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CLINICAL PROGRAMS OF STEM CELL THERAPIES FOR LIVER AND PANCREAS

Giacomo Lanzoni, PhD[1,2,13], **Tsunekazu Oikawa, MD/PhD**[3,13], **Yunfang Wang, MD/PhD**[7,13], **Cai-Bin Cui, MD/PhD**[4,13], **Guido Carpino, MD/PhD**[9,10], **Vincenzo Cardinale, MD**[11], **David Gerber, MD**[4], **Mara Gabriel**[12], **Juan Dominguez-Bendala, PhD**[1], **Mark E. Furth, PhD**[8,14], **Eugenio Gaudio, MD/PhD**[10,14], **Domenico Alvaro, MD/PhD**[11,14], **Luca Inverardi, MD**[1,14], and **Lola M. Reid, PhD**[3,5,6,14]

Giacomo Lanzoni: Giacomo.Lanzoni@gmail.com; Tsunekazu Oikawa: oitsune@gmail.com; Yunfang Wang: wangyf1972@gmail.com; Cai-Bin Cui: cuicb@med.unc.edu; Guido Carpino: guido.carpino@uniroma1.it; Vincenzo Cardinale: v.cardinale80@gmail.com; David Gerber: david_gerber@med.unc.edu; Mara Gabriel: mgabrielconsulting@gmail.com; Juan Dominguez-Bendala: jdominguez2@med.miami.edu; Mark E. Furth: mfurth@wakehealth.edu; Eugenio Gaudio: eugenio.gaudio@uniroma1.it; Domenico Alvaro: domenico.alvaro@uniroma1.it; Luca Inverardi: linverar@med.miami.edu; Lola M. Reid: Lola.M.Reid@gmail.com

[1]Diabetes Research Institute, Miller School of Medicine, University of Miami, Miami, FL. 33136

[2]Department of Histology, Embryology and Applied Biology, University of Bologna, Bologna, Italy

[3]Cell Biology and Physiology, University of North Carolina School of Medicine, Chapel Hill, NC 27599

[4]Department of Surgery, University of North Carolina School of Medicine, Chapel Hill, NC 27599

[5]Program in Molecular Biology and Biotechnology, University of North Carolina School of Medicine, Chapel Hill, NC 27599

[6]Lineberger Cancer Center, University of North Carolina School of Medicine, Chapel Hill, NC 27599

[7]The Stem Cell and Regenerative Medicine Lab, Beijing Institute of Transfusion Medicine, Beijing, PR China, 100850

[8]Wake Forest Innovations, Wake Forest University School of Medicine, Winston-Salem, NC 27157

[9]Department of Health Sciences, University of Rome “ForoItalico”, Rome, Italy

[10]Department of Anatomical, Histological, Forensic Medicine and Orthopedics Sciences, Fondazione Eleonora Lorillard Spencer Cenci, Sapienza University, Rome, Italy

[11]Department of Scienze e Biotecnologie Medico-Chirurgiche, Fondazione Eleonora Lorillard Spencer Cenci, Sapienza University, Rome, Italy

[12]MGabriel Consulting, 3621 Sweeten Creek Road, Chapel Hill, NC 27514

Abstract

Corresponding author: Lola M. Reid, Department of Cell Biology and Physiology and Program in Molecular Biology and Biotechnology, CB# 7038, UNC School of Medicine, Glaxo, Rm. 32-36, Chapel Hill, NC 27599-7038. Tel: 919-966-0346; Fax: 919-966-6112. Lola.M.Reid@gmail.com.
[13]co-equal first authors
[14]co-equal senior authors

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Regenerative medicine is transitioning into clinical programs utilizing stem/progenitor cell therapies for repair of damaged organs. We summarize those for liver and pancreas, organs that share endodermal stem cell populations, biliary tree stem cells (hBTSCs), located in peribiliary glands: they are precursors to hepatic stem/progenitors in canals of Hering and to committed progenitors in pancreatic duct glands. They give rise to maturational lineages along a radial axis within bile duct walls and a proximal-to-distal axis starting at the duodenum and ending with mature cells in the liver or pancreas.

Clinical trials have been ongoing for years assessing effects of fetal-liver-derived hepatic stem/progenitors transplanted into the hepatic artery of patients with various liver diseases. Immunosuppression was not required. Control subjects, those given standard of care for a given condition, all died within a year or deteriorated in their liver functions. Subjects transplanted with 100–150 million hepatic stem/progenitor cells had improved liver functions and survival extending for several years. Full evaluations of safety and efficacy of transplants are still in progress. Determined stem cell therapies for diabetes utilizing hBTSCs remain to be explored but are likely to occur following ongoing preclinical studies.

In addition, mesenchymal stem cells (MSCs) and hematopoietic stem cells (HSCs) are being used for patients with chronic liver conditions or with diabetes. MSCs have demonstrated significant effects through paracrine signaling of trophic and immune-modulatory factors, and there is limited evidence for inefficient lineage restriction into mature parenchymal or islet cells. HSCs' effects are primarily via modulation of immune mechanisms.

Introduction

Stem cell therapies for diseased solid organs are an important potential modality of regenerative medicine. In this review we focus on prospects for such therapies for liver and pancreas utilizing determined stem cell subpopulations giving rise to these organs[1–6], In addition, mesenchymal stem cells (MSCs) and/or hematopoietic stem cells (HSCs) are being used for patients with either liver diseases or with diabetes[7–14]. Stem cell therapies for liver conditions are being used for acute liver failure, fulminant hepatitis, inborn errors of metabolism, hepatitis viruses, liver toxins, alcohol consumption, autoimmunity and metabolic disorders such as non-alcoholic steato-hepatitis (NASH). Together, diabetes and these liver diseases and conditions constitute a major medical burden, one being addressed by clinical trials of cell therapies using stem cells or mature cells, and that collectively indicate a promising future of regenerative medicine strategies for these patients[15–18].

Categories of Stem Cells giving Rise to Liver and Pancreas

Stem cells and their descendants, committed progenitors, are capable of sustained proliferation and differentiation into specialized cells[19]. The crucial defining distinction of stem cells is their ability to self-renew, i.e. to maintain indefinitely a population with identical properties through symmetric and asymmetric cell divisions[20, 21]. Progenitors play a transitory role in amplification of a cell population during development or regeneration. When the self-renewal capacity of precursors cannot be rigorously ascertained, or when both stem cells and progenitors are involved in a biological process, investigators often use the term stem/progenitor cells.

Stem cells in the first stages of developing mammalian embryos have the remarkable capacity to produce all of the body's cell types and are termed pluripotent[22]. Embryonic stem (ES) cells can remain pluripotent during extensive expansion as established cell lines[23–26]. The self-renewal potential of ES cells appears virtually unlimited, although the accumulation of spontaneous mutations and chromosomal rearrangements eventually

degrades their practical utility[27]. A remarkable finding, one with enormous implications for regenerative medicine and human genetics, is that pluripotent stem cells similar to ES cells can be generated through reprogramming of mature somatic cells by introduction of small sets of defined genetic factors[28, 29]. These are termed induced pluripotent stem (iPS) cells. In principal, ES and iPS cells are sources of stem cells to treat any tissue or organ. Moreover, autologous therapies with iPS cells theoretically should not require immune suppression[30–32]. However, clinical trials with ES and iPS cells face challenges due to the tumorigenic potential of residual undifferentiated cells resulting from difficulties in their lineage restriction to a desired adult fate. Such challenges have short-circuited clinical trials as occurred for Geron (Menlo Park, CA)[33, 34]. In 2013 Geron officials transferred all cell therapy programs to Biotime (Alameda, CA). ViaCyte (San Diego, CA) plans clinical trials for diabetes using encapsulated cells to minimize tumorigenicity and immunogenicity but at the expense of introducing an artificial barrier to physiological functioning[35]. Lineage restriction of ES or iPS cells to a specific fate comes at a price: it requires weeks of treatments with expensive soluble signals and matrix components, resulting in a formidable economic challenge to the clinical uses of these stem cells. Apart from these major concerns for the use of ES and iPS cells in cell therapy, the cells can still provide medical benefits by enabling the creation of in vitro models of human disease to facilitate drug discovery[36].

