STEM CELLS®

TRANSLATIONAL AND CLINICAL RESEARCH

Concise Review: Cell Therapies for Hereditary Metabolic Liver Diseases—Concepts, Clinical Results, and Future Developments

TOBIAS CANTZ,[a,b] AMAR DEEP SHARMA,[b,c] MICHAEL OTT[b,d]

Key Words. Liver • Cellular therapy • Hepatic stem cells • Progenitor cells • Hepatocyte differentiation • Pluripotent stem cells • Clinical translation

ABSTRACT

The concept of cell-based therapies for inherited metabolic liver diseases has been introduced for now more than 40 years in animal experiments, but controlled clinical data in humans are still not available. In the era of dynamic developments in stem cell science, the "right" cell for transplantation is considered as an important key for successful treatment. Do we aim to transplant mature hepatocytes or do we consider the liver as a stem/progenitor-driven organ and replenish the diseased liver with genetically normal stem/progenitor cells? Although conflicting results from cell tracing and transplantation experiments have recently emerged about the existence and role of stem/progenitor cells in the liver, their overall contribution to parenchymal cell homeostasis and tissue repair is limited. Accordingly, engraftment and repopulation efficacies of extrahepatic and liver-derived stem/progenitor cell types are considered to be lower compared to mature hepatocytes. On the basis of these results, we will discuss the current clinical cell transplantation programs for inherited metabolic liver diseases and future developments in liver cell therapy. STEM CELLS *2015;33:1055–1062*

[a]Translational Hepatology and Stem Cell Biology, Cluster of Excellence REBIRTH, [b]Department of Gastroenterology, Hepatology and Endocrinology, Hannover Medical School, Hannover, Germany; [c]Junior Research Group MicroRNA in Liver Regeneration, Cluster of Excellence REBIRTH, Hannover Medical School, Hannover, Germany; [d]TWINCORE, Centre for Experimental and Clinical Infection Research, Hannover, Germany

Correspondence: Michael Ott, M.D., TWINCORE, Centre for Experimental and Clinical Infection Research, Feodor-Lynen Str. 7, 30625 Hannover, Germany. Telephone: +495115327120; Fax: +495115327186; e-mail: Ott.Michael@mh-hannover.de

Received September 24, 2014; accepted for publication November 7, 2014; first published online in STEM CELLS *EXPRESS* December 19, 2014.

1066-5099/2014/$30.00/0

http://dx.doi.org/10.1002/stem.1920

HEPATOCYTES OR STEM CELLS: WHICH CELL DRIVES PARENCHYMAL LIVER MAINTENANCE AND REGENERATION OF THE LIVER?

The long prevailing view in liver physiology and pathophysiology has localized a hepatic stem/progenitor cell compartment at or near the canals of Hering, in periductular glands and in extrahepatic bile duct structures [1–3]. Some researchers found proof that biliary tree-associated stem/progenitor cells countinuously generate hepatocytes and bile duct cells and maintain the normal turnover of parenchymal liver cells [4]. According to the "streaming theory," stem/progenitor cell-derived hepatocytes continuously migrate from the periportal areas toward central vein structures, mature and express differential metabolic activities. Cell fate tracing experiments have attributed Sox9 expressing cells with stem/progenitor properties in the liver as well as in several other epithelial organs [5–7]. More recent data challenge this view and provide evidence for a more complex situation in the liver.

Following acute injury small cells, which express the WNT ligand receptor Lgr5, emerge in the liver and generate hepatocytes [8]. These cells were identified randomly throughout the liver and could not be traced in the noninjured liver. Although the $Lgr5^+$ cells, which coexpress Krt19 and Sox9, could still be derived from a (not identified) biliary lineage-derived stem/progenitor cell in the liver, other studies indicate that mature hepatocytes can dedifferentiate and acquire properties of a liver stem/progenitor cell including the expression of progenitor and biliary lineage-associated cell surface markers. "Knockdown" of the Hippo/Yes-associated protein (YAP) signalling pathway in hepatocytes resulted in emergence of cells with liver stem/progenitor characteristics. Upon restauration of YAP expression, at least some progenitor cells turned back to the mature hepatocyte phenotype indicating a bidirectional role of the Hippo/YAP pathway. Isolated hepatocyte-derived stem/progenitor-like cells in this study demonstrated self-renewal and engraftment capacity at a single cell level [9]. The process of dedifferentiation after liver injury and subsequent redifferentiation in the regenerative phase has also been observed for human hepatocytes in a combined human/mouse chimeric liver transplantation and cell fate tracing animal model [10]. The appearance of dedifferentiated hepatocyte-derived cells expressing stem/progenitor markers through a process, which mimics epithelial to mesenchymal transition in tumor tissues, could thus be viewed as a response to escape mature hepatocyte specific injuries such as viral diseases (hepatitis B and C) or cytochrome P450-related toxicities.

