HHS Public Access

Author manuscript

Curr Transplant Rep. Author manuscript; available in PMC 2018 December 01.

Published in final edited form as:

Curr Transplant Rep. 2017 December ; 4(4): 280–289. doi:10.1007/s40472-017-0165-6.

Clinical Hepatocyte Transplantation: What Is Next?

James E. Squires[1,§], **Kyle A. Soltys**[2], **Patrick McKiernan**[1], **Robert H. Squires**[1], **Stephen C Strom**[3], **Ira J Fox**[4], and **Alejandro Soto-Gutierrez**[5,§]

[1]Division of Pediatric Gastroenterology, Hepatology, and Nutrition, Children's Hospital of Pittsburgh of UPMC, Pittsburgh, PA, United States

[2]Thomas E. Starzl Transplant Institute, Children's Hospital of Pittsburgh of UPMC, Pittsburgh, PA, United States

[3]Karolinska Institutet, Department of Laboratory Medicine, Division of Pathology, Stockholm, Sweden

[4]Department of Surgery, Children's Hospital of Pittsburgh of UPMC, and McGowan Institute for Regenerative Medicine, University of Pittsburgh, Pittsburgh, Pennsylvania, USA

[5]Department of Pathology, University of Pittsburgh, Pittsburgh, Pennsylvania, USA

Abstract

Purpose of review—Significant recent scientific developments have occurred in the field of liver repopulation and regeneration. While techniques to facilitate liver repopulation with donor hepatocytes and different cell sources have been studied extensively in the laboratory, in recent years clinical hepatocyte transplantation (HT) and liver repopulation trials have demonstrated new disease indications and also immunological challenges that will require the incorporation of a fresh look and new experimental approaches.

Recent findings—Growth advantage and regenerative stimulus are necessary to allow donor hepatocytes to proliferate. Current research efforts focus on mechanisms of donor hepatocyte expansion in response to liver injury/preconditioning. Moreover, latest clinical evidence shows that important obstacles to HT include optimizing engraftment and limited duration of effectiveness, with hepatocytes being lost to immunological rejection. We will discuss alternatives for cellular rejection monitoring, as well as new modalities to follow cellular graft function and near-to-clinical cell sources.

Summary—HT partially corrects genetic disorders for a limited period of time and has been associated with reversal of ALF. The main identified obstacles that remain to make HT a curative

[§]Corresponding authors **Proofs and contact information**: Alejandro Soto-Gutierrez, MD, PhD Department of Pathology, University of Pittsburgh 200 Lothrop Street, 423 Biomedical Science Tower Pittsburgh, PA 15261, USA, Phone: (412) 648-0064; Fax: (412) 648-1916, als208@pitt.edu**And/or** James E. Squires, MD, MS, Department of Hepatology Children's Hospital of Pittsburgh, 4401 Penn Ave. Pediatric Gastroenterology, 3rd Floor, Pittsburgh, PA 15224 United States, Phone: (412) 692-5180, james.squires2@chp.edu.

Compliance with Ethical Guidelines

Conflict of Interest

Robert Squires, Kyle Soltys, Patrick McKiernan, Stephen Strom, Ira Fox, and Alejandro Soto-Gutierrez declare no conflict of interest.

Human and Animal Rights and Informed Consent

This article does not contain any studies with human or animal subjects performed by any of the authors.

approach include improving engraftment rates, and methods for monitoring cellular graft function and rejection. This review aims to discuss current state-of-the-art in clinical HT and provide insights into innovative approaches taken to overcome these obstacles.

Keywords

Hepatocytes; autologous hepatocytes; hepatocyte rejection monitoring; liver preconditioning

Introduction

Hepatocyte transplantation (HT) has for some time been seen as a promising potential alternative to orthotopic liver transplantation (OLT) expanding the therapeutic approach to a collection of liver diseases. Advantages of cell transplantation compared to whole-organ replacement include a less invasive procedure, the ability of multiple recipients to benefit from a single donor, the capacity to cryopreserve cells for long-term storage and use at later time points, improved theoretical safety profile as graft rejection reverts the patient to their pretransplant state, and the inherent preservation of the native liver so as to enable potential recovery in cases such as acute liver failure from acetaminophen induced liver injury (1). In general, ideal candidate conditions for HT therapy include those where the hepatic scaffolding and microenvironment are preserved and vasculature remains intact. Certain monogenic diseases of the liver exemplify such conditions where there is primary hepatic expression of single gene defects without significant parenchymal damage. In such instances, HT is suggested to enable the replacement of a critical mass of metabolically normal cells with a functioning gene to support appropriate metabolic processes. Acute liver failure (ALF) represents another opportunity for HT therapy. Here, HT aims to temporarily improve liver function and allow the natural regenerative capacity of the liver to proceed or to stabilize and bridge affected patients to more traditional liver transplant. Indeed, published experiences of HT in the treatment of liver-based metabolic diseases (2–16) and ALF (17–21) have shown promising early results; however, only partial correction of metabolic disorders has been achieved and HT has not shown to reliably circumvent the need for traditional organ transplant in ALF (5, 6, 8, 12, 14, 21–25). Thus, in many ways HT has yet to live up to its expectations. This is underscored by the declining number of active hepatocyte transplantation programs (26, 27).

Identifying an ample source of hepatocytes for transplant, optimizing cell quality and storage, maximizing engraftment, controlling rejection episodes, and the ability to monitor the function and rejection of transplanted cells in vivo constitute the main challenges preventing broader implementation of HT into clinical treatment algorithms.

Hepatocyte cell transplantation involves supplementing approximately 5–10% of the native liver volume with human hepatocytes harvested from donor livers (28, 29). HT has been performed in many clinical conditions with varying success. (Table 1) A recent review of the clinical outcomes of the first 100 patients treated with HT has been published (30) and an additional 43 patient experiences have been reported (26). While a detailed clinical report is beyond the scope of this article, it is noted that significant barriers persist limiting broader HT implementation (26, 31, 32) explicitly highlighted in a recent report that included a

preclinical and clinical approach (25).The identification of these impediments has led to recent advancements looking to overcome the limitations.

The aim of this report is to critically evaluate the current state of HT, underscoring important advancements that have occurred in the expanding field of liver cell transplantation and discuss new exciting technologies that have the potential to change the landscape of liver replacement once and for all.

Improving Human Hepatocytes Quality

A growing interest in cell therapies has enabled multiple avenues of research investigating the benefits in treating a variety of liver diseases. The use of fully differentiated human hepatocytes is preferred to alternative cell sources, such as stem cells and their derivatives, due to minimizing the concern for cancerous transformation and their poor ability to function as primary human hepatocytes (33–36). Currently, the primary sources of hepatocytes for HT remains livers that have been denied for OLT, surplus tissue resulting from reduce-graft transplants, and normal tissue resulting from benign tumor resection procedures (37–39). Recent efforts looking to expand the sources of human hepatocytes have shown that explanted diseased livers might represent a valuable source of metabolically competent human hepatocytes (40). Regardless of the source, other small studies have shown that a variety of factors affect the yield and viability of the hepatocytes isolated from these donor livers, sometimes with conflicting results, and multiple factors are often present within a single donor specimen (39, 41–48). These findings, combined with the knowledge that the quality of isolated hepatocytes is directly correlated with clinical outcomes after HT, has led investigators to search for strategies to optimize the cell product in hepatocyte isolation. A recent investigation, utilizing over 1000 samples, has greatly expanded the understanding of the interplay among various factors affecting viability and yield of isolated human hepatocytes (49). (Table 2) The investigators then used the data to generate algorithms for the prediction of isolated hepatocyte viability and yield which have been made publicly available (www.klinikum.uni-uenchen.de/Chirurgische-Klinik-und-Poliklinik-Grosshadern/de/0700-forschung/ag-leberregeneration/core-facility/Qualitaetsrechner_Hepatozyten.html). While predictive modeling is provocative, ultimate hepatocyte isolation success is determined by validated methodology and protocols. Therefore, modifications aimed at improving the quality of isolated human hepatocytes are constantly being explored.

