Hepatol Int (2017) 11:54–69
DOI 10.1007/s12072-016-9757-y

REVIEW ARTICLE

# Hepatocyte-like cells derived from induced pluripotent stem cells

**Namita Roy-Chowdhury[1,2,3] · Xia Wang[1,2] · Chandan Guha[2,4] · Jayanta Roy-Chowdhury[1,2,3]**

Received: 21 March 2016 / Accepted: 19 July 2016 / Published online: 17 August 2016


**Abstract** The discovery that coordinated expression of a limited number of genes can reprogram differentiated somatic cells to induced pluripotent stem cells (iPSC) has opened novel possibilities for developing cell-based models of diseases and regenerative medicine utilizing cell reprogramming or cell transplantation. Directed differentiation of iPSCs can potentially generate differentiated cells belonging to any germ layer, including cells with hepatocyte-like morphology and function. Such cells, termed iHeps, can be derived by sequential cell signaling using available information on embryological development or by forced expression of hepatocyte-enriched transcription factors. In addition to the translational aspects of iHeps, the experimental findings have provided insights into the mechanisms of cell plasticity that permit one cell type to transition to another. However, iHeps generated by current methods do not fully exhibit all characteristics of mature hepatocytes, highlighting the need for additional research in this area. Here we summarize the current approaches and achievements in this field and discuss some existing hurdles and emerging approaches for improving iPSC differentiation, as well as maintaining such cells in culture for increasing their utility in disease modeling and drug development.

**Keywords** iPS cells · Reprogramming · Hepatocyte-like cells · Directed differentiation · Cellular models of disease · Cell transplantation

## Introduction

Hepatocytes perform a variety of diverse but interrelated functions including protein synthesis, energy metabolism, detoxification of endogenous and exogenous substances and biliary excretion. Inherited abnormalities of any one of the thousands of genes preferentially expressed in the liver can lead to an inherited metabolic disorder. Availability of a cellular platform for modeling these disorders and to using such platforms to assist drug discovery would be a great boon to pharmacological development. If iHep cells could be produced in large quantities and at a reasonable cost, they could be utilized in bioartificial liver assist devices that could provide life support during acute liver failure, while awaiting the availability of a donor liver. Orthotopic or auxiliary liver transplantation can rescue patients with both acute and chronic liver failure, as well as a large number of liver-based metabolic diseases. However, liver transplantation requires considerable resources and expertise and is dependent on the immediate availability of donor livers. For this reason, many investigators have explored the transplantation of isolated primary hepatocytes as a minimally invasive alternative to liver transplantation, either as a bridge to whole organ transplantation or as a definitive treatment. Despite some encouraging results, universal application of hepatocyte

✉ Jayanta Roy-Chowdhury
jayanta.roy-chowdhury@einstein.yu.edu

1 Division of Gastroenterology and Hepatology, Department of Medicine, Albert Einstein College of Medicine, 1300 Morris Park Avenue, Bronx, NY 10461, USA

2 Marion Bessin Liver Research Center, Albert Einstein College of Medicine, 1300 Morris Park Avenue, Bronx, NY 10461, USA

3 Department of Genetics, Albert Einstein College of Medicine, 1300 Morris Park Avenue, Bronx, NY 10461, USA

4 Department of Radiation Oncology, Albert Einstein College of Medicine, 1300 Morris Park Avenue, Bronx, NY 10461, USA

transplantation has been thwarted by the severe shortage of donor livers, which are normally prioritized for organ transplantation. Also, the viability of primary hepatocytes after cryopreservation is highly variable. These problems highlight the need for developing an alternative, preferably renewable source of human hepatocytes for use in regenerative medicine. Although tissue stem cells, including hematopoietic, adipose or mesenchymal stem cells, as well as bipotent liver progenitor cells could potentially serve as sources for in vitro differentiation of hepatocyte-like cells, in this review we have focused on methods for reprogramming normal or patient-derived somatic cells into induced pluripotent stem cells (iPSC) and directed differentiation of the iPSCs into hepatocyte-like cells, which are commonly termed iHeps.

## Pluripotent stem cells as a source for developing cellular models of inherited metabolic diseases and application in regenerative medicine

Because pluripotent stem cells (PSC) can proliferate continuously and also differentiate into cells of all three germ layers, they offer the possibility of generating differentiated cells in vitro for disease modeling, drug development and regenerative medicine. Research of human embryonic stem cells provided the initial platform for developing methods for generating differentiated cells. However, application of these cells was constrained by ethical considerations and inability to harvest the cells from patients with inherited diseases. The breakthrough discovery by Takahashi and associates [1] that transient overexpression of only four transcription factors was sufficient to change the epigenetic landscape of differentiated somatic cells, reprogramming them to pluripotent stem cells (iPSC) overcame many of the limitations of embryonic stem cells. As no embryo needed to be destroyed for generating the iPSCs, the ethical concerns were significantly mitigated. From a translational viewpoint, the ability to generate iPSCs from adult somatic cells has opened the possibility of developing cellular models of genetic diseases. In addition, cells differentiated from iPSCs from individual patients with monogenetic diseases could be used for transplantation after correcting the genetic defect, thereby circumventing the need for immune suppression.

## Reprogramming somatic cells to iPSCs

The landmark discovery by Yamanaka and associates that mouse and human skin fibroblasts can be reprogrammed to iPSCs by transiently overexpressing only four pluripotency factors, Oct3/4, Sox2, Klf4 and c-Myc (OSKM), catalyzed a worldwide effort to generate iPSCs from a variety of somatic cells by a number of transfection or transduction methods. Alternative approaches based on expressing specific sets of microRNAs that coordinately modulate the expression of multiple genes are also beginning to emerge.

### Choice of somatic cells for reprogramming

Skin fibroblasts, which were used originally by investigators pioneering the generation of iPSCs continue to be used most frequently by many investigators [1]. Although culturing skin fibroblasts is straightforward, in most cases a skin biopsy is required specifically for this purpose. This minimally invasive procedure is generally safe, but is associated with the potential risk of causing scars or even keloids. Alternative somatic cell types such as peripheral blood cells [2, 3], hair follicle keratinocytes or renal tubular epithelial cells shed in the urine [4–6] can be harvested without such risk and reprogrammed to iPSCs. Under special circumstances, such as during surgery for clinical indications, adipose-derived stem cells [7], neural stem cells [8], hepatocytes [9], keratinocytes [10] and pancreatic islet β cells [11] could be procured without exposing the subject to an additional procedure. Aminiotic cells obtained during diagnostic amniotic fluid aspiration [12], as well as cord blood endothelial cells [13], have been also used for reprogramming into iPSCs. Differentiated cells owe their phenotype to epigenetic modification of their genome, particularly in the promoter/enhancer domains of the genes. During reprogramming to pluripotent cells, the epigenetic marks are mostly effaced. At least in some cases, effacement of the epigenetic marks may not be complete, whereby the epigenetic "memory" is partially retained by the somatic cells. In these cases the derived iPSCs may carry some phenotypic characteristics of the somatic cells, which may hinder their differentiation to desired cell types. On the other hand, conceptually, the partial retention of the parent cell phenotype could even provide a potential benefit in generating the target cell type. The ability of iPSCs to be differentiated to hepatocytes has been reported to differ from donor to donor, and to vary according to the original cell type [14]. For example, iPSCs derived from PBMCs were reported to be more able to be differentiated into iHeps than iPSCs derived from dermal fibroblasts. Similarly, somatic cells of endodermal origin, such as tubular epithelial cells shed in the urine, could be potentially more amenable to differentiation to hepatocytes, which are also endoderm-derived [5, 6]. However, if the reprogramming is complete, at least in theory, iPSCs derived from any cell type should exhibit similar gene expression patterns and should be phenotypically identical. Another issue to consider in selecting the somatic cell source is the probability of these cells to have acquired

genetic lesions or rearrangement prior to their harvesting. For example, epithelial cells derived from umbilical cord blood have minimal exposure to environmental genotoxic agents and should be highly desirable as a starting cell type for reprogramming. However, this has to be weighed against the fact that such cells are usually not available to many patients at the time of diagnosis of their inherited disease. Adipose tissue-derived stem cells or bone marrow hematopoietic stem cells (HSC) have the advantage of having undergone only infrequent cell divisions, reducing their chance of acquiring somatic cell mutations. However, obtaining these cells requires invasive procedures. Peripheral blood mononucleocytes (PBMC) are a convenient source of somatic cells, but random recombinations of the variable, diverse and joining gene segments [*V*(*D*)*J*] in T and B lymphocytes remains a concern. HSCs obtained from blood samples would not be associated with such concerns, but they are normally preset in very small numbers. For skin fibroblasts, in addition to the minor risk of scar or keloid formation, there is a concern about the life-long exposure of the skin cells to ultraviolet irradiation and environmental genotoxins that could potentially result in preexisting acquired genetic lesions, although the frequency of this has not been determined systematically. In all cases, it should be considered that aneuploidy, genetic mutations, deletions, insertions, duplications or rearrangements observed in iPSCs could have preexisted in the somatic cells, or could have been acquired during reprogramming. How frequently this affects the iPSCs and to what extent these genetic abnormalities affect their ability to be differentiated to target cell types remains to be determined.

## Reprogramming methods

The standard approach consists of overexpressing the four Yamanaka reprogramming transcription factors, OCT3/4, KLF4, SOX2 and MYC. An alternative strategy is based on overexpressing a set of microRNAs for coordinated modulation of multiple genes. A brief discussion of these strategies follows (Fig. 1).

## Reprogramming by overexpression of Yamanaka factors

In their pioneering studies, Yamanaka and associates had used retroviral vectors, based on Moloney's murine leukemia virus (MoMuLV). After infection, the RNA genome of these retroviruses give rise to cDNAs that are randomly integrated into the host genome. MoMuLVs infect almost exclusively dividing cells. Expression of the transgenes integrated via MoMuLV vectors are often silenced after the somatic cells are reprogrammed to iPSCs. Subsequently, pluripotency is maintained by the switched gene expression pattern of the reprogrammed cells. To increase the transduction efficiency, another class of recombinant retroviruses, termed lentiviruses, were used because these can infect both dividing and non-dividing cells [15, 16]. Random integration of the proviral genome into host chromosomes is associated with the potential risk of activation of host cell protooncogenes. For this reason, lentiviral vectors were designed with the transcription units flanked by lox sequences, so that the exogenous DNA components could be removed by treating the cells with Cre recombinase after reprogramming is achieved [17]. Removal of the integrated transcription factor genes has also been achieved using the piggyBac (PB) transposition system, which utilizes 13-base pair inverted terminal repeats and the active PB transcriptase for insertion and subsequent excision of the DNA cassette, without altering the host genomic sequence [18]. Because transgene removal by the Cre or PB system may be incomplete, investigators have sought methods that do not require integration of exogenous DNA into the host genome.

