

Chapter 2

Alternative Cell Sources to Adult Hepatocytes for Hepatic Cell Therapy

Eugenia Pareja, María José Gómez-Lechón, and Laia Tolosa

Abstract

Adult hepatocyte transplantation is limited by scarce availability of suitable donor liver tissue for hepatocyte isolation. New cell-based therapies are being developed to supplement whole-organ liver transplantation, to reduce the waiting-list mortality rate, and to obtain more sustained and significant metabolic correction. Fetal livers and unsuitable neonatal livers for organ transplantation have been proposed as potential useful sources of hepatic cells for cell therapy. However, the major challenge is to use alternative cell sources for transplantation that can be derived from reproducible methods. Different types of stem cells with hepatic differentiation potential are eligible for generating large numbers of functional hepatocytes for liver cell therapy to treat degenerative disorders, inborn hepatic metabolic diseases, and organ failure. Clinical trials are designed to fully establish the safety profile of such therapies and to define target patient groups and standardized protocols.

Key words Clinical trials, Fetal hepatocytes, Induced pluripotent stem cells, Neonatal hepatocytes, Pluripotent stem cells, Inborn metabolic errors, Liver transplantation, Hepatocyte transplantation

1 Introduction

Cell-based therapies have been a particularly active research area in recent years, whose objective has been to restore lost organ function. Hepatocyte transplantation (HT) has been considered worldwide a promising alternative to liver transplantation (LT) for a variety of indications, including acute liver failure (ALF) and metabolic liver diseases [1–8]. Cell transplantation offers a number of potential advantages compared with LT. The procedure is considerably less invasive, with less risk of morbidity and mortality. Unlike whole organs, cells can be cryopreserved and stored until needed. So cells are available immediately for both programmed treatment, which can be performed repeatedly for liver-based metabolic disorders, and emergency use in patients with ALF when an organ is not available. However at present, HT is limited by scarce availability of suitable donor liver tissue for hepatocyte isolation. Other major

concerns include the detrimental effects of cryopreservation on the viability and metabolic function of adult hepatocytes [6, 9–11] and, despite encouraging results, long-term sustained therapeutic benefits of adult HT are generally lacking due to allograft rejection. Therefore, the ability to reproducibly generate a well-characterized source of engraftable and functional liver cells has remained a challenge.

New cell-based therapies are being developed to supplement whole-organ liver transplantation, to reduce the waiting-list mortality rate, and to obtain more sustained and significant metabolic correction [4, 12]. Fetal livers [7, 13–16] or neonatal livers [17–19] unsuitable for organ transplantation have been proposed as potential useful sources of hepatic cells for cell therapy. Fetal and neonatal hepatocytes are also less vulnerable to cryopreservation than adult hepatocytes [19, 20]. Notwithstanding, the major challenge for this field is to identify alternative reliable cell sources for transplantation, the equivalent to hepatocytes, which can be derived from reproducible methods. Replacing hepatocytes with hepatocyte-like cells (HLCs) generated from stem cells is an alternative strategy to overcome shortage of hepatocytes. Stem cells that have the potential to be expanded, maintained, and differentiated in cell cultures are promising to help improve the efficacy of hepatic cell-based therapy for treating and repairing damaged tissue in the future [4, 21–23].

Different types of stem cells with hepatic differentiation potential are eligible for generating large numbers of functional hepatocytes for liver cell therapy. These include pluripotent stem cells (PSCs), comprising embryonic stem cells (ESCs), induced pluripotent stem cells (iPSCs), and adult stem cells, such as hepatic progenitor cells (HPCs), amnion-derived stem cells (ASCs), and mesenchymal stem cells (MSCs). Human PSCs-derived hepatocytes are emerging as cell-based systems that may potentially provide a stable source of hepatocytes for multiple applications, including cell therapy. Different protocols have been developed to isolate ESCs and to induce them to form HLCs by mimicking the developmental pathway of the liver during embryogenesis [24, 25]. The recently described induced pluripotent stem cells (iPSCs) might circumvent ethical concerns about embryonic and fetal liver stem cells. iPSC-derived hepatocytes have been shown to be most promising in terms of acquiring a primary tissue-like phenotype and unlimited availability [4, 25, 26]. Although current differentiation protocols of iPSCs to hepatic cells need to be optimized, they can provide a limitless supply of hepatocytes, which implies the additional benefit of being able to provide patient-specific hepatocytes.

In vitro expansion makes it possible to break away from dependency on organ donation and is also compatible with large-scale pharmaceutical production, which provides a real prospect of

bringing hepatic cell-based therapy to any patient in need in any metabolic center. Pharmaceutical development is also the guarantee to conduct proper clinical trials to evaluate both safety and efficacy. Stem or progenitor cells, produced in vitro under good manufacturing practice conditions, are classed as medicinal products in Europe. Ideally, these cell lines would be highly viable preparations with robust hepatic function and engraftment capacity, and well characterized.

In summary, cell-based therapies, particularly those based on stem cells or more differentiated progenitor cells, are promising tools at the service of regenerative medicine to treat degenerative disorders, inborn hepatic metabolic diseases, and organ failure [4, 5, 12, 21]. Nonetheless, critical aspects need to be further addressed, including the long-term safety, tolerability and efficacy of these stem cell-based treatments, as well as their tumorigenic potential. Consequently, it is paramount to conduct larger well-designed clinical trials to fully establish the safety profile of such therapies and to define target patient groups with efficacy assessed by standardized protocols.

2 Clinical Hepatic Cell Therapy Applications

LT is currently the treatment of choice for end-stage liver diseases and life-threatening liver-based metabolic disorders. Inborn errors of metabolism affect around 1/900 live births. This pathology often represents rare conditions characterized by accumulation of metabolic intermediates in organs and physiological fluid. For some metabolic disorders, the risks associated with LT are not justified, and HT could be a less invasive option to improve these patients' long-term outcome [1].

Theoretically, all liver disorders that are attributable to a single gene defect have the potential to benefit from HT. However, only a small number of disorders have been targeted to date. Management of patients with metabolic diseases is very complex and includes orphan medications, specific diets, and special education. Besides life-threatening conditions, one main concern is that long-term intellectual prognosis may be impaired. The quality of life of patients and their family is often poor.

The indication for HT is currently based on disease severity or quality of life, and the goal is to avoid or postpone LT, be it due to inborn genetic metabolism errors or ALF [1, 6, 27].

The most encouraging outcomes of HT have been reported in patients with metabolic liver-based disorders [7, 28]. HT is, therefore, a promising alternative, especially in diseases with a nearly intact liver, but with systemic organ damage. Clinical studies into disorders, such as glycogen storage disorders and urea cycle defects, have already highlighted the corrective capacity of HT to improve

clinical outcomes, and it is reasonable to assume that additional advances with HT can be expected [29–32]. The obtained results are encouraging, but the cell transplantation protocols used in different centers vary. Therefore, outcomes are difficult to compare. The lower surgical risk and fewer consequences of graft loss associated with HT, as opposed to LT, could benefit patients with these diseases. Deciding the best timing for transplantation in these patients is challenging. The general rule is to try to postpone surgery and exposing children to immunosuppressive drugs as much as possible. The first attempt to treat inherited metabolic liver disease with HT was made in the *ex vivo* gene therapy for familial hypercholesterolemia. A trial that involves four patients has demonstrated a slight reduction in plasma cholesterol levels and the persistence of transplanted cells. Although the procedure did not effectively lower LDL levels, the trial established the feasibility of HT and the longevity of transplanted cells [33]. Table 1 summarizes the commonest liver metabolic disorders in which HT has been clinically applied. The results are encouraging, although the cell transplantation protocols used in different centers vary. So outcomes are difficult to compare.

HT has also been envisaged as a useful therapeutic approach for bridging patients to LT, and is indicated for providing metabolic support during ALF and acute on chronic liver failure in which the only hope for survival for most patients is either LT, or facilitating liver regeneration in cases of acute or fulminant hepatic failure or a major resection for metastatic disease [1, 5, 34]. In fact, the first HT clinical trials were reported by Mito and Kusano, who injected isolated human hepatocytes into the spleen of ten Japanese patients with liver cirrhosis or chronic hepatitis [35].

3 Alternative Cell Sources to Adult Hepatocytes

The worldwide shortage of donor livers for HT has prompted the search for alternative cell therapies for intractable liver diseases (Fig. 1). Current sources of liver tissue are mainly adult organs rejected for transplantation, normally of marginal quality, such as severe steatosis, prolonged cold ischemia time, and older donors. Despite improvements in hepatocyte isolation methods, it is well known that the mature hepatocytes obtained from these livers often show poor and insufficient functional quality and viability [4, 21, 36]. Therefore, other sources of human hepatic tissue for hepatocyte isolation have been explored. One interesting alternative to the adult liver is the use of fetal livers or neonatal livers for unsuitable organ transplantation as potential sources of good-performing hepatic cells. Although their use in cell transplantation has been poorly explored, they have several advantages compared to adult liver cells: availability, proliferative capacity,

Table 1
Clinical indications of hepatocyte transplantation

Inborn hepatic metabolic diseases	Clinical case	Follow up after HT	Reference
Crigler–Najjar syndrome type 1	10-year-old female. 7.5 × 10 ⁹ hepatocytes (5 % liver mass)	Reduction of phototherapy Reduction of bilirubin levels Excretion of conjugated bilirubin OLT, 4 years after HT	[136]
	8-year-old female. 7.5 × 10 ⁹ hepatocytes	40 % Reduction of bilirubin levels OLT, 20 months after HT	[29]
	9-year-old boy. 7.5 × 10 ⁹ hepatocytes (5 % liver mass)	30 % Reduction of bilirubin levels Inadequate phototherapy → OLT 5 months after HT	[137]
	18-month-old. 4.3 × 10 ⁹ hepatocytes	40 % reduction bilirubin to 7 months. OLT at 8 months	[138]
	3-year-old girl. 2.1 × 10 ⁹ hepatocytes	No clear benefit. OLT at 18 months	[138]
	9-year-old female. 6.1 × 10 ⁹ hepatocytes (4 % liver mass)	30 % reduction of serum bilirubin. OLT 6 months after HT	[139]
	1-year-old female. 2.6 × 10 ⁹ hepatocytes. (8.6 % liver mass)	25 % reduction of serum bilirubin. OLT 4 months after HT	[139]
	7-year-old female. 1.4 × 10 ⁹ hepatocytes (<1 % liver mass)	40 % reduction of serum bilirubin levels after HT and 50 % reduction in phototherapy. OLT after 11 months	[140]
	11-year-old male. 7.2 × 10 ⁹ hepatocytes	20 % reduction of bilirubin levels. OLT	[141]
Urea cycle defects	7-month-old female. 6.7 × 10 ⁹ hepatocytes. (17 % liver mass)	Decrease of bilirubin levels (25 mg/day to 14) 50 % reduction of phototherapy	[31]