Recent introduction of liberase, an enzyme used in pancreatic islet cell isolation, and *N*-acetylcysteine (NAC), an antioxidant with multiple hepatoprotective properties, into established isolation protocols has enabled improvements in overall isolation success from 40% to 70% (33, 50, 51). Additional strategies such as machine perfusion techniques and perfusate selection have been shown to dramatically increase cell yields (52, 53). Moreover, machine perfusion organ reconditioning has proven valuable in improving cell viability and function of ischemic livers and experimentally on fatty livers (53–56). Alternatively, the addition of polyethylene glycol to hypothermic preservation solutions has been shown to improve hepatocyte survival following successful isolation (57–59). Ultimately, definitive assessment of hepatocyte quality is critical prior to proceeding with HT. Novel approaches

with the capacity to quickly and affordably assess a variety of hepatic functions, as a reflection of quality, are enabling more personalization of HT preparations (26, 60, 61). Future work aimed at the standardization of the procedural techniques ranging from isolation through cryopreservation, thawing, and functional assessment of hepatocytes prior to HT will enable the incorporation of various center experiences into longitudinal studies advancing the field of HT in humans.

Improving Transplanted Hepatocytes Engraftment and Repopulation

After decades of experimental and clinical trials, we now know that 1) a selective growth advantage over native hepatocytes and 2) regenerative stimuli are necessary to allow donor hepatocytes to proliferate after hepatocyte transplantation. Engraftment describes the process by which transplanted hepatocytes translocate from the sinusoidal space into the recipient liver plates following disruption of the sinusoidal endothelium and integrate into the host liver parenchyma. This process happens soon after the vascular delivery of hepatocytes into the native liver and can last for a few days, but remains relatively inefficient with potential loss of >70% of infused cells (62). Once hepatocytes have engrafted in the liver sinusoids, transplanted hepatocytes should be induced to repopulate the liver parenchymal by providing growth advantage and a regenerative stimulus. Published reports describe a range of variability (6, 11, 15, 26) and efforts to provide donor cells a selective growth advantage over the host liver include partial hepatectomy (26, 63) (short-term regenerative stimulus), preoperative portal vein occlusion (32, 64, 65) (short-term regenerative stimulus), and native liver irradiation (26, 66–69) (growth advantage). However, it is acknowledged that some of these techniques, such as major hepatectomy and chemotherapeutic irradiation, carry unacceptably high risks for human patients limiting their use (13, 32, 33, 68, 69). Since the mechanism of these approaches are essentially different; providing either regeneration stimulus or growth advantage, clinical approaches that seek to deliver both are desirable to maximize the potential of donor hepatocytes to repopulate livers.

Segmental portal embolization, by causing transitional ischemia, provides a strong regenerative stimulus and has demonstrated good regeneration capacity with acceptable risk (13, 70). In this procedure, a balloon is positioned in the left portal vein just beyond the bifurcation. It is inflated so as to occlude the left portal vein but allows transportal infusion of cells into the right lobe of the liver through the side port of the endovascular sheath (32); consequently, there is a contraction of remaining liver volume in which the transplanted hepatocytes may engraft limiting the donor cell mass that can be delivered. Volumetric portal embolization (VPE) is a newly described procedure aimed at addressing this barrier to engraftment (71). The procedure, which consists of partial and random distal embolizations of presinusoidal vessels, demonstrated the ability to induce appropriate regenerative signals yet preserved the total liver parenchyma enabling a small long-term increase in the number of transplanted cells and the efficacy of engraftment (71).

To address the problem of cellular engraftment and repopulation of donor hepatocytes after cell transplantation, liver-directed radiation has been suggested as an alternative to facilitate cellular repopulation by inhibiting host hepatocyte proliferation and inducing postmitotic hepatocyte death, allowing donor hepatocytes to preferentially proliferate and repopulate the

irradiated host liver (25). The timing of radiation preconditioning of the recipient liver related to HT can also affect engraftment. Delaying HT by 24 hours after preparative hepatic irradiation in mice was shown to enhance engraftment (72) and a shortened interval from irradiation to cell transplant resulted in a lower than anticipated engraftment rate in human studies (25).

Another approach to solve the engraftment and repopulation challenge is by utilizing gene therapy as a tool to select and provide a repopulating advantage (73). Nygaard and colleagues elegantly showed that by introducing a therapeutic transgene (coagulation factor 9) with a selection marker (shRNA, that makes cells resistant to a small-molecule inhibitor of fumarylacetoacetate hydrolase) into transplanted hepatocytes, they could pharmacologically improve the transgene expression in a living animal. These liver preconditioning experiments indicate that it is possible to precondition liver cells using gene-editing approaches to facilitate repopulation of transplanted hepatocytes.

Recent investigations have also interrogated the role of the innate immune system in preventing engraftment. Until hepatocytes traverse the endothelium they remain vulnerable to processes which induce rapid immunological clearance. One such process, the instant blood-mediated inflammatory reaction (IBMIR) occurs in which transplanted hepatocytes are recognized by the innate immune system, leading to activation of both complement and coagulation pathways resulting in cell destruction and low engraftment (74, 75). Importantly, traditional immunosuppression has not been shown to ameliorate the IBMIR (76). Strategies aimed at manipulating the coagulation component (74), the inflammatory component (77), or both in combination (78) of the IBMIR have been shown to improve engraftment.

An alternative approach to prevent activation of the IBMIR following HT is to implant the donor cell mass outside of the portal circulation. One strategy involves the encapsulation of the donor cells in microbeads which provide an immune-protective coating while enabling the maintenance of necessary metabolic functions (75, 79–82). This approach is more amenable to the management of acute liver failure and recent work establishing animal models and developing mechanisms to create banked, cryopreserved microbeads for emergency use are potential advancements (83, 84). Additional techniques used to evade the host immune system include tissue-engineering strategies whereby liver mass constructs are assembled *ex vivo* and then inserted (usually into the peritoneal cavity) to provide a functional support system (85–87). Unfortunately, these approaches have been limited by overall functional mass efficiency; however, recent advancements have demonstrated higher efficiency as well as a reduction in initial cell function loss (88). Collectively, these advancements make the goal of bioengineered liver units for transplantation potentially more attainable.

Improving Transplanted Hepatocyte Survival and Monitoring

Historically, HT has only partially corrected genetic disorders with the longest registered/published clinical improvement of about 2 years after transplantation (89). A recent report by our group indicates that a combination of radiation preconditioning and segmental portal embolization is effective to improve engraftment and repopulation of transplanted

hepatocytes; however, the long-term survival of the graft was hampered by rejection (25). With HT, it is difficult to identify transplanted cells by biopsy, which is routinely performed in solid organ transplant recipients. Thus, it is difficult to know definitively whether the donor cells are being rejected until it is too late to intervene.

Traditional organ transplantation requires regular allograft monitoring for the development of complications such as cell-mediated and antibody-mediated rejection in addition to operational tolerance, all of which assist in the immunosuppressive management of the transplant recipient. Currently, no consensus exists regarding optimal immunosuppressive regimens in HT, with most centers adopting or slightly modifying their institutional protocols for solid organ transplant (1, 2, 5, 6, 12, 15, 26, 29, 90). However, it is becoming clear that the immune responses to HT differ considerably from solid organ transplant and refined immunosuppression strategies are needed to improve the clinical outcome (91).

