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Review Article

Cellular therapies in liver and pancreatic diseases ☆

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ABSTRACT

Over the past two decades, developments in regenerative medicine in gastroenterology have been greatly enhanced by the application of stem cells, which can self-replicate and differentiate into any somatic cell. The discovery of induced pluripotent stem cells has opened remarkable perspectives on tissue regeneration, including their use as a bridge to transplantation or as supportive therapy in patients with organ failure. The improvements in DNA manipulation and gene editing strategies have also allowed to clarify the physiopathology and to correct the phenotype of several monogenic diseases, both in vivo and in vitro. Further progress has been made with the development of three-dimensional cultures, known as organoids, which have demonstrated morphological and functional complexity comparable to that of a miniature organ. Hence, owing to its protean applications and potential benefits, cell and organoid transplantation has become a hot topic for the management of gastrointestinal diseases. In this review, we describe current knowledge on cell therapies in hepatology and pancreatology, providing insight into their future applications in regenerative medicine.

1. Introduction

Acute liver failure (ALF) and end-stage chronic liver disease (ESCLD) represent a significant health and economic burden worldwide [1,2]. Liver transplantation (LT) remains the only curative treatment in these conditions, but the shortage of donors limits the possibility of offering this option to all patients, resulting in increased wait-list mortality; in addition, the periprocedural complications and the consequences of long-term immunosuppressive therapy are motivations to explore curative alternatives [3,4]. However, the attempts to administer hepatocytes to patients with ESCLD and ALF have produced controversial results [5,6].

The discovery of stem cells (SCs) has been a milestone in modern medicine. SCs can self-replicate and be converted into any somatic cell type following specific stimulation [7]. The conversion of a somatic cell into an induced pluripotent stem cell (iPSC) was first described in 2006 [8]. Additionally, the potential to manipulate SCs DNA and the development of three-dimensional SCs-derived cultures, known as organoids, have opened new doors to regenerative medicine [9,10].

In this review, we describe current knowledge on cell therapies in hepatology and pancreatology, with an additional focus on their use in understanding the pathogenetic mechanisms of diseases.

2. Stem cells main features and types

SCs are relatively undifferentiated cells characterized by 3 properties: self-renewal, clonality, and the ability to differentiate into other cell types [11,12]. Based on their differentiation ability, they can be divided into totipotent, pluripotent, multipotent, oligopotent, and unipotent SCs. Totipotent SCs can differentiate into all cell types, giving rise to both embryonic and extraembryonic tissues [13]. Pluripotent SCs can form all the 3 germ layers (ectoderm, endoderm, and mesoderm), and include embryonic stem cells (ESCs) and iPSCs [14]. Multipotent and oligopotent SCs have a narrower differentiation capacity, the former give rise to cells within a specific germ layer, and the latter to different cells within a specific tissue; finally, unipotent SCs can differentiate into only a single cell type [15]. Based on the developmental stage, SCs can be divided into ESCs, foetal SCs (FSCs), and adult SCs (ASCs). ESCs are pluripotent SCs derived from the inner cell mass of the blastocyst. FSCs are found in blood and hemopoietic organs in early pregnancy, as well as in somatic organs, amniotic fluid, and placenta throughout gestation [16], and are considered multipotent SCs. ASCs, also

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known as somatic stem cells or tissue stem cells, can be isolated in most tissues and persist during the whole life; ASCs are involved in tissue maintenance and repair in response to injury. Mesenchymal stem/stromal cells (MSCs) [17], hematopoietic SCs (HSCs), liver SCs (LSCs), pancreatic SCs, gut SCs, epidermal SCs and neuronal SCs are example of ASCs [7,18].

3. Cellular therapies in hepatobiliary diseases

In recent decades, cell transplantation has been studied in several areas of hepatology. Although primary hepatocytes were the first to be tested, due to the limitations related to their use and effectiveness, they have been rapidly replaced by SCs, which have gained increasing attention in this field.