(continued)

Table 1
(continued)

Inborn hepatic metabolic diseases	Clinical case	Follow up after HT	Reference
<i>Ornithine transcarbamylase (OTC)</i>	5-year-old boy. 1×10^9 hepatocytes	Ammonia levels normalized. Died from pneumonia 42 days after HT	[142]
	10-month-old male. 4×10^9 hepatocytes (22 % liver mass)	Normalization of ammonia and glutamine levels on normal protein diet. OLT at 6 months of age	[30]
	1-day-old male. 1.74×10^9 hepatocytes (5 % liver mass)	Decrease of ammonia levels and increase in serum urea. Auxiliary partial OLT performed at 7 months of age	[143]
	14-month-old boy. 3.5×10^9 hepatocytes	Blood ammonia level decreased. Urea levels increased. OLT 6 months after HT	[144]
	6-h-old male. 0.64×10^9 hepatocytes (4 % liver mass)	No metabolic crises occurred in the neonatal period. Died at 4 months of age by norovirus infection	[17, 18]
	9-days-old male. 0.56×10^9 hepatocytes (4 % liver mass)	Blood ammonia level decreased. Protein intake increased. OLT	[17, 18]
<i>Carbamoylphosphate synthetase type 1 (CPSI) deficiency</i>	12-year-old girl. 0.87×10^9 hepatocytes (2 % liver mass)	Blood ammonia level decreased. Urea levels increase. Died from septic shock 30 days after HT	[31]
<i>Citrullinemia</i>	2.5-month-old male. 1.37×10^9 hepatocytes	Normalization of ammonia. Urea production and protein intake increased. OLT	[17, 18]
	3-year-old female. 1.46×10^9 hepatocytes (3 % liver mass)	Normalization of ammonia. 40% increase in urea. Increased protein intake 10 months after HT. OLT	[17, 18]
<i>Argininosuccinatelyase (ASL) deficiency</i>	2-year-old-female. 3×10^9 hepatocytes	Decrease of citrulline and ammonia at 2 weeks to 6 months	[145]
	3.5-year-old female. 3×10^9 hepatocytes (10 % liver mass)	Decrease of ammonia levels. Metabolic control Psychomotor catch-up. OLT after 18 months	[146]

(continued)

Table 1
(continued)

Inborn hepatic metabolic diseases	Clinical case	Follow up after HT	Reference
Glycogen storage disease type I			
<i>Type I a</i>	47-year-old female. 2×10^9 hepatocytes (1 % liver mass)	Improved blood glucose control. Decrease in blood lactate concentration. G6Pase enzyme activity in liver biopsy 8 weeks after HT	[147]
	6-year-old female. 2.35×10^9 hepatocytes (17 % liver mass)	Improved blood glucose, cholesterol, and triglycerides control. Decrease of blood lactate concentration. No hypoglycemic episodes	[31]
<i>Type I b</i>	18-year-old male. 0.17×10^9 hepatocytes (6 % liver mass)	Improved blood glucose control. Decreased blood lactate concentration. G6pase enzyme activity in liver biopsy at 7 months	[148]
Refsum disease	4 year-old female. 2.1×10^9 hepatocytes (5 % liver mass)	40% decrease of pipecolic acid 18 months after HT	[149]
Phenylketonuria	6-year-old male. 2.58×10^9 hepatocytes	70% reduction in phenylalanine level. Half-life of phenylalanine decreased from 41.6 to 19.1 h	[150]
Tyrosinemia type 1	45-day-old male. 0.66×10^9 hepatocytes (3.5 % liver mass)	Improvement in clotting factors levels. Bilirubin levels decreased. OLT at 4 months	[31]
Factor VII deficiency	3-month-old male. 1.1×10^9 hepatocytes (4 % liver mass)	70% decrease of their requirements for recombinant FVII (rFVIIa). OLT at 7 months	[138]
	3-year-old male. 2.2×10^9 hepatocytes (3 % liver mass)	70% decrease of requirements of recombinant FVII (rFVIIa). OLT at 8 months	[138]
	3-month-old male. 3×10^9 (unpub)	OLT	[138]
Primary hyperoxaluria	1.3-year-old-female. 2.1×10^9 hepatocytes	Plasma oxalate continuously decreased. OLT at 12 months	[151]

(continued)

Table 1
(continued)

Inborn hepatic metabolic diseases	Clinical case	Follow up after HT	Reference
Familial hypercholesterolaemia	28-year-old female. 1.1×10^9 hepatocytes	Up to 20 % decrease of LDL, cholesterol and Apo B	[152]
	12-year-old male. 1.3×10^9 hepatocytes	No effect	[152]
	7-year-old female. 1.0×10^9 hepatocytes	Up to 20 % decrease of LDL, cholesterol, and Apo B	[152]
	41-year-old female. 3.2×10^9 hepatocytes	Minor effect	[152]
	11-year-old female. 1.5×10^9 hepatocytes	Up to 20 % decrease of LDL, cholesterol, and Apo B	[152]
Progressive familial intrahepatic cholestasis (PFIC)	32 month old. 0.3×10^9 hepatocytes	No benefit, cirrhosis established. OLT at 5 months	[138]
	16 month old. 0.3×10^9 hepatocytes	No benefit, cirrhosis established. OLT at 14 months	[138]

good adaptation and integration capacity into the host liver, and plasticity for fetal hepatic cells. PSCs may offer many advantages as an alternative source of cells for cell therapy. They are readily available and may be effectively expanded in vitro or in vivo, and differentiated into hepatocytes. ESCs cells have also been suggested to possibly be more resistant to cryopreservation and less immunogenic. The equivalent to ESCs in gene expression are iPSCs, which can be generated from human skin by the retroviral transduction of transcription factors [37] to generate suitable populations of human hepatocytes for personalized hepatic cell therapy. Ex vivo gene-corrected patient-specific iPSCs lines can also be used for autologous transplantation as a therapeutic option [4].

The challenges associated with the clinical use of stem cells are considerable. For example, perfecting methods to the scale-up production of hepatocytes from stem cells is a difficult, but necessary, step because vast numbers of cells are required for transplants to be effective. Before basic stem cell research can be translated into clinical practice with patients, it must be first rigorously tested and validated in preclinical studies.

3.1 Fetal Hepatocytes

Fetal liver [7, 13–15, 38] has been considered a potential suitable alternative cell source for hepatic cell therapy. Traditional hepatocyte isolation techniques for low-gestational-age fetal livers are based on the mechanical disruption of tissue into small fragments, and then incubation with collagenase for the digestion of

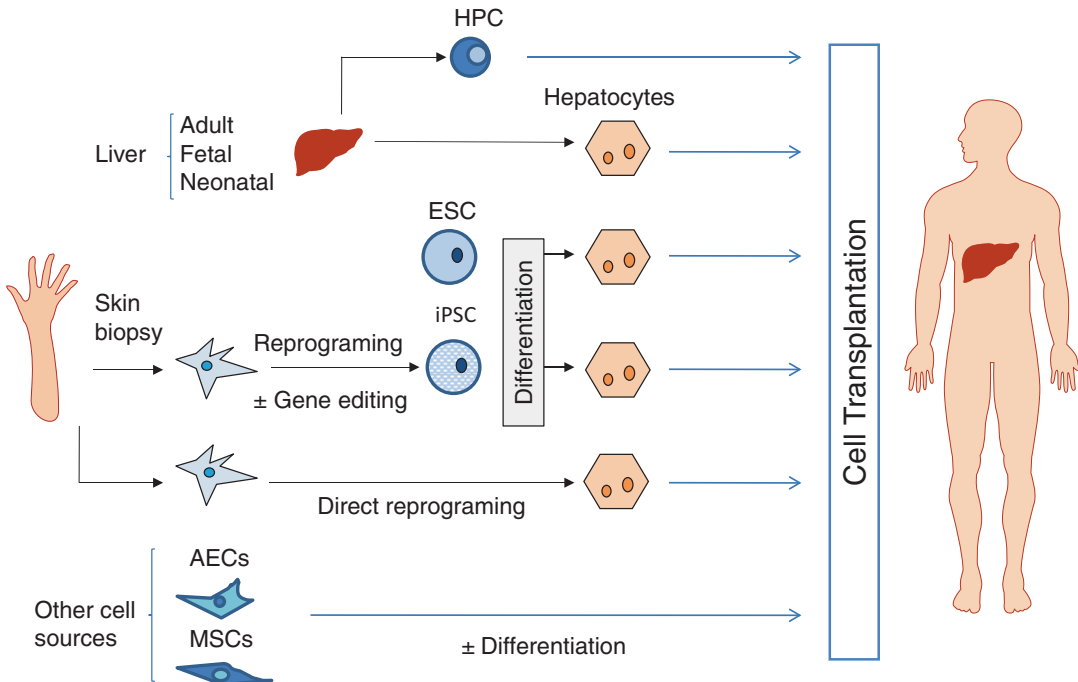


Fig. 1 Cell sources for hepatic cell therapy. Hepatocytes can be obtained from fetal livers, or unused neonatal or adult livers for OLT. Embryonic stem cells (hESC) are available and can be expanded and differentiated into hepatocytes. Induced pluripotent stem cells (iPSCs), generated from human skin cells, can be differentiated into human hepatocytes for personalized hepatic cell therapy. Other stem cell sources can also be used

connective tissue, which yields only a fraction of the number of available cells. Therefore, the clinical application of fetal hepatocytes from 12-week gestation livers is restricted given the difficulty of obtaining large numbers of fetal liver cells. For example, to transplant a quantity of cells that corresponds to 2% of the liver mass in a newborn, around 30 of these fetal livers are needed [8]. However, a five-step method for donated tissue from gestation weeks 18 to 22 has been reported, in which a portal vein perfusion technique resulted in wide viabilities [38]. Briefly, the liver was perfused in situ for 10 min at 37 °C with a buffered solution that contained chelating agent ethylenediamine tetraacetic acid (EGTA) to loosen the desmosomal cell–cell junctions. Thereafter the liver was perfused for 10 min with the solution without EGTA to prepare tissue for digestion by collagenase perfusion with a calcium-containing solution for 7–10 min until the organ became soft and tissue started to disintegrate. Any undigested tissue was removed by filtration and the cell suspension was washed 2–3 times by low-speed centrifugation. The procedure yielded between 3.8 and 13×10^7 cells per gram of liver tissue, and an average cell viability of 78%, as determined by the trypan blue exclusion method [38].