Adenoviruses are DNA viruses that infect cells via the Coxsackie adenovirus receptor (CAR) [19] and recombinant adenoviral vectors can transduce a wide range of cells without integration, although integration may occur rarely [20]. Because CAR is expressed at a lower level in human cells than in rodent cells, generating human iPSCs using these vectors is much less efficient than reprogramming mouse cells [21]. Non-viral DNA vectors used for reprogramming include the transfection of "minicircles" generated by removing the bacterial components of plasmids [22]. The minicircles remain in cells for a longer duration than do conventional plasmids. To deal with the problem of rapid loss of plasmids from dividing cells, the Yamanaka laboratory has introduced episomal plasmids containing the Epstein-Barr nuclear antigen-1 (EBNA1) sequences. Whereas conventional plasmids are diluted and lost during cell division [23], the OriP/EBNA1 plasmids can replicate during cell division for about six cycles, before they are lost from the cells [24], whereby a single transfection is sufficient for reprogramming. A set of three plasmids that express OCT3/4, SOX2, KLF4, L-Myc and Lin28, in addition to a shRNA that suppresses p53 expression [25] can be introduced into somatic cells in a single step to generate iPSCs. Because DNA-based methods are associated with a minute risk of integration into the host genome, other investigators have developed RNA-based methods of reprogramming.

Reprogramming by transfecting mRNA encoding five pluripotency factors was first reported by Plews et al. [26]. The RNA is generated by in vitro transcription and then modified to reduce cellular interferon response that can rapidly destroy the exogenous RNA. Replacement of

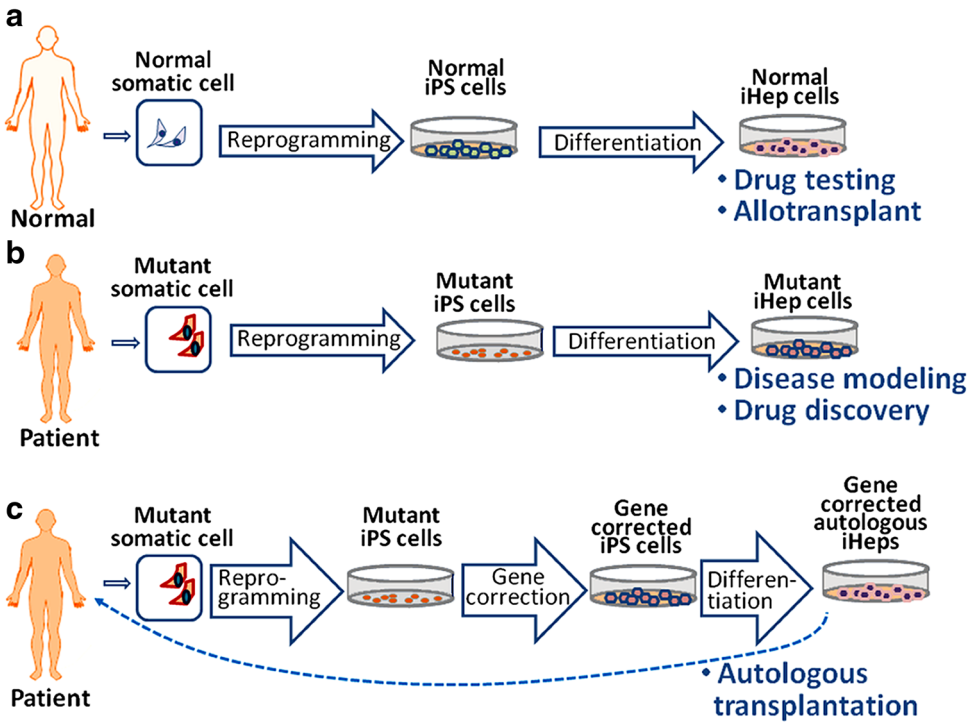


**Fig. 1** Potential uses for iPSC-derived hepatocyte-like cells (iHeps). **a** Somatic cells obtained from normal subjects can be reprogrammed by expressing OCT3/4, SOX2, KLF4, and MYC. The normal iPSC are subjected to multistep directed differentiation to generate iHep cells. After characterizing the iHep cells for hepatocyte-specific gene expression and functions, they may be utilized potentially for (1) drug development, particularly for toxicity testing, which will require a high level of in vitro maturation of the iHeps and (2) transplantation into allogeneic but HLA-matched donors, which will be facilitated by the establishment of iPSC "banks". **b** Somatic cells from patients with inherited diseases of the liver may be reprogrammed to iPSCs carrying the disease-causing mutations and then differentiated to iHeps. These iHeps can serve as (1) cellular models of inherited liver-based diseases to help in the understanding of disease mechanisms and prognosis for patients carrying individual mutations and (2) a platform for discovery of drugs for novel therapies of those disorders. **c** Somatic cells derived from individual patients with liver-based inherited metabolic disorders may be reprogrammed to iPSCs, which are genetically corrected, by gene transfer or homologous recombination augmented by targeted DNA breaks using ZFN, TALEN or CRISPR technologies. The genetically corrected iPSCs could be then differentiated to iHeps that could be autografted into the donor individual, thereby obviating the need for immunosuppression

cytidine and uridine residues by 5-methylcytidine and pseudouridine, respectively, ameliorates the interferon response. Additionally, interferon binding to its receptor is inhibited by expressing an interferon receptor mimetic, B18R/B19R. Efficiency of this system is increased by adding internal ribosomal entry sequences, strong translational initiation signals and a polyA signal at the 3′UTR [27]. Despite these modifications, repeated transfection of the RNA is required, which may be injurious to some primary cell types. Another highly efficient RNA-based reprogramming strategy is based on recombinant Sendaï virus (Hemagglutinating Virus of Japan, HVJ). This single-stranded RNA virus of the paramyxovirus family differs from other currently used recombinant viral vectors as the entire replicative cycle of the Sendai virus occurs outside the nucleus, thereby virtually eliminating the risk of integration of the exogenous genes into the host genome. Transfer of the pluripotency transcription factors by a single infection with a set of three recombinant Sendaï viruses results in a high frequency of reprogramming of human primary somatic cells [28–31].

Finally, reprogramming has been achieved without using any form of nucleic acids. In this strategy the reprogramming pluripotency proteins (OSKM) are introduced into the cells. To deliver the proteins across the cell membranes, they are tagged with arginine or lysine-enriched "cell penetrating peptides" (CPP) [32, 33], such as a peptide fragment of the human immunodeficiency virus transactivator of transcription (HIV-TAT). The need to transfer large amounts of the protein and a short half life of the transcription factors in the dividing cells limits the efficiency of this method [6].

## Reprogramming methods based on overexpression of microRNAs

Reprogramming of somatic cells with microRNAs (miRNAs) represents the first departure from reprogramming

based on pluripotency factors. miRNAs can regulate cell function by modulating the expression of multiple genes simultaneously. miRNAs enriched in embryonic stem cells (ESC) may be involved in maintaining pluripotency [34, 35]. The *miR302/367* cluster, which is highly expressed in ESCs, has been used to augment transcription factor-based reprogramming of somatic cells [36]. This cluster is transcribed as a single polycistronic transcript from intron 8 of the Larp 7 gene on chromosome 3 and is processed into five miRNAs [37], four of which (*miR301a, b, c* and *d*) have identical seed sequences. Expression of the *miR302/367* cluster, which is highly conserved across species, upregulates the pluripotency transcription factors, OCT3/4 and SOX2. In turn, this cluster activates endogenous OCT3/4 expression after the cell is reprogrammed to iPSC. The five miRs derived from this cluster target TGFβ receptor 2, promote E-cadherin expression, accelerate mesenchymal-to-epithelial transition and promote cell division [38]. Retrovirus-mediated expression of the *miR302/367* cluster is sufficient to reprogram murine and human somatic cells [39]. Chromatin remodeling is required for reprogramming. Inhibition of histone deacetylase 2 by valproic acid markedly enhances *miR302/367*-mediated reprogramming [36, 38, 39].

## Directed differentiation of iPSCs into hepatocyte-like cells

iPSC-derived hepatocyte-like cells (iHep) can help understand the mechanisms underlying inherited metabolic liver diseases, serve as a platform for studying the toxicity and efficacy of drugs and be a renewable cell source for regenerative therapies of human liver diseases. iHeps generated from patients with several monogenic liver-based metabolic diseases have been reported to reflect specific aspects of the corresponding disorders and have suggested therapeutic targets [40].

Factors that need to be taken into account in designing strategies for differentiating iPSCs to iHeps include the extracellular matrix, coculture with other cell types, and the media and supplements to provide cell signaling. The majority of differentiation protocols use undefined matrices such as Matrigel [41, 42], although defined matrix components such as collagen [43], laminin [44], fibronectin [45] and vitronectin [46] have been identified as effective. These defined components, singly or in combinations [47], are being introduced for iHep differentiation. Approaches to stepwise differentiation of iPSCs are informed by advances in understanding the molecular events at various stages of embryonic development [48, 49]. In mammals, the embryonic ventral foregut gives rise to the liver. Therefore, a number of current differentiation methods of embryonic stem cells and iPSCs for generating hepatocyte-like cells have been based on stepwise induction of definitive endoderm, differentiation to hepatic progenitors and finally, maturation to hepatocyte-like iHep cells [50]. The first step of differentiating pluripotent stem cells to definitive endoderm cells is critical in hepatocyte differentiation. The critical element of this step includes exposure to the transforming growth factor β (TGF-β) superfamily members activin A and bone morphogenic protein 4 (BMP4) [51–54]. A short exposure to Wnt3a, which interacts with activin A, increases the proportion of cells that are differentiated to definitive endoderm [55]. Combination of fibroblast growth factors (FGF) and BMP4 promotes definitive endoderm cell commitment later in embryonic development [56, 57]. The effect of factors involved in early mammalian development is inhibited by fetal bovine serum in the culture medium [58]. During embryonic development of definitive endoderm, marker genes that are expressed include SRY (sex determining region Y)-box 17 (Sox17) and forkhead box A2 (Foxa2). This gene expression pattern specifies foregut endoderm, which subsequently differentiates to pancreatic and hepatic cells [59].