3.1. Hepatocytes

Up to 80% of the liver mass consists of hepatocytes, cells with a pivotal role in the metabolic functions of the liver [19]. In case of acute liver injury or surgical resection, the hepatocytes can induce liver regeneration by stimulating the proliferation of themselves and of other cell types in the liver; however, when liver damage is severe or chronic, their function is impaired and maladaptive [20]. Based on these considerations, allogenic hepatocyte transplantation (HT) has been regarded as a potential alternative to LT, being less invasive and costly, repeatable, and available as needed, because hepatocytes can be cryopreserved [21]. In addition, a single donor liver can be used for multiple recipients, as the number of cells required to achieve clinical benefits is about 5–10% of the liver mass.

However, the rate of hepatocyte engraftment after transplantation is low, estimated at around 0.5% of the recipient's liver mass. This is due to both cells' quality and immune rejection, which eliminates up to 70% of engrafted cells within the first 24 h after transplantation [22]. For this reason, repeated infusions are required.

Hepatocytes have been isolated either from human livers unsuitable for LT, or from liver segments available after split LT, using a three-step collagenase perfusion technique originally developed by Berry and Friend [23,24]. With this technique, the native liver of the recipient remains in situ, allowing for its potential recovery and, eventually, for gene therapy [21].

Several routes of hepatocyte administration have been reported. The preferred one is intraportal infusion, especially in patients with acute and metabolic conditions [24]. However, this procedure is associated with a transient increase in portal pressure, thus the use of the splenic artery should be preferred in patients with portal hypertension. Intraperitoneal infusion has also been reported, yet is burdened with a low survival rate of the hepatocytes due to the lack of an anchor site and the interference of the host immune response [25]. To overcome these limitations, several animal studies were conducted to assess the safety and the efficacy of alginate-encapsulated human hepatocyte microbeads transplantation through the portal vein, which also prevents the host immune system response and reduces the associated haemorrhage risk [26,27].

Although HT has proven to be a safe technique, its clinical efficacy is still debated, mainly for the limitations related to its use; in fact, the effects of HT usually last less than one year on account of allograft rejection and low rate of cell engraftment [6], as well as to the thawing-induced damage occurring after cryopreservation, which decreases cell viability and functioning [28]. Finally, the availability of liver donors is limited, and they are often suboptimal, limiting the possibility to isolate good-quality cells [23].

3.2. Stem cells

To overcome the limitations of HT, SCs have recently emerged as an alternative source for cell transplantation and liver regeneration [29].

Under specific culture conditions, SCs can be prompted to differentiate into hepatocyte-like cells (HLCs) and cholangiocytes [30–32]. The most frequently used SCs for regenerative medicine applications are MSCs, HSCs, and LSCs. ESCs. More recently, iPSCs have aroused great interest in the field of tissue engineering and regenerative medicine due to their pluripotent activity; up to date, they have just been the object of in vitro and in vivo animal studies [33].

3.2.1. Mesenchymal stem/stromal cells

MSCs are adult multipotent SCs able to differentiate in many types of cells, such as osteoblasts, chondrocytes, and adipocytes [34]. MSCs can be isolated from bone marrow [35], but also from other tissues, such as adipose tissue [36], synovial membrane [37], umbilical cord [38], and placenta [39]. The properties of MSCs include immunomodulation, homing, trans-differentiation, rapid expansion in vitro, and a low risk of tumorgenicity and of immunogenicity since they lack the expression of major histocompatibility complex (MHC) class II antigens. All these characteristics make these cells suitable for regenerative therapy [40].