Cell mediated rejection has been shown to result in allograft loss after liver cell transplantation (2). Tools used in the monitoring of whole or partial liver allografts, such as circulating liver enzymes, are impractical in the setting of HT where only a minority of liver cells constitute the graft (26, 92). Additional serum biomarkers that may be more disease specific, such as bilirubin in Crigler-Najjar or amino acids and ammonia in metabolic diseases, have not been sensitive enough to detect rejection before damage to the allograft is irreversible (25). Routine liver biopsy may be of little use given the random nature of engraftment and the resulting variability in the distribution of donor cells (25, 92). Recent work has proposed that anti-donor activity, as measured by an allospecific CD154+ assay to detect recipient cytotoxic memory T cells, may be a possible strategy to monitor for early evidence of rejection in the setting of HT (25, 93–95). Furthermore, the assay correlated well with a response to a change in therapy, thus potentially identifying a solution to one of the major obstacles in the field of HT (25). Additionally, donor-specific antibody (DSA) driven rejection has recently evolved into an established pathology in solid organ liver transplant (96). The development and contribution of donor-specific antibodies (DSA) in HT is still very much unknown. The presence of *de novo* DSA following HT has been temporarily associated with graft loss (9, 25) and in one reported case was associated with the peak measurement of the immune reactivity index score that has been shown to enable the prediction rejection (25). Ultimately, a more robust understanding of the immunological responses induced by HT is needed to help guide therapeutic regimens that will enable extended cell graft survival and broader application of HT to patients who will benefit from this promising therapy. Investigators have looked at specific immune stimulatory and inhibitory signals regulating the innate immune response in HT. Researchers investigating the role of CD47, a member of the Ig superfamily which provides a protective signal against phagocytotic activity of macrophages, recently reported on the contribution of donor CD47 in the regulation of T-cell alloresponses in hepatocyte transplantation. Thru a collection of innovative experiments, they were able to show the important role that CD47 plays in the control of not only T-cell alloresponses but also tolerance induction following HT (97). This work, combined with other published works, adds to the building evidence that CD47 incompatibility may induce both innate and adaptive immune mediated rejection of transplanted hepatocytes (97–101).

Additional strategies that are being explored to enable post-transplant cell monitoring include the use of non-invasive imaging techniques such as magnetic resonance imaging (MRI). These MRI-based cell tracking methods have demonstrated the ability to locate nano- or micro-particle tagged hepatocytes *in vivo*. Importantly, newer technologies, including the use of cells labeled with micron-sized iron oxide particles (MPIO), have shown the capacity to detect single cells by MRI (102–105). Recently, investigators were able to show that porcine hepatocytes could successfully be labeled with MPIO and still maintain their multitude of metabolic functions (106). However, a recurrent challenge is the ability to quantify the number of cells being detected and to confirm that the signal detected is actually an engrafted hepatocyte and not uptake by kuppfer cells or macrophages after hepatocytes have been destroyed. Additional imaging modalities including Cherenkov illumination imaging (CLI), photoacoustic imaging (PAI), surface enhanced Raman imaging (SERI), and theranostic imaging represent further strategies for *in vivo* cell transplantation monitoring (107–109). Very recently, the thyroidal sodium iodide symporter (NIS) gene was used to visualize transplanted hepatocytes. HCs were transduced ex vivo with the Slc5a5 (NIS) gene under the control of the thyroxine-binding globulin promoter. NIS-transduced hepatocytes could robustly concentrate radiolabeled iodine *in vitro*. NIS-transduced hepatocytes were readily imaged *in vivo* by single-photon emission computed tomography, and this demonstrated for the first time noninvasive 3-dimensional imaging of regenerating tissue (110). While challenges such as the capability to assess for viability and safety in tagged cells remain, the ability to monitor cell location after transplant would be an important advancement in the development of human regenerative medicine therapies.

So, What's next for Clinical Hepatocyte Transplantation?

Clinical trials of hepatocytes transplantation have only demonstrated that is a safe procedure. Questions regarding transplantation of adequate cell numbers to produce clinically satisfactory repopulated liver mass for the different monogenic liver diseases remain. Given the short-term graft survival and immunological hurdles that have been identified in the latest hepatocyte allotransplantation trials, autologous-gene-corrected and fully functional hepatocytes would be ideal. Despite progress in advancing the differentiation of human stem cells into hepatocytes *in vitro*, cells that replicate the ability of human primary adult hepatocytes to proliferate and completely replace livers for clinical applications has not been achieved. Ultimately, clinical transplantation of autologous liver cells will require the generation of high numbers of liver cells with functionality equal to primary human hepatocytes. Based on the observations that *in vivo* maturation has been confirmed by genome-wide analysis when human hepatocyte-derived cells were transplanted in the livers of FRG (immunocompromised Fah-deficient mouse) mice for 9 months, it is possible that animals could be used as *in vivo* bioreactors to mature and biofabricate large amounts of functional human hepatocytes for transplantation. One could imagine future scenarios where liver tissue could be collected from patients with monogenic diseases and gene correction could be achieved *in vitro* using the CRISPR/CAS9 system. Those cells which successfully underwent functional gene correction could then be selected and amplified *in vivo* using xenograft animal models to grow hepatocytes. A critical mass of modified, mature hepatocytes would be produced which would enable the original patient/donor to receive an

autologous hepatocyte transplantation of their own genetically corrected cells. Hurdles to this approach would need to be overcome, including exposing patients to risky surgical resections and quickening the process of gene correction and selection as isolated human hepatocytes are known to undergo rapid dedifferentiation *in vitro*.

Several, alternative cell sources including induced pluripotent stem cells (iPSC) and immunomodulatory human amnion epithelial cells (hAEC), as well as others have been developed and studied. (Table 3) To be therapeutically effective, these surrogate hepatocytes must retain the ability to perform the complex metabolic functions and the proliferation capacity of primary human hepatocytes. Ideally, they are also autologous so as to eliminate the need for immunosuppression and the potential for rejection (111).

Protocols have been developed to differentiate human pluripotent stem cells into a fetal hepatocyte-like cells (HLC) (112) with studies suggesting that *in vivo*, these cells could go on to develop a more adult-like phenotype (113–115). Moreover, protocols have been developed that have enabled the reprogramming of somatic cells into inducible pluripotent stem cells (116, 117). The ability to reprogram individual patient's somatic cells, such as skin fibroblasts, into iPSCs and then re-direct them to develop into HLC has several advantages over other cell sources. These include the generation of an infinitely expandable population of cells and the ability to circumvent immunologic rejection following transplantation. Furthermore, advances in gene editing technologies have facilitated the possibility of correcting the specific genetic anomaly that induce disease and subsequently engineer disease-free autologous cells for re-introduction via HT (118). These technologies are already being tested in murine xenograft models whereby human HLC are being differentiated from iPSCs, engrafted into livers of immunosuppressed mice, and then used to study physiology as well as develop new pharmacologic therapies (119, 120).

hAECs are fetal-derived cells isolated from the amnion membrane in full-term human placentas. Several recent developments have renewed interest in these cells, particularly as it relates to their use in managing liver disease. hAECs are available without the accompanied ethical or religious concerns. Furthermore, in contrast to other sources of pluripotent cells, they do not express telomerase and have not been found to be immortal or tumorigenic when transplanted. They have been reported to express genes normally found in mature liver and, because of an immune-privileged status, might enable the avoidance of immunosuppression in the recipient (121). Prior studies have demonstrated the feasibility of hAEC transplant in patients with lysosomal storage disorders (122–124) and recent work in animal models suggest additional benefit in managing both congenital metabolic disease and liver fibrosis (125).