The next step toward differentiation to hepatocyte lineage is the induction of hepatic progenitor cells or hepatoblasts. This is accomplished in vitro by adding a cocktail of growth factors, of which hepatocyte growth factor (HGF) is the most critical [60, 61]. The transcription factor hepatocyte nuclear factor 4-alpha (HNF4α) is initially expressed in the embryo in the developing hepatic diverticulum and its expression increases during liver development. HNF4α expression marks the differentiation of definitive endoderm cells toward the hepatocyte lineage in vitro [62]. Alpha-fetoprotein (AFP) expression is an important marker of primitive hepatocytes [63].

In the final step, the hepatocyte progenitors are matured to hepatocyte-like iHep cells by attempting to recapitulate the physiological environment during perinatal and neonatal life. Commonly, the maturation step in vitro includes exposure of the cells to oncostatin M (interleukin-6 family cytokine), in combination with glucocorticoids [64]. With the differentiation to iHeps, the cells acquire hepatocyte-like morphology and accumulate glycogen. Some hepatocyte-specific functions, including albumin and apolipoprotein synthesis, and urea production, reach near-adult levels in late fetal life. Other functions, such as UGT1A1-mediated bilirubin glucuronidation and alpha-1 antitrypsin synthesis are minimal in neonates and are stimulated by perinatal events, such as increased portal blood flow, alteration of blood oxygen tension after the closure of the ductus venosus, elevated plasma glucagon and catecholamine levels, and probably a host of other changes, such as introduction of food and bacterial

colonization of the bowels. Perinatal changes in the epigenetic landscape of DNA is central in establishing the gene expression pattern of adult hepatocytes [65]. Some isoforms of UDP-glucuronosyltransferase and sulfotransferases exhibit a second peak at adolescence, coincident with the surge of sex hormones. Incompleteness of the knowledge of mechanisms of perinatal hepatocyte maturation is an obstacle to the final maturation of iHeps. Commonly used markers of iHep maturation include the expression of albumin, CK18, cytochrome p450 enzymes (CyP), SERPINA1 (α1-antitrypsin, ATT), asialoglycoprotein receptor 1 (ASGPR1), C/EBPα, UGT1A1 and Prox1. Standard functional assays used to compare the extent of similarity of the iHeps to primary hepatocytes include urea production, indocyanin green uptake and clearance, low-density lipoprotein (LDL) uptake, albumin and ATT secretion into the medium and inducible cytochrome P450 activity. Although cryopreserved primary hepatocytes are usually used for comparison in these in vitro assays, there is a significant variability in the gene expression and function of primary human hepatocytes from batch to batch [66]. Cell culture components used in various laboratories for iHep differentiation have been reviewed [50].

## Improving differentiation of iPSC to hepatocyte-like cells (iHeps)

Despite the effort of many investigators, currently available techniques do not achieve the differentiation of iHeps to the level of normal adult primary hepatocytes. It is often stated in literature that the phenotype of iHeps is similar to that of fetal or neonatal hepatocytes. In our experience, although iHeps exhibit several characteristics of fetal hepatocytes, they also express some genes, such as *UGT1A1* and *SERPINA1*, which are expressed at very low levels in neonatal hepatocytes. Many hepatocyte-preferred genes are expressed in iHeps at much lower levels than in primary hepatocytes. The lack of full maturation of the iHeps can affect, to different extents, their utility (a) as a platform for pharmacological testing, (b) as pathophysiological models for inherited liver-based disorders and (c) in cell-transplantation-based liver regeneration. These application-specific issues have been discussed under separate headings.

Another hurdle in the application of iHeps is that like primary hepatocytes, iHep cells deteriorate rapidly in culture with loss of expression of liver-specific genes. Some of the recent advances toward improving the differentiation and maintenance of iHeps are discussed below.

## Providing postnatal signals

While several current protocols lead to iHep maturation to the level at which they express secreted proteins, such as albumin and alpha-1 antitrypsin, inducible expression of detoxification enzymes, such as cytochrome P450 has lagged behind the levels found in mature primary hepatocytes by several logs. Notably, the expression of many hepatocyte-specific genes is at a low level at birth and increases during postnatal development. In many cases, the rapid maturation of the gene expression pattern is mediated by nuclear receptors. For example, the nuclear pregnane X receptor (PXR) is critical to xenobiotic sensing and induction of proteins involved in all phases of detoxification, including CYP3A4, a phase I oxidative enzyme, glutathione S-transferase, a phase II enzyme and phase III transport, uptake and efflux proteins such as OATP2 (SLC02A1) and the P-glycoprotein (MDR1) [67]. Avior et al. argued that a possible reason for the failure of most current differentiation protocols to achieve iHep maturation beyond the level of fetal hepatocytes is that the signals provided during the stepwise differentiation process do not include those that the hepatocytes are exposed to during the postnatal period [68]. There is a dramatic postpartum shift of the liver from placental to enteral nutrition [69]. Fatty acids in breast milk become the primary source of energy. The fetal intestinal contents are basically sterile in utero and colonization by the gut microbiota occurs after onset of feeding. Consequently, the fetal liver becomes exposed to bacterially derived secondary bile salts, such as lithocholic acid. Lithocholic acid is an activator of the nuclear pregnane X receptor (PXR), which in turn controls the expression cytochrome P450 isoforms, such as CYP2C9 and CYP3A4. Similarly, vitamin K is not transported efficiently across the placenta. Exposure of this fat-soluble vitamin to intestinal bacteria generates menaquinones. Menaquinone-4 is a known activator PXR. Indeed, exposure of iHeps to lithocholic acid at the final stage of maturation induced a dose-dependent expression of PXR, its nuclear localization and expression of its targets, CYP3A4 and CYP2C9 [68]. Importantly, these iHeps exhibited appropriate P450 induction by omeprazole, an aryl hydrocarbon receptor (AhR) agonist, as well as by rifampicin, a PXR agonist. Lithocholic acid is hepatotoxic. It is possible that other PXR activating agents can be used to replace this secondary bile acid in the final step of maturation of the iHeps. Small molecules may also be useful in activating other nuclear receptors. Finally, the expression and activity of nuclear receptors and their target genes should be a part of the evaluation of the extent of iHep maturation [5].

## Forced expression of hepatocyte-enriched transcription factors

Transcription factors that are expressed at various developmental stages have been expressed at various steps of

differentiation of iPSCs to iHeps. Recombinant adenoviral vectors have been used to express SOX17 [70], HEX [71] and HNF4α [72] to improve the differentiation to definitive endoderm, hepatoblasts and iHeps, respectively. Thus, sequential expression of developmental stage-specific transcription factors, or perhaps microRNAs, could potentially improve the phenotype of iHeps.

Many of the components used in media or matrices for iHep differentiation are difficult to adapt to current "good manufacturing practices" (GMP). For application in regenerative medicine, small molecules and chemicals are being explored for moving toward GMP-compatible methods. For example, CHIR99021 has been shown to generate definitive endoderm from iPSCs [73]. Dexamethasone and hydrocortisone-21-hemisuccinate have been used in the final stage of iHep maturation [74]. A number of other small molecules that promote differentiation toward hepatocytes have been identified [75].

With advances in the understanding of hepatocyte maturation, it is likely that improved iHep phenotypes that closely resemble adult primary hepatocytes may be achieved. Until then, it may be useful to develop methods to generate iHeps with characteristics that are specifically suited for specific applications. For example, iHeps with xenobiotic detoxification functions comparable with primary hepatocytes could be generated by transduction of the cells at a late stage of differentiation with the nuclear receptor, constitutive androstane receptor (CAR), which regulates the expression of multiple enzymes mediating the detoxification of endogenous metabolites, drugs, toxins and other xenobiotics [76].

### Prolonged culturing of the iHeps

Hepatocytes in liver chords exist in three-dimensional structures in the native liver matrix. They are connected with neighboring hepatocytes by tight and gap junctions and are in close vicinity of non-parenchymal cells, such as hepatic sinusoidal endothelial cells, stellate cells and Kupffer cells, enabling cross-talk between parenchymal and non-parenchymal cells. The matrix, the neighboring cells, as well as nutrients, growth factors and signaling molecules in the portal blood cooperate to maintain the viability and gene expression characteristics of hepatocytes. Thus, it is not surprising that primary hepatocytes or iHeps do not retain their function in "minimalistic" two-dimensional cultures. In an effort to overcome this hurdle, there is an ongoing effort to mimic the spatial organization of the liver [77]. In three dimensional co-culture systems hepatocytes and hepatic sinusoidal endothelial cells appear to support each other [78]. Coculturing iHep cells with endothelial and stromal cells increases the degree of their maturation by direct contact or through paracrine mechanisms [79]. Similarly, culturing hepatocyte-specific definitive endoderm cells with human umbilical vein endothelial cells and mesenchymal stem cells (MSCs), resulted in three-dimensional cell clusters, in which the iPSC-derived cells expressed AFP, albumin and other hepatocyte-preferred genes. After implantation intracranially, in the small bowel mesentery, or beneath the renal capsule of immunodeficient mice, the cell clusters became vascularized and continued to proliferate for two months. Viability and function of the engrafted cell clusters was evidenced by secretion of human albumin and AAT into the host plasma. The detoxification function of the implants was shown by their cytochrome P450 activity, and improved survival of recipient mice after toxic liver damage. Although implanted cell clusters are not connected to the bile duct system, this model only partially recreates the liver environment. To recreate a structure more completely resembling the liver, whole mouse livers were decellularized by detergent perfusion, and then seeded with isolated hepatocytes and endothelial cells [80, 81]. It is possible that a similar environment would promote the differentiation and increase the longevity of iHeps.

