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The History and Use of Human Hepatocytes for the Study and Treatment of Liver Metabolic Diseases

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1.0 INTRODUCTION: GENERAL LIVER BACKGROUND

The liver is one of the largest, most complex organs in the human body and is essential for life. It is located below the diaphragm within the abdominal pelvic region of the abdomen and weighs approximately 1500–1800 grams. It is made up of a spongy mass of wedge-shaped lobes (2012). Functions of the liver include, but are not limited to: processing of carbohydrates, fats, and proteins, vitamin storage, detoxification of substances in the blood, metabolism, protein synthesis, glycogen storage, decomposition of red blood cells, plasma protein synthesis, clotting factor synthesis, hormone production, bile production and it regulates high volume biochemical reactions (synthesis and breakdown) of complex molecules necessary for normal physiology and homeostasis (2012; Kmiec, 2001).

The liver receives blood inflow from the portal vein and hepatic artery. This arterial and venous blood mix together and travel through the specialized liver sinusoids perfusing the entire organ with blood that eventually will drain into the hepatic vein. The hepatic sinusoids perfuse the liver with both oxygen-rich blood from the hepatic artery and venous blood from the portal vein. The portal vein inflow blood has already circulated through the gut, pancreas, spleen and other associated organs and is enriched with toxins and other substances needed to be handled by the specialized cells of the liver for normal body physiology to occur.

The different lobes of the human liver consist of parenchymal cells known as hepatocytes and various non-parenchymal cells. Hepatocytes make up 80% of the total liver volume

whereas non-parenchymal cells only make up 6.5%; however 40% of the cells within the liver are of the non-parenchymal type (Kmiec, 2001). The walls of the hepatic sinusoid are lined by the following non-parenchymal cells: sinusoidal endothelial cells (SECs), Kupffer cells (KCs), hepatic stellate cells (HSCs) and intrahepatic lymphocytes (IHLs) such as pit cells. Under both normal and pathological conditions there is cross-talk between both hepatocytes and their non-parenchymal cell counterparts through paracrine effects that influence cell behavior and function within the liver microenvironment (Kmiec, 2001).

SECs line the specialized hepatic sinusoid and their major function is to filter the blood that perfuses the entire liver. SECs have specialized fenestrations that allow free diffusion of blood to the surfaces of hepatocytes allowing the parenchymal cells to perform their various functions to maintain normal body physiology (Kmiec, 2001).

KCs are liver tissue specific macrophages that have strong endocytic and phagocytic abilities. They secrete potent mediators of the inflammatory response and therefore play an important role in early phase liver inflammation and innate immune defense (Kmiec, 2001).

HSCs are characterized by their intracytoplasmic fat droplets and well-branched cytoplasmic processes. Under normal liver conditions HSCs store vitamin A, control turnover of liver ECM and regulate sinusoidal contractility and therefore sinusoidal blood flow. When liver damage occurs quiescent HSCs transform to myofibroblast-like cells that play a major role in the development of liver fibrosis and eventually cirrhosis (Kmiec, 2001).

2.0 ORTHOTOPIC LIVER TRANSPLANTATION

The first experimental trials of auxiliary liver transplantation occurred in homotransplantation canine models in 1956 by Goodrich and colleagues (Goodrich et al., 1956). These successful trials laid the groundwork for the first clinical liver transplant in humans to be performed by Thomas E. Starzl in 1963 at the University of Pittsburgh (Starzl et al., 1963). The report described orthotopic liver transplant (OLT) in 3 patients, one of which died on the operating table (patient 1) and the other two survived 22 and 7.5 days respectively with their grafts (patients 2 and 3). Patient 1 was a 3 year old white male with congenital biliary atresia, patient 2 was a 48 year old African American male with Laenne's cirrhosis and a primary hepatoma and patient 3 was a 67 year old white male with progressive jaundice and an intrahepatic duct cell carcinoma. Long term survival in these studies was limited by the lack of an effective immunosuppressive drug. In 1983 the development of immunosuppressive drug azathioprine allowed OLT to become an established treatment for hepatic failure (as reviewed in) (Dhawan et al., 2010). The introduction of cyclosporine and FK506 a few years later further solidified the procedure in the medical community. Table 1 summarizes the liver diseases currently treated by OLT in the United States.

Since the initial 3 OLT surgeries, there has been significant advancement in surgical techniques, immunosuppression and overall patient management to the point where OLT is a routine procedure, albeit with the potential complications and risks associated with a whole organ transplant. Currently, the only curative therapy for acute and chronic liver failure and inborn errors in metabolism is OLT. There are three different types of OLT surgeries

practiced in the United States today: whole liver, reduced liver and liver segment (2012). Whole liver transplants involve removing a patient's entire liver and replacing it with a whole liver from an organ donor, first performed in 1963 (Starzl et al., 1963). Since the liver is known to regenerate *in vivo*, surgeons speculated that smaller patients could be transplanted with organs that could be tailored made in size to the patients and have the graft grow with patients as they grow. This observation led to the establishment of the following two liver transplant techniques. Reduced liver transplants are performed when the surgeon makes a donor liver smaller for the recipient, first performed in 1984 (Bismuth and Houssin, 1984). And finally liver segment transplants are surgeries that allow one cadaveric donor liver to transplant two patients, first performed in 1989 and 1990 (Emond et al., 1989; Ringe et al., 1990). Liver segment transplants can also occur with living donor donations as well. Since partial transplantation of the liver to treat patients is possible it seems likely that cell transplantation may also be possible, if issues with engraftment, proliferation of the graft, immunosuppression and patient management are overcome. Cells can be given to patients with liver failure to allow for metabolic support and potential liver failure reversal. Cells can also be given to patients with inborn errors in metabolism because transplanted cells will be competent in the enzyme or protein that is deficient in the patient with the metabolic disorder.

3.0 CELL TRANSPLANT AS A POTENTIAL THERAPY – REASONS FOR DEVELOPMENT

An important question that needs to be answered is whether or not cell transplant, either primary-cell or stem cell-based, is a viable therapy to treat liver disease. The need for cellular transplant to become a viable therapy to treat liver diseases is obvious. According to the most recent annual report performed by the Organ Procurement and Transplantation Network (OPTN) and the Scientific Registry of Transplant Recipients (SRTR), in 2009 there were almost 26,000 people on the liver transplant waiting list and only a total of 6,320 OLTs performed (Figure 1) (2011). This resulted in 1,723 deaths on the waitlist and 15,682 people on the waitlist at year's end (Figure1) (2011).

Over the course of the past 10 years of collated data, one can see a trend that is continuing. The total number of patients requiring a transplant is staying stagnant and so is the number of total transplants being performed. This is due to the lack of donor livers available for transplant. In order to transplant more patients an alternative source of liver tissue is necessary. When a surgeon performs an OLT there is one donor liver for one patient (in most cases). If cellular transplant can be used to treat patients there can be 1 donor for many recipients, depending on the number of cells necessary to treat the patient and the overall viable cell yield from the isolation. Furthermore, one can increase the amount of tissue used for transplant if cells can be isolated from liver tissues that are normally discarded. Therefore one can identify liver diseases that can be managed or cured by cellular transplant and treat those patients with cells and leave the whole organ transplants solely for diseases that can only be treated by OLT. This would greatly reduce the burden on the liver transplant waiting list and allow surgeons to decrease the number of patients on the waitlist while increasing the number of total transplants performed. Also, if cell transplant can be

shown to manage patient symptoms for certain underlying liver pathologies requiring an OLT, these patients can be given cells as a “bridge to transplant” and be sustained until an organ becomes available for OLT. This procedure would reduce the number of deaths on the transplant waitlist. Moreover, with the emerging fields of human adult stem cell, human embryonic stem cell (hESC) and human induced pluripotent stem cell (hiPSC) research there is the possibility of alternative cell sources to treat liver diseases, which would further reduce the burden on the liver transplant waiting list.

Although OLT is a routine procedure, it still is a major surgery with a high incidence of surgical complications (graft vs. host disease, ischemia/reperfusion injury, graft failure, etc.). Furthermore the expenses associated with the surgery and therapies to maintain the graft for the life of a patient are extremely high. To compound issues further, there are limited donors and the timing of the transplant is critical. In contrast, cell transplantation is less invasive and less costly with fewer complications and risks associated with the procedure. Moreover, cryopreserved cells can be made available on demand from cell banks with cell expansion possible to treat a greater number of patients with the same amount of donor tissue. New cell sources for liver tissue to transplant are also becoming a reality with the discovery of different stem cells (hESCs, hiPSCs and adult stem cells) and the ability to turn these cells into hepatocyte-like cells. These alternative cell sources would solve the problem of the shortage of donor liver tissue to isolate primary hepatocytes from. Taken together this information shows the need to investigate if cellular transplantation to treat different liver diseases is possible. Table 2 summarizes the benefits of cell transplantation over OLT.

4.0 PRIMARY HEPATOCYTE TRANSPLANT RESEARCH – FROM TISSUES TO CELLS IN ANIMAL MODELS

In 1964 investigators performed the first heterotopic partial autotransplantation of pieces of rat livers (Grisham et al., 1964). This crude liver graft was demonstrated to have structure and function when transplanted to the kidney capsule or subcutaneous space. This idea of transplanting pieces of liver was further solidified to be viable when sliced rat liver tissue was autologously transplanted into rats subcutaneously (Hiraoka et al., 1983). These autografts were approximately 1/10 of the original size and found alive in subcutaneous tissue 1 year after transplant. Partial hepatectomy was performed on the rats prior to transplantation to see if this would increase the size of the graft, however this did not lead to an increase at the end of the 1 year study. Therefore if pieces of liver can be transplanted and shown to have function why can’t cells be transplanted and function as well?