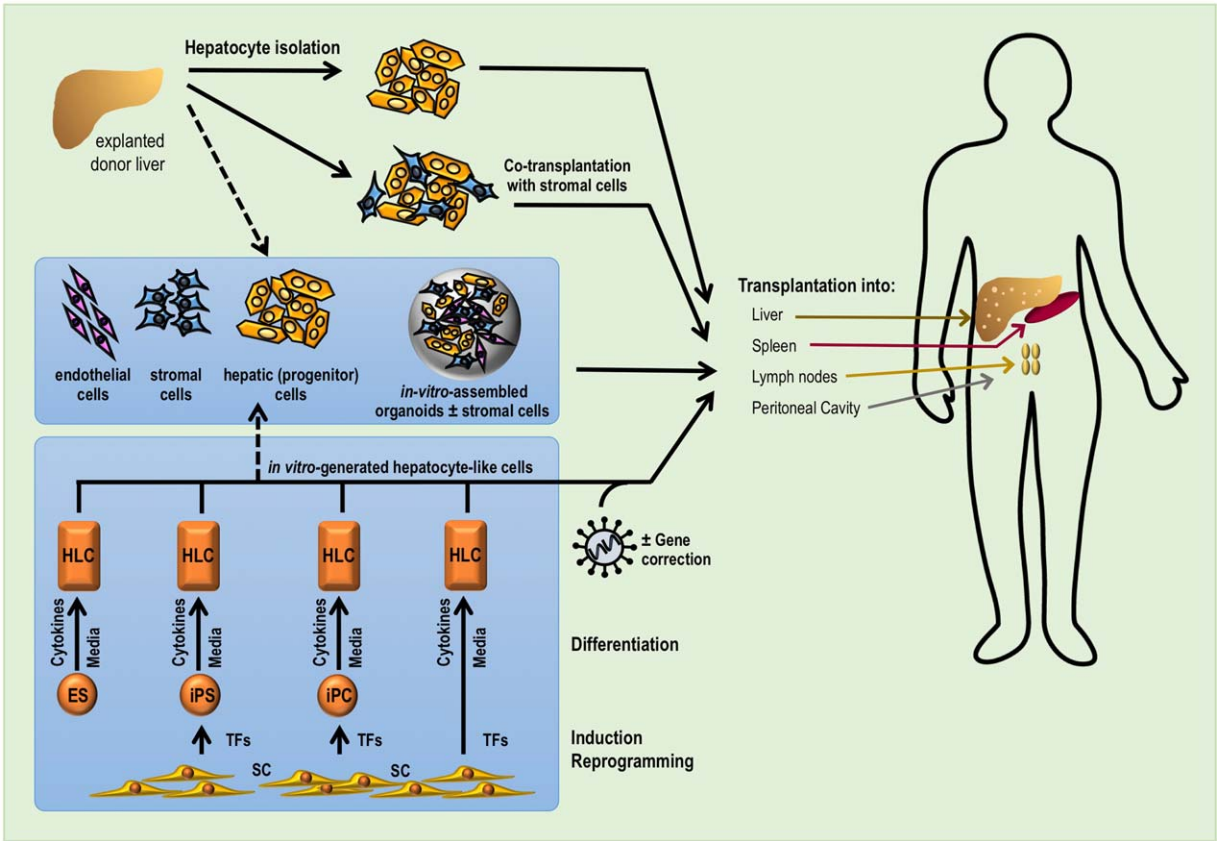

Figure 1. Concept of hepatic cell transplantation for inherited metabolic liver diseases. Primary hepatocytes can be isolated from explanted donor organs and used as allogeneic cell transplants (for references see Table 1). Engraftment in the host liver can be achieved by delivering these cells via branches of the portal vein. Furthermore, several extrahepatic tissues has been studied as more easily accessible transplantation site, such as the spleen [21], the peritoneal cavity, or the lymph nodes [22], and interestingly, cotransplantation with stromal cells may support higher engraftment efficiencies [23]. As alternative sources for cell transplants, ES cells can be directly differentiated into HLC [24]. SCs such as fibroblasts are induced by TFs to become iPS cells which can be also differentiated into HLCs [25]. Somatic cells can be partially programmed by transduction of TFs into iPCs, which have bipotential (hepatocyte-like cells and cholangiocyte-like cells) differentiation capacity. Somatic cells can be directly converted by TFs into hepatocyte-like cells and further differentiated by cytokines and media. More complex three-dimensional aggregates can be generated from hepatic progenitor cells [8] or can be assembled exploiting iPS-derived hepatic cells, stromal cells, and endothelial cells [26]. Abbreviations: ES, embryonic stem; HLC, hepatocyte-like cell; iPC, induced progenitor cell; iPS, induced pluripotent stem; SC, somatic cell; TF, transcription factors.

Regardless of the nature and phenotype of the stem/progenitor cells, the overall contribution to liver regeneration of hepatic cells, which are not derived from hepatocytes, has been questioned. It is long known that the liver regenerates by replication of mature hepatocytes after loss of liver tissue and acute injuries [11–13]. During chronic injuries, in which replication capacity of mature hepatocytes are exhausted or chemically blocked, small oval-shaped cells expressing stem cell and biliary lineage markers appear in periportal areas [14–16]. It was hypothesized that these biliary lineage-associated oval cells (rats) or progenitor-like cells (mice) regenerate the liver via differentiation into hepatocytes. Cells fate tracing models, however, have demonstrated that nonhepatocyte cells in the liver do not significantly contribute to hepatocyte regeneration in acute as well as various chronic liver injury models [17–19]. These findings argue against a functional stem cell compartment as almost all hepatocytes were derived from resident hepatocytes independent from the injury applied to the mouse livers. Additionally, all of these studies did not find evidence for stem/progenitor contribution to hepatocyte maintenance in normal livers. The results have recently been confirmed in mouse/mouse and human/mouse chimeric liver transplantation models [10, 20].

Engraftment and Repopulation in Chimeric Liver Transplantation Models

After intraportal or intrasplenic injection, engraftment and integration into the hepatic cords of primary mature hepatocytes has been demonstrated in many animal species and humans (Fig. 1). Hepatocytes are entrapped into liver sinusoids and cross the endothelial barrier most likely by mechanical forces. The transplanted cells connect to neighboring hepatocytes by re-expression of gap junction proteins, survive long term, and respond to growth stimuli [27]. Some evidence exist that cotransplantation of stromal cells facilitates the process of hepatocyte engraftment and proliferation most likely by the release of cytokines and growth factors [23]. Engraftment in the adult liver similar to mature hepatocytes has also been demonstrated for dedifferentiated hepatocytes, fetal hepatoblasts, embryonic and induced pluripotent stem (iPS)-