## iHeps as a platform for pharmacological testing

Hepatocytes are the primary site of metabolic transformation of lipids mediated by the cytochrome P450 family of monooxygenases, which are also involved in the phase I transformation of drugs, toxins and other xenobiotics. Phase II of detoxification, mediated by conjugation reactions, and Phase III, which comprises transport of the metabolites out of cells are also carried out primarily by hepatocytes. Drugs or their intermediary metabolites can be toxic to the liver, which makes drug-induced liver injury (DILI) a leading cause of acute liver injury and post-market drug withdrawals. This makes hepatotoxicity and drug metabolism a major focus of pharmaceutical development. The low concordance between animal and clinical studies, and low metabolic activity of human hepatoma cell lines, make primary hepatocytes an essential platform for drug metabolism and toxicity studies. However, primary hepatocytes are scarce because of a shortage of donor livers, they rapidly lose metabolic functions in vitro and exhibit a significant batch-to-batch variation. iHeps could potentially fill this void. However, as mentioned above, most currently used methods for differentiation of human pluripotent stem cells do not express adequate levels of inducible cytochrome P450 enzymes, such as CYP3A4. Thus, in a recent drug toxicity screening study using hPSC-derived hepatocytes, poor correlation was shown with primary human hepatocytes, resulting in $R^2$ of 0.49 [82]. Employing postpartum cues of hepatocyte maturation, such as

lithocholic acid and vitamin $K_2$ in the final step of iHep maturation, other investigators have obtained a significant increase in expression and nuclear localization of PXR, resulting in a 70-fold increase in CYP3A4 expression [68]. Comparison of the 50 % toxic concentrations (TC50) of these iHeps with primary human hepatocytes yielded a coefficient of correlation of 0.94. The iHeps exhibited steatosis, apoptosis, and cholestasis upon exposure to nine known hepatotoxins, confirming their ability to produce predictive results. These studies and other ongoing research demonstrate the possibility of using iHeps as a reproducible and renewable cellular platform for evaluation of drug metabolism and toxicity.

## iHeps as pathophysiological models for inherited liver-based disorders

Because iHeps can be generated from individuals of all ages from a wide variety of cell sources, they offer the opportunity to generate cellular models to understand the pathophysiology of diseases and testing of therapies at the level of "personalized medicine". For this purpose, the iHeps must be differentiated enough to express the specific hepatocyte function that is deranged in the inherited disorder, and the mutant iHep must recapitulate the main characteristics of the inherited disorder. An important goal of generating cellular models of "monogenetic" disorders is to explore the interaction of the disease causing mutation with known and yet to be discovered functional variation of other genes. Such interaction may underlie the spectrum of the phenotypic presentation of diseases caused by a specific genetic lesion. For example, the most common mutation of the *SERPINA1* gene causing α1-antitrypsin (AAT) deficiency can manifest as severe liver disease, severe pulmonary emphysema, a combination of the two, or no obvious clinical disorder at all. Variability of disease severity is also common in primary hyperoxaluria type 1, Wilson's disease and primary hemochromatosis. Therefore, it is important to generate iHeps that not only exhibit the disease-causing mutation, but also represent the gene expression repertoire of primary hepatocytes as much as possible. Similarly, when generating cellular platforms for infectious diseases, the goal may be to discover novel mechanisms of interaction of the pathogens with cell surface or intracellular molecules that may offer therapeutic targets. Again, as these novel interactions are unknown, it is important to produce iHeps that are as differentiated toward primary hepatocytes as possible.

iHeps have been generated from patients with AAT deficiency (ATD), familial hypercholesterolemia (abnormalities of low-density lipoprotein receptor, LDLR), autosomal dominant hypercholesterolemia (gain of function mutation of proprotein convertase subtilisin kexin type 9, PCSK9), Wilson's disease (*ATP7B* deficiency), familial transthyretin amyloidosis (FTA), glycogen storage disease type 1a (GSD1A, glucose-6-phosphatase deficiency), Crigler-Najjar syndrome type 1 (CN1, *UGT1A1* deficiency) and primary hyperoxaluria-1 (PH1, *AGXT* deficiency). Also wildtype iHeps are being employed in the research on infectious diseases.

The most common mutation that causes ATD gives rise to a misfolded mutant AAT termed ATZ. The mutant protein is secreted inefficiently and the low plasma concentration of the AAT results in lung disease due to the uninhibited action of leucocyte elastase. On the other hand about 20 % of patients carrying the same ATZ mutation develop clinically recognizable liver disease. In the latter group, the mutant ATZ protein accumulates in hepatocytes, giving rise to globules of polymerized ATZ. iHeps derived from an ATD patient with liver disease showed excess AAT content compared with normal [83]. To determine whether iHeps derived from patients with lung disease or liver disease presentation can model the corresponding clinical type, iHep from somatic cells of two individuals carrying the ATZ mutation were used, one with pulmonary disease and another with liver disease [84]. iHeps derived from the ATD patient with liver disease exhibited slower secretion and clearance of the mutant ATZ protein, compared with iHeps from a patient with no known liver disease or a normal individual. Thus, iHeps could model the different clinical presentations in patients carrying the identical ATZ mutation [84]. iHeps from ATD patients were used as a platform for a blind large-scale high-throughput drug screening study utilizing 96-well immunofluorescence readers and the Johns Hopkins Drug Library. Five clinically approved drugs were identified that reduced AAT accumulation in the patient-specific iHeps. Also, the ATZ mutation could be corrected in the patient iPSCs using the TALEN (transcription activator-like effector nuclease) technology. iHeps derived from the genomically corrected iPSCs were functional and did not accumulate the mutant ATZ [85].

Wilson's disease is caused by mutations in the *ATP7B* gene, encoding an ATPase, which is involved in copper removal from hepatocytes. iHEPs generated from Wilson's disease patients exhibited this defect. The disease phenotype was rescued by *ATP7B* gene transfer using a lentiviral vector or by addition of curcumin, which acts as a chaperone drug [86].

iHeps derived from patients with familial hypercholesterolemia type IIa, caused by mutations in the LDLR gene [87], exhibited deficiency of LDL internalization [83] and was used to demonstrate the effect of the cholesterol-lowering drug Lovastatin [88].

PCSK9 is a protein secreted by hepatocytes that binds to the extracellular domain of LDLR, thereby targeting it to the lysosomal pathway for degradation, which disrupts its recycling to the cell surface [89]. Gain of function mutations (GOF) of PCSK9 causes hypercholesterolemia, whereas its loss of function (LOF) mutations are associated with hypobetalipoproteinemia. Urinary sample-derived iHeps were generated from patients carrying GOF or LOF [6]. iHeps with GOF mutations exhibited reduced LDL uptake, which was ameliorated by Prevastatin treatment. In contrast, iHeps carrying LOF mutation exhibited increased LDL uptake. Thus, iHeps represent a useful model to decipher the effects of PCSK9 mutations.

iHeps have been generated also from patients with glucose-6-phosphatase deficiency (GSD1a). These cells showed excess accumulation of glycogen and lipids, which reproduced the pathology in hepatocytes of GSD1a patients [83].

Familial amyloid polyneuropathy (FAP), also termed transthyretin-related hereditary amyloidosis, results from genetic lesions of the transthyretin gene (*TTR*), which is expressed in hepatocytes. This disorder causes deposition of the amyloidogenic variants of transthyretin, particularly in the nerves. In the end stage, heart and kidney are also affected. iPSCs derived from FAP patients were differentiated into iHeps, as well as neurons and cardiomyocytes [90]. The iHeps expressed the mutant transthyretin. Exposure of the derived neurons and cardiomyocytes to the mutant transthyretin caused oxidative stress and cell death in neurons [90].

In addition, modeling inherited disorders, iHeps have been used as a platform for investigating infectious diseases caused by malaria parasites and hepatitis C virus (HCV). Differentiation of iPSCs to the stage of hepatic progenitor cells was sufficient to make them susceptible to plasmodium strains, such as *P. berghei, P. yoelii* and *P. vivax* [91]. Further maturation to the iHep stage using small molecules permitted infection by *P. falciparum.* Furthermore, plasmodium infection was reduced in this model by treatment with primaquine [91]. Importantly, this approach may permit evaluation of the efficacy of various drugs in the treatment of malaria in various ethnic groups with polymorphic variations.

Several laboratories are exploring the use of iHeps in HCV research. iHEPs were shown to express CD81, scavenger receptor class B type I, claudin-1 and occludin, the receptors required for HCV infection [92]. HCV replicated within the iHeps. Anti-CD81 antibody reduced HCV entry and interferon reduced viral replication in these cells [91]. iHeps had the ability to sustain the entire HCV life cycle [47]. Innate immune response was evidenced by upregulation of the IL-28B expression and TNF-α secretion. iHEPs infected with HCV genotype 2a released HCV virions capable of infecting HuH-7.5 cells [47]. In addition to these studies on iHep cells in culture, ability of iHep cells to support HCV infection in vivo has been demonstrated by transplanting human iHep cells into mice. The transplanted iHep cells proliferated and further matured in the murine host livers over 3 months. The resulting iHEPs could be infected by HCV in vivo [93].

## iHeps in cell-transplantation-based liver regeneration

Clinical trials of hepatocyte transplantation have provided encouraging results for the treatment of inherited liver-based disorders or as a bridge to liver transplantation while awaiting organ donors for patients with acute liver failure [94, 95]. One of the major hurdles to the universal application of hepatocyte transplantation is the scarcity of donor organs as a source of primary hepatocytes. Because iHep cells could be a potentially renewable source of human hepatocytes, several laboratories have explored the possibility of transplanting these cells into the liver of experimental animals. iHeps offer the attractive possibility of being generated from somatic cells of the recipient, so that a perfect tissue match could be obtained without the need for immunosuppression. In the case of patients with inherited disorders, this approach would require gene correction of the patient-derived iPSCs. In a proof-of-principle study, iPSCs derived from an ATD patient was gene-corrected using the zinc finger nuclease technology and iHeps derived from these cells was transplanted into a mouse model of liver injury [96]. The gene-corrected iHeps remained functional after transplantation and did not give rise to tumors. However, generating iHeps from individuals is both time consuming and expensive. With that in mind, banks of iPSC lines with an extensive range of HLA profiles are being developed with the participation of many nations [European Bank for induced pluripotent Stem Cells (EBiSC), Kyoto University Stem Cell Bank, Japan, StemBANCC, EU, HipSci, UK and the Coriell Institute, USA]. An iPSC bank could potentially provide HLA-compatible normal iPSCs for individual recipients in a short time and at a much lower cost.