In summary, the cumulative experience in HT has demonstrated that while partial corrections of metabolic liver disease can be achieved, long-term cures have been elusive. Acute liver failure has been successfully managed with HT and remains an attractive area for therapeutic expansion. Many barriers to HT have been identified and continued scientific and patient directed efforts will be required to identify new cell sources for transplant, enhance engraftment, optimize safety, and improve the outcome thus enabling a broader implementation of this therapy in the treatment of patients with liver disorders.

Acknowledgments

Financial Disclosure: This work was supported by grants from NIH, RO1 AI122369 to I.J.F and DK099257 to A.S.-G., and the Pittsburgh Liver Research Center Seed Grant to J.E.S. and A.S.-G.

Abbreviations

OLT	orthotopic liver transplantation
HT	Hepatocyte transplantation
ALF	Acute liver failure
MPIO	micron-sized iron oxide particles
CLI	Cherenkov illumination imaging
PAI	photoacoustic imaging
SERI	surface enhanced Raman imaging
iPSC	inducible pluripotent stem cells
ESC	embryonic stem cells
hAEC	human amnion epithelial cells
MSC	mesenchymal stem cells

References

Papers of particular interest, published recently, have been highlighted as:

• Of importance

•• Of major importance

1. Dhawan A, Puppi J, Hughes RD, Mitry RR. Human hepatocyte transplantation: current experience and future challenges. Nat Rev Gastroenterol Hepatol. 2010; 7:288–298. [PubMed: 20368738]
2. Allen KJ, Mifsud NA, Williamson R, Bertolino P, Hardikar W. Cell-mediated rejection results in allograft loss after liver cell transplantation. Liver Transpl. 2008; 14:688–694. [PubMed: 18433045]
3. Darwish AA, Sokal E, Stephenne X, Najimi M, de Goyet Jde V, Reding R. Permanent access to the portal system for cellular transplantation using an implantable port device. Liver Transpl. 2004; 10:1213–1215. [PubMed: 15350017]
4. Dhawan A, Mitry RR, Hughes RD. Hepatocyte transplantation for liver-based metabolic disorders. J Inherit Metab Dis. 2006; 29:431–435. [PubMed: 16763914]
5. Dhawan A, Mitry RR, Hughes RD, Lehec S, Terry C, Bansal S, Arya R, et al. Hepatocyte transplantation for inherited factor VII deficiency. Transplantation. 2004; 78:1812–1814. [PubMed: 15614156]
6. Fox IJ, Chowdhury JR, Kaufman SS, Goertzen TC, Chowdhury NR, Warkentin PI, Dorko K, et al. Treatment of the Crigler-Najjar syndrome type I with hepatocyte transplantation. N Engl J Med. 1998; 338:1422–1426. [PubMed: 9580649]
7. Grossman M, Rader DJ, Muller DW, Kolansky DM, Kozarsky K, Clark BJ 3rd, Stein EA, et al. A pilot study of ex vivo gene therapy for homozygous familial hypercholesterolaemia. Nat Med. 1995; 1:1148–1154. [PubMed: 7584986]

8. Horslen SP, McCowan TC, Goertzen TC, Warkentin PI, Cai HB, Strom SC, Fox IJ. Isolated hepatocyte transplantation in an infant with a severe urea cycle disorder. Pediatrics. 2003; 111:1262–1267. [PubMed: 12777539]

9. Jorns C, Nowak G, Nemeth A, Zemack H, Mork LM, Johansson H, Gramignoli R, et al. De Novo Donor-Specific HLA Antibody Formation in Two Patients With Crigler-Najjar Syndrome Type I Following Human Hepatocyte Transplantation With Partial Hepatectomy Preconditioning. Am J Transplant. 2016; 16:1021–1030. [PubMed: 26523372]

10. Lysy PA, Najimi M, Stephenne X, Bourgois A, Smets F, Sokal EM. Liver cell transplantation for Crigler-Najjar syndrome type I: update and perspectives. World J Gastroenterol. 2008; 14:3464–3470. [PubMed: 18567072]

11. Meyburg J, Das AM, Hoerster F, Lindner M, Kriegbaum H, Engelmann G, Schmidt J, 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. [PubMed: 19295306]

12. Muraca M, Gerunda G, Neri D, Vilei MT, Granato A, Feltracco P, Meroni M, et al. Hepatocyte transplantation as a treatment for glycogen storage disease type 1a. Lancet. 2002; 359:317–318. [PubMed: 11830200]

13. Puppi J, Tan N, Mitry RR, Hughes RD, Lehec S, Mieli-Vergani G, Karani J, et al. Hepatocyte transplantation followed by auxiliary liver transplantation--a novel treatment for ornithine transcarbamylase deficiency. Am J Transplant. 2008; 8:452–457. [PubMed: 18211511]

14. Sokal EM, Smets F, Bourgois A, Van Maldergem L, Buts JP, Reding R, Bernard Otte J, 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. [PubMed: 12973120]

15. Stephenne X, Najimi M, Sibille C, Nassogne MC, Smets F, Sokal EM. Sustained engraftment and tissue enzyme activity after liver cell transplantation for argininosuccinate lyase deficiency. Gastroenterology. 2006; 130:1317–1323. [PubMed: 16618422]

16. Stephenne X, Najimi M, Smets F, Reding R, de Ville de Goyet J, Sokal EM. Cryopreserved liver cell transplantation controls ornithine transcarbamylase deficient patient while awaiting liver transplantation. Am J Transplant. 2005; 5:2058–2061. [PubMed: 15996260]

17. Bilir BM, Guinette D, Karrer F, Kumpe DA, Krysl J, Stephens J, McGavran L, et al. Hepatocyte transplantation in acute liver failure. Liver Transpl. 2000; 6:32–40. [PubMed: 10648575]

18. Fisher RA, Bu D, Thompson M, Tisnado J, Prasad U, Sterling R, Posner M, et al. Defining hepatocellular chimerism in a liver failure patient bridged with hepatocyte infusion. Transplantation. 2000; 69:303–307. [PubMed: 10670643]

19. Khan AA, Habeeb A, Parveen N, Naseem B, Babu RP, Capoor AK, Habibullah CM. Peritoneal transplantation of human fetal hepatocytes for the treatment of acute fatty liver of pregnancy: a case report. Trop Gastroenterol. 2004; 25:141–143. [PubMed: 15682663]

20. Schneider A, Attaran M, Meier PN, Strassburg C, Manns MP, Ott M, Barthold M, et al. Hepatocyte transplantation in an acute liver failure due to mushroom poisoning. Transplantation. 2006; 82:1115–1116. [PubMed: 17060866]

21. Strom SC, Chowdhury JR, Fox IJ. Hepatocyte transplantation for the treatment of human disease. Semin Liver Dis. 1999; 19:39–48. [PubMed: 10349682]

22. Habibullah CM, Syed IH, Qamar A, Taher-Uz Z. Human fetal hepatocyte transplantation in patients with fulminant hepatic failure. Transplantation. 1994; 58:951–952. [PubMed: 7940741]

23. Horslen SP, Fox IJ. Hepatocyte transplantation. Transplantation. 2004; 77:1481–1486. [PubMed: 15239608]

24. Mito M, Kusano M, Kawaura Y. Hepatocyte transplantation in man. Transplant Proc. 1992; 24:3052–3053. [PubMed: 1466053]

••25. Soltys KA, Setoyama K, Tafaleng EN, Soto Gutierrez A, Fong J, Fukumitsu K, Nishikawa T, et al. Host conditioning and rejection monitoring in hepatocyte transplantation in humans. J Hepatol. 2016 Hepatocyte transplantation can potentially be used to treat genetic liver disorders but its application in clinical practice has been impeded by inefficient hepatocyte engraftment and the inability to monitor rejection of transplanted liver cells. In this study, we first show in non-human primates that pretreatment of the host liver with radiation improves the engraftment of transplanted liver cells. We then used this knowledge in a series of clinical hepatocyte transplants

in patients with genetic liver disorders to show that radiation pretreatment and rejection risk monitoring are safe and, if optimized, could improve engraftment and long-term survival of transplanted hepatocytes in patients.