Determined stem cells, called “adult stem cells” by the lay press, occur in fetal and postnatal tissues but are restricted to lineages defined by a germ layer (ectoderm, mesoderm or endoderm)[19]. Determined stem cells for liver and pancreas comprise multiple subpopulations of biliary tree stem cells (hBTSCs), found in peribiliary glands (PBGs) throughout the biliary tree. These give rise to hepatic stem cells (hHpSCs) and hepatoblasts (hHBs), found intrahepatically in or near the canals of Hering[37–39]. The hBTSCs are precursors also to pancreatic stem cells (hPSCs) in the hepato-pancreatic common duct that lineage restrict to committed progenitors in pancreatic duct glands (PDGs)[4]. These stem cells can replenish mature cells lost through normal turnover or injury and disease. Their proliferation and differentiation are regulated tightly to ensure life-long maintenance of appropriate numbers of both stem/progenitors and mature cells. This regulation is controlled by intrinsic genetic programs and by extrinsic cues from soluble signals working synergistically with extracellular matrix components within the microenvironments of stem cell niches[40, 41]. Signals in niches help to maintain stem cells in a quiescent state, designated G_0[42], with cycling occurring slowly except for physiological demands to replace mature cells. Although often described as having lesser expansion capacity than ES or iPS cells, hHpSCs and hBTSCs can self-renew extensively. They are easily isolated from normal tissue of any age donors and can be cultured under wholly defined, serum-free conditions for months with more than 25–30 population doublings within 8 weeks, and through more than 40 population doublings in ~12 weeks, corresponding to greater than one trillion-fold (1×10^{12}) potential expansion[1, 4, 43, 44].

Studies by C. Habibullah and associates indicate that hHpSCs and their descendants, hHBs, can be effective in treating patients with liver disease[17, 45–47] when transplanted via the hepatic artery. The safety and potential advantages of transplanting cells through the hepatic artery was demonstrated in prior studies using bone marrow-derived stem cells[48]. Clinical studies of hBTSCs have yet to occur, but preclinical research is ongoing, and clinical trials are anticipated to occur within a few years.

Mesenchymal stem cells or MSCs can be isolated from tissues such as bone marrow, adipose tissue, umbilical cord tissue or amniotic fluid[11, 49]. These cells can be found in the perivascular compartment of most (all?) organs, including the liver[50, 51]. They can be expanded *ex vivo* for multiple passages, but not indefinitely, in standard media supplemented with serum, though serum-free formulations better suited for clinical

applications have now been developed [52]. MSCs can be efficiently lineage restricted to any mesodermal fate (e.g. bone, cartilage, fat) but only inefficiently to endodermal or ectodermal fates[49, 53–56]. MSCs can differentiate into immature hepatocyte-like or islet-like cells but with such low efficiency that they are not a practical source for clinical products[57, 58]. The demonstrated mechanism of actions of MSCs for liver or pancreatic diseases comprise trophic or immune-modulatory regulation[11, 54, 59–61]. Paracrine effects of MSCs in regeneration are widely recognized,[60]. Despite the extensive use of MSCs in research models and clinical trials, significant ambiguity remains regarding their identity and the specific factors most critical to their role in tissue repair and organogenesis[62].

This review will focus on current clinical programs using determined stem cells, MSCs or HSCs, and the findings from these compared to results with mature cells, hepatocytes for liver or pancreatic islets for diabetes. Details of the clinical programs, summaries from cryopreservation and grafting technologies, and a more extensive list of references are given in the online supplement.

Embryonic Development of Liver and Pancreas

Definitive endoderm derives from ES cells through effects of a number of key transcription factors, including Goosecoid, MIXL1, SMAD2/3, SOX 7, and SOX17[63]. During early embryonic development, endoderm subsequently segregates into foregut (lung, thyroid)[64, 65], stomach[66], mid-gut (pancreas, biliary tree and liver)[67], and both foregut and hindgut (intestine)[68], through effects of specific combinations of transcription factors. Those dictating the mid-gut organs include SOX9, SOX17, FOXA1/FOXA2, ONECUT2/OC-2 and others[69–72].

Organogenesis of liver and pancreas occurs with outgrowths at anlage on either side of the duodenum and extending and ramifying into the branching biliary tree structure. The ends of the biliary tree engage in the cardiac mesenchyme to form liver [73] and retroperitoneally in aorta-induced pancreatic mesenchyme to form pancreas[74]. The biliary tree branch closest to the duodenum forms ventral pancreas; the next major branch forms the cystic duct extending to form gallbladder; and the final branches within the liver form large intralobular bile ducts (Figure 1A). On the other side, the anlage forms a duct that extends to form dorsal pancreas. The formation of intestine incorporates a twisting motion that swings the ventral pancreas to the other side where it merges with the dorsal pancreas to form the complete pancreatic organ. The liver cannot swing to the opposite side, due to its size and connections into the cardiac mesenchyme. As a result, the liver and the ventral pancreas share the hepato-pancreatic common duct connecting to the duodenum at the major papilla (the ampulla of Vater), while the dorsal pancreas connects to the duodenum via the accessory pancreatic duct at the minor papilla (Figure 1B).

Organization of Stem Cell Niches for Liver and Pancreas

All tissues are comprised of maturational lineages of cells consisting of epithelial-mesenchymal cell partnerships beginning with epithelial stem cells (e.g. hHpSCs) partnered with mesenchymal stem/progenitors (e.g. angioblasts). These yield cellular descendants maturing coordinately and generating epithelial-mesenchymal partners changing step-wise with respect to their morphology, ploidy, growth potential, gene expression and other phenotypic traits, and regulated via gradients of soluble signals and matrix components that are defined in many regions of the lineages but only partially in the stem cell niches[43, 44, 75–77]. That which is known is summarized in Tables S1 and S2.

Lineage tracing studies have been done by Lemaigre[78], Furuyama[79], Kawaguchi[80] and others demonstrating that a population of SOX9+ cells gives rise to liver, biliary tree and

pancreas. Those of Swenson[81] found that acinar cells derive from a single stem cell, whereas islets derive from more than one[81]. Importantly, stem cells are found in postnatal liver[43, 82] but are very rare in postnatal pancreas[83–85]. This paradox has been clarified recently with the discovery that pancreatic stem cells are not in the pancreas but rather in the biliary tree, particularly in the hepato-pancreatic common duct.[4].

Regeneration of liver and pancreas derives, in part, from proliferation of mature cells[83, 86] and, in part, from stem/progenitors[6, 87–93]. However, the limited proliferative ability of mature parenchymal cells or islets implicates stem/progenitors as logical cell sources for clinical studies. More detailed presentations on the phenotypic traits of the biliary tree,[5] pancreas,[4] and liver [18] have been given in prior publications. The net sum of the phenotypic traits and activities of cells at the sequential maturational lineage stages yields the functions of the composite tissue.

Stem/Progenitor Cell Niches

Stem cells and progenitor cells reside in discrete locations called niches, each with a unique environment[41](Figures 2–4). The niches for the mid-gut organs include: peribiliary glands (PBGs) in the extrahepatic and intrahepatic biliary tree[1, 2, 4, 39]; the ductal plates in fetal and neonatal livers[37, 78]; the canals of Hering, derived from ductal plates and found in pediatric and adult livers[37–39, 90, 94]; and the pancreatic duct glands (PDGs), reservoirs of committed progenitors[4, 95–97]. These niches form a network that is continuous throughout the biliary tree. The network has anatomical connections from biliary tree directly into the canals of Hering[2], the site of intrahepatic stem cells, and to the PDGs, reservoirs of committed progenitors within the pancreas[4]. *In situ* studies provide hints, but not yet proof, that the network may begin with Brunner's Glands (Carpino, Lanzoni, et al., unpublished data), found uniquely in the duodenum between the portals to the dorsal pancreatic duct and the hepato-pancreatic duct[98] (Figure 4).

Characteristics of the Biliary Tree Stem Cells *In Situ*

Niches consists of PBGs found within bile duct walls (intramural glands) or tethered to the surface of bile ducts (extramural glands) of the biliary tree[99]. PBGs occur in highest frequencies at branching points of the biliary tree; the greatest numbers are present in the hepato-pancreatic common duct and, secondarily, in the large intrahepatic bile ducts[5]. Other than anatomical and histological findings from the pioneering studies of Nakanuma and associates[99–101], nothing is known of the roles of the extramural PBGs. Each PBG contains a ring of cells at its perimeter and is replete with mucous production (PAS-positive material) in its center. The cells in the ring are phenotypically homogeneous in the PBGs in some sites (e.g. hepato-pancreatic common duct, large intrahepatic bile ducts) but are quite heterogeneous in other sites (e.g. cystic duct, hilum, common duct)[1, 2, 4]. The variations in phenotypic traits of the cells implicate maturational lineages for which there are two axes[2, 4, 6]:

- A radial axis[1, 4] starting with high numbers of primitive stem cells (characterized by elevated expression of pluripotency genes, co-expression of transcription factors relevant to both liver and pancreas, and expression of other stem cell markers) located in PBGs near the fibromuscular layer in the interior of the bile ducts, and ends with mature cells towards the bile duct lumens. The radial axis near the liver yields mature parenchymal cells (Figure 3); that near the pancreas yields mature pancreatic cells; and that in between yields mature biliary epithelial cells.
- A proximal-to-distal axis[1, 2, 4, 5] starts with high numbers of primitive stem cells in PBGs near the duodenum, and progresses along the length of the bile ducts to

mature cells with proximity to liver or to cells with pancreatic markers when near the pancreas (Figure 4;Table S2).