Apart from the cost of generation of iHeps, a major hurdle of application of these cells in regenerative medicine is the inefficient engraftment and a low level of subsequent proliferation that is required for significant repopulation of the liver. Primary hepatocytes in the host liver are highly capable of proliferating in response to proliferative stimuli, such as partial hepatectomy. Because the hepatocytes in a majority of inherited liver-based metabolic disorders retain normal proliferative capacity, the transplanted iHep cells not only need to penetrate

through the sinusoidal endothelial barrier to engraft, they need to compete with the host hepatocytes to repopulate the liver. To provide a proliferative advantage to the transplanted cells, most of the published studies of iHep transplantation were performed in mice in which there is a severe loss of host hepatocytes because of a genetic abnormality of modification. For example, mice transgenically expressing urinary plasminogen activator in the hepatocytes (uPA-mice) or mice with fumarylacetoacetate hydrolase deficiency ($FAH^{-/-}$) have massive death of host hepatocytes, which stimulates compensatory proliferation of the transplanted hepatocytes. Transplantation of human iHep cell in immunodeficient uPA mice resulted in up to 15 % liver repopulation 100 days after transplantation [93]. But as discussed above, most inherited disorders are not associated with such spontaneous attrition of host liver cells. Therefore, some preparative manipulations of host cells are required to provide a proliferative advantage to the transplanted cells. One preparative regiment developed for use in rat and mouse transplant recipients consists of administering retrorsine, a plant alkaloid that inhibits hepatocyte replication and 70 % hepatectomy to stimulate cell division. Using this preparative approach in recipient immunosuppressed analbuminemic rats, significant repopulation of the liver with human iHeps was achieved, as indicated by the levels of human serum albumin appearing in the recipient plasma [97]. Another preparative regimen that has potential for clinical translation consists of X-irradiation of a portion of the recipient liver followed by hepatocyte or iHep transplantation. Hepatocyte growth factor (HGF) is used to stimulate the proliferation of hepatocytes. In experimental animals this can be achieved conveniently by a single injection of an adenoviral vector expressing human HGF. This approach results in repopulation of the irradiated segment of the liver by the transplanted non-irradiated hepatocytes, while the remaining liver remains unperturbed. Transplantation of normal rat hepatocytes into Ugt1a1-deficient jaundiced Gunn rats (a model of human Crigler–Najjar syndrome type 1) using this preparative method resulted in normalization of serum bilirubin levels [98]. When this preparative regimen was used in Gunn rat recipients, which were immunosuppressed with daily tacrolimus administration, transplantation of human iHeps resulted in progressive reduction of serum bilirubin by up to 60 % of the baseline levels and a significant repopulation of the X-irradiated liver lobe was observed. Excretion of bilirubin glucuronides in the bile of the recipient Gunn rats confirmed the function of the transplanted iHep cells in vivo [99].

There has been a significant discordance among the extents of iHep engraftment and liver repopulation observed in different laboratories. Several laboratories have reported different levels of repopulation of mouse or rat livers with transplanted iHeps [5, 93, 97, 99–103]. However, Haridass et al. found no significant repopulation after iHep transplantation. Because of the difference in approaches employed by the various laboratories, it is difficult to pinpoint the cause of the variable results. Engraftment and subsequent proliferation of the transplanted cells are two different but interrelated processes that could be affected by the method of differentiation of the iPSCs to iHeps, as well as the animal model used for transplantation. In several reports of successful repopulation with iHeps [5, 93, 97, 99], the method described by Basma et al. [97] was used with or without minor modifications. Human iHep transplantation has been tested mainly in rodents. Two types of models have been employed: (1) mice with genetic disorders that cause attrition of the host hepatocytes, e.g. transgenic mice expressing uPA from albumin or Mup promoter [93]; $Fah^{-/-}$ mouse models of tyrosinemia; and a transgenic mouse model of AAT deficiency liver disease, expressing a mutant human SERPINA1 (PiZ mice) [5]; (2) Mice or rats with induced liver injury, such as treatment with retrorsine [97], $CCl_4$ [100], thioacetamide [102], diethylnitrosamine [103] or preparative irradiation of a liver lobe [99]. In most cases, the evaluation of liver engraftment has been limited to immunohistochemical staining of the liver for human proteins and measurement of human serum proteins, e.g. albumin in the recipient serum, but Chen et al. [99] have also reported amelioration of a liver-specific function in the Gunn rat model of inherited jaundice, and Carpentier et al. [93] have demonstrated infection with hepatitis C virus after repopulation of *MUP-uPA*/SCID/Bg mouse livers with human iHep. In most cases, serum human albumin levels in the recipient rats or mice was not commensurate with the level of repopulation measured by immunohistochemical staining. Exceptions to this were studies where human embryonic cell-derived iHeps were transplanted into retrorsine-pretreated analbuminemic rats [97] or human iPS cell-derived iHeps were transplanted into *MUP-uPA*/SCID/Bg mice [93]. In the latter case, although the level of repopulation varied from <1 to up to 20 %, serum human albumin levels were proportional to the extent of repopulation determined by immunohistochemical staining. Carpentier et al. [93] point out that they transplanted human iHeps in *MUP-uPA*/SCID/Bg mice at the age of 5–8 months, at which time the mouse livers are still healthy, compared with livers in the more commonly used *ALB-uPA*/SCID model.

Although it is beyond the scope of this review, other investigators have transdifferentiated fibroblasts without first reprogramming them to iPSCs [104–106]. Du et al. overexpressed the hepatic fate conversion factors HNF1A, HNF4A, and HNF6 and the maturation factors ATF5, PROX1, and CEBPA in fibroblasts [104]. Transplantation

**Table 1** A "wish-list" for general and application-specific characterization of iHeps

| Application | Gene expression/function | Purpose/rationale | References |
|---|---|---|---|
| General evaluation: expression of genes encoding proteins localized in specific organelles | (a) Sinuosidal surface proteins: LDLR, ASGPR, SLCO1B1, NTCP<br>(b) Canalicular surface proteins: ABCC2 (MRP2), ABCB11 (BSEP)<br>(c) Endoplasmic reticulum proteins: UGT1A1, UGT1A6, UGT2B4, UGT2B10, CYP genes<br>(d) Cytosolic/cytoplasmic proteins: GSTA1, FABP1, SULT2A1, KRT18, KRT19<br>(e) Golgi apparatus: ATP7B, MAN1A1<br>(f) Mitochondrial: PCK2 (PEPCK)<br>(g) Nuclear: HNF4α, CEPBA, NR1H2 & NRNH3 (LXR), NR2B1 & NR2B2 (RXR α) PROX1<br>(h) Secretory proteins: ALB, AFP, APOA1, APOA2, HGF, TTR, SERPIN1A1, coagulation Factor V | Expression of these genes should be determined at the mRNA level and/or protein level to evaluate extent of differentiation of the iHeps toward adult human hepatocytes | [5, 93, 97, 99–103] |
| General evaluation: cell function | (a) Secretion of albumin and alpha-1 antitrypsin (AAT) into the media<br>(b) Indocyanin green (ICG) uptake and clearance<br>(c) Glycogen content<br>(d) Urea secretion into the media (preferably incorporation of isotopically labeled ammonia ($^{15}ND_3$) into urea) [108]<br>(e) P450 activity | (a)Albumin and AAT are expressed and secreted almost exclusively by hepatocytes<br>(b) ICG is taken up by hepatocytes via OATP1B1 (encoded by *SLO1B1*) and pumped out by canalicular MRP2 (encoded by *ABCC2*)<br>(c) Glycogen synthesis is an energy-consuming process catalyzed by a series of enzymes<br>(d) Urea synthesis is a hepatocyte-specific function mediated by urea cycle enzymes. However, urea secretion does not prove the activity of the entire urea cycle, and may result from arginase activity alone [108]<br>(e) Cytochrome P450 s are heme-containing oxidoreductases that mediate bile salt production from cholesterol and phase I detoxification of drugs | [5, 93, 97, 99–103] |
| Drug development | (a) Sinusoidal uptake proteins, e.g. SLC01B1, SLC01B1<br>(b) Glutatione-*S*-transferases (binding of organic anions, as well as glutathione conjugation<br>(c) The nuclear receptor PXR<br>(d) Phase I detoxification proteins—Cytochrome P450 isoforms: CYP1A2, CYP2A6, CYP2B6, CYP2C8, CYP2D6, CYP2E1, CYP3A4, CYP3A5, CYP3A7, CYP7A1<br>Inducibility of CYP3A4, PXR expression<br>(e) Phase II detoxification enzymes: UGT1A1, SULT2A1<br>(f) Phase III detoxification proteins: MRP2, ABCC2, MRP3, MDR1 | Drugs undergo a multi-step process for hepatic detoxification:<br>(a) Uptake at the sinusoidal surface<br>(b) Storage within hepatocytes<br>(c) Phase I detoxification consisting of simple modifications, most commonly mediated by P450 enzymes, which are coordinately regulated by the nuclear receptor PXR<br>(d) Following initial modification mediated by P450, the intermediary metabolites undergo Phase II or conjugation reactions<br>(e) Detoxified metabolites are pumped out into the bile canaliculus or to sinusoidal blood by canalicular ATP-binding cassette transporters | [68] |

**Table 1** continued

| Application | Gene expression/function | Purpose/rationale | References |
|---|---|---|---|
| Disease modeling | (a) Expression of the gene of interest in the wildtype iHep control<br>(b) Expression of the mutant gene (when missense mutations exist)<br>(c) Expression of other known genes involved the metabolic pathway<br>(d) Expression of genes that are hypothesized to modify the phenotype (e.g. genes involved in autophagy, ubiquitination, Neddylation, etc.) | (a) A set of well-differentiated wildtype iHep is essential for comparison<br>(b) The mutant protein could be toxic or dysfunctional because of configurational changes. Thus, these genes or their products could be potential therapeutic targets<br>(c) Inhibition or induction of the expression of a different gene in the metabolic pathway could modify the disease phenotype<br>(d) Gene products affecting protein processing, folding or degradation could offer potential therapeutic targets | [83–93] |
| Transplantation | (a) Mitochondria: morphology and function:<br>Number of mitochondria per cell and their morphology are assessed by electron microscopy and fluorescent staining<br>Expression of POLG, TFAM<br>Mitochondrial proteins: Atp5g, Atp8 and Ucp2<br>(b) ATP content and oxygen consumption<br>(c) Expression of cMET, EGFR, FGFR, β1-integrin and β-catenin | (a) The process of engraftment and more importantly, subsequent proliferation of iHeps consume energy, requiring high ATP supply provided by mitochondria<br>*POLG* and *TFAM* are encoded by nuclear genes but regulate mitochondrial gene transcription<br>Mitochondrial proteins Atp5g, Atp8 and Ucp2 can be used as markers of abundance of mitochondria per iHep<br>(b) Direct measurement of ATP content and oxygen consumption are indicators of mitochondrial function<br>(c) Cell surface receptors HGF (cMet), EGF (EGFR), FGF4 (FGFR) are essential for the proliferation of hepatocytes. β1-Integrin and β-catenin are required for hepatocyte proliferation | [5, 68, 93, 97, 99–105, 110, 111, 112] |

of these cells into immunodeficient transgenic mice that conditionally express uPA (Tet-uPA/Rag2$^{-/-}$/γc$^{-/-}$) resulted in up to 30 % liver repopulation, along with plasma levels of human serum albumin exceeding 300 μg/ml [104]. In another study, Zhu et al. partially reprogrammed fibroblasts toward pluripotency and then differentiated them to endoderm progenitor cells (iMPC-EPCs) and subsequently to hepatocyte-like cells. Transplantation of these cells in *Fah*$^{-/-}$ *Rag2*$^{-/-}$ mice resulted in up to 2 % liver repopulation in 6 months [105].