Two important studies that paved the way for hepatocyte transplants becoming a potential reality to treat liver diseases occurred in the pancreas and diabetes field. In 1967 a method for the isolation of intact islet of Langerhans from rat pancreas was reported (Lacy and Kostianovsky, 1967) and then in 1972 these rat islets were transplanted into diabetic rats (Ballinger and Lacy, 1972). This cell transplant improved blood glucose levels though intraperitoneal or subcutaneous injection of the cells, thus demonstrating that transplanted cells can function within a recipient. Now a procedure to isolate viable hepatocytes needed to be discovered in order for the hepatocyte transplant field to push forward. This was

accomplished in 1969 by Berry and Friend, who described a high-yield preparation of isolated rat liver parenchymal cells using a collagenase perfusion method (Berry and Friend, 1969). Since then this procedure has been refined and optimized with human tissue. Gramignoli and colleagues have developed a purified tissue dissociation enzyme mixture for the isolation of human hepatocytes where the isolated cells can be used in a clinical transplantation setting (Gramignoli et al., 2011).

Now that it is possible to isolate highly viable and functioning human hepatocytes, researchers need to demonstrate that the isolated cells can be transplanted into liver metabolic disease animal models and function enough to improve blood chemistries and overall symptoms associated with disease. In 1970 cells from a clonal strain of a rat hepatoma were transplanted subcutaneously into Gunn rats that are deficient in the UGT1A1 enzyme (Rugstad et al., 1970b). This enzyme deficiency causes animals to become hyperbilirubinemic and jaundiced. The Gunn rat is a useful model for the human counterpart, Crigler-Najjar Type 1 (CN-1). This disease is characterized by the inability to conjugate bilirubin. Bilirubin is formed during the breakdown of hemoglobin from senescent erythrocytes or any other cells that contain hemoproteins. Patients suffering from this disorder experience altered senses, poor coordination, slurring of speech, weakness, brain damage and possible coma due to high levels of circulating unconjugated bilirubin. Although the cells transplanted were not hepatocytes, they had been previously shown to metabolize bilirubin *in vitro* (Rugstad et al., 1970a). This was the first report to demonstrate transplanted cells can provide metabolic support, in this case helping to reduce bilirubin levels in rats modeling a known human metabolic disease. Matas and colleagues further developed this research in the CN-1 rat model by transplanting mechanically isolated liver fragments and demonstrating that they too reduce bilirubin levels in blood serum (Matas et al., 1976). Finally in 1977 the first actual hepatocyte transplant occurred when Carl Groth and colleagues isolated rat hepatocytes and transplanted them via the portal vein into the Gunn rat (Groth et al., 1977). They observed a 21% decrease in bilirubin levels at day 10 with a maximum reduction of 35% at day 28. An important caveat of this experiment was the administration of cyclophosphamide for immunosuppression that allowed the cell graft to survive better post transplant.

Further evidence that cell transplantation can be used to treat metabolic disease occurred when cryopreserved human hepatocytes were attached to collagen-coated microcarriers and injected IP into athymic-UGT1A1 deficient Gunn rats and athymic-analbuminemic Nagase rats (Moscioni et al., 1989). These Nagase rats are deficient in albumin synthesis. Animals were made athymic in order to be deficient in T-cells to help with stabilization of the graft. Cells formed aggregates on the surface of the pancreas. Gunn rats excreted bilirubin glucuronides (conjugated bilirubins) in bile for 30 days and overall serum bilirubin levels were reduced. Nagase rats had plasma albumin levels increased from a low of 0.024 mg/ml to a high of 4.8 mg/ml 8 days post transplant and remained near this high level for the duration of the 30 day experiment. This study was one of the first to demonstrate that human cells have the ability to engraft after transplant and function metabolically. More importantly this study demonstrated that when 1–2% liver mass is transplanted, a 15 fold increase in albumin was observed and when 2–4% if liver mass is transplanted, albumin was increased

to 60% of a normal value. Thus, low levels of engraftment of proficient cells resulted in much larger increases in the correction of the metabolic defect than expected. These and many other studies cited below support the hypothesis that low-level engraftment of proficient cells, less than 10% of liver mass, may be sufficient to substantially correct the symptoms of metabolic liver disease. This hypothesis still provides the scientific support for cellular therapy of liver disease.

A study performed on the Eizai hyperbilirubinemic rat, which models the human Dubin-Johnson Syndrome, demonstrated that transplanted fully competent rat hepatocytes can engraft and successfully integrate with the hepato-biliary system. These rats have impaired canalicular excretory transport of organic anions, bile acid glucuronides and sulfate conjugates therefore causing conjugated hyperbilirubinemia. Animals were treated with intraportal injections of wild type rat hepatocytes along with a 68% partial hepatectomy to promote hepatic proliferation *in vivo*. This therapy resulted in reduction of serum bilirubin level and bile excretion became possible demonstrated by biliary transport of indocyanine and sulfobromophthalein into bile. Furthermore it shows that hepatic transport of bile acid conjugates can be restored by hepatocyte transplant. Additionally, it is a second report that showed only a transplant of 1–2% of liver mass results in a 50% reduction in bilirubin levels. It appears that one does not need large engraftment to make a major physiological impact within the transplant recipient.

Multi-locational allogenic hepatocyte transplant for the treatment of ascorbic acid deficiency in rats was shown to be possible (Nakazawa et al., 1996). Cells were transplanted in the portal vein, spleen omentum and mesentery. Ascorbic acid was found in the serum of recipients up to 3 months after transplant. This report demonstrates that transplanted hepatocytes do not need to engraft into the liver architecture to provide metabolic support.

Further evidence that hepatocyte transplant is a viable method to treat liver metabolic disease occurred when investigators corrected a mouse model of progressive familial intrahepatic cholestasis type-3 (PFIC-3) (De Vree et al., 2000). Patients that have PFIC-3 have a mutation in the MDR3 gene that encodes the hepatocanalicular phospholipid translocator that results in the absence of phospholipids in the bile. This absence causes chronic bile salt-induced damage to hepatocytes and bile duct epithelium. The mdr2-knockout mouse is the animal model for the human disease. Transplanted mdr2-competent hepatocytes repopulated the liver and were shown to restore phospholipid secretion and diminish liver pathology. More importantly, liver engraftment was shown to be enhanced by administering a high cholate diet to further intensify the liver damage. The thought here is that enhancing liver damage will give the transplanted cells more room to engraft in the liver. However, the animals given a high cholate diet developed multiple hepatic tumors and biliary phospholipid secretion decreased, most likely due to the diet causing enhanced pathology. Animals fed the control diet had slower repopulation; however these transplant recipients were still able to completely abrogate pathology without tumor formation. Overall, these data demonstrate that hepatocyte transplantation could be useful to treat PFIC in humans.

Reduction of serum cholesterol in Watanabe rabbits has also been accomplished by porcine hepatocyte transplant (Gunsalus et al., 1997). This is the animal model for the human homozygous familial hypercholesterolemia, an LDL-receptor defect. Investigators demonstrated that the transplanted cells initially localize within the hepatic sinusoids and can over time migrate out of vessels and integrate into the parenchyma. These engrafted cells provided functional LDL receptors to lower serum cholesterol by 30–60% for the 100 day study. This report determined that they transplanted 10% of liver mass, which resulted in 1–5% survival of hepatocytes. Therefore 0.1–0.5% of liver mass corrects cholesterol by 30–60%, thus one does not need large engraftment to correct disease phenotype.

Wilson's disease was corrected by hepatocyte transplant in a rat model (Irani et al., 2001). Long-Evans Cinnamon rats have excessive accumulation of copper within hepatocytes and therefore, are a good model of human Wilson's disease. These animals have the atp7b gene mutation, an ATP-dependent copper transporter, which results in copper cytotoxicity and decreased biliary excretion of copper. The investigators pretreated all rat recipients with retrorsine to inhibit proliferation of native hepatocytes and gave the animals a partial hepatectomy to promote liver repopulation. The liver was shown to have repopulation of transplanted rat cells. This repopulation was <25% at 6 weeks, 26–40% at 4 months and 74–100% at 6 months and longer. The highly repopulated animals restored biliary copper excretion and lowered intrahepatic copper levels. Furthermore the liver histology was completely normal compared to extensive damage in non-transplanted animals. Wilson's disease was also shown to be reversed using hepatocyte transplant as an early intervention to treat the underlying disease pathologies (Malhi et al., 2002b). Rat hepatocytes were transplanted into the same rat modeled mentioned previously via intrasplenic injections. 71% of the transplanted animals became highly repopulated. Copper levels were virtually normal in 60% of these animals and the remaining 40% had substantially less copper compared to control non-transplanted animals. There were positive changes in ceruloplasmin levels; bile copper excretion capacity and liver histology were maintained as expected with the decreases in liver copper levels. This report demonstrated the efficacy of cell transplant to treat inborn errors in metabolism at an early age before extensive liver damage occurs.

Rat hepatocytes have been shown to engraft, survive and proliferate in rat livers that have CCl_4 and phenobarbital-induced cirrhosis (Gagandeep et al., 2000). This demonstrates that cell transplant is a viable option to treat liver failure and cirrhosis. This was further corroborated when rat hepatocytes were shown to rescue rats with D-galactosamine-induced acute liver failure (Baumgartner et al., 1983). Intrasplenic injections of cells and liver cell culture supernatants were shown to lead to a survival rate of 47.1% and 42.9% respectively. Injections were performed 20–28 hours after poisoning. These reports demonstrated that hepatocytes themselves, and the soluble factors that they release, improve survival of the recipient either through engrafting within the liver to provide metabolic support or by stimulating liver regeneration of the recipient's native cells or both

Cell transplant has also been shown to treat chronic liver failure as well. A rat model of CCl_4 and phenobarbital-induced chronic liver failure utilized intrasplenic injection of rat hepatocytes to treat the disease (Kobayashi et al., 2000). The rats in this model display

irreversible liver cirrhosis. Animals receiving the cells showed significant improvements in liver function and prolonged survival compare to non-transplanted controls. These data further demonstrated the utility of hepatocyte transplant to treat liver disease.

