Zhang et al., 2012)) may carry the risk of viral transmission to the recipient. MSCs were shown to be permissive for herpes simplex virus and cytomegalovirus infections in vitro, but not for Epstein–Barr virus infection (Sundin et al., 2006). Parvovirus B19 was detected in cultured MSCs of 5% of healthy donors and varicella zoster virus in 11% (Rollin et al., 2007), suggesting that a risk is present. Furthermore, viral transmission of parvovirus B19 to bone marrow cells was demonstrated in vitro (Sundin et al., 2008). However, patients transplanted with allogeneic B19-positive MSCs did not develop viremia or symptomatic B19 infection. Sundin et al. concluded that B19-positive MSCs carry a low pathogenicity risk in these highly immunosuppressed individuals (Sundin et al., 2008); however no information was available on herpes simplex virus and cytomegalovirus transmission by MSCs in vivo. In this context, it seems prudent to screen both recipient and MSC donor for parvovirus B19, herpes simplex virus and cytomegalovirus as they can lead to dramatic infections in immunosuppressed patients.

Conclusions

MSC transplantation has recently gained widespread enthusiasm particularly in the perspective to use them to treat acute and chronic liver diseases. Currently, there are conflicting data in small animals regarding MSC efficiency to sustain liver function and to reduce inflammation. Crucial points governing success of MSC therapeutic potential seem to be the type of liver injury as well as the subpopulation of MSC chosen (Waterman et al., 2010). Unwanted effects such as myofibroblastic transdifferentiation of the transplanted MSCs may be influenced by subpopulation of MSCs, the route of injection as well as the recipient. Time frame of treatments might be important as well, i.e. injected MSCs may directly participate to fibrogenesis during the "injury" phase and accelerate healing process during the "resolution" phase (Kisseleva and Brenner, 2012). Only few reports in large animals showed that MSCs can decrease the severity of acute liver failure, and to our best knowledge, experimental liver fibrosis in large animals is not an established model. Nevertheless, several clinical trials have been initiated, mainly in patients with no options for other treatments. Few studies were published, and most of them had poorly defined endpoints, rendering therapeutic benefits difficult to evaluate. A common finding in these studies was that MSC infusions reduce MELD score in patients with end-stage liver disease. The most likely mechanism that could explain this favorable effect appears to be the ability of MSCs to reduce inflammation and to promote endogenous repair rather than to directly replace hepatocytes.

To date, safety studies in humans do not have extended follow-up in particular with respect to the evaluation of MSC tumorigenic potential. Investigations described in this review were certainly validated by institutional ethics committees; however, not only registered- and university affiliated-investigators use MSCs in humans for clinical applications (Anon., 2012), therefore it remains important to obtain national regulatory agency approval to warrant maximal safety in the clinical environment (Prockop and Olson, 2007). Questions regarding efficacy, safety and mechanisms of action may be answered by a highly regulated large-scale multicenter study. In conclusion, the prospect of using MSCs as cell therapy for treating liver diseases is encouraging but will require an improved understanding of the mechanism behind their therapeutic effects and a stronger validation in preclinical and clinical settings.

Authorship

RM: research design and writing of the manuscript.

YM: research design, writing of the manuscript and critical revision for important intellectual content.

PM: research design and critical revision for important intellectual content.

CG: research design, writing of the manuscript and critical revision for important intellectual content.

LB: research design, writing of the manuscript and critical revision for important intellectual content.

Funding: no funds were received.

Conflict of interest: the authors disclose no conflict of interest.

References

Abdel Aziz, M.T., et al., 2007. Therapeutic potential of bone marrow-derived mesenchymal stem cells on experimental liver fibrosis. Clin. Biochem. 40 (12), 893–899.

Aggarwal, S., Pittenger, M.F., 2005. Human mesenchymal stem cells modulate allogeneic immune cell responses. Blood 105 (4), 1815–1822.

Aguilar, S., et al., 2007. Murine but not human mesenchymal stem cells generate osteosarcoma-like lesions in the lung. Stem Cells 25 (6), 1586–1594.

Amer, M.E., et al., 2011. Clinical and laboratory evaluation of patients with end-stage liver cell failure injected with bone marrow-derived hepatocyte-like cells. Eur. J. Gastroenterol. Hepatol. 23 (10), 936–941.

Amin, M., et al., 2006. Evaluation of bone marrow-derived stem cells in hepatocyte regeneration in patients with end stage liver disease. Liver Int. 26, 21–22.

Anon., 2012. The darker side of stem cells. Nature 483 (7387), 5.

Augello, A., et al., 2007. Cell therapy using allogeneic bone marrow mesenchymal stem cells prevents tissue damage in collagen-induced arthritis. Arthritis Rheum. 56 (4), 1175–1186.

Aurich, I., et al., 2007. Functional integration of hepatocytes derived from human mesenchymal stem cells into mouse livers. Gut 56 (3), 405–415.