To circumvent the initial hurdle of engraftment of the transplanted hepatocytes into the vascularized liver plates, Tekebe et al. [107] generated self-assembling three-dimensional "liver buds" by coculturing human iPSC-derived hepatic endoderm cells with human umbilical vein endothelial cells (HUVEC) and human mesenteric stem cells. Transplanting multiple "liver buds" on the mesentery of transgenic mice expressing tyrosine kinase rescued the recipients from Ganciclovir-induced liver failure. However, although these organoids form bile canaliculi between hepatocytes, these are not connected to bile ducts, precluding their exocrine function. Also, it is unclear whether the vasculature endothelium generated from the HUVECS will acquire the characteristic fenestrated morphology of liver sinusoidal endothelial cells.

Clearly, the differentiation of pluripotent cells is still a work in progress. Incremental improvements continue to be provided by work from various laboratories. A limited set of surrogate markers that could be global indicators of maturation of iHeps has not been defined. Therefore, critical assessment of the improvements reported by various laboratories require the availability of extensive data on the characteristics of the iHeps. Until the technology advances to a level where a single protocol is able to produce iHeps that are suitable for all different applications, it is practical to identify a set of general characteristics that should be

evaluated for all applications, and additional sets that are particularly important for specific types of application of iHeps. A "wish list" of general and application-specific characterization of iHeps is given in Table 1.

For future application of iHeps in regenerative medicine, the iPSCs and the iHeps will need to be produced under GMP conditions. Extensive safety studies will need to be performed both in small animals, such as rats and mice, and in larger and longer-lived recipients, preferable in non-human primates. iPSCs have been generated from rhesus monkey [108] and pigtail macaque [109] fibroblasts and differentiated to functional iHeps.

In summary, the availability of methods for reprogramming human somatic cells to iPSCs and directed differentiation of the iPSCs to iHeps has opened the opportunities for generating personalized and renewable hepatocyte-like cells for modeling of inherited as well as infectious diseases. These cells can serve as a platform for drug discovery. Although there has been some success in repopulating the livers of rodent models of human diseases with the iHep cells, initial engraftment and subsequent proliferation of the transplanted iHeps remains low. Thus, directed differentiation of the iPSCs toward the mature hepatocyte phenotype requires further refinement. It is likely, however, that the transplanted iHep cells will continue to mature in vivo. Research by many groups worldwide continues to produce creative solutions for eventually overcoming the existing hurdles to applying iHeps to pathophysiological and pharmacological research and using the cells for personalized regenerative medicine.

**Compliance with ethical standards**

**Funding** This work was funded in part by the following Grants: 1PO1 DK 096990-01 (to JRC, PD: D. Perlmutter); RO1 DK092469 (to NRC), P30 DK041296-28 (to JRC, A. PD: Wolkoff) R01 DK100490-01A1 (to JRC, PI: DA Shafritz); New York Stem Cell Foundation CO26440 (to JRC); R01 DK064670, R33 CA121051 (to CG).

**Conflict of interest** Namita Roy-Chowdhury, Xia Wang, Chandan Guha and Jayanta Roy-Chowdhury declare that they have no conflict of interest.

**Ethical approval** This article does not contain any studies with human participants or any studies with animals performed by any of the authors.

## References

1. Takahashi K, Yamanaka S. Induction of pluripotent stem cells from mouse embryonic and adult fibroblast cultures by defined factors. Cell 2006;126(4):663–676. doi:10.1016/j.cell.2006.07.024
2. Loh YH, Hartung O, Li H, Guo C, Sahalie JM, Manos PD, et al. Reprogramming of T cells from human peripheral blood. Cell Stem Cell 2010;7(1):15–19. doi:10.1016/j.stem.2010.06.004
3. Staerk J, Dawlaty MM, Gao Q, Maetzel D, Hanna J, Sommer CA, et al. Reprogramming of human peripheral blood cells to induced pluripotent stem cells. Cell Stem Cell 2010;7(1):20–24. doi:10.1016/j.stem.2010.06.002
4. Zhou T, Benda C, Duzinger S, Huang Y, Li X, Li Y, et al. Generation of induced pluripotent stem cells from urine. J Am Soc Nephrol 2011;22(7):1221–1228. doi:10.1681/ASN.2011010106
5. Sauer V, Tchaikovskaya T, Wang X, Li Y, Zhang W, et al. Human urinary epithelial cells as a source of engraftable hepatocyte-like cells using stem cell technology. Cell Transplant 2016. doi:10.3727/096368916X692014
6. Si-Tayeb K, Idriss S, Champon B, Caillaud A, et al. Urine-sample-derived human induced pluripotent stem cells as a model to study PCSK9-mediated autosomal dominant hypercholesterolemia. Dis Model Mech 2016;9(1):81–90
7. Sun N, Panetta NJ, Gupta DM, Wilson KD, Lee A, Jia F, et al. Feeder-free derivation of induced pluripotent stem cells from adult human adipose stem cells. Proc Natl Acad Sci USA 2009;106(37):15720–15725. doi:10.1073/pnas.0908450106
8. Kim JB, Greber B, Arauzo-Bravo MJ, Meyer J, Park KI, Zaehres H, et al. Direct reprogramming of human neural stem cells by OCT4. Nature 2009;461:643–649. doi:10.1038/nature08436
9. Liu H, Ye Z, Kim Y, Sharkis S, Jang YY. Generation of endoderm-derived human induced pluripotent stem cells from primary hepatocytes. Hepatology 2010;51(5):1810–1819. doi:10.1002/hep.23626
10. Aasen T, Raya A, Barrero MJ, Garreta E, Consiglio A, Gonzalez F, et al. Efficient and rapid generation of induced pluripotent stem cells from human keratinocytes. Nat Biotechnol 2008;26(11):1276–1284. doi:10.1038/nbt.1503
11. Bar-Nur O, Russ HA, Efrat S, Benvenisty N. Epigenetic memory and preferential lineage-specific differentiation in induced pluripotent stem cells derived from human pancreatic islet beta cells. Cell Stem Cell 2011;9(1):17–23. doi:10.1016/j.stem.2011.06.007
12. Li H, Collado M, Villasante A, Strati K, Ortega S, Canamero M, et al. The Ink4/Arf locus is a barrier for iPS cell reprogramming. Nature 2009;460:1136–1139. doi:10.1038/nature08290
13. Haase A, Olmer R, Schwanke K, Wunderlich S, Merkert S, Hess C, et al. Generation of induced pluripotent stem cells from human cord blood. Cell Stem Cell 2009;5(4):434–441. doi:10.1016/j.stem.2009.08.021
14. Kajiwara M, Aoi T, Okita K, et al. Donor-dependent variations in hepatic differentiation from human-induced pluripotent stem cells. Proc Natl Acad Sci USA 2012;109(31):12538–12543
15. Kitamura T, Koshino Y, Shibata F, Oki T, Nakajima H, Nosaka T, et al. Retrovirus-mediated gene transfer and expression cloning: powerful tools in functional genomics. Exp Hematol 2003;31(11):1007–1014
16. Picanco-Castro V, de Sousa Russo-Carbolante EM, Tadeu Covas D. Advances in lentiviral vectors: a patent review. Recent Pat DNA Gene Seq 2012;6(2):82–90
17. Soldner F, Hockemeyer D, Beard C, Gao Q, Bell GW, Cook EG, et al. Parkinson's disease patient-derived induced pluripotent stem cells free of viral reprogramming factors. Cell 2009;136(5):964–977. doi:10.1016/j.cell.2009.02.013
18. Woltjen K, Michael IP, Mohseni P, Desai R, Mileikovsky M, Hamalainen R, et al. piggyBac transposition reprograms fibroblasts to induced pluripotent stem cells. Nature 2009;458:766–770. doi:10.1038/nature07863
19. Roy-Chowdhury J, Horwitz MS. Evolution of adenoviruses as gene therapy vectors. Mol Ther 2002;5:340–344
20. Stephen SL, Sivanandam VG, Kochanek S. Homologous and heterologous recombination between adenovirus vector DNA and chromosomal DNA. J Gene Med 2008;10(11):1176–1189. doi:10.1002/jgm.1246