Many studies have shown MSCs' ability to differentiate into HLCs in vitro and in vivo when processed with a combination of several growth factors and cytokines, and co-cultured with liver cells [40,41]. Even though HLCs can support liver function, these cells still show markers of MSCs and appear to have a lower activity than adult hepatocytes [42,43]. Thereby, MSCs' beneficial role in liver diseases may be due to their paracrine and immunomodulatory properties, rather than to their differentiation potential [19]. Indeed, after the infusion, MSCs reach the injured site and produce various bioactive molecules, including growth factors that promote cell regeneration and neoangiogenesis [44–46]; in particular, they suppress T cell maturation, promote regulatory T cell differentiation, inhibit B cells proliferation and lead to the formation of M2 type macrophages that release anti-inflammatory cytokines [44,47]. MSCs exert antifibrotic effects both directly acting on hepatic stellate cells (HSCs) and producing soluble factors (e.g. transforming growth factor β, prostaglandin E2, interleukin 10) that suppress immune cell activity reducing the extracellular matrix synthesis [44,45,48,49]. Several routes of administration have been used in liver disease: peripheral vein, hepatic artery, portal vein, intrahepatic and intrasplenic injection. However, when infused by the peripheral venous route, MSCs become trapped in the lungs on the first pass due to their size, limiting their possibility to reach the liver [50].

The open questions on the use of MSCs are the choice of the optimal injection route, the timing of the injection, and the number of cells to be injected. Further preclinical and clinical studies are needed to standardize their use in order to improve therapeutic efficacy.

3.2.2. Hematopoietic stem cells

Since the liver participates in haematopoiesis during the foetal development, being the major responsible for erythropoiesis, especially in the first trimester of pregnancy, HSCs have been evaluated as an alternative form of cell transplantation for the treatment of liver disease [19]. HSCs are characterized by the expression of the surface markers $CD34^{+}/CD133^{+}$; they can be isolated from bone marrow, peripheral blood or umbilical cord blood, and can differentiate into any blood lineage [51]. Data from animal studies demonstrate that HSCs may play a significant role in hepatic regeneration [52,53]. Yannaki et al. have reported that HSCs

primed with granulocyte colony-stimulating factor (G-CSF) migrate to the site of injury, promote tissue regeneration, and induce hepatocyte formation [54]. The exact mechanisms by which HSCs can improve liver function are still unclear; although several studies have shown HSCs' ability to differentiate into hepatocytes [55,56], the potential role of HSCs in hepatic regeneration in mice models seems to be related to their fusion with host hepatocytes, rather than their trans-differentiation into hepatocytes [57,58]. Moreover, HSCs may play a paracrine role by secreting cytokines and growth factors that stimulate liver regeneration and neoangiogenesis [59].

Therefore, although these mechanisms are not yet fully elucidated, HSCs still hold great promise in the liver tissue regeneration field.

3.2.3. Liver stem cells (hepatobiliary bipotent stem cells)

During liver development at foetal stage, hepatoblasts originating from the foregut endoderm give rise to both hepatocytes and biliary epithelial cells. Hepatoblasts are considered the liver FSCs population due to their bidirectional differentiation potential [60]. Another type of LSCs is adult liver stem/progenitor cells (LPCs) known as "oval" cells in mice [61]. In case of liver damage, activated LPCs exhibit self-renewing and bipotent properties, having the ability to generate both hepatocytes and cholangiocytes [62]. LPCs are located in SCs niches, such as ductal plates in foetal and neonatal livers, canals of Hering in paediatric and adult livers, and in peribiliary glands and in crypts of adult gallbladder epithelium [63]. Several studies have shown their ability to differentiate into mature hepatocytes, cholangiocytes, and pancreatic islets in vitro, highlighting their regenerative potential in the field of liver cell therapy [64–66].