Hepatocytes have been shown to provide metabolic support, in a glucose-related function, when transplanted to rats that have received a 90% hepatectomy (Demetriou et al., 1988). Rat hepatocytes were attached to collagen coated microcarriers and were transplanted IP 3 days prior to surgery. Non-transplanted animals died within 48 hours from hypoglycemia. Rats that received cells had significantly higher blood glucose levels and 40% of them survived longer than 28 days. All control rats died within 5 days. When cells were injected IP immediately after the surgery all rats became hypoglycemic and died within 48 hours, suggesting that engraftment is required for the transplanted hepatocytes to provide metabolic support and improve survival.

Primary mouse hepatocyte transplantation improved the phenotype and extended survival in a murine model of intermediate maple syrup urine disease (Skvorak et al., 2009a; Skvorak et al., 2009b). This was also true in a mouse model of tyrosinemia type-1 using both mouse (Overturf et al., 1997) and human hepatocytes (Azuma et al., 2007). Table 3 summarizes some of the hepatocyte transplants that have been performed to treat different liver diseases within relevant animal models. This table includes the disease treated, transplant site, donor cell species and recipient species.

5.0 INTEGRATION AND INTERACTION OF TRANSPLANTED CELLS WITHIN RECIPIENT

The integration and interaction of transplanted hepatocytes in the host has been determined in a rat model (Koenig et al., 2005). When cells are transplanted via the portal vein they reach the distal portal spaces and sinusoids within 1 hour after injection. During this process they occlude the portal vein flow and thus increase the portal pressure and cause initial damage of the recipient liver. This was inferred from increases in AST, ALT and LDH 1–2 hours after transplant. The cells then traverse the endothelial barrier through mechanical disruption. The transplanted cells lose their membrane-bound gap junctions facilitated by connexin-32 down-regulation during the migration through the sinusoids. Integration of the graft in the liver parenchyma and bile canalicular activity requires up to five days and is associated with connexin-32 up-regulation. MMP-2 degrades the ECM in close proximity to donor cells, which allows space for cell proliferation of the graft. Cells that remain in the portal vein are removed by polymorphonuclear leukocytes and macrophages.

Primary hepatocytes have been transplanted via many different avenues. These include: peritoneal cavity, omentum, mesentery, spleen, portal vein, kidney capsule and lung.

6.0 METHODS TO INCREASE REPOPULATION – GIVING DONOR CELLS PROLIFERATIVE ADVANTAGE

There have been many studies that have investigated ways to increase repopulation of the recipient liver with donor hepatocytes. Methods to increase repopulation include: genetic

manipulation of the recipient through delivery of a virus expressing urokinase plasminogen activator to breakdown the ECM and give the transplanted cells more space to engraft (Azuma et al., 2007), growth factor administration in concordance with cell transplantation (VEGF) to help increase migration past endothelial cells (Shani-Peretz et al., 2005), pretreatment with partial hepatectomy to stimulate expression of soluble factors in the body that lead to liver regeneration, pretreatment with retrorsine which inhibits proliferation of the native hepatocytes through alkylation of DNA without extensive toxicity and allows transplanted cells to be the only cells that can proliferate, pretreatment with CCl_4 or radiation to damage the host liver to allow transplanted cells to have a growth advantage over native cells, administration of vasodilators to promote migration through liver sinusoids, immunosuppression (Groth et al., 1977), preconditioning with radiation and ischemia-reperfusion (Malhi et al., 2002a) to damage native cells and promote proliferation of transplanted cells through oxidative DNA damage and finally hepatic stimulatory substances obtained from regenerating porcine livers (Jiang et al., 1993). Table 4 summarizes some of the methods to increase donor cell repopulation and the species of the donor and recipient. Humanized mouse models for the study and treatment of liver disease represent a novel tool for researchers. These models are more extensively reviewed by Strom and colleagues (Strom et al., 2010).

Some liver-based inborn errors in metabolism may provide a built in selective growth advantage of donor cells that may make the liver more susceptible to donor hepatocyte engraftment and repopulation. This is due to the nature of the disease and the associated hepatocellular damage to native cells. These diseases include: tyrosinemia, progressive familial cholestasis type 2 and 3, Wilson's disease and alpha-1-antitrypsin disease.

7.0 HEPATOCYTE TRANSPLANTS IN HUMANS – THE CLINICAL EXPERIENCE

Hepatocyte transplantation was transitioned to the clinic to investigate if patients would respond to this new therapy after showing promise in various liver-based metabolic disease and liver failure animal models. Intraportal injection is the preferred method of delivery for clinical hepatocyte transplantation; however alternative sites include the spleen, pancreas, peritoneal cavity and subrenal capsule. Transplantation of cells through the portal vein can cause portal hypertension and formation of hepatocyte thrombi; however this can be minimized by limiting the number of cells per infusion and the rate at which the cells are delivered. To increase the total number of cells given to a patient multiple infusions can be performed.

Initially researchers believed transplantation of cells would not be as immunogenic as OLT; however this appears not to be the case. There is no consensus as to what is the best immunosuppressive protocol but most transplants follow the protocol used for OLT, which includes combination of tacrolimius (with or without steroids) and monoclonal antibodies, such as interleukin 2 receptor antibodies.

In animals, engraftment can be assessed rather easily by ensuring the transplanted cells have a unique reporter protein the recipient liver is lacking. Transplanted cells can then be

identified by ICC, in situ hybridization, real time RT-PCR or flow cytometry. In humans, it is more difficult to assess engraftment. Indirect evidence includes positive changes in the symptoms of the patients and changes in enzyme activity. For example patients with urea cycle disorders show extended decreases in serum ammonia levels and patients with CN-1 show extended decreases in serum bilirubin levels after hepatocyte transplant. Direct evidence includes the identification of donor sex chromosomes or HLA antigens in cases of donor-recipient sex mismatch or HLA mismatch. In patients that fit this category who receive eventual OLT, livers can be obtained to perform ICC, in situ hybridization or real time RT-PCR to test for the presence of donor-derived cells.

Theoretically, all patients that are afflicted by liver-based metabolic disorders resulting from a mutation within an important metabolic protein can benefit from hepatocyte transplant. Transplanted cells will be genetically competent in whatever protein is mutated within the patient and when they repopulate the recipient liver they will provide metabolic support that results in the symptoms of the disease being alleviated. The indication for hepatocyte transplantation is currently based on severity of disease or quality of life, with the aim being to avoid or postpone liver transplantation whether due to genetic defects or acute or chronic liver failure (Dhawan et al., 2010). The following paragraphs will describe all reports of clinical hepatocyte transplants that have occurred worldwide since 1993. Most transplants have been performed with adult hepatocytes; however some have been performed with hepatocytes isolated from livers of aborted fetuses (fetal hepatocytes). The first report of adult hepatocyte transplant occurred when Mito and colleagues treated 9 patients with cirrhosis and 1 patient with chronic active hepatitis (Mito, 1993). In these studies, autotransplantation was performed. Cells were isolated from the patients with cirrhosis and their own cells were tranplsnated back into them to provide metabolic support. Investigators reported the presence of cells in the spleens of 8 patients up to 11 months post transplant. One patient’s ascites resolved. In 1997, Strom and colleagues were the first to report the use of allogenic hepatocytes for the cellular therapy of liver disease (Strom et al., 1997b). In these initial studies, investigators were looking to bridge terminal liver failure patients to OLT. Liver failure in these five patients was caused by alpha 1-antitrypsin (A1AT) deficiency, hepatitis C, total parenteral nutrition / sepsis, acute dilantin toxicity, and hepatitis B. Three patients saw full recovery after being successfully bridged to OLT. One patient died due to complications associated with an intracranial bleed. The final patient succumbed to their condition when life support was elected to be terminated by their family.

CN-1 is characterized by patients who experience hyperbilirubinemia caused by a mutation in the UDP-glucuronosyltransferase 1A1 (UGT1A1) gene. This gene encodes the enzyme that is responsible for conjugation of bilirubin to glucuronide for excretion in urine and bile. Since bilirubin crosses the blood-brain barrier, high levels of circulating bilirubin cause brain damage (kernicterus). Bilirubin is formed from the breakdown of hemoproteins, such as hemoglobin. Bilirubin is water insoluble and therefore cannot be cleared from the body unless it becomes water soluble. Glucuronidation of the propionic acid carboxyls of bilirubin disrupts hydrogen bonding and makes the molecule water soluble and rapidly excretable in the bile. Except for liver transplantation, the only therapy for CN-1 patients is phototherapy. Patients must be exposed for 6–12 hrs per day, beneath lamps that emit a blue light (420–470 nm). This light penetrates the skin and converts some of the bilirubin to photo-activated

isomers (photobilirubin and lumirubin) that are more water-soluble than the parent compound and more readily excreted. This therapy becomes less and less effective as the patients become older as their surface area to body mass ratio decreases, and changes in the thickness of the skin also make phototherapy less effective. Currently the only curative treatment for CN-1 is OLT. However, since 1998, 10 patients ranging from 1 to 11 years have been treated with 15 million to 7.5 billion hepatocytes (1 patient received CD326+ fetal hepatocytes) (Allen et al., 2008; Ambrosino et al., 2005; Darwish et al., 2004; Dhawan et al., 2006; Fox et al., 1998; Khan et al., 2008b; Lysy et al., 2008; Meyburg et al., 2010). Patients saw a 20–50% reduction in serum bilirubin, increased the amount of conjugated bilirubin and decreased their phototherapy. Only 1 of 10 patients did not benefit from the transplant. However no patients were completely corrected with cells alone. All patients were ultimately referred for whole organ transplant.