21. Zhou W, Freed CR. Adenoviral gene delivery can reprogram human fibroblasts to induced pluripotent stem cells. Stem Cells 2009;27(11):2667–2674. doi:10.1002/stem.201
22. Jia F, Wilson KD, Sun N, Gupta DM, Huang M, Li Z, et al. A nonviral minicircle vector for deriving human iPS cells. Nat Methods 2010;7(3):197–199. doi:10.1038/nmeth.1426
23. Gonzalez F, Barragan Monasterio M, Tiscornia G, Montserrat Pulido N, Vassena R, Batlle Morera L, et al. Generation of mouse-induced pluripotent stem cells by transient expression of a single nonviral polycistronic vector. Proc Natl Acad Sci USA 2009;106(22):8918–8922. doi:10.1073/pnas.0901471106
24. Yu J, Hu K, Smuga-Otto K, Tian S, Stewart R, Slukvin II, et al. Human induced pluripotent stem cells free of vector and transgene sequences. Science 2009;324(5928):797–801. doi:10.1126/science.1172482
25. Okita K, Nakagawa M, Hyenjong H, Ichisaka T, Yamanaka S. Generation of mouse induced pluripotent stem cells without viral vectors. Science 2008;322(5903):949–953. doi:10.1126/science.1164270
26. Plews JR, Li J, Jones M, Moore HD, Mason C, Andrews PW, et al. Activation of pluripotency genes in human fibroblast cells by a novel mRNA based approach. PLoS One 2010;5(12):e14397. doi:10.1371/journal.pone.0014397
27. Warren L, Manos PD, Ahfeldt T, Loh YH, Li H, Lau F, et al. Highly efficient reprogramming to pluripotency and directed differentiation of human cells with synthetic modified mRNA. Cell Stem Cell 2010;7(5):618–630. doi:10.1016/j.stem.2010.08.012
28. Tokusumi T, Iida A, Hirata T, Kato A, Nagai Y, Hasegawa M. Recombinant Sendai viruses expressing different levels of a foreign reporter gene. Virus Res 2002;86(1–2):33–38
29. Fusaki N, Ban H, Nishiyama A, Saeki K, Hasegawa M. Efficient induction of transgene-free human pluripotent stem cells using a vector based on Sendai virus, an RNA virus that does not integrate into the host genome. Proc Jpn Acad Ser B Phys Biol Sci 2009;85(8):348–362
30. Macarthur CC, Fontes A, Ravinder N, Kuninger D, Kaur J, Bailey M, et al. Generation of human-induced pluripotent stem cells by a nonintegrating RNA Sendai virus vector in feeder-free or xeno-free conditions. Stem Cells Int 2012:564612;. doi:10.1155/2012/564612
31. Nishimura K, Sano M, Ohtaka M, Furuta B, Umemura Y, Nakajima Y, et al. Development of defective and persistent Sendai virus vector: a unique gene delivery/expression system ideal for cell reprogramming. J Biol Chem 2011;286(6):4760–4771. doi:10.1074/jbc.M110.183780
32. Frankel AD, Pabo CO. Cellular uptake of the tat protein from human immunodeficiency virus. Cell 1988;55(6):1189–1193
33. Ziegler A, Nervi P, Durrenberger M, Seelig J. The cationic cell-penetrating peptide CPP(TAT) derived from the HIV-1 protein TAT is rapidly transported into living fibroblasts: optical, biophysical, and metabolic evidence. Biochemistry 2005;44(1):138–148. doi:10.1021/bi0491604
34. Wang Y, Baskerville S, Shenoy A, Babiarz JE, Baehner L, Blelloch R. Embryonic stem cell-specific microRNAs regulate the G1-S transition and promote rapid proliferation. Nat Genet 2008;40(12):1478–1483. doi:10.1038/ng.250
35. Wang Y, Blelloch R. Cell cycle regulation by MicroRNAs in embryonic stem cells. Cancer Res 2009;69:4093–4096. doi:10.1158/0008-5472.CAN-09-0309
36. Judson RL, Babiarz JE, Venere M, Blelloch R. Embryonic stem cell-specific microRNAs promote induced pluripotency. Nat Biotechnol 2009;27:459–461. doi:10.1038/nbt.1535
37. Card DA, Hebbar PB, Li L, Trotter KW, Komatsu Y, Mishina Y, et al. Oct4/Sox2-regulated miR-302 targets cyclin D1 in human embryonic stem cells. Mol Cell Biol 2008;28(20):6426–6438. doi:10.1128/MCB.00359-08
38. Liao B, Bao X, Liu L, Feng S, Zovoilis A, Liu W, et al. MicroRNA cluster 302-367 enhances somatic cell reprogramming by accelerating a mesenchymal-to-epithelial transition. J Biol Chem 2011;286(19):17359–17364. doi:10.1074/jbc.C111.235960
39. Anokye-Danso F, Trivedi CM, Juhr D, Gupta M, Cui Z, Tian Y, et al. Highly efficient miRNA-mediated reprogramming of mouse and human somatic cells to pluripotency. Cell Stem Cell 2011;8(4):376–388. doi:10.1016/j.stem.2011.03.001
40. Park IH, Arora N, Huo H, Maherali N, Ahfeldt T, Shimamura A, et al. Disease-specific induced pluripotent stem cells. Cell 2008;134(5):877–886. doi:10.1016/j.cell.2008.07.041
41. Sullivan GJ, Hay DC, Park IH, et al. Generation of functional human hepatic endoderm from human induced pluripotent stem cells. Hepatology 2010;51(1):329–335
42. Takebe T, Sekine K, Enomura M, et al. Vascularized and functional human liver from an iPSC-derived organ bud transplant. Nature 2013;499:481–484
43. Nagamoto Y, Tashiro K, Takayama K, et al. The promotion of hepatic maturation of human pluripotent stem cells in 3D co-culture using type I collagen and Swiss 3T3 cell sheets. Biomaterials 2012;33(18):4526–4534
44. Takayama K, Kawabata K, Nagamoto Y, et al. 3D spheroid culture of hESC/hiPSC-derived hepatocyte-like cells for drug toxicity testing. Biomaterials 2013;34(7):1781–1789
45. Asgari S, Pournasr B, Salekdeh GH, et al. Induced pluripotent stem cells: a new era for hepatology. J Hepatol 2010;53(4):738–751
46. Rowland TJ, Miller LM, Blaschke AJ, et al. Roles of integrins in human induced pluripotent stem cell growth on Matrigel and vitronectin. Stem Cells Dev 2010;19(8):1231–1240
47. Schwartz RE, Fleming HE, Khetani SR, Bhatia SN. Pluripotent stem cell-derived hepatocyte-like cells. Biotechnol Adv 2014;32(2):504–513
48. Irion S, Nostro MC, Kattman SJ, Keller GM. Directed differentiation of pluripotent stem cells: from developmental biology to therapeutic applications. Cold Spring Harb Symp Quant Biol 2008;73:101–110. doi:10.1101/sqb.2008.73.065
49. Murry CE, Keller G. Differentiation of embryonic stem cells to clinically relevant populations: lessons from embryonic development. Cell 2008;132(4):661–680. doi:10.1016/j.cell.2008.02.008
50. Han S, Bourdon A, Hamou W, Dziedzic N, Goldman O, Gouon-Evans V. Generation of functional hepatic cells from puripotent stem cells. J Stem Cell Res Ther 2012;1–7. doi:10.4172/2157-7633.S10-008
51. Touboul T, Hannan NR, Corbineau S, Martinez A, Martinet C, Branchereau S, et al. Generation of functional hepatocytes from human embryonic stem cells under chemically defined conditions that recapitulate liver development. Hepatology 2010;51(5):1754–1765. doi:10.1002/hep.23506
52. Yamanaka Y, Ralston A. Early embryonic cell fate decisions in the mouse. Adv Exp Med Biol 2010;695(01/2010):1–13. doi:10.1007/978-1-4419-7037-4_1
53. Vallier L, Touboul T, Chng Z, Brimpari M, Hannan N, Millan E, et al. Early cell fate decisions of human embryonic stem cells and mouse epiblast stem cells are controlled by the same signalling pathways. PLoS One 2009;4(6):e6082. doi:10.1371/journal.pone.0006082
54. Teo AK, Ali Y, Wong KY, Chipperfield H, Sadasivam A, Poobalan Y, et al. Activin and BMP4 synergistically promote formation of definitive endoderm in human embryonic stem cells. Stem Cells 2012;30(4):631–642. doi:10.1002/stem.1022
55. Toivonen S, Lundin K, Balboa D, Ustinov J, Tamminen K, Palgi J, et al. Activin A and Wnt-dependent specification of human definitive endoderm cells. Exp Cell Res 2013;319(17):2535–2544. doi:10.1016/j.yexcr.2013.07.007