Aurich, H., et al., 2009. Hepatocyte differentiation of mesenchymal stem cells from human adipose tissue in vitro promotes hepatic integration in vivo. Gut 58 (4), 570–581.

Baertschiger, R.M., et al., 2008. Mesenchymal stem cells derived from human exocrine pancreas express transcription factors implicated in beta-cell development. Pancreas 37 (1), 75–84.

Baertschiger, R.M., et al., 2009. Fibrogenic potential of human multipotent mesenchymal stromal cells in injured liver. PLoS One 4 (8), e6657.

Banas, A., et al., 2007. Adipose tissue-derived mesenchymal stem cells as a source of human hepatocytes. Hepatology 46 (1), 219–228.

Banas, A., et al., 2008. IFATS collection: in vivo therapeutic potential of human adipose tissue mesenchymal stem cells after transplantation into mice with liver injury. Stem Cells 26 (10), 2705–2712.

Bartholomew, A., et al., 2002. Mesenchymal stem cells suppress lymphocyte proliferation in vitro and prolong skin graft survival in vivo. Exp. Hematol. 30 (1), 42–48.

Beckermann, B.M., et al., 2008. VEGF expression by mesenchymal stem cells contributes to angiogenesis in pancreatic carcinoma. Br. J. Cancer 99 (4), 622–631.

Beerheide, W., et al., 2002. Downregulation of beta2-microglobulin in human cord blood somatic stem cells after transplantation into livers of SCID-mice: an escape mechanism of stem cells? Biochem. Biophys. Res. Commun. 294 (5), 1052–1063.

Bernardo, M.E., et al., 2007. Human bone marrow derived mesenchymal stem cells do not undergo transformation after long-term in vitro culture and do not exhibit telomere maintenance mechanisms. Cancer Res. 67 (19), 9142–9149.

Bian, Z.Y., et al., 2010. Human mesenchymal stem cells promote growth of osteosarcoma: involvement of interleukin-6 in the interaction between human mesenchymal stem cells and Saos-2. Cancer Sci. 101 (12), 2554–2560.

Boomsma, R.A., Geenen, D.L., 2012. Mesenchymal stem cells secrete multiple cytokines that promote angiogenesis and have contrasting effects on chemotaxis and apoptosis. PLoS One 7 (4), e35685.

Campard, D., et al., 2008. Native umbilical cord matrix stem cells express hepatic markers and differentiate into hepatocyte-like cells. Gastroenterology 134 (3), 833–848.

Cao, H., et al., 2012. Therapeutic potential of transplanted placental mesenchymal stem cells in treating Chinese miniature pigs with acute liver failure. BMC Med. 10, 56.

Caplan, A.I., 1991. Mesenchymal stem cells. J. Orthop. Res. 9 (5), 641–650.

Carvalho, A.B., et al., 2008. Bone marrow multipotent mesenchymal stromal cells do not reduce fibrosis or improve function in a rat model of severe chronic liver injury. Stem Cells 26 (5), 1307–1314.

Casiraghi, F., et al., 2013. Multipotent mesenchymal stromal cell therapy and risk of malignancies. Stem Cell Rev. 9 (1), 65–79.

Chamberlain, J., et al., 2007. Efficient generation of human hepatocytes by the intrahepatic delivery of clonal human mesenchymal stem cells in fetal sheep. Hepatology 46 (6), 1935–1945.

Chang, Y.J., et al., 2009. Mesenchymal stem cells facilitate recovery from chemically induced liver damage and decrease liver fibrosis. Life Sci. 85 (13–14), 517–525.

Corcione, A., et al., 2006. Human mesenchymal stem cells modulate B-cell functions. Blood 107 (1), 367–372.

Cousin, B., et al., 2009. Adult stromal cells derived from human adipose tissue provoke pancreatic cancer cell death both in vitro and in vivo. PLoS One 4 (7), e6278.

da Silva Meirelles, L., Chagastelles, P.C., Nardi, N.B., 2006. Mesenchymal stem cells reside in virtually all post-natal organs and tissues. J. Cell Sci. 119 (Pt 11), 2204–2213.

da Silva Meirelles, L., et al., 2009. MSC frequency correlates with blood vessel density in equine adipose tissue. Tissue Eng. Part A 15 (2), 221–229.

Dahl, J.A., et al., 2008. Genetic and epigenetic instability of human bone marrow mesenchymal stem cells expanded in autologous serum or fetal bovine serum. Int. J. Dev. Biol. 52 (8), 1033–1042.

Dalakas, E., et al., 2010. Bone marrow stem cells contribute to alcohol liver fibrosis in humans. Stem Cells Dev. 19 (9), 1417–1425.

Dasari, V.R., et al., 2010a. Upregulation of PTEN in glioma cells by cord blood mesenchymal stem cells inhibits migration via downregulation of the PI3K/Akt pathway. PLoS One 5 (4), e10350.

Dasari, V.R., et al., 2010b. Cord blood stem cell-mediated induction of apoptosis in glioma downregulates X-linked inhibitor of apoptosis protein (XIAP). PLoS One 5 (7), e11813.