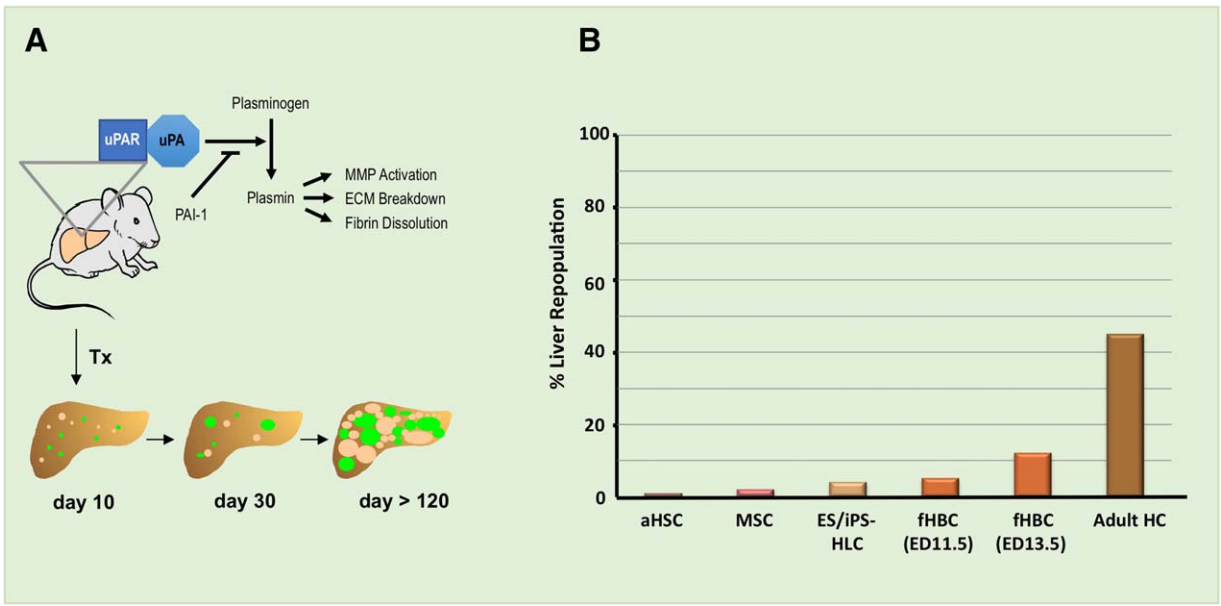

Figure 2. Competitive repopulation fitness of various human cell sources in heterozygous immunodeficient uPA mice. **(A)**: alb-uPAtg mice express the uPA in hepatocytes, resulting in activation of metalloproteinases, breakdown of extracellular matrix, and fibrin dissolution. In heterozygous alb-uPAtg mice, a few endogenous hepatocytes (light brown) could silence the transgene and compete with transplanted enhanced green fluorescent protein (EGFP)-transgenic cells (green) to repopulate the liver. **(B)**: Extent of liver tissue repopulation subject to various cell transplant sources. aHSC (1×10^6 cells, $n = 21$ animals); MSC (1×10^6 cells, $n = 23$ animals); ES/iPS-HLC (1×10^6, $n = 9$ animals); fHBC (ED11.5d) (1×10^6 cells, $n = 7$ animals); fHBC (ED13.5d) (5×10^5 cells, $n = 4$ animals; 1×10^6 cells, $n = 4$ animals); and adult HC (5×10^5 cells, $n = 5$ animals; 1×10^6 cells, $n = 5$ animals). Results were partially adapted from Haridass et al. [31]. Abbreviations: aHSC, adult murine hematopoietic stem cell; ECM, extracellular matrix; ES/iPS-HLC, murine embryonic or induced pluripotent stem cell-derived hepatocyte-like cell; fHBC (ED 11.5d), murine fetal hepatoblasts at day 11.5 post conception; HC, murine hepatocyte; MMP, matrix metalloproteinase; MSC, murine mesenchymal stromal cell derived from bone marrow; uPA, urokinase-type plasminogen activator.

derived hepatocyte-like cells (HLCs) as well as hepatic cells derived from direct reprogramming protocols [9, 24, 28–30]. In most of the studies, engraftment and repopulation of transplanted cells has been demonstrated in genetic liver repopulation ($Fah^{-/-}$ and alb-$uPA^{tg+/+}$) model mice, after acute toxic liver injuries (carbon tetrachloride and galactosamine) or in mice, which have been treated with hepatocyte proliferation blocking compounds (retrorsine and monocrotaline). These models provide space for growth of a few initially engrafted cells or even a selective advantage for transplanted cells to repopulate the liver and may thus overestimate the "engraftment and repopulation fitness" of a particular cell type. In a competitive liver repopulation mouse model (heterozygous alb-$uPA^{tg+/-}$ mice, Fig. 2A), engraftment and repopulation capacities of mature hepatocytes, however, were always superior to early and late hepatoblasts as well as embryonic stem (ES)- and iPS cell-derived HLCs [31]. Any of the tested postnatal nonhepatocytes, such as hematopoietic stem cells or mesenchymal stromal cells, did not engraft long term and did not repopulate the liver. The data suggest that stem/progenitor-derived cells should be forced in vitro to mimic primary hepatocytes as close as possible for proper engraftment and repopulation (Fig. 2B).

Rat hepatoblasts and human/rat liver-derived stromal cells were shown to engraft and gradually replace resident hepatocytes in the normal liver [28, 32]. In our own experiments, this phenomenon has not been observed for mouse and human hepatoblasts or stromal cells after transplantation into noninjured livers of mice so far. Signals in the liver, which maintain continuous growth and termination of proliferation, should be identified to support this concept.

Clinical Experience with Hepatocyte Transplantation in Hereditary Metabolic Liver Disease

From the clinical point of view, transplantation of hepatocytes or hepatocyte-like cells may represent an alternative to orthotopic liver transplants for the correction of genetic disorders resulting in metabolically deficient states. The aim of hepatocyte transplantation in metabolic disease is to partially replace the missing function without the need to replace the whole organ. More than 30 children and adults, who received liver cell therapy for metabolic liver disease, are reported in literature [33–35]. Clinical therapies up to now have been performed by infusing fresh or cryopreserved primary hepatocyte suspensions, which were isolated from donated organs. The availability of high-quality liver tissue for cell isolation, however, has slowed the widespread application of this therapy. Furthermore, the clinical situation of target patients is rarely immediately life threatening, and often acceptable conventional therapies are available. Therefore, the potential benefit must be carefully weighed against any possible complications, such as side effects from immunosuppression, hepatocyte embolization of the pulmonary vascular system, sepsis, or hemodynamic instability.

The results of hepatocyte transplantation for several metabolic liver diseases have been encouraging with demonstrable therapeutic effects, although long-term correction of metabolic deficiencies in the majority of cases has not been reported. Therapeutic benefit has been reported in a girl with Crigler-Najjar syndrome type I, which is a recessively inherited metabolic disorder characterized by severe unconjugated hyperbilirubinemia [36]. Isolated hepatocytes were infused through the portal vein and partially corrected plasma

Table 1. Clinical hepatocyte transplantations in patients with inherited metabolic liver diseases