Regarding the hepatic markers of cells, the human fetal liver obtained during the first 12 weeks of gestation mainly exhibits cells that express hematopoietic [39, 40] and endothelial cell markers [41], while the majority of cells express hepatic markers appear early in the second trimester [42]. Nonetheless, the resulting cell preparations from the fetal liver late in the second trimester phenotypically show, in addition to hepatic progenitors, cells that express typical mesenchymal stem cells and hematopoietic stem cells markers [38, 43]. The percentage of fetal liver cells that express proliferation markers is 45 times higher than the percentage of adult hepatocytes that express these markers [38]. Indeed, a larger number of cells positive for the epithelial cell adhesion molecule (EpCAM), a transmembrane glycoprotein that is expressed in hepatic progenitors, have been described in fetal livers [43]. So even though human fetal liver cells are not likely to be routinely used for clinical liver cell repopulation, future trends will focus on the potential of fetal liver stem/progenitor cells to repopulate the liver [44].

Fetal hepatocytes are immature and hepatocytic traits depend on the gestational age. It has been reported that 20–22-week gestation fetal hepatocytes perform liver-specific functions at levels comparable to those of their adult counterpart, while the values of liver-specific functions in cells from 16 to 18 gestation weeks are very low [20]. Based on current knowledge, drug-metabolizing enzymes are expressed at negligible or low levels in fetal liver [45, 46]. Moreover, the fetal expression patterns of CYP450 enzymes considerably vary. Although substantial developmental changes in the hepatic expression in both CYP2C9 and CYP2E1 occur, the expressions of CYP2C19, CYP3A5, and CYP3A7 remain relatively constant in the fetal liver [45, 46], with CYP3A7 accounting for up to 50% of total fetal hepatic CYP450 content [47]. Regarding conjugating enzymes, the expression of UDP-glucuronosyltransferase 1A1 (UGT1A1), the major enzyme responsible for bilirubin glucuronidation, is absent or very weak in the fetal liver [46, 48]. However, UGT1A6, UGT1A9, and UGT2B7 isozymes have already been seen to be expressed in the fetal liver, but develop slowly during the postnatal period [49, 50]. Some authors have proposed the hepatic maturation of fetal hepatocytes in primary culture to generate fully differentiated hepatocytes for clinical use [51, 52]. Fetal hepatocytes, with a high regenerative capacity, have shown a mature hepatic phenotype, established by gene expression profiling, and functional integration within the first few weeks after transplantation into the host liver [13, 14].

Clinical studies that used fetal hepatocytes have suggested that they are a potentially useful source of cells for clinical therapy. In agreement with this, a 1994 study showed that fetal hepatocytes injected intraperitoneally into ten patients with fulminant hepatic failure induced the recovery of some of them, with neurological improvement and lowered ammonia and bilirubin levels recorded,

with no procedure-related complications [15]. There has been a case report of a patient diagnosed as acute fatty liver of pregnancy. After delivery, the patient progressed to grade IV encephalopathy and did not improve despite intensive clinical management measures. After the infusion of human fetal hepatocytes, she completely recovered [16]. Another case report of a patient with clinical end-stage chronic liver failure, who received two intrasplenic infusions of freshly isolated fetal liver cells, obtained an improved MELD (Model for End-Stage Liver Disease) score, with no signs of encephalopathy within the first 18 months after cell transplantation [38]. Intrasplenic fetal liver cell infusion in patients with end-stage chronic liver disease, who are on the waiting list for liver transplantation, has also been recently reported to induce a positive effect on both clinical scores and encephalopathy [53]. A recent study has suggested that rat fetal hepatocytes may have antifibrotic properties when transplanted into damaged livers, which would have the dual benefit of supporting parenchymal regeneration while targeting the scarring component of chronic liver disease [54]. This may have two unresolved explanations: this finding is a general phenomenon, seen using human fetal hepatocytes, particularly in the chronic liver injury setting; fetal cells could be less immunogenic compared with adult hepatocytes.

3.2 Neonatal Hepatocytes

Livers from neonatal donors are not normally used for transplantation because of their small size and the technical difficulties of performing vascular sutures. However, a high yield of good functional quality hepatocytes can be isolated from neonatal liver tissue [17–19]. Liver tissue is enzymatically dissociated using a two-step collagenase perfusion technique. Major liver vessels are cannulated and perfused at 37 °C at a flow rate of 100 ml/min with a calcium-free buffered salt solution that contains 0.5 mM chelating agent EGTA. Then the liver is perfused with a calcium-containing collagenase buffered salt solution for 10 min to digest the extracellular matrix [9, 19]. After digestion, liver tissue is disrupted, and suspended hepatocytes are purified by filtration and low-speed centrifugation [19]. The hepatocyte number and cell viability are assessed by the trypan blue exclusion technique and are usually higher than 90%. The average hepatocyte yield is $15\text{--}20 \times 10^6$ hepatocytes per gram of liver tissue [19] (Table 2).

Neonatal hepatocytes show similar hepatic functionality to an adult liver. The ureogenic capability of neonatal hepatocytes is comparable to that reported for adult hepatocytes [9, 19] (Table 2). In fact, Meyburg et al. [18] transplanted neonatal hepatocytes from the same donor to four different pediatric patients to treat urea cycle disorders with considerable beneficial therapeutic effects. Regarding the expression of CYP450 enzymes, significant increases in expression to mature levels for most enzymes occur only after birth [45, 46]. Moreover, functional CYP450s in neonatal

Table 2

Donor and hepatocyte isolation details and quality assessment of the hepatocytes from adult and neonatal livers after cryopreservation and thawing (C/T)

	Adult hepatocytes	Neonatal hepatocytes
Donor's age	48 ± 28 years	35 ± 5 weeks of gestation
Cold ischemia time (h)	7.1 ± 4.5	1.9 ± 0.5
Cell viability fresh (%)	79 ± 24	87 ± 4
Yield × 10 ⁶ (viable cells/g liver)	8 ± 4	21 ± 14
Characterization after C/T		
Cell viability (%)	63 ± 8	82 ± 5
Attachment efficiency (%)	47 ± 12	64 ± 13
Ureogenic rate (nmol/min × 10 ⁶ cells)	1.6 ± 0.9	1.3 ± 0.5
% EpCAM cells	1.0 ± 0.8	6.1 ± 4.2
% Apoptotic cells	7.2 ± 3.3	3.6 ± 1.4

hepatocytes display a similar balance to that in the adult liver [19, 55]. A recent study has revealed that CYP3A4 and CYP2C9 give the highest activity values in all the neonatal hepatic cell preparations, followed by CYP2A6, CYP2B6, and CYP2E1 with lower levels, and finally by CYP2C19 and CYP2D6 with the lowest activity values, where CYP1A2 was almost undetectable [19] (Fig. 2). The relatively strong CYP3A4 activity noted in neonatal hepatocytes is a good indication of their metabolic competence because the drugs commonly used in standard immunosuppression for HT (e.g., tacrolimus, cyclosporine, or prednisone) are metabolized by this enzyme. Previous reports have reported similar patterns of human hepatic CYP enzymes in the neonatal liver [45, 56, 57]. Some drug-metabolizing enzymes do not become active until a certain age is reached and, conversely, others appear to be higher compared with adults.

Glucuronidation activity in neonates has been described to develop from minimal to almost adult levels within months of birth and has shown extensive interindividual variability [46, 48]. Particularly, the expression of UGT1A1 in humans is modulated in a developmental fashion and reaches adult levels by 14 weeks of postnatal life [46, 48]. In the study of Tolosa et al. [19], the expression of UGT1A1 and UGT1A9 was detected in all the hepatocyte preparations, while enzymatic activity was measured only in some preparations, probably due to lack of maturity of the enzymes [46, 48]. However, isoforms UGT1A6 and UGT2B7 showed considerable activity in all the neonatal preparations, thus providing them with a detoxification capability by the conjugation and elimination of hydrophobic compounds [19].

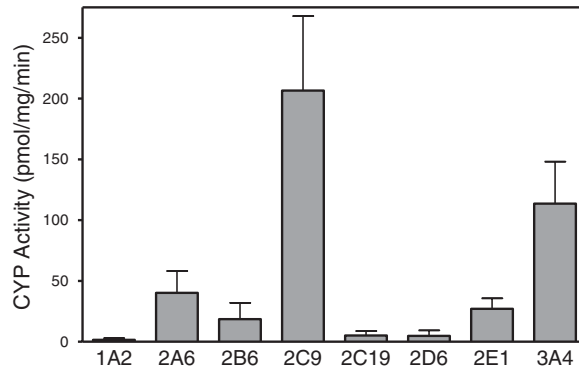


Fig. 2 CYP450 activities in neonatal hepatocytes. The drug detoxification competence of hepatocytes was assessed after cryopreservation by measuring the activities of the major CYP450 enzymes responsible for the oxidative metabolism of drugs in the human liver

Postnatal human livers as fetal livers have been shown to contain pluripotent hepatic progenitors, which are EpCAM+ [43, 58, 59]. If compared to the adult liver, the hepatocyte isolation procedure in neonatal livers provides cell suspensions with a higher proportion of hepatic progenitor cells (EpCAM+ staining), which can also participate in the regeneration of liver parenchyma after transplantation (Table 2). These results could imply a key advantage, particularly for neonatal hepatocytes used as a source of high quality cells to improve hepatic cell therapy applicability. Another major issue for neonatal human hepatocytes is that they are diploid [60] and can pass through the usual mitotic cycles several times, and they maintain their initial ploidy, while the liver cells polyploidization process is generally considered to indicate terminal differentiation and senescence leading to diminished replication capacity [61]. Therefore, the larger number of progenitor cells and diploidy maintenance through several mitotic cycles confer excellent advantages to the hepatic cells isolated from early neonatal livers over adult ones to help improve engraftment, proliferation, and long-term survival in the host liver.