26. Gramignoli R, Vosough M, Kannisto K, Srinivasan RC, Strom SC. Clinical hepatocyte transplantation: practical limits and possible solutions. Eur Surg Res. 2015; 54:162–177. [PubMed: 25633583]
27. Khan Z, Strom SC. Hepatocyte Transplantation in Special Populations: Clinical Use in Children. Methods Mol Biol. 2017; 1506:3–16. [PubMed: 27830542]
28. Huppert SS, Campbell KM. Emerging advancements in liver regeneration and organogenesis as tools for liver replacement. Curr Opin Organ Transplant. 2016; 21:581–587. [PubMed: 27755169]
29. Jorns C, Ellis EC, Nowak G, Fischler B, Nemeth A, Strom SC, Ericzon BG. Hepatocyte transplantation for inherited metabolic diseases of the liver. J Intern Med. 2012; 272:201–223. [PubMed: 22789058]
30. Hansel MC, Gramignoli R, Skvorak KJ, Dorko K, Marongiu F, Blake W, Davila J, et al. The history and use of human hepatocytes for the treatment of liver diseases: the first 100 patients. Curr Protoc Toxicol. 2014; 62:14, 12, 11–23. [PubMed: 25378242]
31. Hughes RD, Mitry RR, Dhawan A. Current status of hepatocyte transplantation. Transplantation. 2012; 93:342–347. [PubMed: 22082820]
32. Soltys KA, Soto-Gutierrez A, Nagaya M, Baskin KM, Deutsch M, Ito R, Shneider BL, et al. Barriers to the successful treatment of liver disease by hepatocyte transplantation. J Hepatol. 2010; 53:769–774. [PubMed: 20667616]
33. Bartlett DC, Newsome PN. A Modified Protocol for the Isolation of Primary Human Hepatocytes with Improved Viability and Function from Normal and Diseased Human Liver. Methods Mol Biol. 2017; 1506:61–73. [PubMed: 27830545]
34. Lo B, Parham L. Ethical issues in stem cell research. Endocr Rev. 2009; 30:204–213. [PubMed: 19366754]
35. Strom S, Fisher R. Hepatocyte transplantation: new possibilities for therapy. Gastroenterology. 2003; 124:568–571. [PubMed: 12557161]
36. Tsiaoussis J, Newsome PN, Nelson LJ, Hayes PC, Plevris JN. Which hepatocyte will it be? Hepatocyte choice for bioartificial liver support systems. Liver Transpl. 2001; 7:2–10. [PubMed: 11150414]
37. Horner R, Kluge M, Gassner J, Nosser M, Major RD, Reutzel-Selke A, Leder AK, et al. Hepatocyte Isolation After Laparoscopic Liver Resection. Tissue Eng Part C Methods. 2016; 22:839–846. [PubMed: 27481660]
38. Li AP. Human hepatocytes: isolation, cryopreservation and applications in drug development. Chem Biol Interact. 2007; 168:16–29. [PubMed: 17270162]
39. Vondran FW, Katenz E, Schwartlander R, Morgul MH, Raschzok N, Gong X, Cheng X, et al. Isolation of primary human hepatocytes after partial hepatectomy: criteria for identification of the most promising liver specimen. Artif Organs. 2008; 32:205–213. [PubMed: 18201288]
40. Kleine M, Riemer M, Krech T, DeTemple D, Jager MD, Lehner F, Manns MP, et al. Explanted diseased livers - a possible source of metabolic competent primary human hepatocytes. PLoS One. 2014; 9:e101386. [PubMed: 24999631]
41. Alexandre E, Cahn M, Abadie-Viollon C, Meyer N, Heyd B, Mantion G, Cinqualbre J, et al. Influence of pre-, intra- and post-operative parameters of donor liver on the outcome of isolated human hepatocytes. Cell Tissue Bank. 2002; 3:223–233. [PubMed: 15256871]
42. Alexandrova K, Griesel C, Barthold M, Heuft HG, Ott M, Winkler M, Schrem H, et al. Large-scale isolation of human hepatocytes for therapeutic application. Cell Transplant. 2005; 14:845–853. [PubMed: 16454359]
43. Gramignoli R, Tahan V, Dorko K, Skvorak KJ, Hansel MC, Zhao W, Venkataramanan R, et al. New potential cell source for hepatocyte transplantation: discarded livers from metabolic disease liver transplants. Stem Cell Res. 2013; 11:563–573. [PubMed: 23644508]
44. Hewes JC, Riddy D, Morris RW, Woodrooffe AJ, Davidson BR, Fuller B. A prospective study of isolated human hepatocyte function following liver resection for colorectal liver metastases: the effects of prior exposure to chemotherapy. J Hepatol. 2006; 45:263–270. [PubMed: 16635536]

45. Kawahara T, Toso C, Douglas DN, Nourbakhsh M, Lewis JT, Tyrrell DL, Lund GA, et al. Factors affecting hepatocyte isolation, engraftment, and replication in an in vivo model. Liver Transpl. 2010; 16:974–982. [PubMed: 20677288]

46. Lloyd TD, Orr S, Patel R, Crees G, Chavda S, Vadyar H, Berry DP, et al. Effect of patient, operative and isolation factors on subsequent yield and viability of human hepatocytes for research use. Cell Tissue Bank. 2004; 5:81–87. [PubMed: 15241003]

47. Richert L, Alexandre E, Lloyd T, Orr S, Viollon-Abadie C, Patel R, Kingston S, et al. Tissue collection, transport, isolation procedures required to optimize human hepatocyte isolation from waste liver surgical resections A multilaboratory study. Liver Int. 2004; 24:371–378. [PubMed: 15287861]

48. Serralta A, Donato MT, Orbis F, Castell JV, Mir J, Gomez-Lechon MJ. Functionality of cultured human hepatocytes from elective samples, cadaveric grafts and hepatectomies. Toxicol In Vitro. 2003; 17:769–774. [PubMed: 14599475]

••49. Lee SM, Schelcher C, Laubender RP, Frose N, Thasler RM, Schiergens TS, Mansmann U, et al. An algorithm that predicts the viability and the yield of human hepatocytes isolated from remnant liver pieces obtained from liver resections. PLoS One. 2014; 9:e107567. Isolated human primary hepatocytes are an essential in vitro model for basic and clinical research. For successful application as a model, isolated hepatocytes need to have a good viability and be available in sufficient yield. This study identifies donor characteristics, intra-operative factors, tissue processing and cell isolation parameters that affect the viability and yield of human hepatocytes. By developing an acessible algorithm, projected viability can be determined even before isolation of hepatocytes, so that donors that result in high viability and yield can be identified. [PubMed: 25313881]