Disease Sex/age	Cells (total)	Cells/kg per body weight $\times$ 10	Outcome	References
Urea cycle disorders				
Ornithine transcarbamylase activity				
Male/1 day	10.5×10^9	3.1	Ammonia levels ↓, OLT after 6 months	[42]
Male/14 months	2.4×10^9	0.24	Ammonia levels ↓, OLT after 6 months	[44]
Male/1 day	1.6×10^9	0.4	Ammonia levels ↓, OLT after 7 months	[46]
Male/5 years	1.7×10^9	NA	Ammonia levels ↓, death after 43 days	[47]
Male/6 hours	0.6×10^9	0.18	Ammonia levels ↓, death after 4 months	[45]
Male/4 days	0.6×10^9	0.16	Ammonia levels ↓, waiting for OLT	[45]
Citrullinemia				
Female/4 years	1.5×10^9	0.1	Ammonia levels ↓	[45]
Carbamoylphosphate synthase deficiency				
Male/10 weeks	1.37×10^9	0.23	Ammonia levels ↓	[45]
Argininosuccinate lyase deficiency				
Female/3.5 years	4.7×10^9	0.35	Ammonia levels ↓ psychomotor catch- up	[41]
Infantile Refsum disease				
Female/4 years	2.0×10^9	0.2	Pipecolic acid ↓	[39]
Phenylketonuria				
Male/6 years	2.5×10^9	NA	Phenylalanine ↓	[48]
FVII deficiency				
Male/3 years	2.2×10^9	0.14	FVII consumption↓, OLT after 7 months	[40]
Male/3 month	1.09×10^9	0.18	FVII consumption ↓, OLT after 8 months	[40]
Glycogen storage disease type I a and Ib				
Female/47 years (type Ia)	2.0×10^9	0.04	Control of blood glucose levels	[38]
Male/18 years (type Ib)	6×10^9	0.17	Control of blood glucose levels	[49]
Crigler-Najjar syndrome type I				
Female/9 years	6×10^9	0.16	35% reduction in serum bilirubin levels, OLT after 6 months	[50]
Female/7 years	1.4×10^9	NA	40% reduction in serum bilirubin levels, OLT after 11 months	[51]
Female/1 year	2.6×10^9	0.35	65% reduction in serum bilirubin levels, OLT after 4 months	[50]
Female/10 years	7.5×10^9	0.2	50% reduction in serum bilirubin levels, OLT after 4 years	[36]
Male/9 years	7.5×10^9	NA	50% reduction in serum bilirubin levels, OLT after 5 months	[37]
Female/3 years	2.1×10^9	NA	30% reduction in serum bilirubin levels	[35, 52]
Male/1.5 years	4.3×10^9	NA	30% reduction in serum bilirubin levels, OLT after 8 months	[35, 52]

Abbreviations: NA, not announced; OLT, orthotopic liver transplantation.

bilirubin levels for more than 11 months. Similarly, a 9-year-old boy received 7.5×10^9 hepatocytes, infused via the portal vein, which resulted in a decrease in of bilirubin levels from 530 ± 38 μmol/l (mean $\pm$ SD) before to 359 ± 46 μmol/l [37] after transplantation. These cases demonstrate efficacy and safety, however, a single course of cell application seems not sufficient to correct Crigler-Najjar syndrome type I completely.

Promising results have also been obtained in a 47-year-old woman suffering from glycogen storage disease type 1a, an inherited disorder of glucose metabolism resulting from mutations in the gene encoding the hepatic enzyme glucose-6-phosphatase [38]. ABO blood group-compatible hepatocytes (2×10^9) were infused into the portal vein. Nine months after cell transplantation, her metabolic situation had clearly improved. Successful hepatocyte transplantation has also been achieved in a 4-year-old girl with infantile Refsum disease, an inborn error of peroxysome metabolism, leading to increased levels of serum bile acids and the formation of abnormal bile acids [39]. A total of 2×10^9 hepatocytes from a male donor were given during eight separate intraportal infusions. Abnormal bile acid production (for instance pipecolic acid) had decreased by 40% after 18 months. Hepatocyte transplantation has been used successfully to treat inherited factor VII deficiency [40]. Two brothers (aged 3 months and 3 years) received infusions of 1.1 and 2.2×10^9 ABO-matched hepatocytes into the inferior mesenteric vein. Transplantation improved the coagulation defect and decreased the necessity for exogenous factor VII to approximately 20% of that prior to cell therapy. As with the other metabolic liver diseases, hepatocyte transplantation has been shown to provide a partial and temporary correction of urea cycle defects. Sustained response was reported in a patient with argininosuccinate lyase deficiency after repeated hepatocyte transplantation. Engraftment of the transplanted cells was analyzed in repeated liver biopsies for more than 12 months by fluorescence in situ hybridization for the Y-chromosome and by measurement of tissue enzyme activity [41]. Other patients showed clinical improvement, reduced ammonia levels, and increased production of urea [35, 42–45]. A summary of well-documented and published hepatocyte transplantations in various inherited metabolic liver diseases is presented in Table 1.

Based on these initial results, two major programs, which are sponsored by biotech companies, currently evaluate safety and efficacy of liver cell therapy for inherited metabolic liver disease in controlled clinical trials. The Cytonet program uses cryopreserved isolated primary human hepatocytes for intraportal application in patients with urea cycle disorders. The Promethera program isolates and propagates a liver stromal cell for transplantation in various hereditary metabolic diseases.

The Cytonet Program

The Cytonet investigational medicinal product "Human Heterologous Liver Cells" (HHLivC) is being developed as an advanced therapy medicinal product for the treatment of urea cycle disorders, a rare disorder, which is characterized by inherited deficiencies of enzymes involved in the urea cycle. HHLivC consists of a cryopreserved dispersion of liver cells prepared for intraportal administration. HHLivCs are isolated from nontransplantable donor organs and refined in a manufacturing process under Good Manufacturing Practice conditions [53]. For long-term storage in the vapor phase over liquid nitrogen, the final study medication is prepared with a cryopreservation solution and subsequently filled in single final medication bags. The study medication is infused via the portal vein through branches of the inferior or superior mesenteric vein. Viable liver cells per kilogram body weight (3×10^8) are infused in equal fractions over period of 6 days. After initial applications of HHLivCs in four patients with urea cycle disorders with promising results, a total of 21 patients with ornithine transcarbamylase (OTC), carbamoylphosphat-synthetase I, and argininosuccinate synthase deficiencies at the age of 0–5 years have subsequently been recruited for two pivotal studies (CCD02 and CCD05) in Germany, U.S. and Canada [45]. Primary efficacy endpoints as defined by the incidence of severe (>500 μM) and moderate (>250 μM) hyperammonemic events are compared with matched historical controls ($n = 63$ patients). Performance of the urea cycle cannot be easily assessed in vivo, since in vitro enzyme activity does not always reflect in vivo metabolic flux in urea cycle deficient (UCD) patients [54]. Furthermore, established biochemical markers, such as plasma urea, ammonia or glutamine concentrations, do not reliably indicate the functioning of the urea cycle because the plasma concentrations of these metabolites also depend on other factors, mainly the diet, conservative therapy, or trigger factors such as infections. Therefore, direct outcome parameters, which are unaffected by concomitant treatments and diet, are desirable to determine the effectiveness of HHLivC therapy in vivo. An in vivo ^{13}C-ureagenesis assay, which is able to directly measure the patient's metabolic capability to produce urea from oral sodium acetate, was developed. This test was adopted for the studies based on published protocols [55, 56] and is used as an additional efficacy endpoint in studies CCD02 and CCD05. To allow patients access to the new treatment option that enables prevention or delay of hyperammonemic crises in this pediatric population with a high medical need, the market approval application was submitted to the European Medicines Agency in December 2013 based on currently available interim data.