Neonatal liver cells seem less vulnerable to cryopreservation effects than adult hepatocytes and can be stored in liquid nitrogen with no significant loss of viability and function postthawing [6, 19]. Apoptosis has been identified as an important cause of freshly isolated and banked hepatocyte death, with mitochondria as the key players in the initiation of apoptosis and in cryopreservation-induced cell damage [62]. Neonatal hepatocytes present smaller apoptotic and necrotic cell numbers after thawing if compared with adult ones [19] (Table 2). These findings are quite likely related to the fact that the mitochondrial function is not substantially altered by cryopreservation in neonatal cells [17]. In addition, a higher expression of adhesion molecules (β 1-integrin, β -catenin, and

e-cadherin), and better attachment efficiency and cell survival have been reported after thawing [19]. One major problem commonly found after HT is the small number of transplanted cells found in the graft. Both the membrane integrity and adhesion molecules involved in cell–cell and cell–extracellular matrix adhesion mechanisms need to be preserved for engraftment into the host liver to be a success [63]. In agreement with these facts, there are reports which indicate that transplanted newborn [64] and fetal [65] rat hepatocytes, and neonatal mouse hepatocytes [66], can integrate, expand, and differentiate by producing more colonies in the host liver than adult liver cells, irrespectively of the recipient's age. This suggests an improved engraftment and survival advantage of transplanted neonatal hepatocytes in the host liver as opposed to adult hepatocytes. In summary, high functional hepatocytes can be particularly obtained from neonatal livers, which can extend the pool of available organs for cell transplantation.

3.3 Embryonic Stem Cells

PSCs may offer many advantages as an alternative cell source for transplantation because of their availability and ability to be expanded *in vitro* or *in vivo*. Stem cells may have the potential to produce a more sustained significant metabolic correction but must be shown to be effective in controlled trials. Stem cells for cellular therapy of liver disease have been extensively reviewed [3, 21, 26, 67–69].

ESCs are self-renewing PSCs that can be isolated from the inner mass of a blastocyst. Since the source of ESCs needs the blastocyst to be destroyed, some ethical concerns have arisen. These cells proliferate extensively *in vitro*, differentiate into derivatives of all three germ layers, and express a number of characteristic markers (Oct4, SSEA-4, TRA-1-60, and TRA-1-81) with high levels of telomerase activity [70]. Studies on liver development have identified crucial signals for the hepatic lineage, which include Activin A, FGF, BMP, HGF, Oncostatin M, and dexamethasone [4, 71, 72]. From all this, different protocols have been developed to differentiate ESCs into HLCs. The obtained phenotype seems to come closer to fetal hepatocytes than adult ones [73] although *in vivo*, these cells could mature and achieve a more similar phenotype to adult cells. In this sense, different attempts, which include their culture in 3D systems [74], have been recently described.

The potential to differentiate human ESCs *in vitro* and to provide an unlimited source of hepatocytes for use in biochemical research and the treatment of liver disease are most promising [75]. Although animal models have provided encouraging results [21], the clinical application of ESCs has always been associated with practical and ethical concerns. Therapeutically useful differentiated ESCs must be safe (i.e., nontumorigenic) and need to contribute to liver function *in vivo*. However, the potential of ESCs and their differentiated progeny to generate spontaneous tumors is

of particular concern for clinical applications. Initial studies have shown that ESCs injected in their undifferentiated state into mice resulted in teratoma formation, which killed the animals [76], while no teratoma was produced when differentiated cells were injected. Yet some reports have indicated tumor formation after the transplantation of ESC-derived hepatic cells despite predifferentiation [77, 78], and transplanted cells containing a number of undifferentiated ESCs [79]. Other reports have shown that transplantation of highly differentiated cells does not entail the development of tumors [80], thus suggesting that steering ESCs to an appropriate state could be an important step for safe and effective cell therapies. To this end, well-defined methods to reduce the tumorigenicity of transplanted cells, including protocols for complete terminal differentiation and the very strict elimination of undifferentiated ESCs from transplanted cells, should be established [81, 82].

3.4 Induced Pluripotent Stem Cells

iPSCs are human somatic cells that have been reprogrammed to a pluripotent state. They are the equivalent to ESCs in gene expression, but can be reliably derived from adult tissue and have been shown to differentiate efficiently into HLCs [23, 83]. Although many protocols have been defined to differentiate iPSCs, they still differ from adult hepatocytes [84], and different strategies such as their culture in a 3D system [85] have been described to increase their maturity. On top of this, the latest advances made in gene editing technologies have vigorously endorsed the possibility of obtaining disease-free autologous cells from patient-specific iPSCs (Fig. 3). Fascinating progress has been made with the demonstration of the ability of human iPSCs to form small liver organoids with metabolic function when cocultured with endothelial and mesenchymal cells [86].

Human iPSCs provide a unique opportunity to generate live cellular models of patient-specific diseases for personalized cell therapy and for screening candidate pharmacological molecules (Fig. 3) [87]. For monogenic metabolic disorders, the use of iPSCs would enable the study of the effect of mutations on the differentiation and/or function of cells. The liver engraftment potential and regenerative capabilities of human iPSC-derived hepatic cells *in vivo* have been recently demonstrated [88].

Personalized cell therapy using iPSCs would avoid rejection and, thus, immunosuppression. However, there is still some controversy about the immunogenicity of these cells, which should be assessed before these autologous cells are clinically used [89]. On top of this, such a therapeutic approach would require correcting the genetic anomalies that induce the disease. In this sense, several strategies, such as the use of lentivirus, Zinc Finger Nucleases, or transcription activator-like effector nucleases, have been assessed [87] (Fig. 3). Targeted gene correction of pathological mutations

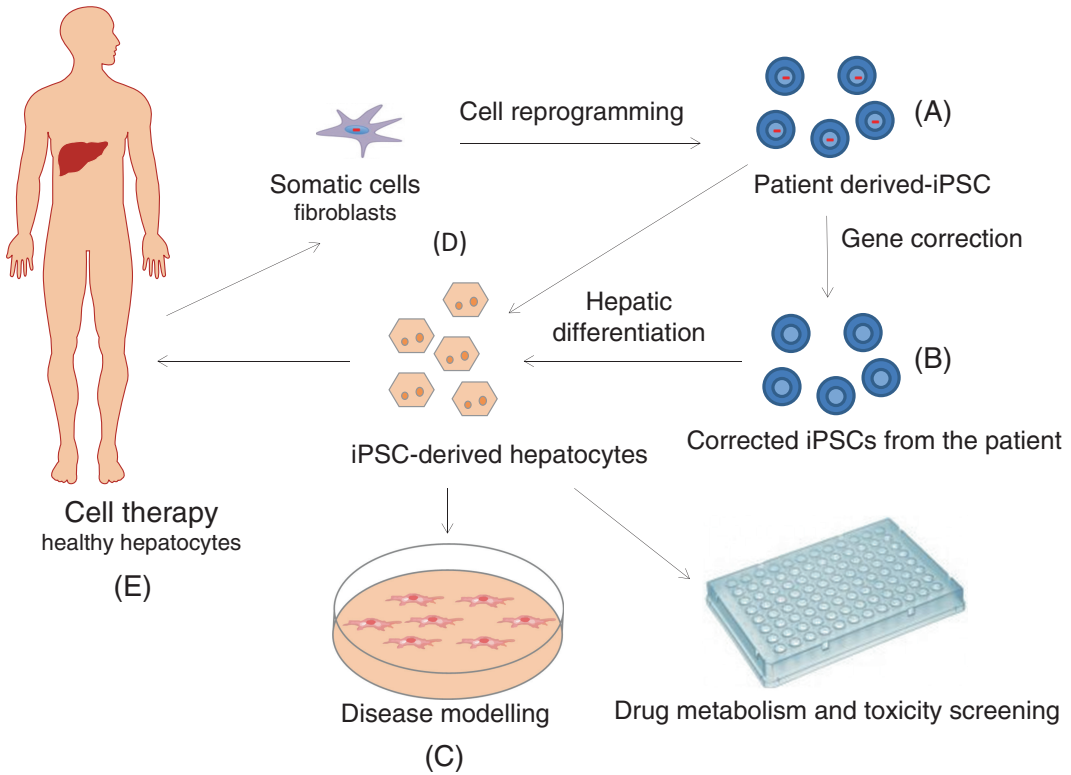


Fig. 3 Modeling liver diseases using induced pluripotent stem cells (iPSCs)-derived hepatocytes. The iPSCs derived from human skin cells of patients with genetic liver diseases (A), by generating ex vivo gene-corrected patient-specific iPSC lines (B), can be used for disease modeling (C) under in vitro simple cell culture conditions. They can be further reprogrammed to generate human hepatocytes (D) and can be used for autologous transplantation as a therapeutic option (E)

in patient iPSCs is promising for both regenerative medicine and in vitro disease modeling. The differentiation derivatives from corrected patient iPSCs have the potential to be employed in autologous cell therapy. However, it is essential to ensure that gene editing procedures do not introduce any unexpected mutation. To this end, more efficient and safer gene correction strategies, as well as more robust whole-genome sequencing tools, may be needed to generate mutation-free iPSCs before being therapeutically applied in the future.

3.5 Direct Reprogrammed Cells

The development of protocols that differentiate PSCs into HLCs has been limited by the need for information about the mechanisms implicated in the functional maturation of the human liver after birth [90]. If cells are directly reprogrammed, this limitation may be evaded. Moreover, this strategy would be presumably less tumorigenic, provided that integration-free gene delivery methods are used [87]. Different publications have demonstrated the production of highly functional human HLCs using direct

reprogramming approaches [91, 92]. Yet their use for cell-based therapies remains unclear because the currently available technologies to generate them and to increase their proliferative ability are incompatible with *in vivo* use [90].

3.6 Other Potential Cell Sources

Liver damage or loss of liver mass can extensively stimulate regenerative capacity until the hepatic mass has been restored by the proliferation of mature parenchymal liver cells [93, 94]. However when liver regeneration is impaired, hepatic progenitor cells (HPCs), with the bipotential capability of generating both biliary epithelia and hepatocytes, become active and replace damaged cells [95]. Organoids have also been derived from human livers by the EpCAM selection of bipotential hepatic epithelial cells [96]. Recently, different studies in animals have suggested that hepatocytes supply all the parenchyma's regenerative capacity [4, 97, 98], although other recent studies in mice have shown the importance of HPCs in liver injury recovery [4, 99]. A key issue would be to determine if studies in rodents can be extrapolated to human disease.