The PBGs throughout the biliary tree retain a portion of the cells (~2–4%) as stem cells until the connection with the canals of Hering[2], the sites with the highest oxygen levels in the liver; presumably oxygen is a trigger for rapid maturation to adult parenchymal cells. In the other direction, the PBGs within the hepato-pancreatic common duct connect directly into the pancreatic duct glands (PDGs)[4]; strikingly and for unknown reasons, the stem cell features are lost immediately upon transition into the pancreatic ducts such that only committed progenitors seem to remain. It is assumed, but as yet unknown, whether the maturational lineages involve migration of cells. The network provides a biological framework for ongoing organogenesis of liver, biliary tree and pancreas throughout life.

Further details on the phenotypic traits support the interpretation of maturational lineages. The stem cells near fibromuscular layers co-express endodermal transcription factors essential for liver and pancreas formation (e.g. SOX9, SOX17, PDX1). They express pluripotency genes (NANOG, OCT4, SOX2, KLF4), other stem cell markers (NCAM, CD133, CXCR4, SALL4), and indicators of proliferation (e.g. Ki67). They do not express markers of mature cells (e.g. insulin, albumin)[2, 102].

The first intermediate stages activate first expression of leucine-rich repeat-containing G protein coupled receptor 5 (LGR5) and then epithelial cell adhesion molecule (EpCAM). Subsequent stages involve retention of key endodermal transcription factors (e.g. PDX1 or SOX17, but not both) in the nucleus. With increasing proximity to the bile duct lumen and also in proximity to either liver or to pancreas, the expression of pluripotency genes fades away; other stem cell traits are progressively lost (e.g. LGR5 or CD133 or SALL4); and mature markers increase (e.g. albumin or insulin—which one depends on proximity to liver or to pancreas, respectively). Huch et al [91], also found evidence for LGR5 expression on hepatic stem cells; expansion of LGR5+ cells *ex vivo* with an agonist, R-spondin, and the transplantation *in vivo* resulted in formation of liver and bile duct tissue.

In summary, stem cells and progenitors in the biliary tree and their descendants mature along pathways defined embryologically and persisting throughout life in the form of maturational lineages along a radial axis and a proximal (duodenum)-to-distal (organ) axis. Future investigations must determine if the maturational axes are mediated by cellular migration. A schematic of the proximal-to-distal axis is given in Figure 4 and further details in Table S2.

Ex Vivo Studies of Hepatic and Biliary Tree Stem Cells

Isolation of hHpSCs and hHBs from livers of all donor ages can be performed by selection for cells positive for expression of epithelial cell adhesion molecule, EpCAM[43]. EpCAM+ cells can be subdivided into hHpSCs by secondary selection for neural cell adhesion molecule, NCAM (CD56) versus into hHBs by secondary selection for intercellular cell adhesion molecule, ICAM-1 (CD54)[82]. The hHpSCs constitute approximately 1% (0.5% to 1.5%) of the total liver cell population throughout life in the livers of donors from fetuses to elderly adults. The hHBs constitute more than 80% of the parenchyma in fetal livers declining to 0.01% of the adult parenchyma. Unlike mature parenchymal cells, hHpSCs and hHBs survive extended periods of ischemia, allowing collection even several days after cardiac arrest[103]. The hHpSCs and hHBs express other stem/progenitor markers such as CD133 (prominin), CD44 (hyaluronan receptors), aldehyde dehydrogenase (ALDH)[104], telomerase[105], and hedgehog proteins[75]. They are small (hHpSCs=7–9 μm; hHBs=10–12 μm), less than half the size of mature parenchymal cells (diploid ones=18–22 μm), and

express weak or negligible levels of adult liver-specific functions (e.g. albumin, cytochrome P450s, or transferrin).

The hBTSCs are also tolerant of ischemia. Their concentration in biliary tree is higher than that of hHpSCs and hHBs in liver. The PBGs in most biliary tree regions contain 2–4% stem cells, and those in the hepato-pancreatic common duct are the richest of all with 5–9% stem cells in the PBGs. Surface markers usable for immunoselection can be EpCAM or LGR5 for some of them, but the majority are negative for EpCAM[2, 4] and for LGR5 (Oikawa and Reid, unpublished observations). Studies are ongoing to assess the efficacy of immunoselection for other surface markers (e.g. NCAM, CD44).

The hHpSCs, hBTSCs, hHBs and committed hepatic and pancreatic progenitors can be isolated also by culture selection in Kubota's Medium[106], a serum-free medium formulation tailored for endodermal stem/progenitors and their mesenchymal stem/progenitor cell partners[1, 43, 44, 106, 107]. It is comprised of any rich basal medium with low calcium (~0.3 mM), no copper, selenium (10^{-10}M), zinc (10^{-12}M), insulin (~5 μg/ml), transferrin/Fe (~5 μg/ml), high density lipoprotein (~10 μg/ml), and a defined mixture of purified free fatty acids bound to highly purified albumin. Notably, the medium contains no cytokines or growth factors. Mature cells do not survive in Kubota's Medium; only stem/progenitors survive[44, 106]. Given the focus of the review on clinical programs, a summary of culture studies is not presented here but is available in various publications[43, 44, 108, 109]. Images of cultures of hBTSCs and hHpSCs are provided in Figure 5.

Clinical Programs

The liver, biliary tree and pancreas are endodermal organs central to handling processing of food, glycogen and lipid metabolism, detoxification of xenobiotics, regulation of energy needs, and synthesis of diverse factors ranging from digestive enzymes (e.g. amylase, trypsin), endocrine signals (e.g. insulin, glucagon), coagulation proteins to carrier proteins (e.g. AFP, albumin, transferrin). The integrity of the body depends heavily on liver, biliary tree, and pancreatic functions, and failure in any of them, especially the liver, results in rapid death.

Clinical Programs in Cell Therapies for Liver (Table 1)

The only curative treatment for advanced liver disease is liver transplantation. However, this treatment is limited by severe shortage of donor organs, the physical demands of the complicated surgery, risks of severe complications, and high costs (typically ~$150,000 to $180,000 for transplant and first year medical follow-up). These limitations drive interests to explore cell therapies using transplantation of mature hepatocytes, MSCs or determined stem cells. Those on transplantation with mature hepatocytes are presented in the online supplement.

Determined Stem Cells—The only trials completed with transplants of determined stem cells have been those conducted by Drs. Habibullah, Habeeb and their associates at the Liver Institute in Hyderabad, India[17, 45–47, 110, 111]. These investigators focused on patients with biliary atresia, inborn errors of metabolism (Crigler-Najjar), non-alcoholic steatohepatitis (NASH), viral cirrhosis (HCV, HBV), alcoholic cirrhosis, and drug toxicity. Each year, approximately 150,000 patients die of liver cirrhosis in India. Patients with advanced stages of liver disease and very high MELD scores were candidates for this cell therapy program. To date, more than 280 patients have been enrolled, but the findings from most of these studies are not yet published.

It was learned that for stem cell populations, it was preferable to transplant via the hepatic artery, a strategy proved safe in preliminary studies with transplantation of bone marrow-derived cells[48]. When done with EpCAM+ cells, it resulted in up to ~20–30% engraftment[17, 46, 47, 111]. This procedure proved safe, as assessed by ultrasound indicating a persistence of echotexture, no focal lesions, and without abnormal changes in the size of the hepatic artery. Fetal liver-derived EpCAM+ cells (hHpSCs and hHBs) were marked with Tc99m-Hexamethylpropyleneamine Oxime and injected; most of the marked cells remained within the liver lobe injected. Also, most of the patients had grade 2 to grade 3 esophageal varices before the transplants, and the majority showed reduction in the varices grading from 3 to 1. Because 2–3 months were required to observe effectiveness of transplants, patients near death were not considered as candidates. A requirement for all trials was a life expectancy of ~5–6 months. Remarkably, immune suppression was not required, although donors and recipients were not matched for histocompatibility antigens. These early studies have been published[17, 45–47, 111]. A representative early publication concerned a trial of 25 subjects and 25 controls with decompensated liver cirrhosis due to various causes. Subjects received fetal liver-derived EpCAM+ cell infusions into the liver via the hepatic artery. At a 6-month follow-up, multiple diagnostic and biochemical parameters showed clear improvement, and there was a significant decrease ($p < 0.01$) in the mean MELD scores.

The clinical trials were completed in June, 2012, and the results were used to apply for regulatory approval in India. The application remains under review. Details on the long-term outcomes of these patients are not yet available, and, of course, these publications are needed to clarify the potential merits of these strategies. Validation of these early findings with many more studies is needed to clarify the efficacy of such treatments for dysfunctional liver conditions.