50. Bartlett DC, Hodson J, Bhogal RH, Youster J, Newsome PN. Combined use of N-acetylcysteine and Liberase improves the viability and metabolic function of human hepatocytes isolated from human liver. Cytotherapy. 2014; 16:800–809. [PubMed: 24642019]

51. Bhogal RH, Hodson J, Bartlett DC, Weston CJ, Curbishley SM, Haughton E, Williams KT, et al. Isolation of primary human hepatocytes from normal and diseased liver tissue: a one hundred liver experience. PLoS One. 2011; 6:e18222. [PubMed: 21479238]

52. Izamis ML, Calhoun C, Uygun BE, Guzzardi MA, Price G, Luitje M, Saeidi N, et al. Simple Machine Perfusion Significantly Enhances Hepatocyte Yields of Ischemic and Fresh Rat Livers. Cell Med. 2013; 4:109–123. [PubMed: 25431743]

53. Izamis ML, Perk S, Calhoun C, Uygun K, Yarmush ML, Berthiaume F. Machine perfusion enhances hepatocyte isolation yields from ischemic livers. Cryobiology. 2015; 71:244–255. [PubMed: 26188080]

54. Bruinsma BG, Sridharan GV, Weeder PD, Avruch JH, Saeidi N, Ozer S, Geerts S, et al. Metabolic profiling during ex vivo machine perfusion of the human liver. Sci Rep. 2016; 6:22415. [PubMed: 26935866]

55. Nativ NI, Yarmush G, So A, Barminko J, Maguire TJ, Schloss R, Berthiaume F, et al. Elevated sensitivity of macrosteatotic hepatocytes to hypoxia/reoxygenation stress is reversed by a novel defatting protocol. Liver Transpl. 2014; 20:1000–1011. [PubMed: 24802973]

56. Yarmush G, Santos L, Yarmush J, Koundinyan S, Saleem M, Nativ NI, Schloss RS, et al. Metabolic Flux Distribution during Defatting of Steatotic Human Hepatoma (HepG2) Cells. Metabolites. 2016; 6

57. Duret C, Moreno D, Balasiddaiah A, Roux S, Briolotti P, Raulet E, Herrero A, et al. Cold Preservation of Human Adult Hepatocytes for Liver Cell Therapy. Cell Transplant. 2015; 24:2541–2555. [PubMed: 25622096]

58. Puts CF, Berendsen TA, Bruinsma BG, Ozer S, Luitje M, Usta OB, Yarmush ML, et al. Polyethylene glycol protects primary hepatocytes during supercooling preservation. Cryobiology. 2015; 71:125–129. [PubMed: 25936340]

59. Jorns C, Gramignoli R, Saliem M, Zemack H, Mork LM, Isaksson B, Nowak G, et al. Strategies for short-term storage of hepatocytes for repeated clinical infusions. Cell Transplant. 2014; 23:1009–1018. [PubMed: 25199147]

60. Bonora-Centelles A, Donato MT, Lahoz A, Pareja E, Mir J, Castell JV, Gomez-Lechon MJ. Functional characterization of hepatocytes for cell transplantation: customized cell preparation for each receptor. Cell Transplant. 2010; 19:21–28. [PubMed: 19796502]
61. Gramignoli R, Tahan V, Dorko K, Venkataramanan R, Fox IJ, Ellis EC, Vosough M, et al. Rapid and sensitive assessment of human hepatocyte functions. Cell Transplant. 2014; 23:1545–1556. [PubMed: 24702711]
62. Weber A, Groyer-Picard MT, Franco D, Dagher I. Hepatocyte transplantation in animal models. Liver Transpl. 2009; 15:7–14. [PubMed: 19109838]
63. Sigal SH, Rajvanshi P, Gorla GR, Sokhi RP, Saxena R, Gebhard DR Jr, Reid LM, et al. Partial hepatectomy-induced polyploidy attenuates hepatocyte replication and activates cell aging events. Am J Physiol. 1999; 276:G1260–1272. [PubMed: 10330018]
64. Abdalla EK. Portal vein embolization (prior to major hepatectomy) effects on regeneration, resectability, and outcome. J Surg Oncol. 2010; 102:960–967. [PubMed: 21165999]
65. Furrer K, Tian Y, Pfammatter T, Jochum W, El-Badry AM, Graf R, Clavien PA. Selective portal vein embolization and ligation trigger different regenerative responses in the rat liver. Hepatology. 2008; 47:1615–1623. [PubMed: 18395841]
66. Guha C, Parashar B, Deb NJ, Sharma A, Gorla GR, Alfieri A, Roy-Chowdhury N, et al. Liver irradiation: a potential preparative regimen for hepatocyte transplantation. Int J Radiat Oncol Biol Phys. 2001; 49:451–457. [PubMed: 11173140]
67. Guha C, Sharma A, Gupta S, Alfieri A, Gorla GR, Gagandeep S, Sokhi R, et al. Amelioration of radiation-induced liver damage in partially hepatectomized rats by hepatocyte transplantation. Cancer Res. 1999; 59:5871–5874. [PubMed: 10606225]
68. Koenig S, Yuan Q, Krause P, Christiansen H, Rave-Fraenk M, Kafert-Kasting S, Kriegbaum H, et al. Regional transient portal ischemia and irradiation as preparative regimen for hepatocyte transplantation. Cell Transplant. 2011; 20:303–311. [PubMed: 20719089]
69. Yamanouchi K, Zhou H, Roy-Chowdhury N, Macaluso F, Liu L, Yamamoto T, Yannam GR, et al. Hepatic irradiation augments engraftment of donor cells following hepatocyte transplantation. Hepatology. 2009; 49:258–267. [PubMed: 19003915]
70. Dagher I, Boudechiche L, Branger J, Coulomb-Lhermine A, Parouchev A, Sentilhes L, Lin T, et al. Efficient hepatocyte engraftment in a nonhuman primate model after partial portal vein embolization. Transplantation. 2006; 82:1067–1073. [PubMed: 17060856]
71. Pourcher G, El-Kehdy H, Kanso F, Groyer-Picard MT, Gaillard M, Trassard O, Blazsek I, et al. Volumetric Portal Embolization: A New Concept to Improve Liver Regeneration and Hepatocyte Engraftment. Transplantation. 2016; 100:344–354. [PubMed: 26757049]
72. Kabarriti RZW, Yaffe H, Liu L, Asp P, Tome WA, et al. Delaying transplantation by 24 hours after preparative hepatic irradiation enhances engraftment and proliferation of transplanted hepatocytes in mouse liver. 90:S174–S175. Int J Radiat Oncol. 2014
73. Nygaard S, Barzel A, Haft A, Major A, Finegold M, Kay MA, Grompe M. A universal system to select gene-modified hepatocytes in vivo. Sci Transl Med. 2016; 8:342ra379.
74. Gustafson E, Asif S, Kozarcanin H, Elgue G, Meurling S, Ekdahl KN, Nilsson B. Control of IBMIR induced by fresh and cryopreserved hepatocytes by low molecular weight dextran sulfate versus heparin. Cell Transplant. 2016
75. Lee CA, Dhawan A, Smith RA, Mitry RR, Fitzpatrick E. Instant Blood-Mediated Inflammatory Reaction in Hepatocyte Transplantation: Current Status and Future Perspectives. Cell Transplant. 2016; 25:1227–1236. [PubMed: 26996786]
76. Han B, Lu Y, Meng B, Qu B. Cellular loss after allogenic hepatocyte transplantation. Transplantation. 2009; 87:1–5. [PubMed: 19136883]
77. Hayashi C, Ito M, Ito R, Murakumo A, Yamamoto N, Hiramatsu N, Fox IJ, et al. Effects of edaravone, a radical scavenger, on hepatocyte transplantation. J Hepatobiliary Pancreat Sci. 2014; 21:919–924. [PubMed: 25205207]
78. Asif S, Ekdahl KN, Fromell K, Gustafson E, Barbu A, Le Blanc K, Nilsson B, et al. Heparinization of cell surfaces with short peptide-conjugated PEG-lipid regulates thromboinflammation in transplantation of human MSCs and hepatocytes. Acta Biomater. 2016; 35:194–205. [PubMed: 26876877]