HPCs isolated from human fetal liver are less immunogenic, highly propagative, and more challenging for cryopreservation than adult ones [100]. Fetal liver stem cells have been identified as a transition between embryonic cells and adult ones, which is mostly nonteratogenic [101]. During the third trimester of fetal development, plasticity to form liver parenchyma cells renders fetal HSCs an excellent resource for cellular therapeutic approaches [100].

Mesenchymal stromal cells (MSCs), formerly known as mesenchymal stem cells, are multipotent nonhematopoietic adult stem cells that have been isolated from a variety of tissues, including bone marrow and adipose tissue [102, 103]. They can readily differentiate into a variety of cell types, including osteoblasts, chondrocytes, adipocytes, and hepatocytes [104]. In the last decade, several studies have reported the plasticity of MSCs toward a functional hepatocyte-like phenotype, which suggests the potential for clinical applications. Based on knowledge about the onset of hepatogenesis and liver development [104], different strategies have been attempted to induce hepatogenic trans-differentiation of MSCs into functional HLCs. They are based on either the use of a cocktail of exogenous factors or multistep trans-differentiation protocols by sequential exposure to several factors, which reflect their temporal expression during *in vivo* hepatogenesis [102, 105–108]. Preclinical and clinical studies have suggested that MSC transplantation can moderately restore liver function in liver diseases [105, 109, 110]. Yet the benefits of MSCs have been suggested to be related more with the expression of immunomodulatory factors than engraftment and subsequent hepatic function of the transplanted cells [111]. In fact, MSCs have been reported to be a therapeutic option for inflammatory conditions. Moreover, the efficacy of MSCs, when cocultured with hepatocytes, provides combined hepatic and anti-inflammatory therapy in ALF [110].

Human amnion epithelial cells (hAECs) isolated from term placenta are an important cell source for generating HLCs. Cells isolated from the placenta have been the subject of intense research because many of these cells express characteristics of multipotent or even PSCs. hAECs are abundant, have similar surface markers and gene expression profiles to those reported for human ESCs and MSCs, respectively, and differentiate into lineages of the three embryonic germ layers, including the HLCs that originate from the endoderm [112, 113]. Under defined culture conditions, hAECs can adopt hepatic characteristics [113–115]. Cells isolated from amnion also have some unique properties compared to some other stem cell sources, in that they are isolated from a tissue that is normally discarded following birth, they are quite plentiful and easily isolated, and they do not produce tumors when transplanted. Cells isolated from the amnion may be a uniquely useful and non-controversial stem cell source [113, 116]. Preclinical studies in animal models have shown that hAECs are able to correct the characteristic biochemical imbalances of liver diseases with minimal manipulation, which would encourage the isolation and banking of these cells to be used in the clinical practice for transplantations of patients with liver disease [116].

As a general consideration, the safety, engraftment, and functionality of HLCs are key issues that will need more work before the full potential of using stem cells to treat end-stage liver failure can be achieved.

4 Cell Cryopreservation

Cryopreservation and banking allow cells to be stored for a long period of time until they are required for both scheduling and emergency treatments, which thus improves clinical outcomes. Cryopreserved cells have some advantages in that they are constantly available, and extensive quality testing can be performed to determine suitability for transplantation, and to customize cell preparations for each receptor and sterility testing [9, 117, 118]. On the one hand, cryopreservation is accomplished by controlling the freezing rate in a solution that contains a permeable cryoprotectant, usually DMSO, and involves a thawing method. On the other hand, it is known that current cryopreservation and thawing procedures cause detrimental effects on the viability and functionality of adult human hepatocytes. Thus upon thawing, they are often unsuitable for clinical use [6, 9–11]. Better cell storage is, therefore, a priority, and refinements of freezing protocols to better preserve hepatocyte functionality in order to improve the performance of single cryopreserved cells after thawing have been implemented [6, 9–11]. Alternatively, it has been reported that microencapsulation techniques have the potential to protect

hepatocytes from cryoinjury, which would not only allow the efficient recovery of functional and morphological integrity after thawing, but prevent immune cell-mediated rejection upon transplantation into allogeneic recipients [119, 120]. Cryopreservation methods of microencapsulated cells with variable effectiveness have been reported. Recently, optimized protocols to produce alginate-microencapsulated adult human hepatocytes suitable for clinical transplantation, which could be adapted to encapsulate fetal, neonatal hepatocytes or stem cell sources, have been established [121, 122]. Indeed, intraperitoneal transplantation of microencapsulated hepatocytes in animal ALF models has provided promising outcomes, which indicates that this strategy should be suitable for emergency treatments in conditions such as ALF [121, 122].

Similarly, optimized cryopreservation protocols for single cell suspensions of HLCs derived from stem cells are needed for wide clinical use. Thus, successful HLCs cryopreservation to retain wide viability, and their hepatic differentiated status, would allow their banking, which would, in turn, offer the advantage of having cells readily and limitlessly available for clinical purposes. As for hepatocytes, it has been recently reported that functional HLCs derived from hAECs can be microencapsulated within alginate without losing viability or function *in vitro*, and could likely be banked for clinical use [115].

5 Clinical Trials

Based on the initial and heartening results obtained in liver cell therapy, different programs, sponsored mainly by biotech companies, currently evaluate the safety and efficacy of liver cell transplantation in controlled clinical trials.

The Cytonet program uses cryopreserved cells for intraportal administration. This program consists of a dispersion of cryopreserved liver cells prepared from nontransplantable organs and refined under Good Manufacturing Practice conditions, and their application in patients with urea cycle disorders [123, 124]. In order to determine the effectiveness of this therapy, an *in vivo* ¹³C-ureagenesis assay has also been proposed [123, 125]. Furthermore, an extracorporeal liver assist device system has been developed by Vital Therapies for acute-on-chronic liver failure [124]. The Promethera program employs liver-derived progenitor cells that are expanded *in vitro* and injected into patients with various hereditary metabolic diseases [123, 124]. Although preclinical studies in animals have shown that these cells are able to engraft and proliferate in the recipient liver [126], the clinical outcome of the first transplantation in a 3-year-old girl with OTC deficiency has not yet been reported [127]. The use of EpCAM⁺ cells to treat different liver diseases has been assessed at the Liver Institute in

Hyderabad in India. A representative early publication has revealed that 25 patients with liver cirrhosis of different etiologies were infused with human fetal liver-derived stem cells (EpCAM+), who obtained improved mean MELD scores [128]. However, details on these patients' long-term outcomes are still not available, and further information is needed to elucidate the potential qualities and efficacy of these strategies [129].

Currently, more than 20 clinical trials focus on treating liver diseases with MSCs [129–131]. In this sense, Peng et al. [134] showed good short-term efficacy, but long-term outcomes were not marked when autologous bone marrow MSCs were transplanted in patients with liver failure caused by hepatitis B. Although studies of cell-based therapies in cirrhosis are very heterogeneous in terms of the types of infused cells, it has been evidenced that some forms of cell-based therapies may transiently improve liver function in some patients with cirrhosis [132–135]. However, a recent randomized controlled trial that used autologous bone marrow MSCs transplantation offered no beneficial effect in cirrhotic patients [132]. So, although the outlook of using MSCs as cell therapy to treat liver diseases is encouraging, a better understanding of the mechanism that underlies their therapeutic effects, and a better validation in preclinical and clinical settings, are required [131].

The current challenge of these second-generation liver cell transplantation strategies is to develop reliable differentiation protocols that confer sufficient maturity to differentiated cells for widespread clinical use, in addition to the evaluation of the immunogenicity, toxicity, and tumorigenicity of cells [4, 124].

References

1. Dhawan A, Puppi J, Hughes RD, Mitry RR (2010) Human hepatocyte transplantation: current experience and future challenges. *Nat Rev Gastroenterol Hepatol* 7:288–298
2. Fisher RA, Strom SC (2006) Human hepatocyte transplantation: worldwide results. *Transplantation* 82:441–449
3. Fitzpatrick E, Mitry RR, Dhawan A (2009) Human hepatocyte transplantation: state of the art. *J Intern Med* 266:339–357
4. Forbes SJ, Gupta S, Dhawan A (2015) Cell therapy for liver disease: from liver transplantation to cell factory. *J Hepatol* 62:S157–S169
5. Hansel MC, Gramignoli R, Skvorak KJ, Dorko K, Marongiu F, Blake W, Davila J, Strom SC (2014) The history and use of human hepatocytes for the treatment of liver diseases: the first 100 patients. *Curr Protoc Toxicol* 62:14.12.11–14.12.23
6. Jorns C, Ellis EC, Nowak G, Fischler B, Nemeth A, Strom SC, Ericzon BG (2012) Hepatocyte transplantation for inherited metabolic diseases of the liver. *J Intern Med* 272:201–223
7. Pietrosi G, Vizzini GB, Gruttadauria S, Gridelli B (2009) Clinical applications of hepatocyte transplantation. *World J Gastroenterol* 15:2074–2077
8. Puppi J, Strom SC, Hughes RD, Bansal S, Castell JV, Dagher I et al (2012) Improving the techniques for human hepatocyte transplantation: report from a consensus meeting in London. *Cell Transplant* 21:1–10
9. Bonora-Centelles A, Donato MT, Lahoz A, Pareja E, Mir J, Castell JV, Gomez-Lechon MJ (2010) Functional characterization of hepatocytes for cell transplantation: customized cell preparation for each receptor. *Cell Transplant* 19:21–28
10. Stephenne X, Najimi M, Sokal EM (2010) Hepatocyte cryopreservation: is it time to change the strategy? *World J Gastroenterol* 16:1–14