Familial hypercholesterolemia (FH) is characterized by patients with abnormally high levels of low density lipoprotein (LDL) in their blood serum. Patients generally have mutations in the LDLR gene that encodes the LDL receptor protein. LDLR is responsible for removing LDL from the circulation. Heterozygous FH can lead to early onset cardiovascular disease around the age of 30 to 40 and can be treated with statins, bile acid sequestrants or other hypolipidemic agents. On the other hand, homozygous FH can cause severe cardiovascular disease in early childhood and often does not respond to conventional therapies and may have to be treated with LDL apheresis or eventual OLT. In 1995 Grossman and colleagues transplanted 5 patients from the ages of 7 to 41 with 1 to 3.2 billion hepatocytes (Grossman et al., 1995). Researchers saw up to a 20% reduction in LDL in 3 patients. The other 2 patients did not respond to therapy.

Factor VII deficiency is a disorder caused by the absence of this coagulation factor, which is one of the critical components of the coagulation cascade that results in the clotting of blood. Patients that have this deficiency have a mutation within the factor VII protein that makes it non-functional. Only homozygotes or compound heterozygotes (2 different mutations within the gene) develop hemorrhagic syndrome, heterozygotes are asymptomatic. Patients develop intracerebral hemorrhages, hemarthroses, cutaeneous-mucosal hemorrhages or hemorrhages provoked by a surgical intervention. Severity of disease depends on type of mutation within the factor VII gene (Giansily-Blaizot et al., 2004). In 2004 Dhawan et al. transplanted 1 3-month old patient and 1 35-month old patient with 1.1 and 2.2 billion cells respectively (Dhawan et al., 2004). The doctors saw a 70% reduction in factor VII requirements after 6 months; however both patients eventually received an OLT.

Glycogen storage disease is characterized by a defect in the processing of glycogen. This inborn error in metabolism results from a mutation in one of the enzymes responsible for the breakdown of glycogen causing impaired protein activity. One type of glycogen storage disease is caused by a mutation in the G6Pase gene, which is responsible for the process of glycogen degradation and gluconeogenesis in the liver. This mutation prevents the production of free glucose molecules and leads to hypoglycemia, abnormal blood sugar levels between meals without constant feedings, the buildup of lactate, uric acids and triglycerides, hepatomegaly, developmental delay and seizures leading to impaired

breathing, coma and death. Patients with this disorder must adhere to a strict dietary regimen with constant feeding every one to four hours. Children often have a nasogastric feeding tube placed on them for the use of a continuous feeding pump; this is especially true at night. The strict diet is so the body does not receive glucose. If it does, the body will store the glucose as glycogen, which will end up building up in high amounts in hepatocytes because it cannot be broken down. This hyper-glycogen state can cause hepatomegaly and eventual liver damage. OLT is the only known curative treatment. Severity of the disease depends on what gene is mutated and the type of mutation. Two patients have been treated for glycogen storage disease with hepatocyte transplant (Lee et al., 2007; Muraca et al., 2002). Patients were 18 and 47 years old and received 6 and 2 billion cells respectively. They were able to be put on a normal diet and their fasting time increased after transplantation. The 18 year old had normal G6Pase activity up to 7 months after transplant.

Infantile Refsum's disease is characterized by mutations in genes that encode peroxins, which are important proteins for normal peroxisome assembly and function, therefore making this disease a peroxisome biogenesis disorder. The most common mutations for the disease occur in the PEX1, PEX3, PEX6, PEX12 and PEX26 genes and severity of the disease depends on what gene is mutated and where the mutation is within the gene. As a result of mutation, these proteins become less active or completely inactive altogether. Since one of the major functions of the peroxisome is the catabolism of very long chain and branched chain fatty acids, non functioning PEX genes lead to the accumulation of these lipids within cells and tissues. Abnormal peroxisome biogenesis can lead to a reduction of myelin in the central nervous system (CNS) leading to abnormal CNS function and development as well as hepatomegaly, eventually causing liver damage. There is currently no standard treatment for this disease. In 2003 a four year old female received 2 billion hepatocytes to treat her underlying Infantile Refsum's disease (Sokal et al., 2003). She saw a 40% reduction in pipecholic acid at 18 months post transplant, thus demonstrating indirect evidence of hepatocyte engraftment and function.

Progressive familial intrahepatic cholestasis (PFIC) is a disease characterized by defects in genes that encode proteins of biliary epithelial transporters. These defects cause problems in the export of bile out of hepatocytes and eventually lead to progressive cholestasis that presents in early childhood. This leads to eventual hepatic failure and need for OLT. Specifically, PFIC-2 is caused by mutation in the bile salt export pump (BSEP) that results in the retention of bile salts within hepatocytes causing hepatocelluar damage through cholestasis. 2 patients with PFIC-2 were transplanted in 2006 to treat their underlying disease (Dhawan et al., 2006). These patients were 16 and 32 months old and were transplanted with 400 million and 200 million cells respectively. Neither patient showed a biochemical benefit from the transplantation, likely because of the presence of cirrhosis at the time of transplant, and both patients were referred for OLT.

Biliary atresia is a congenital or acquired disease associated with the common bile duct between the liver and the small intestine being blocked or absent leading to progressive cholestasis and conjugated hyperbilirubinemia. The acquired type most often occurs due to an underlying autoimmune disease, this being one of the main causes of rejection after OLT. As mentioned earlier, patients have progressive cholestasis with conjugated

hyperbilirubinemia, which causes classical liver failure symptoms: jaundice, malabsorption with developmental delays, fat-soluble vitamin deficiencies, and hyperlipidemia eventually causing cirrhosis and liver failure. Since the bilirubin is conjugated it cannot cross the blood brain barrier, thus brain damage caused by kernicterus is not a concern. The cause of congenital biliary atresia is unknown and the only curative treatment is OLT. In 2008, 1 1-year old patient was treated for biliary atresia by the transplantation of 12 million CD326+ fetal hepatocytes. The patient saw a 3-fold reduction in total bilirubin and an 8-fold reduction in conjugated bilirubin. Furthermore, ALT levels decreased and hepatobiliary scintigraphy showed an increase in liver cell function at 2 months.

Ornithine Transcarbamylase (OTC) deficiency is a urea cycle disorder characterized by a mutation in the OTC gene that encodes the OTC protein essential for the clearance of ammonia from the blood. Ammonia appears in the blood as a normal toxic breakdown product of the body's use of protein and amino acids. Therefore patients that have a mutation within their OTC gene have low functioning or completely non-functioning OTC enzyme activity leading to hyperammonemia and a buildup of orotic acid. The buildup of orotic acid is due to increasing concentration of carbamoyl phosphate entering the pyrimidine synthesis pathway; if the urea cycle was working properly carbamoyl phosphate concentration would remain constant and thus would not enter this alternative biochemical pathway. Hyperammonemia, if left untreated, can cause seizures, loss of appetite, lack of energy, developmental delay, brain damage, irrational behavior, mood swings, coma or death. Management of OTC is done mainly through the administration of a low protein diet. The only curative treatment is OLT. From 1997 to 2009 6 patients with OTC have been treated for their disease by hepatocyte transplant (Horslen et al., 2003; Meyburg et al., 2009; Puppi et al., 2008; Stephenne et al., 2005; Strom et al., 1997a). Age of patients ranged from 6 hours to 5 years and they received anywhere from 600 million to 9 billion cells. All patients saw a decrease in serum ammonia levels associated with psychomotor improvement and an increase in protein tolerance administered by a normal diet. One patient was confirmed to have 0.5% normal liver OTC activity once his liver was explanted when death occurred at day 42 post transplant. Cause of death in this patient was not due to cell transplant. Some patients were also confirmed to have normal urinary orotic acid concentrations. All surviving patients at the end of the various studies wre listed for OLT.

Argininosuccinate lyase (ASL) deficiency is a urea cycle disorder characterized by a mutation in the ASL gene that encodes the ASL protein essential for the clearance of ammonia from the blood. ASL deficiency leads to hyperammonemia with symptoms and consequences very similar to other urea cycle disorders. ASL is an enzyme that cleaves argininosuccinate to produce a molecule of fumarate and arginine in the urea cycle. A mutation in the ASL protein will lead to elevated levels of argininosuccinate resulting in argininosuccinic aciduria and clearance through the urine. Elevated argininosuccinate in the urine is a main symptom of disease. Although argininosuccinate contains two waste nitrogen molecules normally found in urea, patients still experience urea cycle disorder systems. OLT is the only curative treatment. 1 42-month old patient was transplanted with 4.7 billion hepatocytes to treat the underlying ASL deficiency (Stephenne et al., 2006). The patient saw a decrease in serum ammonia levels associated with psychomotor catch up. Although OLT was performed 18 months post cell transplant, donor cells were still detected in the liver.

Carbamoyl phosphate synthetase (CPS) deficiency type-1 is a urea cycle disorder characterized by a mutation in the CPS gene that encodes the CPS protein essential for the clearance of ammonia from the blood. CPS-1 deficiency leads to hyperammonemia with symptoms and consequences very similar to other urea cycle disorders. CPS-1 is the enzyme that catalyzes the reaction of 2 molecules of ammonia and 2 molecules of bicarbonate to form carbamoyl phosphate. This is the initial reaction in the urea cycle to clear waste nitrogen from the body through the production of urea. Thus patients with mutation in the CPS-1 gene have inactive CPS-1 enzyme activity resulting in ammonia accumulation in the serum. OLT is the only curative treatment. In 2009, 1 2.5-month old patient was treated for CPS-1 by the transplantation of 1.4 billion hepatocytes (Meyburg et al., 2009). The patient saw a decrease in serum ammonia levels and an increase in urea production for 11 months. At the end of the study the patient was still on the OLT waiting list.