Deeg, H.J., 2007. How I treat refractory acute GVHD. Blood 109 (10), 4119–4126.

di Bonzo, L.V., et al., 2008. Human mesenchymal stem cells as a two-edged sword in hepatic regenerative medicine: engraftment and hepatocyte differentiation versus profibrogenic potential. Gut 57 (2), 223–231.

Di Nicola, M., et al., 2002. Human bone marrow stromal cells suppress T-lymphocyte proliferation induced by cellular or nonspecific mitogenic stimuli. Blood 99 (10), 3838–3843.

Dmitrewski, J., et al., 1996. Recurrence of primary biliary cirrhosis in the liver allograft: the effect of immunosuppression. J. Hepatol. 24 (3), 253–257.

Dominici, M., et al., 2006. POSITION PAPER. Minimal criteria for defining multipotent mesenchymal stromal cells. The International Society for Cellular Therapy position statement. Cytotherapy 8 (4), 315–317.

Duncan, A.W., Dorrell, C., Grompe, M., 2009. Stem cells and liver regeneration. Gastroenterology 137 (2), 466–481.

El-Ansary, M., et al., 2012. Phase II trial: undifferentiated versus differentiated autologous mesenchymal stem cells transplantation in Egyptian patients with HCV induced liver cirrhosis. Stem Cell Rev. 8 (3), 972–981.

Erices, A., Conget, P., Minguell, J.J., 2000. Mesenchymal progenitor cells in human umbilical cord blood. Br. J. Haematol. 109 (1), 235–242.

European-Union-Group-on-Ethics, 2004. http://ec.europa.eu/bepa/european-group-ethics/docs/avis19_en.pdf.

Fang, B., et al., 2004. Systemic infusion of FLK1($^{+}$) mesenchymal stem cells ameliorate carbon tetrachloride-induced liver fibrosis in mice. Transplantation 78 (1), 83–88.

Fellous, T.G., et al., 2009. Locating the stem cell niche and tracing hepatocyte lineages in human liver. Hepatology 49 (5), 1655–1663.

Fernandez, M., et al., 1997. Detection of stromal cells in peripheral blood progenitor cell collections from breast cancer patients. Bone Marrow Transplant. 20 (4), 265–271.

Forbes, S.J., et al., 2004. A significant proportion of myofibroblasts are of bone marrow origin in human liver fibrosis. Gastroenterology 126 (4), 955–963.

Friedenstein, A.J., et al., 1968. Heterotopic of bone marrow. Analysis of precursor cells for osteogenic and hematopoietic tissues. Transplantation 6 (2), 230–247.

Friedenstein, A.J., Chailakhjan, R.K., Lalykina, K.S., 1970. The development of fibroblast colonies in monolayer cultures of guinea-pig bone marrow and spleen cells. Cell Tissue Kinet. 3 (4), 393–403.

Friedenstein, A.J., et al., 1974. Precursors for fibroblasts in different populations of hematopoietic cells as detected by the in vitro colony assay method. Exp. Hematol. 2 (2), 83–92.

Friedenstein, A.J., Gorskaja, J.F., Kulagina, N.N., 1976. Fibroblast precursors in normal and irradiated mouse hematopoietic organs. Exp. Hematol. 4 (5), 267–274.

Friedenstein, A.J., Chailakhyan, R.K., Gerasimov, U.V., 1987. Bone marrow osteogenic stem cells: in vitro cultivation and transplantation in diffusion chambers. Cell Tissue Kinet. 20 (3), 263–272.

Fukuchi, Y., et al., 2004. Human placenta-derived cells have mesenchymal stem/progenitor cell potential. Stem Cells 22 (5), 649–658.

Garcia, S., et al., 2010. Pitfalls in spontaneous in vitro transformation of human mesenchymal stem cells. Exp. Cell Res. 316 (9), 1648–1650.

Gerdoni, E., et al., 2007. Mesenchymal stem cells effectively modulate pathogenic immune response in experimental autoimmune encephalomyelitis. Ann. Neurol. 61 (3), 219–227.

Gholamrezanezhad, A., et al., 2011. In vivo tracking of ^{111}In-oxine labeled mesenchymal stem cells following infusion in patients with advanced cirrhosis. Nucl. Med. Biol. 38 (7), 961–967.

Glennie, S., et al., 2005. Bone marrow mesenchymal stem cells induce division arrest anergy of activated T cells. Blood 105 (7), 2821–2827.

Gordon, G.J., et al., 2000. Liver regeneration in rats with retrorsine-induced hepatocellular injury proceeds through a novel cellular response. Am. J. Pathol. 156 (2), 607–619.

Hardjo, M., et al., 2009. Suppression of carbon tetrachloride-induced liver fibrosis by transplantation of a clonal mesenchymal stem cell line derived from rat bone marrow. Cell Transplant. 18 (1), 89–99.