11. Terry C, Dhawan A, Mitry RR, Lehec SC, Hughes RD (2010) Optimization of the cryopreservation and thawing protocol for human hepatocytes for use in cell transplantation. *Liver Transpl* 16:229–237
12. McKiernan P (2013) Liver transplantation and cell therapies for inborn errors of metabolism. *J Inher Metab Dis* 36(4):675–680
13. Bin WT, Ma LM, Xu Q, Shi XL (2012) Embryonic hepatocyte transplantation for hepatic cirrhosis: efficacy and mechanism of action. *World J Gastroenterol* 18:309–322
14. Cantz T, Zuckerman DM, Burda MR, Dandri M, Goricke B, Thalhammer S et al (2003) Quantitative gene expression analysis reveals transition of fetal liver progenitor cells to mature hepatocytes after transplantation in uPA/RAG-2 mice. *Am J Pathol* 162:37–45
15. Habibullah CM, Syed IH, Qamar A, Taher-Uz Z (1994) Human fetal hepatocyte transplantation in patients with fulminant hepatic failure. *Transplantation* 58:951–952
16. Khan AA, Habeeb A, Parveen N, Naseem B, Babu RP, Capoor AK, Habibullah CM (2004) Peritoneal transplantation of human fetal hepatocytes for the treatment of acute fatty liver of pregnancy: a case report. *Trop Gastroenterol* 25:141–143
17. Meyburg J, Alexandrova K, Barthold M, Kafert-Kasting S, Schneider AS, Attaran M et al (2009) Liver cell transplantation: basic investigations for safe application in infants and small children. *Cell Transplant* 18:777–786
18. Meyburg J, Das AM, Hoerster F, Lindner M, Kriegbaum H, Engelmann G et al (2009) One liver for four children: first clinical series of liver cell transplantation for severe neonatal urea cycle defects. *Transplantation* 87:636–641
19. Tolosa L, Pareja-Ibars E, Donato MT, Cortes M, Lopez S, Jimenez N et al (2014) Neonatal livers: a source for the isolation of good-performing hepatocytes for cell transplantation. *Cell Transplant* 23:1229–1242
20. Chinnici CM, Timoneri F, Amico G, Pietrosi G, Vizzini G, Spada M et al (2015) Characterization of liver-specific functions of human fetal hepatocytes in culture. *Cell Transplant* 24:1139–1153
21. Allameh A, Kazemnejad S (2012) Safety evaluation of stem cells used for clinical cell therapy in chronic liver diseases; with emphasize on biochemical markers. *Clin Biochem* 45:385–396
22. Lagasse E, Connors H, Al-Dhalimy M, Reitsma M, Dohse M, Osborne L et al (2000) Purified hematopoietic stem cells can differentiate into hepatocytes in vivo. *Nat Med* 6:1229–1234
23. Si-Tayeb K, Noto FK, Nagaoka M, Li J, Battle MA, Duris C et al (2010) Highly efficient generation of human hepatocyte-like cells from induced pluripotent stem cells. *Hepatology* 51:297–305
24. Duan Y, Ma X, Zou W, Wang C, Bahbahan IS, Ahuja TP et al (2010) Differentiation and characterization of metabolically functioning hepatocytes from human embryonic stem cells. *Stem Cells* 28:674–686
25. Hannan NR, Segeritz CP, Touboul T, Vallier L (2013) Production of hepatocyte-like cells from human pluripotent stem cells. *Nat Protoc* 8:430–437
26. Schwartz RE, Fleming HE, Khetani SR, Bhatia SN (2014) Pluripotent stem cell-derived hepatocyte-like cells. *Biotechnol Adv* 32:504–513
27. Enns GM, Millan MT (2008) Cell-based therapies for metabolic liver disease. *Mol Genet Metab* 95:3–10
28. Sokal EM (2006) Liver transplantation for inborn errors of liver metabolism. *J Inher Metab Dis* 29:426–430
29. Darwish AA, Sokal E, Stephenne X, Najimi M, de Goyet JV, Reding R (2004) Permanent access to the portal system for cellular transplantation using an implantable port device. *Liver Transpl* 10:1213–1215
30. Horslen SP, McCowan TC, Goertzen TC, Warkentin PI, Cai HB, Strom SC, Fox IJ (2003) Isolated hepatocyte transplantation in an infant with a severe urea cycle disorder. *Pediatrics* 111:1262–1267
31. Ribes-Koninckx C, Pareja Ibars E, Agrasot MA, Bonora-Centelles A, Polo Miquel B, Vila Carbo JJ et al (2012) Clinical outcome of hepatocyte transplantation in four pediatric patients with inherited metabolic diseases. *Cell Transplant* 21(10):2267–2282
32. Wan Z, Zhang XG, Liu ZW, Lv Y (2013) Therapeutic liver repopulation for metabolic liver diseases: advances from bench to bedside. *Hepatol Res* 43:122–130
33. Raper SE, Grossman M, Rader DJ, Thoene JG, Clark BJ III, Kolansky DM et al (1996) Safety and feasibility of liver-directed ex vivo gene therapy for homozygous familial hypercholesterolemia. *Ann Surg* 223:116–126
34. Bilir BM, Guinette D, Karrer F, Kumpe DA, Krysl J, Stephens J et al (2000) Hepatocyte transplantation in acute liver failure. *Liver Transpl* 6:32–40
35. Mito M, Kusano M, Kawaaura Y (1992) Hepatocyte transplantation in man. *Transplant Proc* 24:3052–3053
36. Bhogal RH, Hodson J, Bartlett DC, Weston CJ, Curbishley SM, Haughton E et al (2011)

- Isolation of primary human hepatocytes from normal and diseased liver tissue: a one hundred liver experience. *PLoS One* 6:e18222
37. Yamanaka S (2009) A fresh look at iPS cells. *Cell* 137:13–17
 38. Gridelli B, Vizzini G, Pietrosi G, Luca A, Spada M, Gruttadauria S et al (2012) Efficient human fetal liver cell isolation protocol based on vascular perfusion for liver cell-based therapy and case report on cell transplantation. *Liver Transpl* 18:226–237
 39. Hann IM, Bodger MP, Hoffbrand AV (1983) Development of pluripotent hematopoietic progenitor cells in the human fetus. *Blood* 62:118–123
 40. Roy V, Miller JS, Verfaillie CM (1997) Phenotypic and functional characterization of committed and primitive myeloid and lymphoid hematopoietic precursors in human fetal liver. *Exp Hematol* 25:387–394
 41. Migliaccio G, Migliaccio AR, Petti S, Mavilio F, Russo G, Lazzaro D et al (1986) Human embryonic hemopoiesis. Kinetics of progenitors and precursors underlying the yolk sac—liver transition. *J Clin Invest* 78:51–60
 42. Nava S, Westgren M, Jaksch M, Tibell A, Broome U, Ericzon BG, Sumitran-Holgersson S (2005) Characterization of cells in the developing human liver. *Differentiation* 73:249–260
 43. Schmelzer E, Zhang L, Bruce A, Wauthier E, Ludlow J, Yao HL et al (2007) Human hepatic stem cells from fetal and postnatal donors. *J Exp Med* 204:1973–1987
 44. Oertel M (2011) Fetal liver cell transplantation as a potential alternative to whole liver transplantation? *J Gastroenterol* 46:953–965
 45. Hines RN (2007) Ontogeny of human hepatic cytochromes P450. *J Biochem Mol Toxicol* 21:169–175
 46. Hines RN (2012) Developmental expression of drug metabolizing enzymes: impact on disposition in neonates and young children. *Int J Pharm* 452(1–2):3–7
 47. Wrighton SA, Vandenbranden M (1989) Isolation and characterization of human fetal liver cytochrome P450H1p2: a third member of the P450III gene family. *Arch Biochem Biophys* 268:144–151
 48. McCarver DG, Hines RN (2002) The ontogeny of human drug-metabolizing enzymes: phase II conjugation enzymes and regulatory mechanisms. *J Pharmacol Exp Ther* 300:361–366
 49. Miyagi SJ, Milne AM, Coughtrie MW, Collier AC (2012) Neonatal development of hepatic UGT1A9: implications of pediatric pharmacokinetics. *Drug Metab Dispos* 40:1321–1327
 50. Strassburg CP, Strassburg A, Kneip S, Barut A, Tukey RH, Rodeck B, Manns MP (2002) Developmental aspects of human hepatic drug glucuronidation in young children and adults. *Gut* 50:259–265
 51. Weber A, Touboul T, Mainot S, Branger J, Mahieu-Caputo D (2010) Human foetal hepatocytes: isolation, characterization, and transplantation. *Methods Mol Biol* 640:41–55
 52. Wu Y, Shatapathy CC, Minger SL (2009) Isolation, in vitro cultivation and characterization of foetal liver cells. *Methods Mol Biol* 481:181–192
 53. Pietrosi G, Vizzini G, Gerlach J, Chinnici C, Luca A, Amico G et al (2015) Phases I–II matched case-control study of human fetal liver cell transplantation for treatment of chronic liver disease. *Cell Transplant* 24:1627–1638
 54. Yovchev MI, Xue Y, Shafritz DA, Locker J, Oertel M (2014) Repopulation of the fibrotic/cirrhotic rat liver by transplanted hepatic stem/progenitor cells and mature hepatocytes. *Hepatology* 59:284–295
 55. Gomez-Lechon MJ, Castell JV, Donato MT (2008) An update on metabolism studies using human hepatocytes in primary culture. *Expert Opin Drug Metab Toxicol* 4:837–854
 56. Blake MJ, Castro L, Leeder JS, Kearns GL (2005) Ontogeny of drug metabolizing enzymes in the neonate. *Semin Fetal Neonatal Med* 10:123–138
 57. Johnson TN (2003) The development of drug metabolising enzymes and their influence on the susceptibility to adverse drug reactions in children. *Toxicology* 192:37–48
 58. Schmelzer E, Wauthier E, Reid LM (2006) The phenotypes of pluripotent human hepatic progenitors. *Stem Cells* 24:1852–1858
 59. Zhang L, Theise N, Chua M, Reid LM (2008) The stem cell niche of human livers: symmetry between development and regeneration. *Hepatology* 48:1598–1607
 60. Gentric G, Desdouets C, Celton-Morizur S (2012) Hepatocytes polyploidization and cell cycle control in liver physiopathology. *Int J Hepatol* 2012:282430
 61. Celton-Morizur S, Desdouets C (2010) Polyploidization of liver cells. *Adv Exp Med Biol* 676:123–135
 62. Stephenne X, Najimi M, Ngoc DK, Smets F, Hue L, Guigas B, Sokal EM (2007) Cryopreservation of human hepatocytes alters the mitochondrial respiratory chain complex I. *Cell Transplant* 16:409–419