Clinical use of determined stem cells has been done thus far with freshly isolated, minimally manipulated cells; in the future it is likely to be facilitated by large-scale manufacturing of cell populations that will require assessment of their genetic stability. The sourcing of donor cells may be fetal tissues in countries that permit their use. Although stem/progenitors cells can be isolated from pediatric and adult livers, the competition for these organs will preclude them as a practical source. Alternatively, neonatal livers or neonatal or adult biliary tree tissue can be used as sources. They have distinct advantages both ethically and practically. The cells may be utilized directly as isolated or after expansion in culture (subject to additional levels of regulatory review). Grafting strategies in which cells are transplanted as a graft comprised of matrix components such as hyaluronans (discussed in the online supplement) [112, 113] should greatly improve engraftment, minimize ectopic distribution of cells, hasten integration into the tissue and improvement of liver functions. However, grafting strategies have yet to be used with patients.

Even though immunological issues proved minimal in transplants of fetal liver-derived EpCAM+ cells[17, 46, 47, 111], it yet may be desirable to match HLA (major histocompatibility) types of donors and recipients. Given sufficient expansion, it should be possible to bank large numbers of cells from a modest number of carefully selected donors and achieve a beneficial degree of HLA matching for the majority of recipients[114].

Clinical trials now being organized in Europe and Asia will comprise one arm duplicating the trials in India and another utilizing the new grafting strategies. The hope is to provide faster responses in patients with fewer cells and with minimal concerns for ectopic cell distribution.

Clinical Trials with MSCs and HSCs—Background on the field of MSCs is given in the online supplement. The ease of sourcing of MSCs, cryopreservation of MSCs, and

transplantation into patients has resulted in large numbers of clinical trials of MSCs throughout the world. At present over 20 clinical trials have been published on the use of MSCs for treatment of chronic liver diseases caused by hepatitis viruses, alcohol or drugs (Tables S4 and S5; www.clinicaltrials.gov). Most of these are investigations with small numbers of patients (typically under 10)). Thus, they are similar to the clinical trials of hepatocyte transplantation in providing anecdotal evidence or evidence with minimal possibility of statistically validated findings of the efficacy of the treatments. There are a small number with larger patient populations such as one reported by Peng at al.[115] (Clinical-Trials.gov: NCT00956891) involving 53 patients who underwent a single transplantation with autologous MSCs by a vascular route via the peripheral vasculature, the spleen or through the hepatic artery into the liver. The trials comprised treatments with:

a. unfractionated bone marrow or peripheral blood or used cytokines (e.g. G-CSF) to mobilize cells in the bone marrow

b. immunoselected cell populations (CD34+ cells, CD133+ cells) from bone marrow

c. cultured MSCs or cultures treated with growth factors such as hepatocyte growth factor (HGF), epidermal growth factor (EGF) or fibroblast growth factor (FGF).

Transplantations of any of these forms of MSCs or HSCs were found to be safe and significantly improved the quality of life and liver functions. The patient responses occurred within days to weeks, but long-term effects (more than a few months) were not observed. The conclusions are that effects are due to trophic and immune-modulatory factors. Caution and prudence are required to interpret the findings correctly, since most are uncontrolled studies. Therefore, randomized controlled studies are necessary in the future for clarification and validation of these therapies as treatments for liver disease.

Stem Cell Therapies for Patients with Diabetes (Table 2)

Pancreatic islet transplants, one of the oldest forms of cell therapies[116–118], are used for Type 1 diabetes (T1D). At this time, pancreatic islet transplantation is NOT an option for patients with Type 2 Diabetes. Pancreatic islet β-cells are lost due to autoimmune attacks in patients with T1D and are functionally impaired in a subset of patients with Type 2 diabetes (T2D). Thus, replenishment of the β-cell mass represents a major goal of several cell-based therapeutic approaches under development. Immune suppression and/or tolerance induction are essential to protect transplanted islets or residual β-cells[118, 119]. Transplantation of islets is effective at restoring normo-glycemia in patients with T1D[120], achieving a marked improvement in patients' quality of life[121], relieving symptoms of the disease for up to several years,[122] and slowing or preventing disease progression.[123] A major challenge is the scarcity of transplantable islets[124] and their intrinsic variability with respect to islet yield, quality and engraftment potential[125].

Early phase clinical trials of stem cell therapies (www.clinicaltrials.gov) are underway for the treatment of T1D and T2D, focused either on MSCs (Table S6), or hematopoietic stem cells (HSCs, Table S7) or on novel stem cell-based approaches (Table S8). Hopes that MSCs or HSCs might differentiate to β-cells have dimmed, since lineage restriction to functional β-cells is extremely limited, if it occurs at all[126]. Positive effects proved due to immune-modulatory or paracrine signaling mechanisms[126]. MSCs are endowed with a well-characterized immune-suppressive potential with beneficial paracrine and anti-inflammatory activities, and are able to inhibit the autoimmune aggression wreaking havoc on β-cells in T1D. Moreover, they may limit allo-immunity against transplanted β-cells, facilitating autologous regeneration by suppressing inflammation, limiting apoptosis and fibrosis, and stimulating angiogenesis[126–128]

The HSCs possess the ability to reconstitute the hematopoietic compartment, including the immune system. In autologous settings and after partial myelo-ablation, they can “reboot” and re-educate the immune system to a β-cell tolerant state. Further details on clinical trials of HSCs, MSCs and platform strategies for diabetes are given in the Online Supplement.

The derivation of β-cells from ES or iPS cells is a focus of many investigations [117, 118, 120]. Human ES cells can mature into β-like islet cells, but they are not the same as normal ones[119]. Limited reproducibility of the elaborate stepwise protocols with various matrix components and cytokines on different human ES cell lines is still hindering the evaluation of cell products but is approaching cGMP grade[35, 119]. As stated previously, the residual tumorigenic potential of ES cell-derived islets and the question of phenotypic stability are hampering translation of preclinical studies into clinical trials. The use of iPS cells for the clinics faces similar hurdles and additional challenges, since iPS cells are derived typically from non-endodermal tissues, the end products retain partial traits (“memory”) of their ectodermal or mesodermal origins and they can also bear mutations present in the donor sources [129, 130].

In contrast to the supportive roles played by MSCs and HSCs, and to the limitations still extant for ES and iPS cells, determined stem cells offer straightforward potential for future β-cell therapies for patients with diabetes. The *in situ* and *ex vivo* studies of maturational lineages provided evidence of progressive maturation of hBTSCs in PBGs to cells that are NGN3+, PDX1+ committed endocrine progenitors, found in PDGs, and thence to insulin+ β cells[4]. Important advantages of hBTSCs are that they are determined stem cells already programmed to lineage-restrict to a pancreatic fate, thus the maturation into islet cells occurs more efficiently and glucose responsiveness can be reached in only 7–10 days in culture[4]. Contrarily, this does not occur in cultures from ES or iPS cells: they require being transplanted *in vivo* to develop glucose sensitivity[119]. In addition, the hBTSCs can be obtained from an ethically acceptable source, the biliary tree. Preclinical studies are still required to define the yield from biliary tree tissue and to understand the cell doses required for efficacy.

A major concern in applications of islet transplantation is the need of immunosuppression, required to prevent transplant rejection and recurrence of autoimmunity (in the case of T1D) and with possible negative impact on the recipient’s health[131]. We don`t know if hBTSC cell therapy will require immunosuppression, but it might be minimal given that immunosuppression proved unnecessary with fetal liver-derived EpCAM+ cells[111].

A major reservoir of hBTSCs is in the hepato-pancreatic common duct[4] implicating this site as a logical target for transplantation in stem cell therapies for diabetes (Figure S3). This could overcome the existing strategy of using ectopic sites for transplantation of pancreatic precursors in order to avoid handling the pancreas, which can result in pancreatitis. A proposed strategy is to transplant hBTSCs by grafting strategies[113] into or onto the hepato-pancreatic duct wall. If done with freshly isolated hBTSCs purified by immunoselection methods, then the procedure would transfer minimally manipulated cells by an homotopic (same place) and homotypic (same cell type) transplantation procedure. This could ease regulatory issues for future clinical trials. Surgical operations on the biliary tree require a sound understanding of biliary tree and vascular anatomy and should be restricted to specialized centers because of the risk of peri-operative complications. The experience from biliary tract reconstruction suggests that end-to-end anastomosis of large ductal fragments should be avoided, since strictures frequently occur due to a compromised blood supply and potential tension on the repair[132, 133]. The transplantation could theoretically be performed by laparoscopic or by endoscopic strategies ideal in establishing outpatient procedures (Figure S3).

Conclusions

Forms of cell therapies are being utilized for treatment of patients with liver diseases and with diabetes. Cell therapies with mature cells (hepatocytes for liver; pancreatic islets for diabetes) provide acute relief, but their effects are transient, cause complications in the transplantation procedures and require immunosuppression. They are limited also by problems in sourcing of cells and difficulties in cryopreservation.

MSCs are easily sourced, readily cryopreserved, and involve transplantation procedures with minimal, if any, complications. They can offer months to years of alleviation of disease conditions by means of immune-modulatory and paracrine signaling mechanisms but offer evidence of only inefficient lineage restriction to mature hepatic parenchymal cells or β-cells. HSCs are effective as treatments in diseases in which there are aberrations of immune reactions. Again, the evidence indicates little hope for consistent ability to transdifferentiate into mature hepatic or pancreatic cells.