Hauner, H., et al., 1989. Promoting effect of glucocorticoids on the differentiation of human adipocyte precursor cells cultured in a chemically defined medium. J. Clin. Invest. 84 (5), 1663–1670.

Hengstler, J.G., et al., 2005. Generation of human hepatocytes by stem cell technology: definition of the hepatocyte. Expert Opin. Drug Metab. Toxicol. 1 (1), 61–74.

Higashiyama, R., et al., 2007. Bone marrow-derived cells express matrix metalloproteinases and contribute to regression of liver fibrosis in mice. Hepatology 45 (1), 213–222.

Hirschi, K.K., D'Amore, P.A., 1996. Pericytes in the microvasculature. Cardiovasc. Res. 32 (4), 687–698.

Hong, S.H., et al., 2005. In vitro differentiation of human umbilical cord blood-derived mesenchymal stem cells into hepatocyte-like cells. Biochem. Biophys. Res. Commun. 330 (4), 1153–1161.

Horwitz, E.M., et al., 1999. Transplantability and therapeutic effects of bone marrow-derived mesenchymal cells in children with osteogenesis imperfecta. Nat. Med. 5 (3), 309–313.

Hung, S.C., et al., 2005. Mesenchymal stem cell targeting of microscopic tumors and tumor stroma development monitored by noninvasive in vivo positron emission tomography imaging. Clin. Cancer Res. 11 (21), 7749–7756.

in't Anker, P.S., et al., 2003. Mesenchymal stem cells in human second-trimester bone marrow, liver, lung, and spleen exhibit a similar immunophenotype but a heterogeneous multilineage differentiation potential. Haematologica 88 (8), 845–852.

Jacobs, S.A., et al., 2013. Immunological characteristics of human mesenchymal stem cells and multipotent adult progenitor cells. Immunol. Cell Biol. 91 (1), 32–39.

Jiang, Y.H., et al., 2002. Pluripotency of mesenchymal stem cells derived from adult marrow. Nature 418 (6893), 41–49.

Jiang, X.X., et al., 2005. Human mesenchymal stem cells inhibit differentiation and function of monocyte-derived dendritic cells. Blood 105 (10), 4120–4126.

Kanazawa, H., et al., 2011. Bone marrow-derived mesenchymal stem cells ameliorate hepatic ischemia reperfusion injury in a rat model. PLoS One 6 (4), e19195.

Karnoub, A.E., et al., 2007. Mesenchymal stem cells within tumour stroma promote breast cancer metastasis. Nature 449 (7162), 557–563.

Karussis, D., et al., 2010. Safety and immunological effects of mesenchymal stem cell transplantation in patients with multiple sclerosis and amyotrophic lateral sclerosis. Arch. Neurol. 67 (10), 1187–1194.

Khakoo, A.Y., et al., 2006. Human mesenchymal stem cells exert potent antitumorigenic effects in a model of Kaposi's sarcoma. J. Exp. Med. 203 (5), 1235–1247.

Kharaziha, P., et al., 2009. Improvement of liver function in liver cirrhosis patients after autologous mesenchymal stem cell injection: a phase I–II clinical trial. Eur. J. Gastroenterol. Hepatol. 21 (10), 1199–1205.

Kim, J., et al., 2009. Biological characterization of long-term cultured human mesenchymal stem cells. Arch. Pharm. Res. 32 (1), 117–126.

Kim, J.K., et al., 2010. Autologous bone marrow infusion activates the progenitor cell compartment in patients with advanced liver cirrhosis. Cell Transplant. 19 (10), 1237–1246.

Kim, S., et al., 2011. In vivo hepatic differentiation potential of human cord blood-derived mesenchymal stem cells. Int. J. Mol. Med. 27 (5), 701–706.

Kisseleva, T., Brenner, D.A., 2012. The phenotypic fate and functional role for bone marrow-derived stem cells in liver fibrosis. J. Hepatol. 56 (4), 965–972.

Kisseleva, T., et al., 2006. Bone marrow-derived fibrocytes participate in pathogenesis of liver fibrosis. J. Hepatol. 45 (3), 429–438.

Korbling, M., et al., 2002. Hepatocytes and epithelial cells of donor origin in recipients of peripheral-blood stem cells. N. Engl. J. Med. 346 (10), 738–746.

Krampera, M., et al., 2003. Bone marrow mesenchymal stem cells inhibit the response of naive and memory antigen-specific T cells to their cognate peptide. Blood 101 (9), 3722–3729.

Kucerova, L., et al., 2010. Tumor cell behaviour modulation by mesenchymal stromal cells. Mol. Cancer 9, 129.

Kuo, T.K., et al., 2008. Stem cell therapy for liver disease: parameters governing the success of using bone marrow mesenchymal stem cells. Gastroenterology 134 (7), 2111–2121 (2121 e1-3).

Lagasse, E., et al., 2000. Purified hematopoietic stem cells can differentiate into hepatocytes in vivo. Nat. Med. 6 (11), 1229–1234.