63. Pinkse GG, Voorhoeve MP, Noteborn M, Terpstra OT, Bruijn JA, De Heer E (2004) Hepatocyte survival depends on beta1-integrin-mediated attachment of hepatocytes to hepatic extracellular matrix. *Liver Int* 24:218–226
64. Brilliant KE, Mills DR, Callanan HM, Hixson DC (2009) Engraftment of syngeneic and allogeneic endothelial cells, hepatocytes and cholangiocytes into partially hepatectomized rats previously treated with mitomycin C. *Transplantation* 88:486–495
65. Cusick RA, Lee H, Sano K, Pollok JM, Utsunomiya H, Ma PX et al (1997) The effect of donor and recipient age on engraftment of tissue-engineered liver. *J Pediatr Surg* 32:357–360
66. Suzuki A, Taniguchi H, Zheng YW, Takada Y, Fukunaga K, Seino K et al (2000) Proliferative and functional ability of transplanted murine neonatal hepatocytes in adult livers. *Transplant Proc* 32:2370–2371
67. Alison MR, Choong C, Lim S (2007) Application of liver stem cells for cell therapy. *Semin Cell Dev Biol* 18:819–826
68. Haridass D, Narain N, Ott M (2008) Hepatocyte transplantation: waiting for stem cells. *Curr Opin Organ Transplant* 13:627–632
69. Lysy PA, Campard D, Smets F, Najimi M, Sokal EM (2008) Stem cells for liver tissue repair: current knowledge and perspectives. *World J Gastroenterol* 14:864–875
70. Wobus AM, Boheler KR (2005) Embryonic stem cells: prospects for developmental biology and cell therapy. *Physiol Rev* 85:635–678
71. Agarwal S, Holton KL, Lanza R (2008) Efficient differentiation of functional hepatocytes from human embryonic stem cells. *Stem Cells* 26:1117–1127
72. Cai J, Zhao Y, Liu Y, Ye F, Song Z, Qin H et al (2007) Directed differentiation of human embryonic stem cells into functional hepatic cells. *Hepatology* 45:1229–1239
73. Baxter MA, Rowe C, Alder J, Harrison S, Hanley KP, Park BK et al (2010) Generating hepatic cell lineages from pluripotent stem cells for drug toxicity screening. *Stem Cell Res* 5:4–22
74. Takayama K, Kawabata K, Nagamoto Y, Kishimoto K, Tashiro K, Sakurai F et al (2013) 3D spheroid culture of hESC/hiPSC-derived hepatocyte-like cells for drug toxicity testing. *Biomaterials* 34:1781–1789
75. Hay DC, Fletcher J, Payne C, Terrace JD, Gallagher RC, Snoeys J et al (2008) Highly efficient differentiation of hESCs to functional hepatic endoderm requires ActivinA and Wnt3a signaling. *Proc Natl Acad Sci U S A* 105:12301–12306
76. Martin GR (1981) Isolation of a pluripotent cell line from early mouse embryos cultured in medium conditioned by teratocarcinoma stem cells. *Proc Natl Acad Sci U S A* 78:7634–7638
77. Ben-David U, Benvenisty N (2011) The tumorigenicity of human embryonic and induced pluripotent stem cells. *Nat Rev Cancer* 11:268–277
78. Cui L, Shi Y, Zhou X, Wang X, Wang J, Lan Y et al (2013) A set of microRNAs mediate direct conversion of human umbilical cord lining-derived mesenchymal stem cells into hepatocytes. *Cell Death Dis* 4:e918
79. Chinzei R, Tanaka Y, Shimizu-Saito K, Hara Y, Kakinuma S, Watanabe M et al (2002) Embryoid-body cells derived from a mouse embryonic stem cell line show differentiation into functional hepatocytes. *Hepatology* 36:22–29
80. Amariglio N, Hirshberg A, Scheithauer BW, Cohen Y, Loewenthal R, Trakhtenbrot L et al (2009) Donor-derived brain tumor following neural stem cell transplantation in an ataxia telangiectasia patient. *PLoS Med* 6:e1000029
81. Harding J, Mirochnitchenko O (2014) Preclinical studies for induced pluripotent stem cell-based therapeutics. *J Biol Chem* 289:4585–4593
82. Tan Y, Ooi S, Wang L (2014) Immunogenicity and tumorigenicity of pluripotent stem cells and their derivatives: genetic and epigenetic perspectives. *Curr Stem Cell Res Ther* 9:63–72
83. Kondo Y, Iwao T, Nakamura K, Sasaki T, Takahashi S, Kamada N et al (2014) An efficient method for differentiation of human induced pluripotent stem cells into hepatocyte-like cells retaining drug metabolizing activity. *Drug Metab Pharmacokinet* 29:237–243
84. Baxter M, Withey S, Harrison S, Segeritz CP, Zhang F, Atkinson-Dell R et al (2015) Phenotypic and functional analyses show stem cell-derived hepatocyte-like cells better mimic fetal rather than adult hepatocytes. *J Hepatol* 62:581–589
85. Gieseck RL III, Hannan NR, Bort R, Hanley NA, Drake RA, Cameron GW et al (2014) Maturation of induced pluripotent stem cell derived hepatocytes by 3D-culture. *PLoS One* 9:e86372
86. Takebe T, Sekine K, Enomura M, Koike H, Kimura M, Ogaeri T et al (2013) Vascularized and functional human liver from an iPSC-derived organ bud transplant. *Nature* 499:481–484