Citrullinemia is a urea cycle disorder characterized by a mutation in the argininosuccinate synthetase (ASS) gene that encodes the ASS protein essential for the clearance of ammonia from the blood. Citrullinemia leads to hyperammonemia with symptoms and consequences very similar to other urea cycle disorders. ASS is the enzyme that catalyzes the reaction of citrulline and aspartate to form argininosuccinate. Thus patients with mutation in the ASS gene have inactive enzyme activity resulting in ammonia accumulation in the serum. OLT is the only curative treatment. In 2009, 1 36-month old patient was treated for citrullinemia by the transplantation of 1.5 billion hepatocytes (Meyburg et al., 2009). The patient saw a return to normal ammonia blood serum levels as well as a 40% increase in urea production with an increase in protein intake due to dietary changes.

A1AT deficiency is a disorder characterized by a mutation in the A1AT gene that encodes the A1AT protein. This protein is essential for inhibiting a wide variety of proteases, which protects tissues from enzymes of inflammatory cells and thus has anti-inflammatory properties. Mutations in the A1AT gene can lead to decreased A1AT activity and protein misfolding, which results in abnormal accumulation of it within hepatocytes. Disease severity is determined by the type of mutation as well as if the patient is a heterozygous or homozygous mutant. A1AT deficiency can cause emphysema or chronic obstructive pulmonary disease (COPD) as well as various liver disorders and complications. OLT and/or lung transplant is the only curative treatment. In 1997, a 52-year old patient was transplanted with 22 million hepatocytes to bridge him to OLT. Two days after cell transplant, the patient received an OLT and kidney transplant and fully recovered. Wild-type A1AT levels were noted in the patient’s serum prior to OLT, with a peak at 92 mg/dl. These wild-type levels were attributed to the metabolic support of the transplanted cells.

Liver failure is the inability of the liver to perform its normal synthetic and metabolic functions to maintain normal physiology and homeostasis and comes in two forms: acute and chronic. Acute liver failure (ALF) is defined as the rapid development of hepatocellular dysfunction and chronic liver failure usually occurs in the context of cirrhosis (liver fibrosis). Since 1993 the following types of liver failure have been treated by hepatocyte transplant: drug-induced ALF (16 patients; 5 received fetal hepatocytes), viral-induced ALF (11 patients; 1 received fetal hepatocytes), idiopathic ALF (4 patients), mushroom poisoning-induced ALF (1 patient), post surgical ALF (1 patient), ALF induced by acute

fatty liver of pregnancy (1 patient), and ALF induced by total parenteral nutrition / sepsis (1 patient). Routes of hepatocytes transplant in these patients include intraperitoneal, intrasplenic and portal vein. Some patients died from their underlying disease, some survived until an organ became available for OLT, and in rare instances, some of the patient's liver failure resolved.

Tables 5 and 6 summarize all current reports, to the knowledge of this investigator, of clinical hepatocyte transplants performed worldwide since 1993 broken down by disease. Table 5 summarizes the transplants performed on metabolic disease patients. The table includes the specific reference where all data can be found along with the age of patient, total number of cells transplanted, the findings associated with the study and the outcomes of the patients. Table 6 summarizes the transplants performed on liver failure patients. The table includes the specific reference where all data can be found along with the age of patient, total number of cells transplanted, route of delivery and the outcomes of the patients.

8.0 OVERALL CONCLUSIONS

It has been concluded that cell therapy shows promise in reversing clinical symptoms associated with liver failure and many metabolic diseases; however issues with cell source, size, viability, cryopreservation, engraftment, immunosuppression and family compliance with disease specific therapy need to be addressed. Moreover the lack of controlled trials during the clinical hepatocyte transplants performed to date makes it difficult to interpret findings and compare different studies. No patients to date have been completely cured of their disease; however cell transplant has shown the best outcomes in patients with liver-based inborn errors in metabolism. These patients demonstrate obvious positive effects on various disease-specific symptoms. Cell-based therapy in some cases helped to reverse liver failure, however once again the lack of proper controls make it difficult to say patient's liver failure was reversed due to hepatocyte transplant.

Hepatocyte transplant has been shown to have potential to serve as a "bridge to transplant" for both metabolic liver-based diseases and liver failure therefore taking some of the burden off the OLT waiting list and allowing patients to survive longer until an organ for transplant becomes available.

Current sources of hepatocytes for transplant are scarce and tissues that are available are livers that are unused or organs unsuitable for OLT. Livers not suitable for whole organ transplant include tissues with excessive steatosis and extended cold ischemic times. This fact demonstrates the limited supply of donor tissue that can provide high quality cells, thus leading to the transplantation of hepatocytes isolated from marginal livers. To compound issues, isolated primary adult human hepatocytes rarely, if ever, proliferate *in vitro* after cellular isolation. This means isolated cells cannot be expanded to treat more patients.

Taken together, alternative cell sources and new methods to improve cell engraftment and liver repopulation need to be investigated in order to make cell-based therapeutics to treat liver diseases a regular clinical procedure. Alternative cell sources for transplant will increase the number of patients that can be treated and protocols to increase engraftment will allow for fewer cells to be delivered to patients and therefore more patients to be treated

overall. Stem cells, specifically hESCs and hiPSCs, are potentially attractive cell source for hepatocyte transplant because they represent a potential constant and unlimited source of cells. However, concerns for tumorigenicity and the low level of mature liver gene expression currently obtained in stem cell-derived hepatocytes indicate that much needs to improve before stem cells become viable options for clinical therapy.

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References

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]

Ambrosino G, Varotto S, Strom SC, Guariso G, Franchin E, Miotto D, Caenazzo L, Basso S, Carraro P, Valente ML, D'Amico D, Zancan L, D'Antiga L. Isolated hepatocyte transplantation for Crigler-Najjar syndrome type 1. Cell Transplant. 2005; 14:151–157. [PubMed: 15881424]

OPTN / SRTR 2010 Annual Data Report. Department of Health and Human Services, Health Resources and Services Administration, Healthcare System Bureau, Division of Transplantation; Rockville, MD: 2011.

*. Organ Datasource Liver. Health and Resources and Services Administration, U.S. Department of Health & Human Services; 2012. This reference does not have a predefined format and therefore uses the "Generic" format

Azuma H, Paulk N, Ranade A, Dorrell C, Al-Dhalimy M, Ellis E, Strom S, Kay MA, Finegold M, Grompe M. Robust expansion of human hepatocytes in Fah−/−/Rag2−/−/Il2rg−/−mice. Nat Biotechnol. 2007; 25:903–910. [PubMed: 17664939]

Ballinger WF, Lacy PE. Transplantation of intact pancreatic islets in rats. Surgery. 1972; 72:175–186. [PubMed: 4262169]

Baumgartner D, LaPlante-O'Neill PM, Sutherland DE, Najarian JS. Effects of intrasplenic injection of hepatocytes, hepatocyte fragments and hepatocyte culture supernatants on D-galactosamine-induced liver failure in rats. Eur Surg Res. 1983; 15:129–135. [PubMed: 6345168]

Berry MN, Friend DS. High-yield preparation of isolated rat liver parenchymal cells: a biochemical and fine structural study. J Cell Biol. 1969; 43:506–520. [PubMed: 4900611]

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

Bismuth H, Houssin D. Reduced-sized orthotopic liver graft in hepatic transplantation in children. Surgery. 1984; 95:367–370. [PubMed: 6367125]

Darwish AA, Sokal E, Stephenne X, Najimi M, de de Goyet JV, Reding R. Permanent access to the portal system for cellular transplantation using an implantable port device. Liver Transpl. 2004; 10:1213–1215. [PubMed: 15350017]

De Vree JM, Ottenhoff R, Bosma PJ, Smith AJ, Aten J, Oude Elferink RP. Correction of liver disease by hepatocyte transplantation in a mouse model of progressive familial intrahepatic cholestasis. Gastroenterology. 2000; 119:1720–1730. [PubMed: 11113093]

Demetriou AA, Reisner A, Sanchez J, Levenson SM, Moscioni AD, Chowdhury JR. Transplantation of microcarrier-attached hepatocytes into 90% partially hepatectomized rats. Hepatology. 1988; 8:1006–1009. [PubMed: 3047034]

Dhawan A, Mitry RR, Hughes RD. Hepatocyte transplantation for liver-based metabolic disorders. J Inherit Metab Dis. 2006; 29:431–435. [PubMed: 16763914]

Dhawan A, Mitry RR, Hughes RD, Lehec S, Terry C, Bansal S, Arya R, Wade JJ, Verma A, Heaton ND, Rela M, Mieli-Vergani G. Hepatocyte transplantation for inherited factor VII deficiency. Transplantation. 2004; 78:1812–1814. [PubMed: 15614156]

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]

Emond JC, Whitington PF, Thistlethwaite JR, Alonso EM, Broelsch CE. Reduced-size orthotopic liver transplantation: use in the management of children with chronic liver disease. Hepatology. 1989; 10:867–872. [PubMed: 2807168]

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

Fisher RA, Strom SC. Human hepatocyte transplantation: worldwide results. Transplantation. 2006; 82:441–449. [PubMed: 16926585]

Fox IJ, Chowdhury JR, Kaufman SS, Goertzen TC, Chowdhury NR, Warkentin PI, Dorko K, Sauter BV, Strom SC. Treatment of the Crigler-Najjar syndrome type I with hepatocyte transplantation. N Engl J Med. 1998; 338:1422–1426. [PubMed: 9580649]

Gagandeep S, Rajvanshi P, Sokhi RP, Slehria S, Palestro CJ, Bhargava KK, Gupta S. Transplanted hepatocytes engraft, survive, and proliferate in the liver of rats with carbon tetrachloride-induced cirrhosis. J Pathol. 2000; 191:78–85. [PubMed: 10767723]