The only determined stem cell trials to date have tested fetal liver-derived EpCAM+ cells: they have been transplanted via the hepatic artery to achieve sufficient engraftment, and they required a survival of the patient for at least 3 months to exert efficacy. Early experience suggests that the therapies have the potential to offer significant benefit, enabling patients to have additional years of life with higher quality of health due to significant improvements in functions and without the need for ongoing immunosuppressive therapy. These therapies promise to be cost effective and to address unmet medical needs for a variety of disease states in patients of all ages and in various states of health.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Abbreviations

The stem cell or progenitor cell populations are indicated by an acronym which is preceded by a small letter indicating the species:

m	murine
r	rat
h	human
AFP	alpha-fetoprotein
hBTSCs	human biliary tree stem cells
CD133	prominin 1
CFTR	Cystic fibrosis transmembrane conductance regulator
C-PEP	C-peptide
CS-PG	chondroitin sulfate proteoglycan
CXCR4	CXC-chemokine receptor 4
CYP450	Cytochrome p450s
DS-PG	dermatan sulfate proteoglycan
EGF	epidermal growth factor
EpCAM	epithelial cell adhesion molecule
ES cells	embryonic stem cells
FBS	fetal bovine serum
FGF	fibroblast growth factor
FOXA2	forkhead box a2
GAGs	glycosaminoglycans
GCG	Glucagon
GFAP	glial fibrillary acidic protein
HA	hyaluronan
hHB	human hepatoblast
HDM	serum-free, hormonally defined medium
HGF	hepatocyte growth factor
hHpSC	human hepatic stem cells
HNF	hepatocyte nuclear factor
HP-PG	heparin proteoglycan
HS-PG	heparan sulfate proteoglycan

ICAM1	intercellular adhesion molecule-1
INS	insulin
iPS	induced pluripotent stem cell
KRT	cytokeratin
KM	Kubota's Medium
LGR5	leucine-rich repeat-containing G protein coupled receptor 5
MIXL-1	Mix paired-like homeobox gene (expressed in primitive streak in embryos)
MUC6	mucin 6, oligomeric mucus/gel-forming
NASH	non-alcoholic steatohepatitis
NCAM	neural cell adhesion molecule
NGN3	neurogenin 3
PBG	peribiliary gland
PDG	pancreatic duct gland
PDX1	pancreatic and duodenal homeobox 1
PROX1	Prospero homeobox protein 1
SALL4	Sal-like protein 4
SMAD	homologs of the *Drosophila* protein, mothers against decapentaplegic
(MAD)	and the *Caenorhabditis elegans* protein
SMA	alpha-smooth muscle actin
SOX	Sry-related HMG box
VEGF	vascular endothelial cell growth factor

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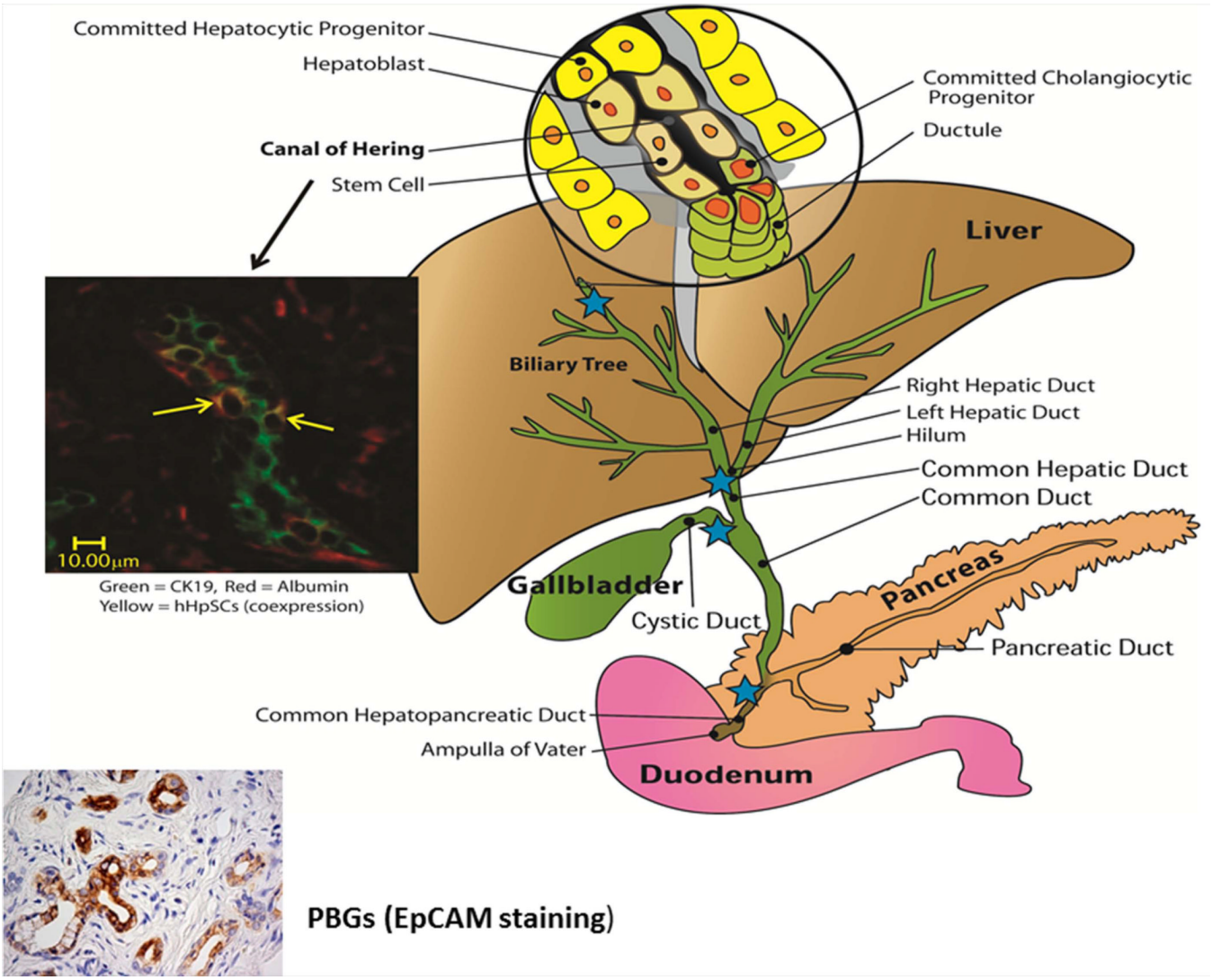