3.2.4. Pluripotent stem cells (embryonic stem cells and induced pluripotent stem cells)

ESCs are pluripotent stem cells derived from the inner cell mass of early blastocyst or morula stage embryos, capable of unlimited, undifferentiated proliferation in vitro. Several studies have demonstrated that ESCs can be induced to differentiate into hepatocytes or cholangiocytes under appropriate culture conditions [67,68]. However, ethical issues, the need for immunosuppressive therapy to avoid rejection, and the risk of tumorigenicity heavily limit their clinical application [8,18,40]. iPSCs share ESCs characteristics of self-renewal and pluripotency but overcome their limitations [31]. iPSCs are pluripotent SCs generated from somatic cells, which are reprogrammed into a pluripotent state, with the ability to differentiate unlimited times [8]. Several studies have reported iPSCs' ability to differentiate into HLCs [69,70]; the protocols adopted for this purpose aim to reproduce the developmental route of the liver during embryogenesis through a three-step mechanism transformation [19,46]. Although HLCs generated from iPSCs exhibit the properties of primary hepatocytes, their phenotype and function resemble those of foetal hepatocytes. Indeed, HLCs express alpha-fetoprotein (AFP) and present immature cytochrome P450 enzyme activity [71]. Moreover, albumin synthesis, urea production, and mitochondrial function are lower than those of primary hepatocytes [72]. iPSCs can also be used to obtain cholangiocytes, which are able to engraft in the mouse liver following retrograde intrabiliary infusion [73].

Currently, iPSCs and HLCs clinical use has several limitations to address, such as tumorigenicity, immunogenicity, long-term safety and efficacy, and the optimal reprogramming process [74].

4. Clinical application of cellular therapies in hepatobiliary diseases

HT has emerged as a potential alternative to LT, especially in the field of inborn errors of metabolism and ALF. Currently, more than 100 patients have been treated with HT worldwide [5]. However, the limitations associated with this technique have led to other cell sources. According to SCs' ability to self-renew and differentiate, as well as to recapitulate the functional and morphological characteristics of a specific tissue, their role as a potential treatment in liver diseases has been investigated, with controversial results. The main evidence comes from the treatment of inborn error of metabolism or monogenic diseases, liver failure, chronic liver disease, and bile duct damage (Fig. 1).

4.1. Inborn errors of metabolism

In patients affected by inborn errors of metabolism, the function of host hepatocytes is altered by gene mutations regarding specific enzymes. Transplanted hepatocytes, containing the functioning version of the altered gene, can theoretically compensate for the defect and ameliorate the patient's metabolic condition [75]. Both adult and foetal hepatocytes have been used to treat inborn errors of metabolism. Fox et al. have reported the first case of HT long-term efficacy in a 10-years-old girl affected by Crigler–Najjar syndrome type I, presenting with severe unconjugated hyperbilirubinemia [76]. The study has demonstrated successful hepatocyte engraftment, and the transplanted cells survived for more than 11 months (Table 1). Up to date, other cases have been reported, showing a reduction in serum bilirubin, with an increase in the conjugated portion [77–83]. HT was also used to treat glycogen storage disease type 1a, urea cycle defects, and phenylketonuria, with significant clinical benefit [84–87]. Based on these studies, HT has been shown to be safe in all treated cases; however, it generally resulted in a partial correction of the disorders, and, more importantly, its efficacy was not sustained over time.

Another promising application of SCs therapy regards monogenic disorders of the liver, for which LT remains the only definitive cure [111]. iPSCs and gene editing have been used to better understand the pathogenesis of the disease and to explore potential therapeutic applications in animal models of alpha-1 antitrypsin deficiency, coagulation factor VII deficiency, infantile Refsum's disease, Wilson's disease, biliary atresia, haemophilia A and familial hypercholesterolaemia [88–97,112–116] (Table 1). However, translation of these findings in large human studies is needed to confirm their successful results.

4.2. Liver failure

ALF and acute-on-chronic liver failure (ACLF) are severe clinical conditions for which, in most cases, LT is the only effective treatment. HT has been evaluated as a bridge to LT in patients on the waiting list (Table 1); beneficial effects on liver injury biomarkers and model for end-stage liver disease (MELD), blood ammonia, cerebral perfusion, and cardiac stability have been reported, as well as a decreased incidence of serious infections [98]. In a small cohort of 8 children with ALF, human hepatocytes microbeads infusion into the peritoneal cavity without immunosuppression allowed to avoid LT in 4 cases, while 3 were successfully bridged to LT [99].