Giansily-Blaizot M, Verdier R, Biron-Adreani C, Schved JF, Bertrand MA, Borg JY, Le Cam-Duchez V, Briquel ME, Chambost H, Pouymayou K, Dutrillaux F, Favier R, Martin-Toutain I, Verdy E, Gay V, Goudemand J, Navarro R, Durin A, d'Oiron R, Lambert T, Pernod G, Barrot C, Peynet J, Bastenaire B, Sie P, Stieltjes N, Torchet MF, de Moerloose P. Analysis of biological phenotypes from 42 patients with inherited factor VII deficiency: can biological tests predict the bleeding risk? Haematologica. 2004; 89:704–709. [PubMed: 15194538]

Goodrich EO Jr, Welch HF, Nelson JA, Beecher TS, Welch CS. Homotransplantation of the canine liver. Surgery. 1956; 39:244–251. [PubMed: 13298971]

Gramignoli R, Green ML, Tahan V, Dorko K, Skvorak KJ, Marongiu F, Zao W, Venkataramanan R, Ellis EC, Geller D, Breite AG, Dwulet FE, McCarthy RC, Strom SC. Development and application of purified tissue dissociation enzyme mixtures for human hepatocyte isolation. Cell Transplant. 2011; 21:1245–1260. [PubMed: 22080793]

Grisham JW, Leong GF, Hole BV. Heterotopic Partial Autotransplantation of Rat Liver: Technic and Demonstration of Structure and Function of the Graft. Cancer Res. 1964; 24:1474–1495. [PubMed: 14221805]

Grossman M, Rader DJ, Muller DW, Kolansky DM, Kozarsky K, Clark BJ 3rd, Stein EA, Lupien PJ, Brewer HB Jr, Raper SE, et al. A pilot study of ex vivo gene therapy for homozygous familial hypercholesterolaemia. Nat Med. 1995; 1:1148–1154. [PubMed: 7584986]

Groth CG, Arborgh B, Bjorken C, Sundberg B, Lundgren G. Correction of hyperbilirubinemia in the glucuronyltransferase-deficient rat by intraportal hepatocyte transplantation. Transplant Proc. 1977; 9:313–316. [PubMed: 405772]

Gunsalus JR, Brady DA, Coulter SM, Gray BM, Edge AS. Reduction of serum cholesterol in Watanabe rabbits by xenogeneic hepatocellular transplantation. Nat Med. 1997; 3:48–53. [PubMed: 8986740]

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]

Hamaguchi H, Yamaguchi Y, Goto M, Misumi M, Hisama N, Miyanari N, Mori K, Ogawa M. Hepatic biliary transport after hepatocyte transplantation in Eizai hyperbilirubinemic rats. Hepatology. 1994; 20:220–224. [PubMed: 8020892]

Hiraoka T, Hagerstrand I, Bengmark S. Attempts at autologous transplantation of sliced liver to the subcutaneous tissue. Eur Surg Res. 1983; 15:37–44. [PubMed: 6341072]

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]

Irani AN, Malhi H, Slehria S, Gorla GR, Volenberg I, Schilsky ML, Gupta S. Correction of liver disease following transplantation of normal rat hepatocytes into Long-Evans Cinnamon rats modeling Wilson's disease. Mol Ther. 2001; 3:302–309. [PubMed: 11273771]

Jiang B, Kasai S, Sawa M, Hirai S, Yamamoto T, Ebata H, Mito M. Beneficial effect of hepatic stimulatory substances on the survival of intrasplenically transplanted hepatocytes. Cell Transplant. 1993; 2:325–329. [PubMed: 8162274]

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]

Khan AA, Parveen N, Mahaboob VS, Rajendraprasad A, Ravindraprakash HR, Venkateswarlu J, Rao P, Pande G, Narusu ML, Khaja MN, Pramila R, Habeeb A, Habibullah CM. Management of hyperbilirubinemia in biliary atresia by hepatic progenitor cell transplantation through hepatic artery: a case report. Transplant Proc. 2008a; 40:1153–1155. [PubMed: 18555137]

Khan AA, Parveen N, Mahaboob VS, Rajendraprasad A, Ravindraprakash HR, Venkateswarlu J, Rao P, Pande G, Narusu ML, Khaja MN, Pramila R, Habeeb A, Habibullah CM. Treatment of Crigler-Najjar Syndrome type 1 by hepatic progenitor cell transplantation: a simple procedure for management of hyperbilirubinemia. Transplant Proc. 2008b; 40:1148–1150. [PubMed: 18555136]

Kmiec Z. Cooperation of liver cells in health and disease. Adv Anat Embryol Cell Biol. 2001; 161:III–XIII. 1–151. [PubMed: 11729749]

Kobayashi N, Ito M, Nakamura J, Cai J, Hammel JM, Fox IJ. Treatment of carbon tetrachloride and phenobarbital-induced chronic liver failure with intrasplenic hepatocyte transplantation. Cell Transplant. 2000; 9:671–673. [PubMed: 11144963]

Koenig S, Stoesser C, Krause P, Becker H, Markus PM. Liver repopulation after hepatocellular transplantation: integration and interaction of transplanted hepatocytes in the host. Cell Transplant. 2005; 14:31–40. [PubMed: 15789660]

Lacy PE, Kostianovsky M. Method for the isolation of intact islets of Langerhans from the rat pancreas. Diabetes. 1967; 16:35–39. [PubMed: 5333500]

Lee KW, Lee JH, Shin SW, Kim SJ, Joh JW, Lee DH, Kim JW, Park HY, Lee SY, Lee HH, Park JW, Kim SY, Yoon HH, Jung DH, Choe YH, Lee SK. Hepatocyte transplantation for glycogen storage disease type Ib. Cell Transplant. 2007; 16:629–637. [PubMed: 17912954]

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]

Malhi H, Gorla GR, Irani AN, Annamaneni P, Gupta S. Cell transplantation after oxidative hepatic preconditioning with radiation and ischemia-reperfusion leads to extensive liver repopulation. Proc Natl Acad Sci U S A. 2002a; 99:13114–13119. [PubMed: 12244212]

Malhi H, Irani AN, Volenberg I, Schilsky ML, Gupta S. Early cell transplantation in LEC rats modeling Wilson's disease eliminates hepatic copper with reversal of liver disease. Gastroenterology. 2002b; 122:438–447. [PubMed: 11832458]

Matas AJ, Sutherland DE, Steffes MW, Mauer SM, Sowe A, Simmons RL, Najarian JS. Hepatocellular transplantation for metabolic deficiencies: decrease of plasms bilirubin in Gunn rats. Science. 1976; 192:892–894. [PubMed: 818706]

Meyburg J, Das AM, Hoerster F, Lindner M, Kriegbaum H, Engelmann G, Schmidt J, Ott M, Pettenazzo A, Luecke T, Bertram H, Hoffmann GF, Burlina A. 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]

Meyburg J, Hoerster F, Schmidt J, Poeschl J, Hoffmann GF, Schenk JP. Monitoring of intraportal liver cell application in children. Cell Transplant. 2010; 19:629–638. [PubMed: 20053320]

Mito M, Kusano M, Sawa M. Hepatocyte transplantation for hepatic failure. Transplant Rev. 1993; 7:35.

Moscioni AD, Roy-Chowdhury J, Barbour R, Brown LL, Roy-Chowdhury N, Competiello LS, Lahiri P, Demetriou AA. Human liver cell transplantation. Prolonged function in athymic-Gunn and athymic-analbuminemic hybrid rats. Gastroenterology. 1989; 96:1546–1551. [PubMed: 2653943]

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

Nakazawa F, Onodera K, Kato K, Sawa M, Kino Y, Imai M, Kasai S, Mito M, Matsushita T, Funatsu K. Multilocational hepatocyte transplantation for treatment of congenital ascorbic acid deficiency rats. Cell Transplant. 1996; 5:S23–25. [PubMed: 8889224]

Overturf K, al-Dhalimy M, Ou CN, Finegold M, Grompe M. Serial transplantation reveals the stem-cell-like regenerative potential of adult mouse hepatocytes. Am J Pathol. 1997; 151:1273–1280. [PubMed: 9358753]

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

Ringe B, Burdelski M, Rodeck B, Pichlmayr R. Experience with partial liver transplantation in Hannover. Clin Transpl. 1990:135–144. [PubMed: 2103139]

Rugstad HE, Robinson SH, Yannoni C, Tashjian AH Jr. Metabolism of bilirubin by a clonal strain of rat hepatoma cells. J Cell Biol. 1970a; 47:703–710. [PubMed: 5497547]

Rugstad HE, Robinson SH, Yannoni C, Tashjian AH Jr. Transfer of bilirubin uridine diphosphate-glucuronyltransferase to enzyme-deficient rats. Science. 1970b; 170:553–555. [PubMed: 4319199]

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

Shani-Peretz H, Tsiperson V, Shoshani G, Veitzman E, Neufeld G, Baruch Y. HVEGF165 increases survival of transplanted hepatocytes within portal radicles: suggested mechanism for early cell engraftment. Cell Transplant. 2005; 14:49–57. [PubMed: 15789662]

Skvorak KJ, Hager EJ, Arning E, Bottiglieri T, Paul HS, Strom SC, Homanics GE, Sun Q, Jansen EE, Jakobs C, Zinnanti WJ, Gibson KM. Hepatocyte transplantation (HTx) corrects selected neurometabolic abnormalities in murine intermediate maple syrup urine disease (iMSUD). Biochim Biophys Acta. 2009a; 1792:1004–1010. [PubMed: 19699299]