The Promethera Program

HepaStem from Promethera Biosciences, a Belgian biotech company, consists of a Heterologous Human Adult Liver Progenitor Cells suspension, which is generated from normal adult human liver tissue. The cells are described as fibroblastic in morphology expressing mesenchymal as well as hepatocytic markers and can be expanded from cultured primary hepatocytes [32, 57]. Preclinical studies in animals have shown safety and engraftment in recipient livers. The first transplantation of HepaStem in a 3-year-old girl suffering from ornithine transcarbamylase (OTC) deficiency showed 3% engraftment after 100 days as determined by the Y chromosome fluorescenc in situ hybridisation (FISH) technique. The clinical outcome of this patient was not reported [58] in the publication. At the annual meeting of the Society for the Study of Inborn Errors of Metabolism (SSIEM) in September, 2014, safety of the treatment was demonstrated in 14 patients with urea cycle defects and in 6 patients with Crigler-Najjar syndrome. Preliminary efficacy data in this phase I study in patients suffering from urea cycle defects have been presented at the SSIEM in Innsbruck this year and showed variable results [59]. Approval for enrollment of patients with urea cycle defects has been granted by Belgian authorities for a phase IIb/III trial in October 2014.

Future Developments to Close the Bottle Necks of Liver Cell Therapies

Clearly, one of the most obvious bottle necks for wide spread distribution of liver cell therapies for metabolic liver diseases is the availability of high-quality cells for transplantation (Fig. 1). Applying more advanced differentiation protocols supported by fine-tuned cytokine and small molecule supplementation or microRNA modulation, the derivation of hepatocyte-like cell from ES and iPS cells has made considerable progress [25, 60–62], although maturity of the cells in various differentiation protocols is still lacking and engraftment after transplantation is low. With iPS technology, the production of unlimited numbers of patient-derived or immunologically compatible hepatocyte-like cells can be envisioned, and genetic engineering technology will allow the correction of disease-causing mutations in patient-derived iPS cells as recently demonstrated [63–65]. For several inherited metabolic liver diseases, such as alpha-1 antitrypsin deficiency, glycogen storage disease type 1a, or familial hypercholesterolemia iPS-derived hepatocyte-like cells, have been developed [65–67]. A detailed review on iPS based disease modelling and transplantation has recently been provided by Ordonez and Goldstein [68].

As proof of concept for safety and applicability of iPS cell-based therapies, iPS cells from a mouse model of hereditary tyrosinemia (fumarylacetoacetate hydrolase deficiency, Fah$^{-/-}$ mice) have been genetically corrected by a lentivirally delivered construct and, such engineered iPS cells have been subjected to tetraploid embryo aggregation experiments [69]. The resulting newborn mice did neither show signs of FAH deficiency nor growth of iPS cell-related tumors and exhibited a normal live span. Recently, major advances in the generation of human iPS cells have been achieved and the elucidation of preferential stoichiometries of the four reprogramming factors [70, 71] inspired the design of suitable polycistronic vectors for the delivery of the reprogramming factors [72]. Importantly, human iPS cells can now be generated by vectors, which do not integrate the transcription factors into the genome of the target cells, or can even be induced by small molecule compounds [73–76]. Although insertional mutagenesis by integrating vectors can be avoided, the cells still carry the risk of "a mutational history," when they are derived from postnatal tissues.

Protocols for the elimination of remaining stem cells in ES and iPS-derived hepatocyte cultures have been developed, but the risk of teratoma formation after transplantation due to residual stem cells remains and provides a hurdle for clinical grade manufacturing. To avoid the state of pluripotency, a partial reprogramming approach has been developed. Those protocols result in the generation of hepatic multipotent progenitor-like cells, which can still be expanded and used for

differentiation toward hepatocytes or cholangiocytes [77]. The direct reprogramming approach attempts to generate cells exhibiting a hepatic phenotype by overexpression of combinations of liver-enriched transcription factors. Several groups have now demonstrated the induction of a mature hepatic phenotype in mouse and human fibroblasts and shown transplantability of the cells in liver repopulation animal models [78–81]. Although many functional features of primary hepatocytes could be demonstrated, fidelity of global gene expression of the so-called iHeps has been questioned [82, 83]. Furthermore, no data are available on genetic stability and tumorigenicity of the cells in transplanted animals. However, the concept of direct reprogramming somatic cells across the respective germ-layer lineages is rather young and similar obstacles need to be overcome for the direct induction of neural cells, cardiac cells, and blood cells [84].

For stable and long-term phenotypic correction in a variety of hereditary metabolic liver diseases, engraftment of the transplanted cells must be improved. A repeated application of cells has been shown to increase the engraftment in animals, a procedure, which has already been incorporated in the Cytonet protocol. Further increase of engraftment can be achieved by pharmacological interventions with vasodilatators, which increase the capacity of liver sinusoids for infused hepatocytes or the application of anti-inflammatory drugs such as indomethacin [85–87].

Various methods and genetic animal models, which harness a selective advantage of transplanted cells over resident cells, are widely available and demonstrate a high degree of stable cellular repopulation after hepatocyte transplantation. Most of these principles, however, are not applicable in humans. A combination of liver irradiation/portal ischemia and portal venous embolization has been successfully tested in rats and nonhuman primates and shown to increase engraftment compared to conventional transplantation [88, 89]. Concerns about long-term consequences in neonates and small children, however, have slowed the translation of these techniques into clinical practice. A recent report from a consensus meeting in London summarized the activities of the major cell transplantation programs, identified new developments, and discussed the challenges for transfer into clinical practice [90].