Le Blanc, K., Ringden, O., 2005. Immunobiology of human mesenchymal stem cells and future use in hematopoietic stem cell transplantation. Biol. Blood Marrow Transplant. 11 (5), 321–334.
Le Blanc, K., et al., 2003. Mesenchymal stem cells inhibit and stimulate mixed lymphocyte cultures and mitogenic responses independently of the major histocompatibility complex. Scand. J. Immunol. 57 (1), 11–20.
Le Blanc, K., et al., 2004. Treatment of severe acute graft-versus-host disease with third party haploidentical mesenchymal stem cells. Lancet 363 (9419), 1439–1441.
Le Blanc, K., et al., 2008. Mesenchymal stem cells for treatment of steroid-resistant, severe, acute graft-versus-host disease: a phase II study. Lancet 371 (9624), 1579–1586.
Lee, K.D., et al., 2004a. In vitro hepatic differentiation of human mesenchymal stem cells. Hepatology 40 (6), 1275–1284.
Lee, R.H., et al., 2004b. Characterization and expression analysis of mesenchymal stem cells from human bone marrow and adipose tissue. Cell. Physiol. Biochem. 14 (4–6), 311–324.
Lee, M.J., et al., 2010. Anti-fibrotic effect of chorionic plate-derived mesenchymal stem cells isolated from human placenta in a rat model of CCl(4)-injured liver: potential application to the treatment of hepatic diseases. J. Cell. Biochem. 111 (6), 1453–1463.
Leu, J.I., Crissey, M.A.S., Taub, R., 2003. Massive hepatic apoptosis associated with TGF-beta 1 activation after Fas ligand treatment of IGF binding protein-1-deficient mice. J. Clin. Invest. 111 (1), 129–139.
Li, H., et al., 2007. Spontaneous expression of embryonic factors and p53 point mutations in aged mesenchymal stem cells: a model of age-related tumorigenesis in mice. Cancer Res. 67 (22), 10889–10898.
Li, C., et al., 2009. Homing of bone marrow mesenchymal stem cells mediated by sphingosine 1-phosphate contributes to liver fibrosis. J. Hepatol. 50 (6), 1174–1183.
Li, J., et al., 2010. 3D PLGA scaffolds improve differentiation and function of bone marrow mesenchymal stem cell-derived hepatocytes. Stem Cells Dev. 19 (9), 1427–1436.
Li, J., et al., 2012. Immediate intraportal transplantation of human bone marrow mesenchymal stem cells prevents death from fulminant hepatic failure in pigs. Hepatology 56 (3), 1044–1052.
Lin, N., et al., 2009. Nerve growth factor-mediated paracrine regulation of hepatic stellate cells by multipotent mesenchymal stromal cells. Life Sci. 85 (7–8), 291–295.
Lin, G., et al., 2010. Effects of transplantation of adipose tissue-derived stem cells on prostate tumor. Prostate 70 (10), 1066–1073.
Lin, H., et al., 2011. Implications of the immunoregulatory functions of mesenchymal stem cells in the treatment of human liver diseases. Cell. Mol. Immunol. 8 (1), 19–22.
Liska, V., et al., 2009. Intraportal injection of porcine multipotent mesenchymal stromal cells augments liver regeneration after portal vein embolization. In Vivo 23 (2), 229–235.
Lyra, A.C., et al., 2007. Feasibility and safety of autologous bone marrow mononuclear cell transplantation in patients with advanced chronic liver disease. World J. Gastroenterol. 13 (7), 1067–1073.
Lyra, A.C., et al., 2010. Infusion of autologous bone marrow mononuclear cells through hepatic artery results in a short-term improvement of liver function in patients with chronic liver disease: a pilot randomized controlled study. Eur. J. Gastroenterol. Hepatol. 22 (1), 33–42.
Makino, S., et al., 1999. Cardiomyocytes can be generated from marrow stromal cells in vitro. J. Clin. Invest. 103 (5), 697–705.
Manousou, P., et al., 2010. Primary biliary cirrhosis after liver transplantation: influence of immunosuppression and human leukocyte antigen locus disparity. Liver Transpl. 16 (1), 64–73.