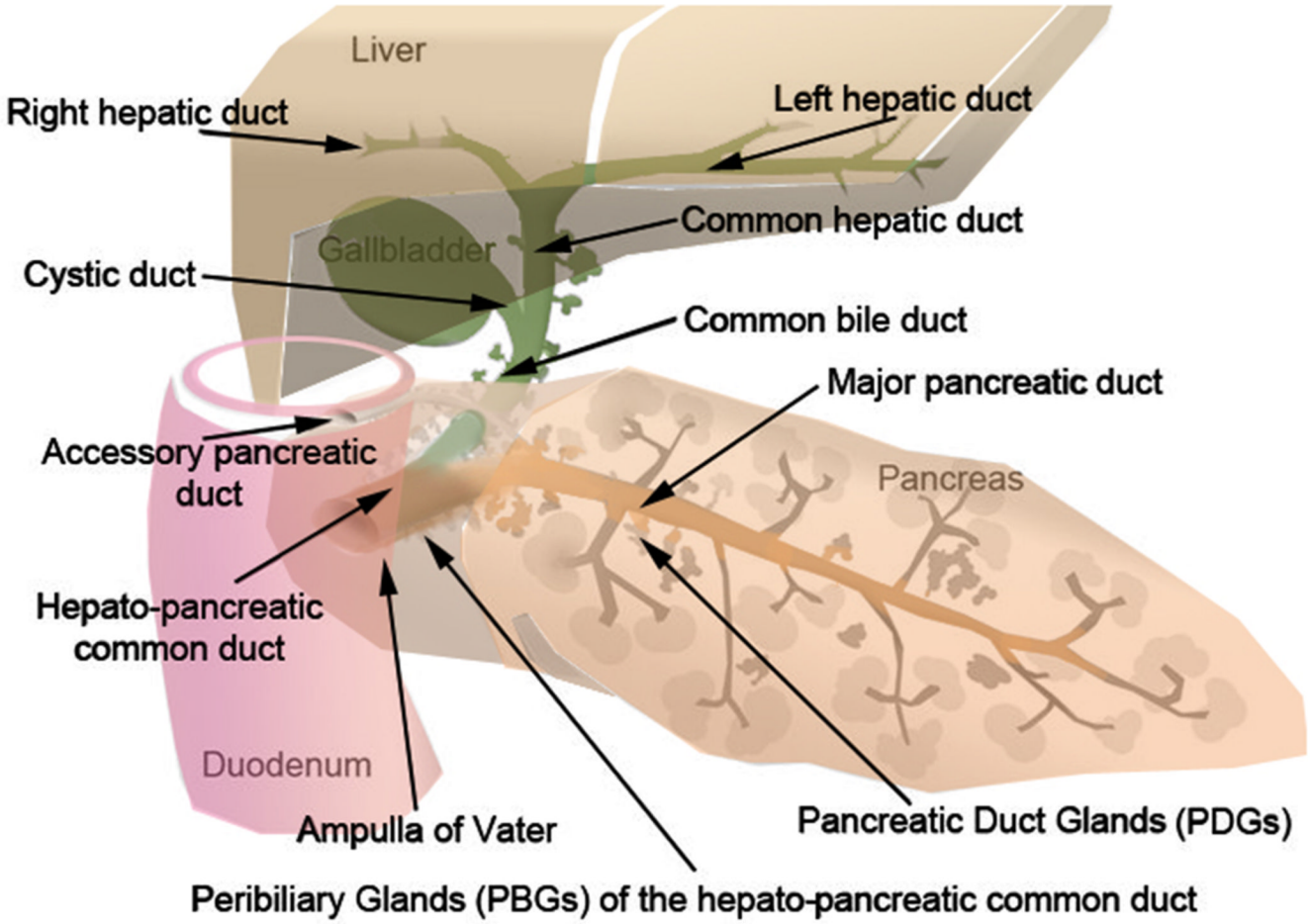


Figure 1. A. Schematic of the biliary tree

The biliary tree connects the liver and the pancreas to the duodenum. The PBGs found throughout the biliary tree are stem cell niches containing stem cells and progenitors and are in especially high numbers at various branching points of the tree (blue stars). Ultimately they connect into the intrahepatic stem cell niches and into the pancreatic duct glands, niches of committed progenitors within the pancreas. Figure modified from one in Turner et al [18].
B. Schematic of the hepato-pancreatic common duct

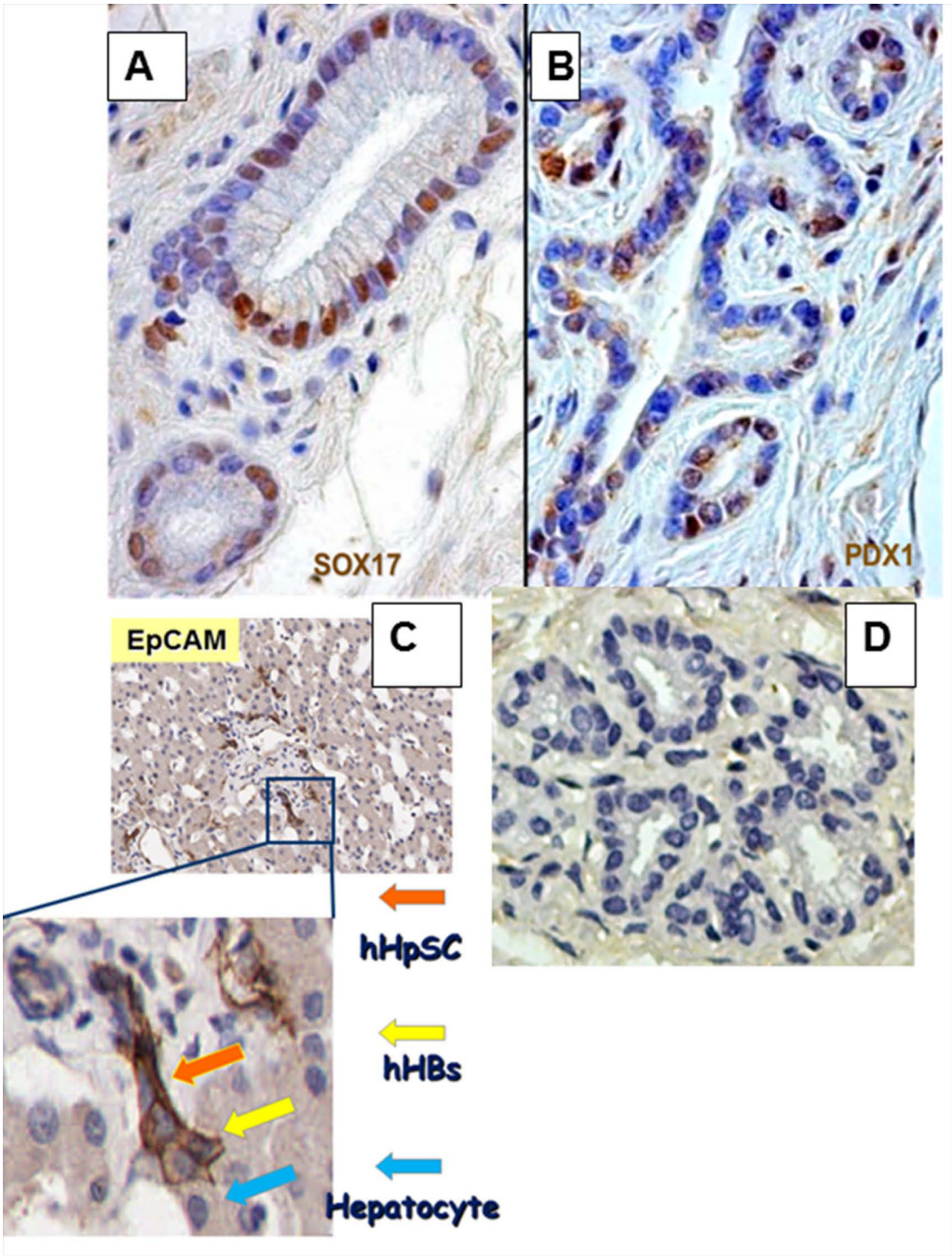


Figure 2. Stem/progenitor cell niches

A and B are peribiliary glands in human biliary tree tissue; sections are stained for SOX17 or PDX1. Note the heterogeneity of cells expressing PDX1 or SOX 17 in these PBGs. Figure modified from one in Cardinale et al.[1]. C. Canals of Hering, stem cell niches for hHpSCs in an adult livers. Figure from Zhang et al, [37]. C1. Enlargement of C and with labeling to show hHpSCs in the canals; hHBs tethered to the ends of the canals of Hering; and hHBs connecting to hepatocytes. D. Pancreatic duct glands (PDGs) containing only committed progenitors stained for SOX2 that is expressed in peribiliary glands but not in pancreatic duct glands. Image is from Wang et al [4].