79. Meier RP, Montanari E, Morel P, Pimenta J, Schuurman HJ, Wandrey C, Gerber-Lemaire S, et al. Microencapsulation of Hepatocytes and Mesenchymal Stem Cells for Therapeutic Applications. Methods Mol Biol. 2017; 1506:259–271. [PubMed: 27830559]
80. Mitry RR, Jitraruch S, Iansante V, Dhawan A. Alginate Encapsulation of Human Hepatocytes and Assessment of Microbeads. Methods Mol Biol. 2017; 1506:273–281. [PubMed: 27830560]
81. Sgroi A, Mai G, Morel P, Baertschiger RM, Gonelle-Gispert C, Serre-Beinier V, Buhler LH. Transplantation of encapsulated hepatocytes during acute liver failure improves survival without stimulating native liver regeneration. Cell Transplant. 2011; 20:1791–1803. [PubMed: 21396154]
82. Teramura Y, Oommen OP, Olerud J, Hilborn J, Nilsson B. Microencapsulation of cells, including islets, within stable ultra-thin membranes of maleimide-conjugated PEG-lipid with multifunctional crosslinkers. Biomaterials. 2013; 34:2683–2693. [PubMed: 23347835]
83. Jitraruch S, Dhawan A, Hughes RD, Filippi C, Lehec SC, Glover L, Mitry RR. Cryopreservation of Hepatocyte Microbeads for Clinical Transplantation. Cell Transplant. 2017
84. Jitraruch S, Dhawan A, Hughes RD, Filippi C, Soong D, Philippeos C, Lehec SC, et al. Alginate microencapsulated hepatocytes optimised for transplantation in acute liver failure. PLoS One. 2014; 9:e113609. [PubMed: 25438038]
85. Ohashi K, Yokoyama T, Yamato M, Kuge H, Kanehiro H, Tsutsumi M, Amanuma T, et al. Engineering functional two- and three-dimensional liver systems in vivo using hepatic tissue sheets. Nat Med. 2007; 13:880–885. [PubMed: 17572687]
86. Uygun BE, Soto-Gutierrez A, Yagi H, Izamis ML, Guzzardi MA, Shulman C, Milwid J, et al. Organ reengineering through development of a transplantable recellularized liver graft using decellularized liver matrix. Nat Med. 2010; 16:814–820. [PubMed: 20543851]
87. Yokoyama T, Ohashi K, Kuge H, Kanehiro H, Iwata H, Yamato M, Nakajima Y. In vivo engineering of metabolically active hepatic tissues in a neovascularized subcutaneous cavity. Am J Transplant. 2006; 6:50–59. [PubMed: 16433756]
88. Zhang S, Zhang B, Chen X, Chen L, Wang Z, Wang Y. Three-dimensional culture in a microgravity bioreactor improves the engraftment efficiency of hepatic tissue constructs in mice. J Mater Sci Mater Med. 2014; 25:2699–2709. [PubMed: 25056199]
89. Fox IJ, Chowdhury JR. Hepatocyte transplantation. Am J Transplant. 2004; 4(Suppl 6):7–13.
90. Ambrosino G, Varotto S, Strom SC, Guariso G, Franchin E, Miotto D, Caenazzo L, et al. Isolated hepatocyte transplantation for Crigler-Najjar syndrome type 1. Cell Transplant. 2005; 14:151–157. [PubMed: 15881424]
91. Oldhafer F, Bock M, Falk CS, Vondran FW. Immunological aspects of liver cell transplantation. World J Transplant. 2016; 6:42–53. [PubMed: 27011904]
92. Castellaneta A, Thomson AW, Nayyar N, de Vera M, Mazariegos GV. Monitoring the operationally tolerant liver allograft recipient. Curr Opin Organ Transplant. 2010; 15:28–34. [PubMed: 19890211]
93. Ashokkumar C, Bentlejewski C, Sun Q, Higgs BW, Snyder S, Mazariegos GV, Abu-Elmagd K, et al. Allospecific CD154+ B cells associate with intestine allograft rejection in children. Transplantation. 2010; 90:1226–1231. [PubMed: 20881665]
94. Ashokkumar C, Shapiro R, Tan H, Ningappa M, Elinoff B, Fedorek S, Sun Q, et al. Allospecific CD154+ T-cytotoxic memory cells identify recipients experiencing acute cellular rejection after renal transplantation. Transplantation. 2011; 92:433–438. [PubMed: 21747326]
95. Ashokkumar C, Talukdar A, Sun Q, Higgs BW, Janosky J, Wilson P, Mazariegos G, et al. Allospecific CD154+ T cells associate with rejection risk after pediatric liver transplantation. Am J Transplant. 2009; 9:179–191. [PubMed: 18976293]
96. Hogen R, DiNorcia J, Dhanireddy K. Antibody-mediated rejection: what is the clinical relevance? Curr Opin Organ Transplant. 2017; 22:97–104. [PubMed: 28060025]
97. Zhang M, Wang H, Tan S, Navarro-Alvarez N, Zheng Y, Yang YG. Donor CD47 controls T cell alloresponses and is required for tolerance induction following hepatocyte allotransplantation. Sci Rep. 2016; 6:26839. [PubMed: 27230788]
98. Ide K, Wang H, Tahara H, Liu J, Wang X, Asahara T, Sykes M, et al. Role for CD47-SIRPalpha signaling in xenograft rejection by macrophages. Proc Natl Acad Sci U S A. 2007; 104:5062–5066. [PubMed: 17360380]