Novel experimental concepts, which recently emerged from animal studies, may provide alternative strategies for cellular therapies of the liver, although none has yet reached a clinical stage. Organ-like structures resembling embryonic liver buds have been developed by self-aggregation of iPS-derived hepatocyte-like cells, endothelial cells, and fibroblasts (Fig. 1). The engineered tissues have been transplanted at extrahepatic sites in mice and shown sustained liver functions and long-term survival [26]. Decellularized liver matrices were successfully repopulated with primary hepatocytes and shown to function in transplantation experiments [91]. Seeding of these acellular liver matrices with gene corrected iPS-derived hepatic cells could lead to autologous liver organ transplantations in patients with inherited metabolic liver diseases. Ectopic hepatocyte transplantations, which were conducted in Japanese patients more than 20 years ago, could again emerge as an alternative strategy to treat inherited metabolic liver [21]. Injection of cells into lymph nodes was sufficient to rescue Fah$^{-/-}$ tyrosinemic mice [22] and could thus be applicable also to other inherited liver diseases.

Summary

Approved protocols for the isolation, cryopreservation, and storage of primary human hepatocytes from donated liver organs have now been developed. Controlled trials with those cells in standardized clinical protocols will soon show, whether hepatocyte infusions can achieve clinical benefits in defined patient populations with hereditary metabolic liver diseases. The engraftment efficacy of primary hepatocytes may already be good enough to show significant clinical benefits in some indications such as “early onset” urea cycle disorders. For other disorders, such as low density lipoprotein (LDL) receptor deficiencies (familial hypercholesterolemia), which also manifest at extrahepatic sites, clinically applicable liver repopulation strategies are needed to increase hepatocyte engraftment and to stably correct the clinical phenotype. Alternative techniques, which are based on tissue engineering approaches or ectopic transplantation of cells, could soon emerge as therapeutic alternatives for affected patients. Finally, cell sources, which are most likely based on ES, iPS, or direct programming technologies, will likely replace the isolated primary cells in the future, if safety and engraftment efficacy can be further increased.

Acknowledgment

TWINCORE is a joint venture between the Hannover Medical School (MHH) and the Helmholtz Centre for Infection Research (HZI).

Author Contributions

T.C.: conception and design and manuscript writing; A.D.S.: manuscript writing and final approval of manuscript; M.O.: conception and design, manuscript writing, and final approval of manuscript.

Disclosure of Potential Conflicts of Interest

The authors indicate no potential conflicts of interest.

References

1 Sigal SH, Brill S, Fiorino AS et al. The liver as a stem cell and lineage system. Am J Physiol 1992;263:G139–G148.

2 Lanzoni G, Oikawa T, Wang Y et al. Concise review: Clinical programs of stem cell therapies for liver and pancreas. Stem Cells 2013;31:2047–2060.

3 Cardinale V, Wang Y, Carpino G et al. The biliary tree—A reservoir of multipotent stem cells. Nat Rev Gastroenterol Hepatol 2012;9:231–240.

4 Cardinale V, Wang Y, Carpino G et al. Multipotent stem/progenitor cells in human biliary tree give rise to hepatocytes, cholangiocytes, and pancreatic islets. Hepatology 2011;54:2159–2172.

5 Furuyama K, Kawaguchi Y, Akiyama H et al. Continuous cell supply from a Sox9-expressing progenitor zone in adult liver, exocrine pancreas and intestine. Nat Genet 2011;43:34–41.