Skvorak KJ, Paul HS, Dorko K, Marongiu F, Ellis E, Chace D, Ferguson C, Gibson KM, Homanics GE, Strom SC. Hepatocyte Transplantation Improves Phenotype and Extends Survival in a Murine Model of Intermediate Maple Syrup Urine Disease. Mol Ther. 2009b

Sokal EM, Smets F, Bourgois A, Van Maldergem L, Buts JP, Reding R, Bernard Otte J, Evrard V, Latinne D, Vincent MF, Moser A, Soriano HE. 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]

Starzl TE, Marchioro TL, Vonkaulla KN, Hermann G, Brittain RS, Waddell WR. Homotransplantation of the Liver in Humans. Surg Gynecol Obstet. 1963; 117:659–676. [PubMed: 14100514]

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]

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]

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

Strom SC, Davila J, Grompe M. Chimeric mice with humanized liver: tools for the study of drug metabolism, excretion, and toxicity. Methods Mol Biol. 2010; 640:491–509. [PubMed: 20645070]

Strom SC, Fisher RA, Rubinstein WS, Barranger JA, Towbin RB, Charron M, Mieles L, Pisarov LA, Dorko K, Thompson MT, Reyes J. Transplantation of human hepatocytes. Transplant Proc. 1997a; 29:2103–2106. [PubMed: 9193547]

Strom SC, Fisher RA, Thompson MT, Sanyal AJ, Cole PE, Ham JM, Posner MP. Hepatocyte transplantation as a bridge to orthotopic liver transplantation in terminal liver failure. Transplantation. 1997b; 63:559–569. [PubMed: 9047152]

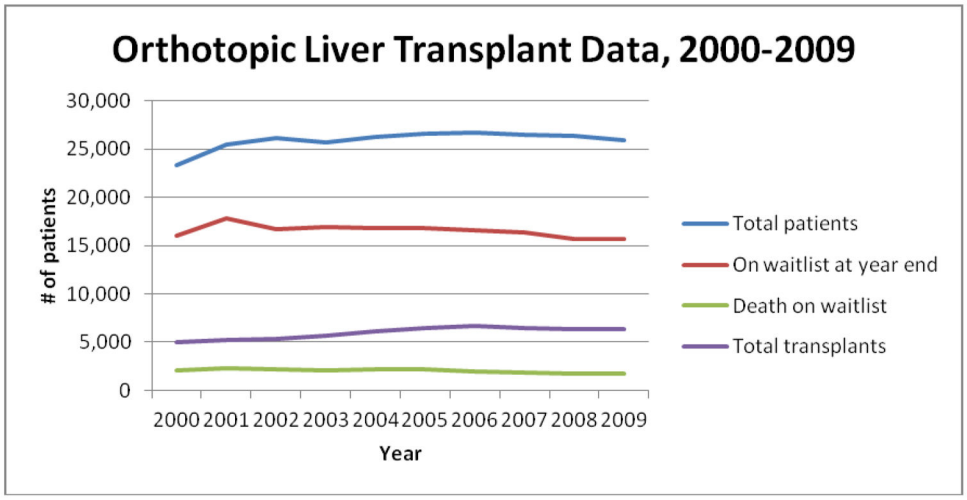

Figure 1. Orthotopic Liver Transplant Data, 2000–2009

Number of total patients on the liver transplant waitlist (blue), number of patients on waitlist at year end (red), number of patient deaths on waitlist (green) and total patients transplanted (purple) from 2000–2009.

Reproduced from the OPTN/SRTR Annual Report.

Table 1
Summary of the Liver Diseases Currently Treated by OLT

Table adapted from the OPTN/SRTR 2010 Annual Data Report (2011).

Liver Diseases Treated by OLT
Non-cholestatic Cirrhosis
Cholestatic Liver Disease/Cirrhosis
Biliary Atresia
Acute Hepatic Necrosis
Metabolic Diseases: Wilson's Disease, Hemochromatosis-Hemosiderosis, Tyrosinemia, Primary Oxalosis, Glycogen Storage Diseases, Hyperlipidemia, Crigler-Najjar Syndrome, Carbamoyl Phosphate Synthase Type-1 Deficiency, Ornithine Transcarbamylase Deficiency, Clotting Factor Deficiencies, Familial Hypercholesterolemia, Infantile Refsum's Disease, Progressive Familial Intrahepatic Cholestasis, Argininosuccinate Lyase Deficiency, Citrullinemia
Malignant Neoplasms
Other: Cystic Fibrosis, Budd-Chiari Syndrome, Neonatal Hepatitis, Congenital Hepatic Fibrosis, Graft vs. Host Disease, Chronic or Acute Liver Failure, Benign Tumor

Table 2
Benefits of Cell Transplantation over OLT

Adapted from Dhawan et al. (Dhawan et al., 2010).

OLT	Cell Tx
One or two patients maximum per one donor liver	Multiple patients can be treated from one donor liver
Major surgery	Less invasive surgery compared to OLT
Organs have to be immediately transplanted	Hepatocytes can be cryopreserved for long term storage and transplantation on demand
Graft failure is life threatening	Graft failure reverts patient to pre-transplantation state; not immediately life threatening
Native liver removed	Native liver remains, allowing for its potential recovery
Curative treatment, but requires patient to be on life-long immunosuppression (in most cases)	Patients can be potentially weaned off immunosuppression if methods become available

Table 3
Hepatocyte Transplants Performed in Relevant Liver Disease Animal Models

Table summarizes some of the hepatocyte transplants that have been performed in relevant liver disease animal models. Information includes disease treated, transplant site, donor cell species, recipient species and reference of paper according to first author last name.

Disease Treated	TX Site	Donor Species	Recipient Species	References
CN-1	SubQ	Rat	Rat	Rugstad et al.(Rugstad et al., 1970a), Matas et al. (Matas et al., 1976)
CN-1	Portal vein	Rat	Rat	Groth et al. (Groth et al., 1977)
CN-1	IP	Human	Rat	Moscioni et al. (Moscioni et al., 1989)
Analbuminemia	IP	Human	Rat	Moscioni et al. (Moscioni et al., 1989)
Dubin Johnson Syndrome	Portal Vein	Rat	Rat	Hamaguchi et al. (Hamaguchi et al., 1994)
Ascorbic Acid Deficiency	Portal vein, spleen, omentum, mesentery	Rat	Rat	Nakazawa et al. (Nakazawa et al., 1996)
PFIC (type 3)	Spleen	Mice	Mice	De Vree et al. (De Vree et al., 2000)
Homozygous familial Hypercholesterolemia	Portal vein	Porcine	Rabbit	Gunsalus et al. (Gunsalus et al., 1997)
Wilson's Disease	Spleen	Rat	Rat	Irani et al. (Irani et al., 2001), Malhi et al. (Malhi et al., 2002b)
Maple Syrup Urine Disease	Direct Liver	Mouse	Mouse	Skvorak et al. (Skvorak et al., 2009a), Skvorak et al. (Skvorak et al., 2009b)
Tyrosinemia Type-1	Spleen	Human	Mouse	Azuma et al. (Azuma et al., 2007)
Tyrosinemia Type-1	Spleen	Mouse	Mouse	Overturf et al. (Overturf et al., 1997)
Acute Liver Failure	Spleen	Rat	Rat	Gagandeep et al. (Gagandeep et al., 2000), Baumgartner et al. (Baumgartner et al., 1983)
Chronic Liver Failure	Spleen	Rat	Rat	Kobayashi et al. (Kobayashi et al., 2000)
Acute Liver Insufficiency	IP	Rat	Rat	Demetriou et al.(Demetriou et al., 1988)

Table 4
Methods to Increase Cellular Engraftment in Relevant Animal Models

Table summarizes some of the methods to increase cellular engraftment in relevant liver disease animal models. Information includes method to increase engraftment, donor cell species, recipient species and reference of paper according to first author last name.

Method to Increase Engraftment	Donor Species	Recipient Species	References
Removal of thymus	Human	Rat	Moscioni et al. (Moscioni et al., 1989)
Partial hepatectomy	Rat	Rat	Hamaguchi et al. (Hamaguchi et al., 1994), Irani et al. (Irani et al., 2001)
Retrorsine	Rat	Rat	Irani et al. (Irani et al., 2001)
CCl_4	Rat	Rat	Gagandeep et al. (Gagandeep et al., 2000)
Phenobarbital	Rat	Rat	Gagandeep et al. (Gagandeep et al., 2000)
D-galactosamine	Rat	Rat	Baumgartner et al. (Baumgartner et al., 1983)
uPA expressing virus	Human	Mouse	Azuma et al. (Azuma et al., 2007)
Immunosuppression	Mouse	Mouse	Groth et al. (Groth et al., 1977)
Radiation	Rat	Rat	Malhi et al. (Malhi et al., 2002a)
Ischemia/Reperfusion	Rat	Rat	Malhi et al. (Malhi et al., 2002a)
Hepatic Stimulatory Substances	Rat	Rat	Jiang et al. (Jiang et al., 1993)

Table 5
Clinical Hepatocyte Transplants in Metabolic Disease Patients Worldwide

Table summarizes all reports of clinical hepatocyte transplants performed worldwide since 1993 to treat various liver-based inborn errors of metabolism and acute liver failure. Table is broken down by each disease that has been treated. Cells transplanted are hepatocytes unless specified otherwise. Adapted from Dhawan et al. (Dhawan et al., 2010).