56. Hansson M, Olesen DR, Peterslund JM, Engberg N, Kahn M, Winzi M, et al. A late requirement for Wnt and FGF signaling during activin-induced formation of foregut endoderm from mouse embryonic stem cells. Dev Biol 2009;330(2):286–304. doi:10.1016/j.ydbio.2009.03.026
57. Xu X, Browning VL, Odorico JS. Activin, BMP and FGF pathways cooperate to promote endoderm and pancreatic lineage cell differentiation from human embryonic stem cells. Mech Dev 2011;128(7–10):412–427. doi:10.1016/j.mod.2011.08.001
58. Johansson BM, Wiles MV. Evidence for involvement of activin A and bone morphogenetic protein 4 in mammalian mesoderm and hematopoietic development. Mol Cell Biol 1995;15(1):141–151
59. Norrman K, Strombeck A, Semb H, Stahlberg A. Distinct gene expression signatures in human embryonic stem cells differentiated towards definitive endoderm at single-cell level. Methods 2013;59(1):59–70. doi:10.1016/j.ymeth.2012.03.030
60. Kamiya A, Kinoshita T, Miyajima A. Oncostatin M and hepatocyte growth factor induce hepatic maturation via distinct signaling pathways. FEBS Lett 2001;492(1–2):90–94
61. Schmidt C, Bladt F, Goedecke S, Brinkmann V, Zschiesche W, Sharpe M, et al. Scatter factor/hepatocyte growth factor is essential for liver development. Nature 1995;373(6516):699–702. doi:10.1038/373699a0
62. Duncan SA, Manova K, Chen WS, Hoodless P, Weinstein DC, Bachvarova RF, et al. Expression of transcription factor HNF-4 in the extraembryonic endoderm, gut, and nephrogenic tissue of the developing mouse embryo: HNF-4 is a marker for primary endoderm in the implanting blastocyst. Proc Natl Acad Sci USA 1994;91(16):7598–7602
63. Gualdi R, Bossard P, Zheng M, Hamada Y, Coleman JR, Zaret KS. Hepatic specification of the gut endoderm in vitro: cell signaling and transcriptional control. Genes Dev 1996;10:1670–1682
64. Kamiya A, Kinoshita T, Ito Y, Matsui T, Morikawa Y, Senba E, et al. Fetal liver development requires a paracrine action of oncostatin M through the gp130 signal transducer. EMBO J 1999;18(8):2127–2136. doi:10.1093/emboj/18.8.2127
65. Thomassin H, Flavin M, Espinas ML, Grange T. Glucocorticoid-induced DNA demethylation and gene memory during development. EMBO J 2001;20(8):1974–1983. doi:10.1093/emboj/20.8.1974
66. Hengstler JG, Brulport M, Schormann W, Bauer A, Hermes M, Nussler AK, et al. Generation of human hepatocytes by stem cell technology: definition of the hepatocyte. Expert Opin Drug Metab Toxicol 2005;1(1):61–74. doi:10.1517/17425255.1.1.61
67. Wang YM, Chai SC, Brewer CT, Chen T. Pregnane X receptor and drug-induced liver injury. Expert Opin Drug Metab Toxicol 2014;10(11):1521–1532. doi:10.1517/17425255.2014
68. Avior Y, Levy G, Zimerman M, Kitsberg D, Schwartz R, et al. Microbial-derived lithocholic acid and vitamin K2 drive the metabolic maturation of pluripotent stem cells-derived and fetal hepatocytes. Hepatology 2015;62:265–278
69. Morelli L. Postnatal development of intestinal microflora as influenced by infant nutrition. J Nutr 2008;138:1791S–1795S
70. Takayama K, Inamura M, Kawabata K, Tashiro K, Katayama K, Sakurai F, et al. Efficient and directive generation of two distinct endoderm lineages from human ESCs and iPSCs by differentiation stage-specific SOX17 transduction. PLoS One 2011;6(7):e21780. doi:10.1371/journal.pone.0021780PONE-D-11-02464
71. Inamura M, Kawabata K, Takayama K, Tashiro K, Sakurai F, Katayama K, et al. Efficient generation of hepatoblasts from human ES cells and iPS cells by transient overexpression of homeobox gene HEX. Mol Ther 2011;19(2):400–407. doi:10.1038/mt.2010.241
72. Takayama K, Inamura M, Kawabata K, Katayama K, Higuchi M, Tashiro K, et al. Efficient generation of functional hepatocytes from human embryonic stem cells and induced pluripotent stem cells by HNF4alpha transduction. Mol Ther 2012;20(1):127–137. doi:10.1038/mt.2011.234
73. Tahamtani Y, Azarnia M, Farrokhi A, et al. Treatment of human embryonic stem cells with different combinations of priming and inducing factors toward definitive endoderm. Stem Cells Dev 2013;22(9):1419–1432
74. Siller R, Greenhough S, Naumovska E, Sullivan GJ. Small-molecule-driven hepatocyte differentiation of human pluripotent stem cells. Stem Cell Rep 2015;4(5):939–952
75. Shan J, Schwartz RE, Ross NT, et al. Identification of small molecules for human hepatocyte expansion and iPS differentiation. Nat Chem Biol 2013;9(8):514–520
76. Funakoshi N, Duret C, Pascussi JM, Blanc P, Maurel P, Daujat-Chavanieu M, et al. Comparison of hepatic-like cell production from human embryonic stem cells and adult liver progenitor cells: CAR transduction activates a battery of detoxification genes. Stem Cell Rev 2011;7(3):518–531. doi:10.1007/s12015-010-9225-3
77. Decaens C, Durand M, Grosse B, Cassio D. Which in vitro models could be best used to study hepatocyte polarity? Biol Cell 2008;100(7):387–398. doi:10.1042/BC20070127
78. Kim Y, Rajagopalan P. 3D hepatic cultures simultaneously maintain primary hepatocyte and liver sinusoidal endothelial cell phenotypes. PLoS One 2010;5:e15456. doi:10.1371/journal.pone.0015456
79. Stevens KR, Ungrin MD, Schwartz RE, Ng S, Carvalho B, Christine KS, et al. InVERT molding for scalable control of tissue microarchitecture. Nat Commun 2013;4:1847. doi:10.1038/ncomms2853
80. Baptista PM, Siddiqui MM, Lozier G, Rodriguez SR, Atala A, Soker S. The use of whole organ decellularization for the generation of a vascularized liver organoid. Hepatology 2011;53(2):604–617. doi:10.1002/hep.24067
81. Uygun BE, Soto-Gutierrez A, Yagi H, Izamis ML, Guzzardi MA, Shulman C, et al. Organ reengineering through development of a transplantable recellularized liver graft using decellularized liver matrix. Nat Med 2010;16:814–820. doi:10.1038/nm.2170
82. Szkolnicka D, Farnworth SL, Lucendo-Villarin B, Storck C, Zhou W, Iredale JP, et al. Accurate prediction of drug-induced liver injury using stem cell-derived populations. Stem Cells Transl Med 2014;3(2):141–148
83. Rashid ST, Corbineau S, Hannan N, et al. Modeling inherited metabolic disorders of the liver using human induced pluripotent stem cells. J Clin Investig 2010;120(9):3127–3136
84. Tafaleng EN, Chakraborty S, Han B, et al. Induced pluripotent stem cells model personalized variations in liver disease resulting from alpha1-antitrypsin deficiency. Hepatology 2015;62(1):147–157
85. Choi SM, Kim Y, Shim JS, et al. Efficient drug screening and gene correction for treating liver disease using patient-specific stem cells. Hepatology 2013;57(6):2458–2468
86. Zhang S, Chen S, Li W, et al. Rescue of ATP7B function in hepatocyte-like cells from Wilson's disease induced pluripotent stem cells using gene therapy or the chaperone drug curcumin. Hum Mol Genet 2011;20(16):3176–3187
87. Brown MS, Goldstein JL. Biomedicine. Lowering LDL—not only how low, but how long? Science 2006;311(5768):1721–1723
88. Cayo MA, Cai J, DeLaForest A, et al. JD induced pluripotent stem cell-derived hepatocytes faithfully recapitulate the

pathophysiology of familial hypercholesterolemia. Hepatology 2012;56(6):2163–2171
89. Seidah NG, Awan Z, Chretien M, Mbikay M. PCSK9: a key modulator of cardiovascular health. Circ Res 2014;114:1022–1036
90. Leung A, Nah SK, Reid W, et al. Induced pluripotent stem cell modeling of multisystemic, hereditary transthyretin amyloidosis. Stem Cell Rep 2013;1(5):451–463
91. Ng S, Schwartz RE, March S, et al. Human iPSC-derived hepatocyte-like cells support Plasmodium liver-stage infection in vitro. Stem cell reports 2015;4(3):348–359
92. Yoshida T, Takayama K, Kondoh M, et al. Use of human hepatocyte-like cells derived from induced pluripotent stem cells as a model for hepatocytes in hepatitis C virus infection. Biochem Biophys Res Commun 2011;416(1–2):119–124
93. Carpentier A, Tesfaye A, Chu V, et al. Engrafted human stem cell-derived hepatocytes establish an infectious HCV murine model. J Clin Investig 2014;124:4953–4964
94. Dhawan A, Strom SC, Sokal E, Fox IJ. Human hepatocyte transplantation. Methods Mol Biol 2010;640:525–534
95. Vogel KR, Kennedy AA, Whitehouse LA, Gibson KM. Therapeutic hepatocyte transplant for inherited metabolic disorders: functional considerations, recent outcomes and future prospects. J Inherit Metab Dis 2014;37(2):165–176
96. Yusa K, Rashid ST, Strick-Marchand H, et al. Targeted gene correction of alpha1-antitrypsin deficiency in induced pluripotent stem cells. Nature 2011;478(7369):391–394
97. Basma H, Soto-Gutierrez A, Yannam GR, et al. Differentiation and transplantation of human embryonic stem cell-derived hepatocytes. Gastroenterology 2009;136(3):990–999
98. Zhou H, Dong X, Kabarriti R, Chen Y, et al. Single liver lobe repopulation with wildtype hepatocytes using regional hepatic irradiation cures jaundice in Gunn rats. PLoS One 2012;7(10):46775
99. Chen Y, Li Y, Wang X, Zhang W, et al. Amelioration of hyperbilirubinemia in Gunn rats after transplantation of human induced pluripotent stem cell-derived hepatocytes. Stem Cell Rep 2015;5(1):22–30
100. Asgari S, Moslem M, Bagheri-Lankarani K, Pournasr B, Miryounesi M, Baharvand H. Differentiation and transplantation of human induced pluripotent stem cell-derived hepatocyte-like cells. Stem Cell Rev 2013;9(4):493–504
101. Woo DH, et al. Direct and indirect contribution of human embryonic stem cell-derived hepatocyte-like cells to liver repair in mice. Gastroenterology 2012;142(3):602–611
102. Li HY, et al. Reprogramming induced pluripotent stem cells in the absence of c-Myc for differentiation into hepatocyte-like cells. Biomaterials 2011;32(26):5994–6005
103. Liu H, Kim Y, Sharkis S, Marchionni L, Jang YY. In vivo liver regeneration potential of human induced pluripotent stem cells from diverse origins. Sci Transl Med 2011;3(82):82ra39
104. Du Y, et al. Human hepatocytes with drug metabolic function induced from fibroblasts by lineage reprogramming. Cell Stem Cell 2014;14(3):394–403
105. Zhu S, et al. Mouse liver repopulation with hepatocytes generated from human fibroblasts. Nature 2014;508(7494):93–97
106. Simeonov KP, Uppal H. Direct reprogramming of human fibroblasts to hepatocyte-like cells by synthetic modified mRNAs. Plos One 2014; e100134
107. Takebe T, et al. Vascularized and functional human liver from an iPSC-derived organ bud transplant. Nature 2013;499(7459):481–484
108. Liu H, Zhu F, Yong J, et al. Generation of induced pluripotent stem cells from adult rhesus monkey fibroblasts. Cell Stem Cell 2008;3(6):587–590
109. Sourisseau M, Goldman O, He W, et al. Hepatic cells derived from induced pluripotent stem cells of pigtail macaques support hepatitis C virus infection. Gastroenterology 2013;145(5):966 e967–969 e967
110. Yua Y, Liua H, Ikedad Y, Amiote BP, Rinaldof P, Duncang SA, Nyberg SL. Stem Hepatocyte-like cells differentiated from human induced pluripotent stem cells: relevance to cellular therapies. Cell Res 2012;9(3):196–207. doi:10.1016/j.scr.2012.06.004
111. Speicher T, Siegenthaler B, Bogorad RL, Ruppert R, Petzold T, Padrissa-Altes S, et al. Knockdown and knockout of β1-integrin in hepatocytes impairs liver regeneration through inhibition of growth factor signalling. Nat Commun 2014;5:3862. doi:10.1038/ncomms4862
112. Padrissa-Altés S, Bachofner M, Bogorad RL, Pohlmeier L, Rossolini T, Böhm F, Liebisch G, Hellerbrand C, Koteliansky V, Speicher T, Werner S. Control of hepatocyte proliferation and survival by Fgf receptors is essential for liver regeneration in mice. Gut 2015;64(9):1444–1453