87. Dianat N, Steichen C, Vallier L, Weber A, Dubart-Kupperschmitt A (2013) Human pluripotent stem cells for modelling human liver diseases and cell therapy. *Curr Gene Ther* 13:120–132
88. Choi SM, Kim Y, Liu H, Chaudhari P, Ye Z, Jang YY (2011) Liver engraftment potential of hepatic cells derived from patient-specific induced pluripotent stem cells. *Cell Cycle* 10:2423–2427
89. Zhao T, Zhang ZN, Rong Z, Xu Y (2011) Immunogenicity of induced pluripotent stem cells. *Nature* 474:212–215
90. Vallier L (2014) Heps with pep: direct reprogramming into human hepatocytes. *Cell Stem Cell* 14:267–269
91. Du Y, Wang J, Jia J, Song N, Xiang C, Xu J et al (2014) Human hepatocytes with drug metabolic function induced from fibroblasts by lineage reprogramming. *Cell Stem Cell* 14:394–403
92. Huang P, Zhang L, Gao Y, He Z, Yao D, Wu Z et al (2014) Direct reprogramming of human fibroblasts to functional and expandable hepatocytes. *Cell Stem Cell* 14:370–384
93. Fausto N, Campbell JS, Riehle KJ (2006) Liver regeneration. *Hepatology* 43:S45–S53
94. Michalopoulos GK, DeFrances MC (1997) Liver regeneration. *Science* 276:60–66
95. Fausto N, Campbell JS (2003) The role of hepatocytes and oval cells in liver regeneration and repopulation. *Mech Dev* 120:117–130
96. Huch M, Gehart H, van Boxtel R, Hamer K, Blokzijl F, Verstegen MM et al (2015) Long-term culture of genome-stable bipotent stem cells from adult human liver. *Cell* 160:299–312
97. Tarlow BD, Pelz C, Naugler WE, Wakefield L, Wilson EM, Finegold MJ, Grompe M (2014) Bipotential adult liver progenitors are derived from chronically injured mature hepatocytes. *Cell Stem Cell* 15:605–618
98. Yanger K, Knigin D, Zong Y, Maggs L, Gu G, Akiyama H et al (2014) Adult hepatocytes are generated by self-duplication rather than stem cell differentiation. *Cell Stem Cell* 15:340–349
99. Shin S, Upadhyay N, Greenbaum LE, Kaestner KH (2015) Ablation of Foxl1-Cre-labeled hepatic progenitor cells and their descendants impairs recovery of mice from liver injury. *Gastroenterology* 148(192–202):e193
100. Habeeb MA, Vishwakarma SK, Bardia A, Khan AA (2015) Hepatic stem cells: a viable approach for the treatment of liver cirrhosis. *World J Stem Cells* 7:859–865
101. Rao MS, Khan AA, Parveen N, Habeeb MA, Habibullah CM, Pande G (2008) Characterization of hepatic progenitors from human fetal liver during second trimester. *World J Gastroenterol* 14:5730–5737
102. Lysy PA, Campard D, Smets F, Malaise J, Mourad M, Najimi M, Sokal EM (2008) Persistence of a chimerical phenotype after hepatocyte differentiation of human bone marrow mesenchymal stem cells. *Cell Prolif* 41:36–58
103. Seo MJ, Suh SY, Bae YC, Jung JS (2005) Differentiation of human adipose stromal cells into hepatic lineage in vitro and in vivo. *Biochem Biophys Res Commun* 328:258–264
104. Duncan SA (2003) Mechanisms controlling early development of the liver. *Mech Dev* 120:19–33
105. Banas A, Teratani T, Yamamoto Y, Tokuhara M, Takeshita F, Osaki M et al (2009) Rapid hepatic fate specification of adipose-derived stem cells and their therapeutic potential for liver failure. *J Gastroenterol Hepatol* 24:70–77
106. Banas A, Teratani T, Yamamoto Y, Tokuhara M, Takeshita F, Quinn G et al (2007) Adipose tissue-derived mesenchymal stem cells as a source of human hepatocytes. *Hepatology* 46:219–228
107. Bonora-Centelles A, Jover R, Mirabet V, Lahoz A, Carbonell F, Castell JV, Gomez-Lechon MJ (2009) Sequential hepatogenic transdifferentiation of adipose tissue-derived stem cells: relevance of different extracellular signaling molecules, transcription factors involved, and expression of new key marker genes. *Cell Transplant* 18:1319–1340
108. Snykers S, Vanhaecke T, Papeleu P, Luttun A, Jiang Y, Vander Heyden Y et al (2006) Sequential exposure to cytokines reflecting embryogenesis: the key for in vitro differentiation of adult bone marrow stem cells into functional hepatocyte-like cells. *Toxicol Sci* 94:330–341, discussion 235–339
109. Kuo TK, Hung SP, Chuang CH, Chen CT, Shih YR, Fang SC et al (2008) Stem cell therapy for liver disease: parameters governing the success of using bone marrow mesenchymal stem cells. *Gastroenterology* 134:2111–2121
110. Yagi H, Parekkadan B, Suganuma K, Soto-Gutierrez A, Tompkins RG, Tilles AW, Yarmush ML (2009) Long-term superior performance of a stem cell/hepatocyte device for the treatment of acute liver failure. *Tissue Eng Part A* 15:3377–3388
111. Banas A, Teratani T, Yamamoto Y, Tokuhara M, Takeshita F, Osaki M 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* 26:2705–2712
112. Ilancheran S, Michalska A, Peh G, Wallace EM, Pera M, Manuelpillai U (2007) Stem cells derived from human fetal membranes display multilineage differentiation potential. *Biol Reprod* 77:577–588
 113. Miki T, Marongiu F, Ellis EC, Dorko K, Mitamura K, Ranade A et al (2009) Production of hepatocyte-like cells from human amnion. *Methods Mol Biol* 481:155–168
 114. Marongiu F, Gramignoli R, Dorko K, Miki T, Ranade AR, Paola Serra M et al (2011) Hepatic differentiation of amniotic epithelial cells. *Hepatology* 53:1719–1729
 115. Vaghjiani V, Vaithilingam V, Saraswati I, Sali A, Murthi P, Kalionis B et al (2014) Hepatocyte-like cells derived from human amniotic epithelial cells can be encapsulated without loss of viability or function in vitro. *Stem Cells Dev* 23:866–876
 116. Strom SC, Skvorak K, Gramignoli R, Marongiu F, Miki T (2013) Translation of amnion stem cells to the clinic. *Stem Cells Dev* 22(Suppl 1):96–102
 117. Donato MT, Lahoz A, Montero S, Bonora A, Pareja E, Mir J et al (2008) Functional assessment of the quality of human hepatocyte preparations for cell transplantation. *Cell Transplant* 17:1211–1219
 118. Gramignoli R, Tahan V, Dorko K, Venkataramanan R, Fox IJ, Ellis EC et al (2014) Rapid and sensitive assessment of human hepatocyte functions. *Cell Transplant* 23:1545–1556
 119. Aoki T, Koizumi T, Kobayashi Y, Yasuda D, Izumida Y, Jin Z et al (2005) A novel method of cryopreservation of rat and human hepatocytes by using encapsulation technique and possible use for cell transplantation. *Cell Transplant* 14:609–620
 120. Kusano T, Aoki T, Yasuda D, Matsumoto S, Jin Z, Nishino N et al (2008) Microencapsule technique protects hepatocytes from cryoinjury. *Hepato Res* 38:593–600
 121. Jitraruch S, Dhawan A, Hughes RD, Filippi C, Soong D, Philippeos C et al (2014) Alginate microencapsulated hepatocytes optimised for transplantation in acute liver failure. *PLoS One* 9:e113609
 122. Sgroi A, Mai G, Morel P, Baertschiger RM, Gonelle-Gispert C, Serre-Beinier V, Buhler LH (2011) Transplantation of encapsulated hepatocytes during acute liver failure improves survival without stimulating native liver regeneration. *Cell Transplant* 20:1791–1803
 123. Cantz T, Sharma AD, Ott M (2015) Concise review: cell therapies for hereditary metabolic liver diseases-concepts, clinical results, and future developments. *Stem Cells* 33:1055–1062
 124. Kadyk LC, Collins LR, Littman NJ, Millan MT (2015) Proceedings: moving toward cell-based therapies for liver disease. *Stem Cells Transl Med* 4:207–210
 125. Tuchman M, Caldovic L, Daikhin Y, Horyn O, Nissim I, Korson M et al (2008) N-carbamylglutamate markedly enhances ureagenesis in N-acetylglutamate deficiency and propionic acidemia as measured by isotopic incorporation and blood biomarkers. *Pediatr Res* 64:213–217
 126. Najimi M, Khuu DN, Lysy PA, Jazouli N, Abarca J, Sempoux C, Sokal EM (2007) Adult-derived human liver mesenchymal-like cells as a potential progenitor reservoir of hepatocytes? *Cell Transplant* 16:717–728
 127. Sokal EM, Stephenne X, Ottolenghi C, Jazouli N, Clapuyt P, Lacaille F (2014) Liver engraftment and repopulation by in vitro expanded adult derived human liver stem cells in a child with ornithine carbamoyltransferase deficiency. *JIMD Rep* 13:65–72
 128. Khan AA, Shaik MV, Parveen N, Rajendraprasad A, Aleem MA, Habeeb MA et al (2010) Human fetal liver-derived stem cell transplantation as supportive modality in the management of end-stage decompensated liver cirrhosis. *Cell Transplant* 19:409–418
 129. Lanzoni G, Oikawa T, Wang Y, Cui CB, Carpino G, Cardinale V et al (2013) Concise review: clinical programs of stem cell therapies for liver and pancreas. *Stem Cells* 31:2047–2060
 130. Margini C, Vukotic R, Brodosi L, Bernardi M, Andreone P (2014) Bone marrow derived stem cells for the treatment of end-stage liver disease. *World J Gastroenterol* 20:9098–9105
 131. Meier RP, Muller YD, Morel P, Gonelle-Gispert C, Buhler LH (2013) Transplantation of mesenchymal stem cells for the treatment of liver diseases, is there enough evidence? *Stem Cell Res* 11:1348–1364
 132. Mohamadnejad M, Namiri M, Bagheri M, Hashemi SM, Ghanaati H, Zare Mehrjardi N et al (2007) Phase I human trial of autologous bone marrow-hematopoietic stem cell transplantation in patients with decompensated cirrhosis. *World J Gastroenterol* 13:3359–3363
 133. Pai M, Zacharoulis D, Milicevic MN, Helmy S, Jiao LR, Levicar N et al (2008) Autologous infusion of expanded mobilized adult bone marrow-derived CD34+ cells into patients

- with alcoholic liver cirrhosis. *Am J Gastroenterol* 103:1952–1958
134. Peng L, Xie DY, Lin BL, Liu J, Zhu HP, Xie C 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* 54:820–828
 135. Xu L, Gong Y, Wang B, Shi K, Hou Y, Wang L et al (2014) Randomized trial of autologous bone marrow mesenchymal stem cells transplantation for hepatitis B virus cirrhosis: regulation of Treg/Th17 cells. *J Gastroenterol Hepatol* 29:1620–1628
 136. Fox IJ, Chowdhury JR, Kaufman SS, Goertzen TC, Chowdhury NR, Warkentin PI et al (1998) Treatment of the Crigler-Najjar syndrome type I with hepatocyte transplantation. *N Engl J Med* 338:1422–1426
 137. Ambrosino G, Varotto S, Strom SC, Guariso G, Franchin E, Miotto D et al (2005) Isolated hepatocyte transplantation for Crigler-Najjar syndrome type I. *Cell Transplant* 14:151–157
 138. Dhawan A, Mitry RR, Hughes RD (2006) Hepatocyte transplantation for liver-based metabolic disorders. *J Inher Metab Dis* 29:431–435
 139. Lysy PA, Najimi M, Stephenne X, Bourgois A, Smets F, Sokal EM (2008) Liver cell transplantation for Crigler-Najjar syndrome type I: update and perspectives. *World J Gastroenterol* 14:3464–3470
 140. Allen KJ, Mifsud NA, Williamson R, Bertolino P, Hardikar W (2008) Cell-mediated rejection results in allograft loss after liver cell transplantation. *Liver Transpl* 14:688–694
 141. Meyburg J, Hoerster F, Schmidt J, Poeschl J, Hoffmann GF, Schenk JP (2010) Monitoring of intraportal liver cell application in children. *Cell Transplant* 19:629–638
 142. Strom SC, Fisher RA, Thompson MT, Sanyal AJ, Cole PE, Ham JM, Posner MP (1997) Hepatocyte transplantation as a bridge to orthotopic liver transplantation in terminal liver failure. *Transplantation* 63:559–569
 143. Puppi J, Tan N, Mitry RR, Hughes RD, Lehec S, Mieli-Vergani G et al (2008) Hepatocyte transplantation followed by auxiliary liver transplantation—a novel treatment for ornithine transcarbamylase deficiency. *Am J Transplant* 8:452–457
 144. Stephenne X, Najimi M, Smets F, Reding R, de Ville de Goyet J, Sokal EM (2005) Cryopreserved liver cell transplantation controls ornithine transcarbamylase deficient patient while awaiting liver transplantation. *Am J Transplant* 5:2058–2061
 145. Fisher RA, Bu D, Thompson M, Tisnado J, Prasad U, Sterling R et al (2000) Defining hepatocellular chimerism in a liver failure patient bridged with hepatocyte infusion. *Transplantation* 69:303–307
 146. Stephenne X, Najimi M, Sibille C, Nassogne MC, Smets F, Sokal EM (2006) Sustained engraftment and tissue enzyme activity after liver cell transplantation for argininosuccinate lyase deficiency. *Gastroenterology* 130:1317–1323
 147. Muraca M, Burlina AB (2005) Liver and liver cell transplantation for glycogen storage disease type IA. *Acta Gastroenterol Belg* 68:469–472
 148. Lee KW, Lee JH, Shin SW, Kim SJ, Joh JW, Lee DH et al (2007) Hepatocyte transplantation for glycogen storage disease type Ib. *Cell Transplant* 16:629–637
 149. Sokal EM, Smets F, Bourgois A, Van Maldergem L, Buts JP, Reding R et al (2003) Hepatocyte transplantation in a 4-year-old girl with peroxisomal biogenesis disease: technique, safety, and metabolic follow-up. *Transplantation* 76:735–738
 150. Stephenne X, Debray FG, Smets F, Jazouli N, Sana G, Tondreau T, Menten R et al (2012) Hepatocyte transplantation using the domino concept in a child with tetrahydropterin nonresponsive phenylketonuria. *Cell Transplant* 21:2765–2770
 151. Beck BB, Habbig S, Dittrich K, Stippel D, Kaul I, Koerber F et al (2012) Liver cell transplantation in severe infantile oxalosis—a potential bridging procedure to orthotopic liver transplantation? *Nephrol Dial Transplant* 27:2984–2989
 152. Grossman M, Rader DJ, Muller DW, Kolansky DM, Kozarsky K, Clark BJ III et al (1995) A pilot study of ex vivo gene therapy for homozygous familial hypercholesterolaemia. *Nat Med* 1:1148–1154

Part II

Experimental Hepatocyte Transplantation

Hepatocyte Transplantation

Methods and Protocols

Stock, P.; Christ, B. (Eds.)

2017, XIII, 365 p. 68 illus., 52 illus. in color., Hardcover

ISBN: 978-1-4939-6504-5

A product of Humana Press