6 Sackett SD, Li Z, Hurtt R et al. Foxl1 is a marker of bipotential hepatic progenitor cells in mice. Hepatology 2009;49:920–929.
7 Shin S, Walton G, Aoki R et al. Foxl1-Cre-marked adult hepatic progenitors have clonogenic and bilineage differentiation potential. Genes Dev 2011;25:1185–1192.
8 Huch M, Dorrell C, Boj SF et al. In vitro expansion of single Lgr5+ liver stem cells induced by wnt-driven regeneration. Nature 2013;494:247–250.
9 Yimlamai D, Christodoulou C, Galli GG et al. Hippo pathway activity influences liver cell fate. Cell 2014;157:1324–1338.
10 Tarlow BD, Pelz C, Naugler WE et al. Bipotential adult liver progenitors are derived from chronically injured mature hepatocytes. Cell Stem Cell 2014;15:605–618.
11 Kang LI, Mars WM, Michalopoulos GK. Signals and cells involved in regulating liver regeneration. Cells 2012;1:1261–1292.
12 Quante M, Wang TC. Stem cells in gastroenterology and hepatology. Nat Rev Gastroenterol Hepatol 2009;6:724–737.
13 Duncan AW, Dorrell C, Grompe M. Stem cells and liver regeneration. Gastroenterology 2009;137:466–481.
14 Grisham JW, Hartroft WS. Morphologic identification by electron microscopy of "oval" cells in experimental hepatic degeneration. Lab Invest 1961;10:317–332.
15 Suzuki A, Zheng YW, Kaneko S et al. Clonal identification and characterization of self-renewing pluripotent stem cells in the developing liver. J Cell Biol 2002;156:173–184.
16 Fausto N. Liver regeneration and repair: Hepatocytes, progenitor cells, and stem cells. Hepatology 2004;39:1477–1487.
17 Malato Y, Naqvi S, Schurmann N et al. Fate tracing of mature hepatocytes in mouse liver homeostasis and regeneration. J Clin Invest 2011;121:4850–4860.
18 Schaub JR, Malato Y, Gormond C et al. Evidence against a stem cell origin of new hepatocytes in a common mouse model of chronic liver injury. Cell Rep 2014;8:933–939.
19 Yanger K, Knigin D, Zong Y et al. Adult hepatocytes are generated by self-duplication rather than stem cell differentiation. Cell Stem Cell 2014;15:340–349.
20 Tarlow BD, Finegold MJ, Grompe M. Clonal tracing of Sox9+ liver progenitors in mouse oval cell injury. Hepatology 2014;60:278–289.
21 Kusano M, Mito M. A study of hepatocellular transplantation. Nihon Rinsho 1992;50:1679-1688.
22 Komori J, Boone L, DeWard A et al. The mouse lymph node as an ectopic transplantation site for multiple tissues. Nat Biotechnol 2012;30:976–983.
23 Joshi M, B Patil P, He Z et al. Fetal liver-derived mesenchymal stromal cells augment engraftment of transplanted hepatocytes. Cytotherapy 2012;14:657–669.
24 Touboul T, Hannan NR, Corbineau S et al. Generation of functional hepatocytes from human embryonic stem cells under chemically defined conditions that recapitulate liver development. Hepatology 2010;51:1754–1765.
25 Sgodda M, Mobus S, Hoepfner J et al. Improved hepatic differentiation strategies for human induced pluripotent stem cells. Curr Mol Med 2013;13:842–855.
26 Takebe T, Sekine K, Enomura M et al. Vascularized and functional human liver from an iPSC-derived organ bud transplant. Nature 2013;499:481–484.
27 Gupta S, Rajvanshi P, Sokhi R et al. Entry and integration of transplanted hepatocytes in rat liver plates occur by disruption of hepatic sinusoidal endothelium. Hepatology 1999;29:509–519.
28 Oertel M, Menthena A, Chen YQ et al. Comparison of hepatic properties and transplantation of Thy-1(+) and Thy-1(−) cells isolated from embryonic day 14 rat fetal liver. Hepatology 2007;46:1236–1245.
29 Schmelzer E, Zhang L, Bruce A et al. Human hepatic stem cells from fetal and postnatal donors. J Exp Med 2007;204:1973–1987.
30 Woo DH, Kim SK, Lim HJ et al. Direct and indirect contribution of human embryonic stem cell-derived hepatocyte-like cells to liver repair in mice. Gastroenterology 2012;142:602–611.
31 Haridass D, Yuan Q, Becker PD et al. Repopulation efficiencies of adult hepatocytes, fetal liver progenitor cells, and embryonic stem cell-derived hepatic cells in albumin-promoter-enhancer urokinase-type plasminogen activator mice. Am J Pathol 2009;175:1483–1492.
32 Najimi M, Khuu DN, Lysy PA et al. Adult-derived human liver mesenchymal-like cells as a potential progenitor reservoir of hepatocytes? Cell Transplant 2007;16:717–728.
33 Fisher RA, Strom SC. Human hepatocyte transplantation: Worldwide results. Transplantation 2006;82:441–449.
34 Fitzpatrick E, Mitry RR, Dhawan A. Human hepatocyte transplantation: State of the art. J Intern Med 2009;266:339–357.
35 Dhawan A, Mitry RR, Hughes RD. Hepatocyte transplantation for liver-based metabolic disorders. J Inherit Metab Dis 2006;29:431–435.
36 Fox IJ, Chowdhury JR, Kaufman SS et al. Treatment of the Crigler-Najjar syndrome type I with hepatocyte transplantation. N Engl J Med 1998;338:1422–1426.
37 Ambrosino G, Varotto S, Strom SC et al. Isolated hepatocyte transplantation for Crigler-Najjar syndrome type 1. Cell Transplant 2005;14:151–157.
38 Muraca M, Gerunda G, Neri D et al. Hepatocyte transplantation as a treatment for glycogen storage disease type 1a. Lancet 2002;359:317–318.
39 Sokal EM, Smets F, Bourgois A et al. Hepatocyte transplantation in a 4-year-old girl with peroxisomal biogenesis disease: Technique, safety, and metabolic follow-up. Transplantation 2003;76:735–738.
40 Dhawan A, Mitry RR, Hughes RD et al. Hepatocyte transplantation for inherited factor VII deficiency. Transplantation 2004;78:1812–1814.
41 Stephenne X, Najimi M, Sibille C et al. Sustained engraftment and tissue enzyme activity after liver cell transplantation for argininosuccinate lyase deficiency. Gastroenterology 2006;130:1317–1323.
42 Horslen SP, McCowan TC, Goertzen TC et al. Isolated hepatocyte transplantation in an infant with a severe urea cycle disorder. Pediatrics 2003;111:1262–1267.
43 Mitry RR, Dhawan A, Hughes RD et al. One liver, three recipients: Segment IV from split-liver procedures as a source of hepatocytes for cell transplantation. Transplantation 2004;77:1614–1616.
44 Stephenne X, Najimi M, Smets F et al. Cryopreserved liver cell transplantation controls ornithine transcarbamylase deficient patient while awaiting liver transplantation. Am J Transplant 2005;5:2058–2061.
45 Meyburg J, Das AM, Hoerster F et al. One liver for four children: First clinical series of liver cell transplantation for severe neonatal urea cycle defects. Transplantation 2009;87:636–641.
46 Puppi J, Tan N, Mitry RR et al. Hepatocyte transplantation followed by auxiliary liver transplantation—A novel treatment for ornithine transcarbamylase deficiency. Am J Transplant 2008;8:452–457.
47 Strom SC, Fisher RA, Thompson MT et al. Hepatocyte transplantation as a bridge to orthotopic liver transplantation in terminal liver failure. Transplantation 1997;63:559–569.
48 Smets FN, Stephenne X, Debray G et al. Hepatocyte transplantation transforms severe phenylketonuria to mild hyperphenylalaninemia. Gastroenterology 2011;140:S967.
49 Lee KW, Lee JH, Shin SW et al. Hepatocyte transplantation for glycogen storage disease type Ib. Cell Transplant 2007;16:629–637.
50 Lysy PA, Najimi M, Stephenne X et al. Liver cell transplantation for Crigler-Najjar syndrome type I: Update and perspectives. World J Gastroenterol 2008;14:3464–3470.
51 Allen KJ, Mifsud NA, Williamson R et al. Cell-mediated rejection results in allograft loss after liver cell transplantation. Liver Transplant 2008;14:688–694.
52 Dhawan A, Puppi J, Hughes RD et al. Human hepatocyte transplantation: Current experience and future challenges. Nat Rev Gastroenterol Hepatol 2010;7:288–298.
53 Alexandrova K, Griesel C, Barthold M et al. Large-scale isolation of human hepatocytes for therapeutic application. Cell Transplant 2005;14:845–853.
54 Yudkoff M, Daikhin Y, Nissim I et al. In vivo nitrogen metabolism in ornithine transcarbamylase deficiency. J Clin Invest 1996;98:2167–2173.
55 Tuchman M, Caldovic L, Daikhin Y et al. N-Carbamylglutamate markedly enhances ureagenesis in N-acetylglutamate deficiency and propionic acidemia as measured by isotopic incorporation and blood biomarkers. Pediatr Res 2008;64:213–217.
56 Ah Mew N, McCarter R, Daikhin Y et al. N-Carbamylglutamate augments ureagenesis and reduces ammonia and glutamine in propionic acidemia. Pediatrics 2010;126:e208–e214.
57 Berardis S, Lombard C, Evraerts J et al. Gene expression profiling and secretome analysis differentiate adult-derived human liver stem/progenitor cells and human hepatic stellate cells. PLoS one 2014;9:e86137.
58 Sokal EM, Stephenne X, Ottolenghi C et al. Liver engraftment and repopulation by

in vitro expanded adult derived human liver stem cells in a child with ornithine carbamoyltransferase deficiency. JIMD Rep 2014; 13:65–72.