99. Navarro-Alvarez N, Yang YG. Lack of CD47 on donor hepatocytes promotes innate immune cell activation and graft loss: a potential barrier to hepatocyte xenotransplantation. Cell Transplant. 2014; 23:345–354. [PubMed: 23394628]
100. Wang H, Madariaga ML, Wang S, Van Rooijen N, Oldenborg PA, Yang YG. Lack of CD47 on nonhematopoietic cells induces split macrophage tolerance to CD47null cells. Proc Natl Acad Sci U S A. 2007; 104:13744–13749. [PubMed: 17699632]
101. Wang H, Wu X, Wang Y, Oldenborg PA, Yang YG. CD47 is required for suppression of allograft rejection by donor-specific transfusion. J Immunol. 2010; 184:3401–3407. [PubMed: 20208011]
102. Heyn C, Ronald JA, Mackenzie LT, MacDonald IC, Chambers AF, Rutt BK, Foster PJ. In vivo magnetic resonance imaging of single cells in mouse brain with optical validation. Magn Reson Med. 2006; 55:23–29. [PubMed: 16342157]
103. Shapiro EM, Sharer K, Skrtic S, Koretsky AP. In vivo detection of single cells by MRI. Magn Reson Med. 2006; 55:242–249. [PubMed: 16416426]
104. Slotkin JR, Cahill KS, Tharin SA, Shapiro EM. Cellular magnetic resonance imaging: nanometer and micrometer size particles for noninvasive cell localization. Neurotherapeutics. 2007; 4:428–433. [PubMed: 17599708]
105. Wu YL, Ye Q, Foley LM, Hitchens TK, Sato K, Williams JB, Ho C. In situ labeling of immune cells with iron oxide particles: an approach to detect organ rejection by cellular MRI. Proc Natl Acad Sci U S A. 2006; 103:1852–1857. [PubMed: 16443687]
106. Roach DR, Garrett WM, Welch G, Caperna TJ, Talbot NC, Shapiro EM. Magnetic cell labeling of primary and stem cell-derived pig hepatocytes for MRI-based cell tracking of hepatocyte transplantation. PLoS One. 2015; 10:e0123282. [PubMed: 25856627]
107. Rodriguez-Porcel M. In vivo imaging and monitoring of transplanted stem cells: clinical applications. Curr Cardiol Rep. 2010; 12:51–58. [PubMed: 20425184]
108. von der Haar K, Lavrentieva A, Stahl F, Scheper T, Blume C. Lost signature: progress and failures in in vivo tracking of implanted stem cells. Appl Microbiol Biotechnol. 2015; 99:9907–9922. [PubMed: 26373727]
109. Wang P, Petrella F, Nicosia L, Bellomi M, Rizzo S. Molecular Imaging of Stem Cell Transplantation for Liver Diseases: Monitoring, Clinical Translation, and Theranostics. Stem Cells Int. 2016; 2016:4058656. [PubMed: 28070195]
110. Hickey RD, Mao SA, Amiot B, Suksanpaisan L, Miller A, Nace R, Glorioso J, et al. Noninvasive 3-dimensional imaging of liver regeneration in a mouse model of hereditary tyrosinemia type 1 using the sodium iodide symporter gene. Liver Transpl. 2015; 21:442–453. [PubMed: 25482651]
111. Rezvani M, Grimm AA, Willenbring H. Assessing the therapeutic potential of lab-made hepatocytes. Hepatology. 2016; 64:287–294. [PubMed: 27014802]
112. Baxter M, Withey S, Harrison S, Segeritz CP, Zhang F, Atkinson-Dell R, Rowe C, et al. Phenotypic and functional analyses show stem cell-derived hepatocyte-like cells better mimic fetal rather than adult hepatocytes. J Hepatol. 2015; 62:581–589. [PubMed: 25457200]
113. Cameron K, Tan R, Schmidt-Heck W, Campos G, Lyall MJ, Wang Y, Lucendo-Villarin B, et al. Recombinant Laminins Drive the Differentiation and Self-Organization of hESC-Derived Hepatocytes. Stem Cell Reports. 2015; 5:1250–1262. [PubMed: 26626180]
114. Duan Y, Catana A, Meng Y, Yamamoto N, He S, Gupta S, Gambhir SS, et al. Differentiation and enrichment of hepatocyte-like cells from human embryonic stem cells in vitro and in vivo. Stem Cells. 2007; 25:3058–3068. [PubMed: 17885076]
115. Hay DC, Fletcher J, Payne C, Terrace JD, Gallagher RC, Snoeys J, Black JR, et al. Highly efficient differentiation of hESCs to functional hepatic endoderm requires ActivinA and Wnt3a signaling. Proc Natl Acad Sci U S A. 2008; 105:12301–12306. [PubMed: 18719101]
116. Takahashi K, Tanabe K, Ohnuki M, Narita M, Ichisaka T, Tomoda K, Yamanaka S. Induction of pluripotent stem cells from adult human fibroblasts by defined factors. Cell. 2007; 131:861–872. [PubMed: 18035408]
117. Yu J, Vodyanik MA, Smuga-Otto K, Antosiewicz-Bourget J, Frane JL, Tian S, Nie J, et al. Induced pluripotent stem cell lines derived from human somatic cells. Science. 2007; 318:1917–1920. [PubMed: 18029452]

118. Tolosa L, Pareja E, Gomez-Lechon MJ. Clinical Application of Pluripotent Stem Cells: An Alternative Cell-Based Therapy for Treating Liver Diseases? Transplantation. 2016; 100:2548–2557. [PubMed: 27495745]

119. Bissig-Choisat B, Wang L, Legras X, Saha PK, Chen L, Bell P, Pankowicz FP, et al. Development and rescue of human familial hypercholesterolaemia in a xenograft mouse model. Nat Commun. 2015; 6:7339. [PubMed: 26081744]

120. Imagawa K, Takayama K, Isoyama S, Tanikawa K, Shinkai M, Harada K, Tachibana M, et al. Generation of a bile salt export pump deficiency model using patient-specific induced pluripotent stem cell-derived hepatocyte-like cells. Sci Rep. 2017; 7:41806. [PubMed: 28150711]

121. Strom SC, Gramignoli R. Human amnion epithelial cells expressing HLA-G as novel cell-based treatment for liver disease. Hum Immunol. 2016; 77:734–739. [PubMed: 27476049]

122. Akle C, McColl I, Dean M, Adinolfi M, Brown S, Fensom AH, Marsh J, et al. Transplantation of amniotic epithelial membranes in patients with mucopolysaccharidoses. Exp Clin Immunogenet. 1985; 2:43–48. [PubMed: 3939973]

123. Bembi B, Comelli M, Scaggiante B, Pineschi A, Rapelli S, Gornati R, Montorfano G, et al. Treatment of sphingomyelinase deficiency by repeated implantations of amniotic epithelial cells. Am J Med Genet. 1992; 44:527–533. [PubMed: 1442900]

124. Scaggiante B, Pineschi A, Sustersich M, Andolina M, Agosti E, Romeo D. Successful therapy of Niemann-Pick disease by implantation of human amniotic membrane. Transplantation. 1987; 44:59–61. [PubMed: 3037739]

125. Miki T. A Rational Strategy for the Use of Amniotic Epithelial Stem Cell Therapy for Liver Diseases. Stem Cells Transl Med. 2016; 5:405–409. [PubMed: 26941361]

Table 1

Experience of hepatocyte transplantation in human diseases

Inborn errors of metabolism	Acute liver failure
Acute intermittent porphyria	Acute fatty liver of pregnancy
a1-Antitrypsin deficiency	Drug induced
Crigler-Najjar syndrome	Idiopathic
Factor VII deficiency	Mushroom poisoning
Familial hypercholesterolemia	Post-surgical
Glycogen storage diseases	Viral
Hemochromatosis-hemosiderosis	
Hyperlipidemia	Other
Infantile Refsum's disease	Biliary atresia
Primary oxalosis	Cirrhosis
Phenylketonuria	
PFIC 2, ABCB11 deficiency, Bile salt exporter pump disease	
Tyrosinemia	
Urea cycle defects	
Ornithine transcarba mylase deficiency	
Argininosuccinate lyase deficiency	
Carbamoylphosphate synthase type 1 deficiency	
Citrullinemia	

PFIC, Progressive familial intrahepatic cholestasis

Table 2

Factors affecting yield and viability of isolated hepatocytes

	Yield	Viability
Fibrosis	↓	↓
Chemotherapy	↑	
Steatosis	↓	↓
Liver enzyme and bilirubin elevation	↓	↓
Weight of perfused liver	↓	
Cold ischemia time	↓	

Ref 49

Table 3

Alternative sources for future cell-based therapies

Cell Type	Potential Indication	Human studies
Mesenchymal Stem Cells	Liver Failure	Yes
	Liver Cirrhosis	
Embryonic Stem Cells	Metabolic Liver Disease	No
	Liver Failure	
Induced pluripotent stem cells	Metabolic Liver Disease	No
	Liver Failure	
Human amnion epithelial cells	Metabolic Liver Disease	Yes
	Liver Cirrhosis	
	Lysosomal Storage Disease	