Similar positive results were also reported by other clinical trials including patients with ALF and ACLF of different aetiologies, using different types of SCs; however, not all the studies could demonstrate an improvement in survival [100–102]. Finally, a meta-analysis evaluated the clinical benefits of SCs therapy in the treatment of ACLF [103], showing a significant reduction in total bilirubin serum levels, an increase in serum albumin, and a significant improvement in MELD score in treated patients, with no significant changes in the international normalized ratio (INR). The study also showed that the use of MSCs may achieve better results than bone marrow-derived mononuclear stem cells (BM-MNCs).

Table 1
In vitro, in vivo, and clinical studies of cell transplantation in liver diseases.

Author	Study design	Clinical setting	Experimental setting	Aim	Cell Source	Route and timing of administration	Results	Limitations
Fox et al. [76]	case report	Crigler-Najjar syndrome	humans (1 pt)	disease therapy	allogenic hepatocytes	portal vein; 3 infusions separated by 4–6 h on the same day	• ↓ Tbil • UGT1A1 activity lasting for 11 mo	• partial correction of defect • time limited efficacy • limited generalizability
Muraca et al. [84]	case report	glycogen storage disease type-1	humans (1 pt)	disease therapy	allogenic hepatocytes	portal vein; 2 infusions of 230′ on the same day	• normal diet • ↑ fasting time up to 9 mo	• partial correction of defect • time limited efficacy • limited generalizability
Meyburg et al. [86]	case series	urea cycle disorders	humans (4 pts)	disease therapy	allogenic hepatocytes	portal vein; the 4 pts received respectively 6, 4, 3, 2 infusions	• ↓ NH3 • ↑ urea • normal urinary orotic acid • metabolic stabilization up to 13 mo	• heterogenicity of cases • partial correction of defect • time limited efficacy
Stephenne et al. [87]	case report	phenylketonuria	humans (1 pt)	disease therapy	allogenic hepatocytes	portal vein; 4 separate infusions in 2 days; another infusion 7.5 mo later	• ↓ blood phenylalanine concentrations • detectable PAH activity lasting for 3 mo	• partial correction of defect • time limited efficacy • limited generalizability
Segeritz et al. [88]	in vitro, case-control	AAT deficiency	2D culture + rats	disease modelling	• AAT HLCs with Z mutation derived from hiPSCs; • AAT derived hiPSC with gene editing	–	• Reproduction of a physiopathological model of AAT deficiency • gene editing rescues mitochondrial disruption and ER misfolding defects in HLCs	only in vitro model
Dhawan et al. [89]	case series	inherited factor VII deficiency	humans (2 pts)	disease therapy	• allogenic hepatocytes	mesenteric vein; pt 1 received 3 infusions; pt 2 received 5 infusions	• ↓ coagulation defect • ↓ in FVII requirement for up to 6 mo	• partial correction of defect • time limited efficacy limited generalizability
Sokal et al. [90]	case report	infantile Refsum's disease	humans (1 pt)	disease therapy	• allogenic hepatocytes	portal vein; 8 separate infusions in 6 days	• ↓ total bile acids • ↓ DHCA • ↓ 40% of pipecholic acid after 18 mo FU	• partial correction of defect • time limited efficacy • limited generalizability
Chen et al. [91]	phase I in vitro; phase II in vivo (transgenic AAT mice expressing the SERPINA1 ZZ genotype	AAT deficiency	2D cultures + rats	disease therapy	hiPSCs committed into HLCs	intra-splenic injections of 1 × 10^6 HLCs	• HLCs 5–10% over total hepatocyte mass at 1 mo • HLCs 20% over total hepatocyte mass at 6 mo • ↑ AAT • transplantation rescued the Z phenotype	• different results of engraftment according to donor cells maturity, host immunity • risk host versus graft reaction
Wei et al. [92]	phase I: in vitro; phase II: in vivo transgenic mice with Atp7b-/-/Rag2-/-/Il2rg-/- genotype	WD	2D culture + rats	disease therapy	hiPSCs committed into HLCs with homozygous or heterozygous ATP7B R778L mutation after gene editing	• intrasplenic injection of 1 × 10^6 HLCs; • HLCs incorporated into WD mice livers at 8 wks post engraftment	• restored ATP7B subcellular location and its trafficking in response to copper overload • recovered copper exportation in cells • ↓ liver inflammation and fibrosis • ↓ hepatic copper accumulation and hepatotoxicity	• low HLCs engraftment efficiency (5%) • no effects on extrahepatic manifestations of WD
Khan et al. [93]	case report	biliary atresia	humans (1 pt)	disease therapy	allogenic human foetal hepatic progenitor cells	• single hepatic artery infusion	• ↓3-fold Tbil; • ↓ 8-fold conjugated bilirubin • ↑ liver cell function at hepatobiliary scintigraphy after 2 mo	• partial correction of defect • time limited efficacy • limited generalizability