Disease: CN-1

Reference	Age of Patient	# Cells TX ($\times 10^9$)	Findings	Outcome
Fox et al. (Fox et al., 1998)	10 years	7.5	↓ 50% bilirubin, UGT1A1 activity in liver	OLT after 4 years
Darwish et al. (Darwish et al., 2004)	8 years	7.5	↓ 40% bilirubin for 6 months	OLT after 20 months
Ambrosino et al. (Ambrosino et al., 2005)	9 years	7.5	↓ 30% bilirubin for a few weeks	OLT after 5 months
Dhawan et al. (Dhawan et al., 2006)	18 months 42 months	4.3 2.1	↓ 40% bilirubin No clear benefit	OLT after 8 months NA
Allen et al. (Allen et al., 2008)	8 years	1.4	↓ 30% bilirubin, ↓ phototherapy	OLT after 11 months
Lysy et al. (Lysy et al., 2008)	9 years 1 year	6.1 2.6	↓ 35% bilirubin for 6 months ↓25% bilirubin, ↓ phototherapy for 4 months	OLT waiting list OLT after 4 months
Khan et al. (Khan et al., 2008b)	2 years	0.015 CD326+ Fetal hepatocytes	↓ 50% bilirubin, ↑ 5-fold conjugated bilirubin	NA
Meyburg et al. (Meyburg et al., 2010)	11 years	7.2	↓ 20% bilirubin	OLT waiting list

Disease: Familial Hypercholesterolemia

Reference	Age of Patient	# Cells TX ($\times 10^9$)	Findings	Outcome
Grossman et al. (Grossman et al., 1995)	Five total w/ages between 7 and 41 years	1.0–3.2	Up to ↓ 20% LDL in 3 patients	NA

Disease: Factor VII Deficiency

Reference	Age of Patient	# Cells TX ($\times 10^9$)	Findings	Outcome
Dhawan et al. (Dhawan et al., 2004)	3 months 35 months	1.1 2.2	↓ 70% rFVII requirement for 6 months ↓ 70% rFVII requirement for 6 months	OLT after 7 months OLT after 8 months

Disease: Glycogen Storage Disease Type-1

Reference	Age of Patient	# Cells TX ($\times 10^9$)	Findings	Outcome
Muraca et al. (Muraca et al., 2002)	47 years	2.0	Normal diet, ↑ fasting time	NA
Lee et al. (Lee et al., 2007)	18 years	6.0	Normal G6Pase activity up to 7 months	NA

Disease: Infantile Refsum's Disease

Reference	Age of Patient	# Cells TX ($\times 10^9$)	Findings	Outcome
Sokal et al. (Sokal et al., 2003)	4 years	2.0	↓ 40% pipecholic acid after 18 months	NA

Disease: Progressive Familial Intrahepatic Cholestasis Type-2				
Reference	**Age of Patient**	**# Cells TX ($\times 10^9$)**	**Findings**	**Outcome**
Dhawan et al. (Dhawan et al., 2006)	32 months 16 months	0.2 0.4	No benefit, cirrhosis established No benefit, cirrhosis established	OLT after 5 months OLT after 14 months

Disease: Biliary Atresia				
Reference	**Age of Patient**	**# Cells TX ($\times 10^9$)**	**Findings**	**Outcome**
Khan et al. (Khan et al., 2008a)	1 year	0.012 CD326+ Fetal hepatocytes	↓ 3-fold total bilirubin, ↓ 8-fold conjugated bilirubin, ↓2.5-fold ALT levels and hepatobiliary scintigraphy showed ↑ liver cell function at 2 months	NA

Disease: Ornithine Transcarbamylase Deficiency				
Reference	**Age of Patient**	**# Cells TX ($\times 10^9$)**	**Findings**	**Outcome**
Strom et al. (Strom et al., 1997a)	5 years	1.0	↓ NH_3, 0.5% of normal liver OTC activity	Death 42 days after TX
Horslen et al. (Horslen et al., 2003)	10 hours	9.0	↓ NH_3, ↑ protein tolerance for a short period	OLT after 6 months
Stephenne et al. (Stephenne et al., 2005)	14 months	2.4	↓ NH_3, ↑ urea, psychomotor improvement	OLT after 6 months
Puppi et al. (Puppi et al., 2008)	1 day	1.6	↓ NH_3, ↑ urea under normal protein diet	APOLT after 7 months
Meyburg et al. (Meyburg et al., 2009)	6 hours 9 days	0.6 0.6	↓ NH_3, ↑ urea, normal urinary orotic acid excretion ↓ NH_3, ↑ protein intake, urinary ortoic acid normal at 6 months	Death 4 months after TX OLT waiting list

Disease: ASL Deficiency				
Reference	**Age of Patient**	**# Cells TX ($\times 10^9$)**	**Findings**	**Outcome**
Stephenne et al. (Stephenne et al., 2006)	42 months	4.7	↓ NH_3, psychomotor catch-up, donor cells detected in liver	OLT after 18 months

Disease: CPS-1 Deficiency				
Reference	**Age of Patient**	**# Cells TX ($\times 10^9$)**	**Findings**	**Outcome**
Meyburg et al. (Meyburg et al., 2009)	2.5 months	1.4	↓ NH_3, ↑ urea for 11 months	OLT waiting list

Disease: Citrullinemia				
Reference	**Age of Patient**	**# Cells TX ($\times 10^9$)**	**Findings**	**Outcome**
Meyburg et al. (Meyburg et al., 2009)	36 months	1.5	Normal NH_3, ↑ 40% urea, ↑ protein intake	NA

Disease: A1AT Deficiency				
Reference	**Age of Patient**	**# Cells TX ($\times 10^9$)**	**Findings**	**Outcome**
Strom et al. (Strom et al., 1997b)	52 years	0.022	Wild type A1AT levels noted in patients serum with a peak at 92 mg/dl	OLT and kidney transplant after 2 days. Full recovery.

Table 6
Clinical Hepatocyte Transplants in Liver Failure Patients Worldwide

Table summarizes all reports of clinical hepatocyte transplants performed worldwide since 1993 to treat various liver-based inborn errors of metabolism and acute liver failure. Table is broken down by each disease that has been treated. Cells transplanted are hepatocytes unless specified otherwise. PV = portal vein; IS = intrasplenic; IP = intraperitoneal. Adapted from Dhawan et al. (Dhawan et al., 2010).

Disease: Drug-induced Acute Liver Failure				
Reference	**Age of Patient**	**# Cells TX ($\times 10^9$)**	**Route of delivery**	**Outcome**
Bilir et al. (Bilir et al., 2000)	32 years 35 years 55 years	1.3 10 39	IS IS IS	Death on day 14 Death on day 20 Death in 6 hours
Strom et al. (Strom et al., 1999)	13 years 43 years	1.0 NA	PV NA	Death on day 4 Death on day 35
Fisher et al. and Strom et al. (Fisher and Strom, 2006; Strom et al., 1997b)	27 years 26 years 21 years 35 years 35 years 51 years	0.03 1.2 0.94 5.4 3.7 3.9	IS IS IS PV PV PV	OLT on day 10 OLT on day 2 Death on day 1 Death on day 18 Full recovery Death on day 3
Habibullah et al. (Habibullah et al., 1994)	32 years 29 years 20 years 20 years 24 years	0.06/kg 0.06/kg 0.06/kg 0.06/kg 0.06/kg Fetal Hepatocytes	IP IP IP IP IP	Death in 30 hours Death in 37 hours Death in 48 hours Full recovery Full recovery

Disease: Viral-induced Acute Liver Failure				
Reference	**Age of Patient**	**# Cells TX ($\times 10^9$)**	**Route of delivery**	**Outcome**
Meyburg et al. (Meyburg et al., 2010)	3 weeks	1.7	PV	Death on day 11
Fisher et al.(Fisher and Strom, 2006)	4 years 54 years	3.4 6.6	PV PV	Death on day 2 Death on day 7
Bilir et al. (Bilir et al., 2000)	29 years 65 years	10 30	PV and IS PV and IS	Death in 18 hours Death on day 52
Strom et al. (Strom et al., 1999; Strom et al., 1997b)	28 years 37 years 43 years 40 years	0.17 0.12 0.73 0.0075	IS IS PV IS	OLT on day 3 Death on day 5 OLT on day 1 Death on day 4 due to intracranial bleed
Fisher et al. (Fisher et al., 2000)	37 years	0.88	IS	Full recovery
Habibullah et al. (Habibullah et al., 1994)	40 years	0.06/kg Fetal Hepatocytes	IP	Death in 13 hours

Disease: Idiopathic Acute Liver Failure				
Reference	**Age of Patient**	**# Cells TX ($\times 10^9$)**	**Route of delivery**	**Outcome**
Fisher et al. (Fisher and Strom, 2006)	3.5 months 23 years 48 years	0.18 0.44 0.75	PV IS PV	OLT on day 1 OLT on day 5 and death on day 13 Death on day 1
Habibullah et al. (Habibullah et al., 1994)	8 years	0.06/kg Fetal Hepatocytes	IP	Full recovery

Disease: Mushroom-poisoning-induced Acute Liver Failure				
Reference	**Age of Patient**	**# Cells TX ($\times 10^9$)**	**Route of delivery**	**Outcome**
Schneider et al. (Schneider et al., 2006)	64 years	4.9	PV	Full recovery

Disease: Postsurgical Acute Liver Failure				
Reference	**Age of Patient**	**# Cells TX ($\times 10^9$)**	**Route of delivery**	**Outcome**
Strom et al. (Strom et al., 1999)	69 years	0.53	IS	Death on day 2

Disease: Acute Liver Failure Induced by Acute Fatty Liver of Pregnancy				
Reference	**Age of Patient**	**# Cells TX ($\times 10^9$)**	**Route of delivery**	**Outcome**
Khan et al. (Khan et al., 2004)	26 years	0.3 Fetal hepatocytes	IP	Full recovery

Disease: Total Parenteral Nutrition / Sepsis				
Reference	**Age of Patient**	**# Cells TX ($\times 10^9$)**	**Route of delivery**	**Outcome**
Strom et al. (Strom et al., 1997b)	6 months	0.05	IS	Death (Life Support Elected to Be Discontinued by Family)