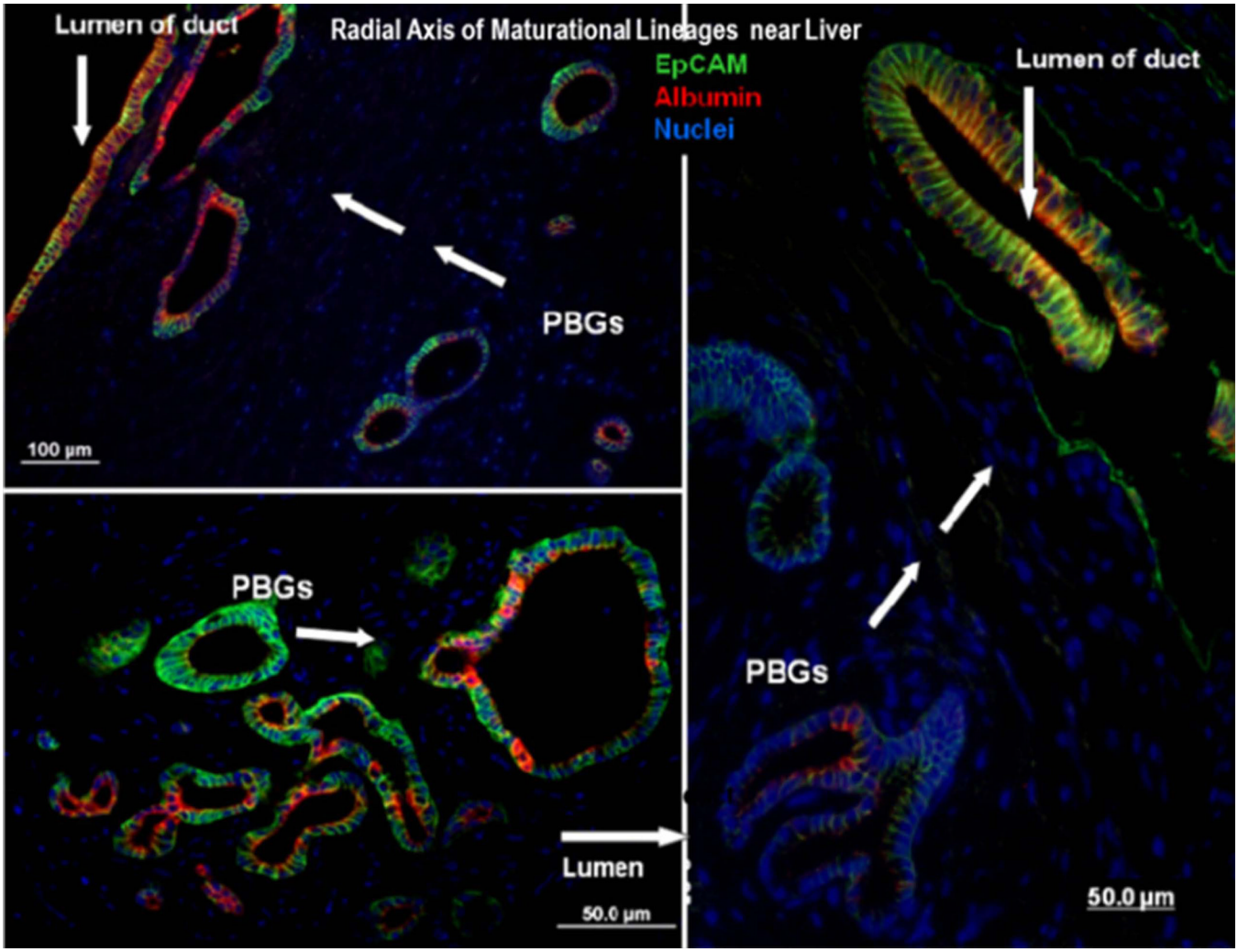


Figure 3. Radial axis maturational lineage near liver

Evidence for a maturational lineage progressing from PBGs near the fibromuscular layer to mature cells at the bile duct lumens. EpCAM is an intermediate marker and albumin a more mature marker for the cells that are maturing towards a liver fate. This occurs in the portion of the biliary tree closest to the liver. Figure reproduced from one in Cardinale et al.[1].

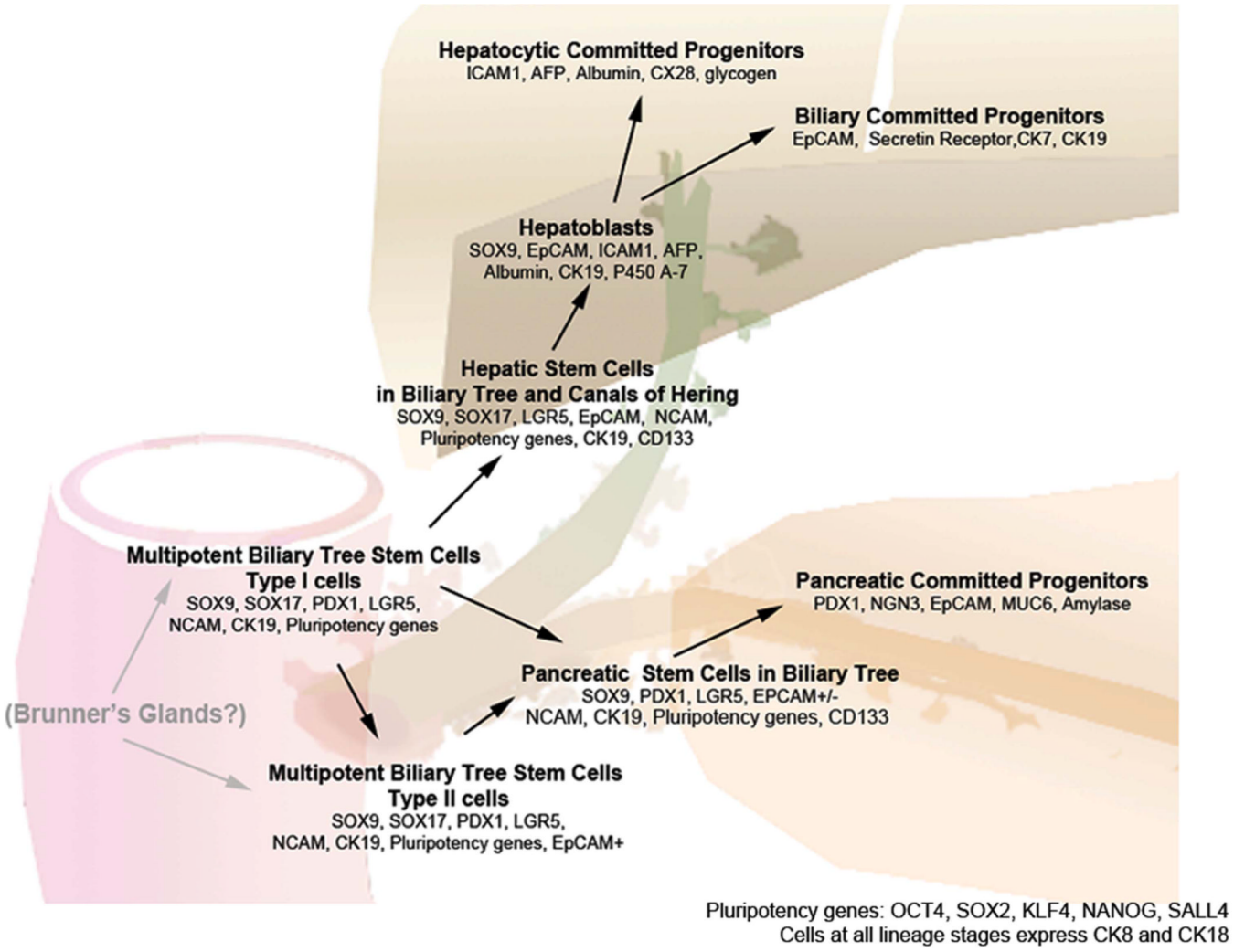
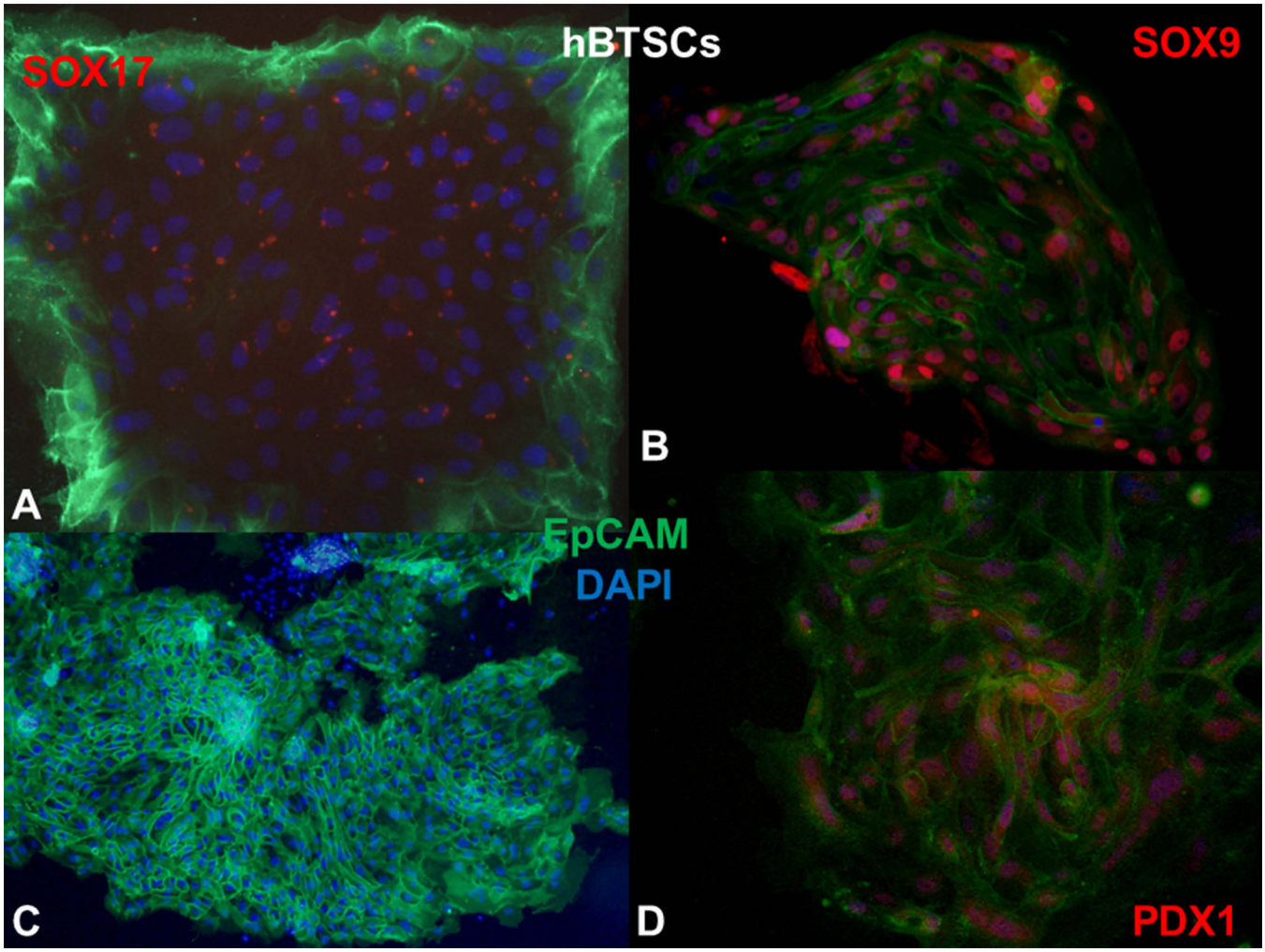


Figure 4. Schematic of the network of stem/progenitor cell niches

Schematic of the network of stem/progenitor cell niches from those in the biliary tree to ones in liver and pancreas. Figure provides a few of the markers on subpopulations of stem cells and progenitors in the proximal-to-distal axis (see Table S2 for more details).