Meisel, R., et al., 2004. Human bone marrow stromal cells inhibit allogeneic T-cell responses by indoleamine 2,3-dioxygenase-mediated tryptophan degradation. Blood 103 (12), 4619–4621.
Mendez-Ferrer, S., et al., 2010. Mesenchymal and haematopoietic stem cells form a unique bone marrow niche. Nature 466 (7308), 829–834.
Miura, M., et al., 2006. Accumulated chromosomal instability in murine bone marrow mesenchymal stem cells leads to malignant transformation. Stem Cells 24 (4), 1095–1103.
Mohamadnejad, M., et al., 2005. Impact of immunosuppressive treatment on liver fibrosis in autoimmune hepatitis. Dig. Dis. Sci. 50 (3), 547–551.
Mohamadnejad, M., et al., 2007. Phase 1 trial of autologous bone marrow mesenchymal stem cell transplantation in patients with decompensated liver cirrhosis. Arch. Iran. Med. 10 (4), 459–466.
Mohsin, S., et al., 2011. Enhanced hepatic differentiation of mesenchymal stem cells after pretreatment with injured liver tissue. Differentiation 81 (1), 42–48.
Morikawa, S., et al., 2009. Prospective identification, isolation, and systemic transplantation of multipotent mesenchymal stem cells in murine bone marrow. J. Exp. Med. 206 (11), 2483–2496.
Najimi, M., et al., 2007. Adult-derived human liver mesenchymal-like cells as a potential progenitor reservoir of hepatocytes? Cell Transplant. 16 (7), 717–728.
Natarajan, A., Wagner, B., Sibilia, M., 2007. The EGF receptor is required for efficient liver regeneration. Proc. Natl. Acad. Sci. U. S. A. 104 (43), 17081–17086.
Nauta, A.J., Fibbe, W.E., 2007. Immunomodulatory properties of mesenchymal stromal cells. Blood 110 (10), 3499–3506.
Nauta, A.J., et al., 2006. Mesenchymal stem cells inhibit generation and function of both $CD34^{+}$-derived and monocyte-derived dendritic cells. J. Immunol. 177 (4), 2080–2087.
Ning, H., et al., 2008. The correlation between cotransplantation of mesenchymal stem cells and higher recurrence rate in hematologic malignancy patients: outcome of a pilot clinical study. Leukemia 22 (3), 593–599.
Nombela-Arrieta, C., Ritz, J., Silberstein, L.E., 2011. The elusive nature and function of mesenchymal stem cells. Nat. Rev. Mol. Cell Biol. 12 (2), 126–131.
Odorfer, K.I., et al., 2011. Hematopoietic bone marrow cells participate in endothelial, but not epithelial or mesenchymal cell renewal in adult rats. J. Cell. Mol. Med. 15 (10), 2232–2244.
Oliveira, P.H., et al., 2011. An appraisal of genetic stability in human mesenchymal stem cells. Portuguese Meeting in Bioengineering.
Ong, S.Y., Dai, H., Leong, K.W., 2006. Inducing hepatic differentiation of human mesenchymal stem cells in pellet culture. Biomaterials 27 (22), 4087–4097.
Ortiz, L.A., et al., 2007. Interleukin 1 receptor antagonist mediates the antiinflammatory and antifibrotic effect of mesenchymal stem cells during lung injury. Proc. Natl. Acad. Sci. U. S. A. 104 (26), 11002–11007.
Oswald, J., et al., 2004. Mesenchymal stem cells can be differentiated into endothelial cells in vitro. Stem Cells 22 (3), 377–384.
Oyagi, S., et al., 2006. Therapeutic effect of transplanting HGF-treated bone marrow mesenchymal cells into CCl4-injured rats. J. Hepatol. 44 (4), 742–748.
Parekkadan, B., et al., 2007a. Mesenchymal stem cell-derived molecules reverse fulminant hepatic failure. PLoS One 2 (9), e941.
Parekkadan, B., et al., 2007b. Immunomodulation of activated hepatic stellate cells by mesenchymal stem cells. Biochem. Biophys. Res. Commun. 363 (2), 247–252.
Peng, L., et al., 2011. Autologous bone marrow mesenchymal stem cell transplantation in liver failure patients caused by hepatitis B: short-term and long-term outcomes. Hepatology 54 (3), 820–828.
Phinney, D.G., Prockop, D.J., 2007. Concise review: mesenchymal stem/multipotent stromal cells: the state of transdifferentiation and modes of tissue repair—current views. Stem Cells 25 (11), 2896–2902.