59 Dobbelaere D, Semts F, McKiernam P et al. Phase I/II multicenter trial of liver derived mesenchymal stem cells (hepastemr) for the treatment of urea cycle disorder and Crigler Najjar syndrome. Interim analysis at 6 months post infusion: *Society for the Study of Inborn Errors of Metabolism (SSIEM)—Annual Symposium*. Vol 37. Innsbruck: J Inherit Metab Dis 2014:S34.

60 Szkolnicka D, Farnworth SL, Lucendo-Villarin B et al. Deriving functional hepatocytes from pluripotent stem cells. Curr Protoc Stem Cell Biol 2014;30:1G 5 1–1G 5 12.

61 Hannan NR, Segeritz CP, Touboul T et al. Production of hepatocyte-like cells from human pluripotent stem cells. Nat Protoc 2013;8:430–437.

62 Möbus S, Yang D, Yuan Q et al. Microrna-199a-5p inhibition enhances the liver repopulation ability of human embryonic stem cell-derived hepatic cells. J Hepatol 2015;62:101–110.

63 Yusa K, Rashid ST, Strick-Marchand H et al. Targeted gene correction of [Agr]1-antitrypsin deficiency in induced pluripotent stem cells. Nature 2011;478:391–394.

64 Eggenschwiler R, Loya K, Wu G et al. Sustained knockdown of a disease-causing gene in patient specific induced pluripotent stem cells using lentiviral vector based gene therapy. Stem Cells Translat Med 2013;2: 641–654.

65 Choi SM, Kim Y, Shim JS et al. Efficient drug screening and gene correction for treating liver disease using patient-specific stem cells. Hepatology 2013;57:2458–2468.

66 Rashid ST, Corbineau S, Hannan N et al. Modeling inherited metabolic disorders of the liver using human induced pluripotent stem cells. J Clin Invest 2010;120:3127–3136.

67 Fattahi F, Asgari S, Pournasr B et al. Disease-corrected hepatocyte-like cells from familial hypercholesterolemia-induced pluripotent stem cells. Mol Biotechnol 2013;54: 863–873.

68 Ordonez MP, Goldstein LS. Using human-induced pluripotent stem cells to model monogenic metabolic disorders of the liver. Semin Liver Dis 2012;32:298–306.

69 Wu G, Liu N, Rittelmeyer I et al. Generation of healthy mice from gene-corrected disease-specific induced pluripotent stem cells. PLoS Biol 2011;9:e1001099.

70 Tiemann U, Sgodda M, Warlich E et al. Optimal reprogramming factor stoichiometry increases colony numbers and affects molecular characteristics of murine induced pluripotent stem cells. Cytometry A 2011;79A: 426–435.

71 Carey BW, Markoulaki S, Hanna JH et al. Reprogramming factor stoichiometry influences the epigenetic state and biological properties of induced pluripotent stem cells. Cell Stem Cell 2011;9:588–598.

72 Warlich E, Kühle J, Cantz T et al. Lentiviral vector design and imaging approaches to visualize the early stages of cellular reprogramming. Mol Ther 2011;19:782–789.

73 Fusaki N, Ban H, Nishiyama A et al. Efficient induction of transgene-free human pluripotent stem cells using a vector based on sendai virus, an RNA virus that does not integrate into the host genome. Proc Jpn Acad Ser B: Phys Biol Sci 2009;85:348–362.

74 Warren L, Manos PD, Ahfeldt T et al. Highly efficient reprogramming to pluripotency and directed differentiation of human cells with synthetic modified mRNA. Cell Stem Cell 2010;7:618–630.

75 Kim D, Kim CH, Moon JI et al. Generation of human induced pluripotent stem cells by direct delivery of reprogramming proteins. Cell Stem Cell 2009;4:472–476.

76 Hou P, Li Y, Zhang X et al. Pluripotent stem cells induced from mouse somatic cells by small-molecule compounds. Science 2013; 341:651–654.

77 Zhu S, Rezvani M, Harbell J et al. Mouse liver repopulation with hepatocytes generated from human fibroblasts. Nature 2014; 508:93–97.

78 Huang P, He Z, Ji S et al. Induction of functional hepatocyte-like cells from mouse fibroblasts by defined factors. Nature 2011; 475:386–389.

79 Huang P, Zhang L, Gao Y et al. Direct reprogramming of human fibroblasts to functional and expandable hepatocytes. Cell Stem Cell 2014;14:370–384.

80 Sekiya S, Suzuki A. Direct conversion of mouse fibroblasts to hepatocyte-like cells by defined factors. Nature 2011;475:390–393.

81 Du Y, Wang J, Jia J et al. Human hepatocytes with drug metabolic function induced from fibroblasts by lineage reprogramming. Cell Stem Cell 2014;14:394–403.

82 Cahan P, Li H, Morris SA et al. Cellnet: Network biology applied to stem cell engineering. Cell 2014;158:903–915.

83 Morris SA, Cahan P, Li H et al. Dissecting engineered cell types and enhancing cell fate conversion via Cellnet. Cell 2014;158:889–902.

84 Pfaff N, Cantz T. From skin to blood: A new member joins the iClub. Cell Stem Cell 2013;13:131–133.

85 Slehria S, Rajvanshi P, Ito Y et al. Hepatic sinusoidal vasodilators improve transplanted cell engraftment and ameliorate microcirculatory perturbations in the liver. Hepatology 2002;35:1320–1328.

86 Bahde R, Kapoor S, Bandi S et al. Directly acting drugs prostacyclin or nitroglycerine and endothelin receptor blocker bosentan improve cell engraftment in rodent liver. Hepatology 2013;57:320–330.

87 Bahde R, Kapoor S, Viswanathan P et al. Endothelin-1 receptor a blocker darusentan decreases hepatic changes and improves liver repopulation after cell transplantation in rats. Hepatology 2014;59:1107–1117.

88 Koenig S, Yuan Q, Krause P et al. Regional transient portal ischemia and irradiation as preparative regimen for hepatocyte transplantation. Cell Transplant 2011;20:303–311.

89 Yannam GR, Han B, Setoyama K et al. A nonhuman primate model of human radiation-induced venocclusive liver disease and hepatocyte injury. Int J Radiat Oncol Biol Phys 2014;88:404–411.

90 Puppi J, Strom SC, Hughes RD et al. Improving the techniques for human hepatocyte transplantation: Report from a consensus meeting in London. Cell Transplant 2012; 21:1–10.

91 Soto-Gutierrez A, Zhang L, Medberry C et al. A whole-organ regenerative medicine approach for liver replacement. Tissue Eng Part C: Methods 2011;17:677–686.