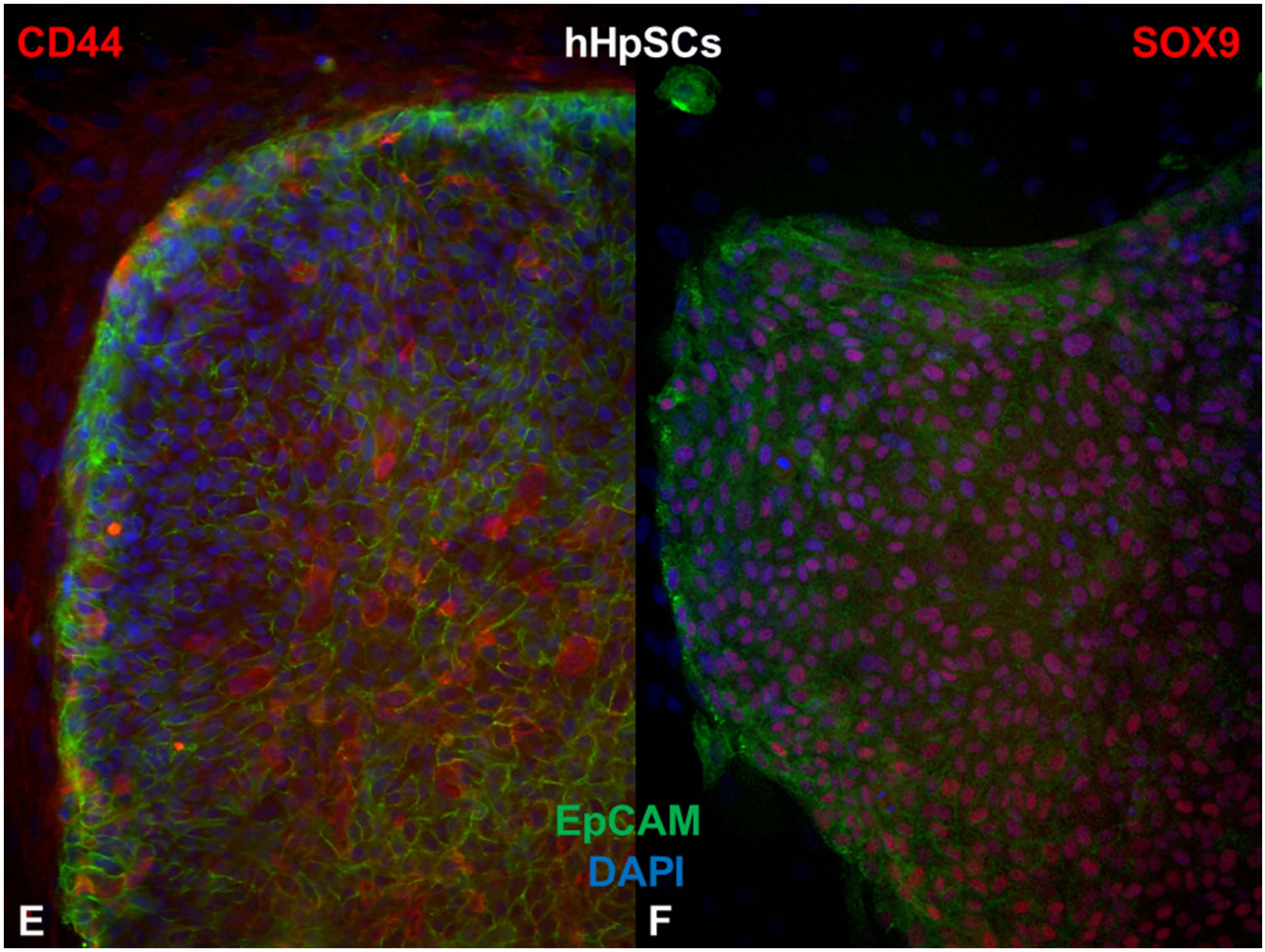


Figure 5. Cultures of human biliary tree stem Cells (hBTSCs) and hepatic stem cells (hHpSCs) under self-replication conditions

A-D: Colonies of human biliary tree stem Cells (hBTSCs); **E-F:** Colonies of hepatic stem cells (hHpSCs). The stem cells have been plated onto culture plastic and in serum-free Kubota's Medium, a medium designed for endodrmal stem cells and progenitors.

Table 1

Conclusions Regarding Ongoing Clinical Trials of Cell Therapies for Liver Diseases and Dysfunctions Cells used

Cells used	Results
Adult Parenchymal Cells--Hepatocyte Transplants (Table S3)	Cells derived from neonatal, pediatric or adult livers
	Delivery by vascular route into portal vein
	Number of patients/trial: see Table S3 in online supplement
	Cell numbers tested: from a few hundred million to billions
	Complications in transplant procedures included emboli formation
	Engraftment efficiencies were typically ~20%. Remainder of the cells either died or distributed ectopically, particularly to the lungs. (unknown significance)
	Immunosuppression required
	Significant Improvement in measured liver functions (e.g. albumin)
	Effects transient. Typically a few months (maximum =a few years) for patients with inborn errors of metabolism. Typically a few days to a few months for acute liver injuries.
Mesenchymal Stem Cells (MSCs) (Tables S4 and S5)	Cells derived from bone marrow, adipose tissue or umbilical cord
	Number of patients in each trial: ~10; a few up to 53 in the United States; larger numbers of patients in trials in China
	Delivery by vascular route through peripheral vasculature, spleen, portal vein, or hepatic artery
	Few if any complications in the transplant procedures
	Immunosuppression not required
	Transient improvements in liver functions and, in the larger trials, in MELD or Child-Pugh Scores
Hepatic Stem Cells (hHpSCs) and Hepatoblasts (hHBs)—EpCAM+ cells	EpCAM+ cells from fetal livers (gestational ages of 16–22 weeks)
	Number of patients in the trials: >280
	Delivery via hepatic artery
	Cell numbers tested ranged from ~100–150 million
	No complications from transplant procedures
	Engraftment efficiencies were typically ~20%. Remainder of the cells either died or distributed ectopically (unknown significance)
	Immunosuppression **not** required
	Significant improvement in MELD or Child-Pugh Scores and in all measures of liver functions
	Effects long-term (>4 years)

Table 2

Conclusions Regarding Ongoing Clinical Trials of Cell Therapies for Diabetes Cells used

Cells used	Results
Adult Beta Cells (Pancreatic islet Transplants)	Islets isolated from the pancreas of adult cadaveric donors
	Delivery by infusion into the portal vein, percutaneous or laparoscopic procedure
	Number of patients: 677 registered in the Collaborative Islet Transplant Registry, CITR. The vast majority of the data collected in the Registry are provided by groups in North America and Europe[134]
	Number of islets transplanted: a minimum of 9000 Islet Equivalents per kilogram, results usually in insulin independence. Most patients receive 2 infusions from different cadaveric donors, resulting in about 900.000 Islet Equivalents per patient
	Few complications in transplant procedures (2% of the patients experience acute bleeding or portal vein thrombosis) <http://www.citregistry.org
	Location of transplanted islets: the liver
	Immunosuppression required
	Significant Improvement: up to 85% of patients remain insulin independent for ~1 year after transplantation; up to 44% remain insulin independent for more than 3 years. Enduring long-term effects: reduction of HbA1c and resolution of severe hypoglycemia
	Effects are transient, the functions of the graft decline with time.
Mesenchymal Stem Cells (MSCs) (Tables S6 and S8)	Cells derived from bone marrow, umbilical cord or adipose tissue
	Number of patients in each trial: See Table S6
	Delivery by vascular route through peripheral vasculature
	Few if any complications in the transplant procedures
	Immunosuppression not required
	Modest improvement
Hematopoietic Stem Cells (HSCs) (Tables S7 and S8)	Bone marrow derived
	Number of patients in the trials: see Table S7
	Delivery by vascular route
	No complications from transplant procedures
	Immunosuppression, consequence of partial myelo-ablation. Partial reboot of the immune system
	Significant improvement
	Effects long-term