Piryaei, A., et al., 2011. Differentiation of bone marrow-derived mesenchymal stem cells into hepatocyte-like cells on nanofibers and their transplantation into a carbon tetrachloride-induced liver fibrosis model. Stem Cell Rev. 7 (1), 103–118.

Pittenger, M.F., et al., 1999. Multilineage potential of adult human mesenchymal stem cells. Science 284 (5411), 143–147.

Popp, F.C., et al., 2007. No contribution of multipotent mesenchymal stromal cells to liver regeneration in a rat model of prolonged hepatic injury. Stem Cells 25 (3), 639–645.

Popp, F.C., et al., 2009. Mesenchymal stem cells as immunomodulators after liver transplantation. Liver Transpl. 15 (10), 1192–1198.

Prockop, D.J., 1997. Marrow stromal cells as stem cells for nonhematopoietic tissues. Science 276 (5309), 71–74.

Prockop, D.J., Olson, S.D., 2007. Clinical trials with adult stem/progenitor cells for tissue repair: let's not overlook some essential precautions. Blood 109 (8), 3147–3151.

Ramasamy, R., et al., 2007. Mesenchymal stem cells inhibit proliferation and apoptosis of tumor cells: impact on in vivo tumor growth. Leukemia 21 (2), 304–310.

Rasmusson, I., et al., 2005. Mesenchymal stem cells inhibit lymphocyte proliferation by mitogens and alloantigens by different mechanisms. Exp. Cell Res. 305 (1), 33–41.

Ringden, O., Nilsson, B., 1985. Death by graft-versus-host disease associated with HLA mismatch, high recipient age, low marrow cell dose, and splenectomy. Transplantation 40 (1), 39–44.

Rollin, R., et al., 2007. Human parvovirus B19, varicella zoster virus, and human herpesvirus-6 in mesenchymal stem cells of patients with osteoarthritis: analysis with quantitative real-time polymerase chain reaction. Osteoarthritis Cartilage 15 (4), 475–478.

Rubio, D., et al., 2005. Spontaneous human adult stem cell transformation. Cancer Res. 65 (8), 3035–3039.

Russo, F.P., et al., 2006. The bone marrow functionally contributes to liver fibrosis. Gastroenterology 130 (6), 1807–1821.

Sacchetti, B., et al., 2007. Self-renewing osteoprogenitors in bone marrow sinusoids can organize a hematopoietic microenvironment. Cell 131 (2), 324–336.

Salazar, K.D., Lankford, S.M., Brody, A.R., 2009. Mesenchymal stem cells produce Wnt isoforms and TGF-beta1 that mediate proliferation and procollagen expression by lung fibroblasts. Am. J. Physiol. Lung Cell. Mol. Physiol. 297 (5), L1002–L1011.

Sato, Y., et al., 2005. Human mesenchymal stem cells xenografted directly to rat liver are differentiated into human hepatocytes without fusion. Blood 106 (2), 756–763.

Schlichenmaier, H., et al., 2002. Expression of cytokeratin 18 during pre- and post-natal porcine lung development. Anat. Histol. Embryol. 31 (5), 273–277.

Schwartz, R.E., et al., 2002. Multipotent adult progenitor cells from bone marrow differentiate into functional hepatocyte-like cells. J. Clin. Invest. 109 (10), 1291–1302.

Secchiero, P., et al., 2010. Human bone marrow mesenchymal stem cells display anti-cancer activity in SCID mice bearing disseminated non-Hodgkin's lymphoma xenografts. PLoS One 5 (6), e11140.

Selmani, Z., et al., 2008. Human leukocyte antigen-G5 secretion by human mesenchymal stem cells is required to suppress T lymphocyte and natural killer function and to induce $CD4^{+}CD25highFOXP3^{+}$ regulatory T cells. Stem Cells 26 (1), 212–222.

Seo, M.J., et al., 2005. Differentiation of human adipose stromal cells into hepatic lineage in vitro and in vivo. Biochem. Biophys. Res. Commun. 328 (1), 258–264.

Sgroi, A., et al., 2011. Interleukin-1 receptor antagonist modulates the early phase of liver regeneration after partial hepatectomy in mice. PLoS One 6 (9), e25442.

Sheng, H., et al., 2008. A critical role of IFNgamma in priming MSC-mediated suppression of T cell proliferation through up-regulation of B7-H1. Cell Res. 18 (8), 846–857.

Slukvin, I.I., Vodyanik, M., 2011. Endothelial origin of mesenchymal stem cells. Cell Cycle 10 (9), 1370–1373.

Snykers, S., et al., 2009. In vitro differentiation of embryonic and adult stem cells into hepatocytes: state of the art. Stem Cells 27 (3), 577–605.

Sotiropoulou, P.A., et al., 2006. Interactions between human mesenchymal stem cells and natural killer cells. Stem Cells 24 (1), 74–85.

Spahr, L., et al., 2013. Autologous bone marrow mononuclear cell transplantation in patients with decompensated alcoholic liver disease: a randomized controlled trial. PLoS One 8 (1), e53719.

Sun, B., et al., 2005. Correlation between melanoma angiogenesis and the mesenchymal stem cells and endothelial progenitor cells derived from bone marrow. Stem Cells Dev. 14 (3), 292–298.

Sun, L., et al., 2009. Mesenchymal stem cell transplantation reverses multiorgan dysfunction in systemic lupus erythematosus mice and humans. Stem Cells 27 (6), 1421–1432.

Sundin, M., et al., 2006. Mesenchymal stem cells are susceptible to human herpesviruses, but viral DNA cannot be detected in the healthy seropositive individual. Bone Marrow Transplant. 37 (11), 1051–1059.

Sundin, M., et al., 2008. Persistence of human parvovirus B19 in multipotent mesenchymal stromal cells expressing the erythrocyte P antigen: implications for transplantation. Biol. Blood Marrow Transplant. 14 (10), 1172–1179.

Suva, D., et al., 2008. In vitro activated human T lymphocytes very efficiently attach to allogenic multipotent mesenchymal stromal cells and transmigrate under them. J. Cell. Physiol. 214 (3), 588–594.

Takashima, Y., et al., 2007. Neuroepithelial cells supply an initial transient wave of MSC differentiation. Cell 129 (7), 1377–1388.

Tan, J., et al., 2012. Induction therapy with autologous mesenchymal stem cells in living-related kidney transplants: a randomized controlled trial. JAMA 307 (11), 1169–1177.

Terai, S., et al., 2006. Improved liver function in patients with liver cirrhosis after autologous bone marrow cell infusion therapy. Stem Cells 24 (10), 2292–2298.

Tobin, L.M., et al., 2013. Human mesenchymal stem cells suppress donor $CD4(^{+})$ T cell proliferation and reduce pathology in a humanized mouse model of acute graft-versus-host disease. Clin. Exp. Immunol. 172 (2), 333–348.

Tolar, J., et al., 2007. Sarcoma derived from cultured mesenchymal stem cells. Stem Cells 25 (2), 371–379.

Tsai, P.C., et al., 2009. The therapeutic potential of human umbilical mesenchymal stem cells from Wharton's jelly in the treatment of rat liver fibrosis. Liver Transpl. 15 (5), 484–495.

Tse, W.T., et al., 2003. Suppression of allogeneic T-cell proliferation by human marrow stromal cells: implications in transplantation. Transplantation 75 (3), 389–397.

Uccelli, A., Moretta, L., Pistoia, V., 2008. Mesenchymal stem cells in health and disease. Nat. Rev. Immunol. 8 (9), 726–736.

Urban, V.S., et al., 2008. Mesenchymal stem cells cooperate with bone marrow cells in therapy of diabetes. Stem Cells 26 (1), 244–253.

van Poll, D., et al., 2008. Mesenchymal stem cell-derived molecules directly modulate hepatocellular death and regeneration in vitro and in vivo. Hepatology 47 (5), 1634–1643.

Vig, P., et al., 2006. The sources of parenchymal regeneration after chronic hepatocellular liver injury in mice. Hepatology 43 (2), 316–324.

Vodyanik, M.A., et al., 2010. A mesoderm-derived precursor for mesenchymal stem and endothelial cells. Cell Stem Cell 7 (6), 718–729.

Wang, X., et al., 2003. Cell fusion is the principal source of bone-marrow-derived hepatocytes. Nature 422 (6934), 897–901.

Waterman, R.S., et al., 2010. A new mesenchymal stem cell (MSC) paradigm: polarization into a pro-inflammatory MSC1 or an Immunosuppressive MSC2 phenotype. PLoS One 5 (4), e10088.

Weiss, M.L., et al., 2006. Human umbilical cord matrix stem cells: preliminary characterization and effect of transplantation in a

rodent model of Parkinson's disease. Stem Cells 24 (3), 781–792.
Xie, Q.P., et al., 2009. Human bone marrow mesenchymal stem cells differentiate into insulin-producing cells upon microenvironmental manipulation in vitro. Differentiation 77 (5), 483–491.
Yan, Y., et al., 2009. Mesenchymal stem cells from human umbilical cords ameliorate mouse hepatic injury in vivo. Liver Int. 29 (3), 356–365.
Yang, Z.F., et al., 2007. Antiinflammatory properties of IL-10 rescue small-for-size liver grafts. Liver Transpl. 13 (4), 558–565.
Zappia, E., et al., 2005. Mesenchymal stem cells ameliorate experimental autoimmune encephalomyelitis inducing T-cell anergy. Blood 106 (5), 1755–1761.
Zhang, W., et al., 2004. Effects of mesenchymal stem cells on differentiation, maturation, and function of human monocyte-derived dendritic cells. Stem Cells Dev. 13 (3), 263–271.
Zhang, Z., et al., 2012. Human umbilical cord mesenchymal stem cells improve liver function and ascites in decompensated liver cirrhosis patients. J. Gastroenterol. Hepatol. 27 (Suppl. 2), 112–120.
Zhao, L., et al., 2012a. Ex vivo-expanded bone marrow mesenchymal stem cells facilitate recovery from chemically induced acute liver damage. Hepatogastroenterology 59 (120).
Zhao, W., et al., 2012b. Intravenous injection of mesenchymal stem cells is effective in treating liver fibrosis. World J. Gastroenterol. 18 (10), 1048–1058.
Zhen, G., et al., 2010. Mesenchymal stem cell transplantation increases expression of vascular endothelial growth factor in papain-induced emphysematous lungs and inhibits apoptosis of lung cells. Cytotherapy 12 (5), 605–614.
Zhou, Y.F., et al., 2006. Spontaneous transformation of cultured mouse bone marrow-derived stromal cells. Cancer Res. 66 (22), 10849–10854.
Zhu, W., et al., 2006. Mesenchymal stem cells derived from bone marrow favor tumor cell growth in vivo. Exp. Mol. Pathol. 80 (3), 267–274.
Zuk, P.A., et al., 2002. Human adipose tissue is a source of multipotent stem cells. Mol. Biol. Cell 13 (12), 4279–4295.
Zvaifler, N.J., et al., 2000. Mesenchymal precursor cells in the blood of normal individuals. Arthritis Res. 2 (6), 477–488.