(*continued on next page*)

Table 1 (*continued*)

Author	Study design	Clinical setting	Experimental setting	Aim	Cell Source	Route and timing of administration	Results	Limitations
Son et al. [94]	phase I in vitro; phase II in vivo trangenic mice with FVIII deficiency	HA	2D culture, 3D culture + rats	disease therapy	hiPSCs committed into ECs expressing FVIII, vWF, CD34+	–	• restored FVIII function • ↑ plasmatic FVIII (12.24%) • linear correlation between transplanted cells and bleeding regression • after 100 days, a network of new capillaries was observed	inhomogeneous engraftment
Tian et al. [95]	in vitro case-control	BA	2D models	disease modelling	hiPSC from BA pts and KO hiPSCs of controls treated with CRISPR/Cas9 to induce BA and controls	–	• ↓ CK7, EpCAM, SOX9, CK19, AE2, and CFTR • ↓ bile ducts formation • ↑ fibrosis deposition • both the pt-iPSCs and the KO-iPSCs showed ↑ YAP • ↓ collagen and YAP by treatment with the anti-fibrogenic drug pentoxifylline	only in vitro model
Omer et al. [96]	in vitro	familial hypercholesterolaemia	2D models	disease therapy	pt-derived Ho-FH iPSCs treated with CRISPR/Cas9 genome editing to correct a 3-base pair homozygous deletion in LDLR exon 4	–	• lovastatin ↑LDLR • sterols ↓ LDLR • genetic correction restored LDLR-mediated endocytosis in FH–HLCs	• only in vitro study • very low level of mature LDLR proteins
Okada et al. [97]	In vitro case control	familial hypercholesterolaemia	2D models	disease therapy	HLCs from hiPSCs of HO-Fh pts. Generation of cultures with homozygous or heterozygous correction of LDLR mutation via CRISPR/Cas9	–	• LDL uptake restored in both types of iPSC-derived HLCs • gene-corrected iPSC-derived HLCs showed little immunogenicity against the host	in vitro study
Strom et al. [98]	prospective controlled trial	ALF and ACLF	humans (9 pts)	disease therapy	allogenic hepatocytes	single splenic artery infusion;	• ↓ NH3 • ↓ AST • normal cerebral perfusion and cardiac stability • 20 mo FU	small sample size
Dhawan et al. [99]	case series	ALF	humans (8 pts)	disease therapy	allogenic hepatocytes	6 pts received a single intraperitoneal infusion; 2 pts	• 4 pts avoided LT • 3 pts successfully bridged to LT	• no demonstration of efficacy • controversial total cells number to be used • too short FU to evaluate safety • different hospitalization time
Lin et al. [100]	RCT	HBV-related ACLF	humans (110 pts)	disease therapy	allogenic BM-MSCs	received 2 infusions intravenous; weekly for 4 wks	• 8 yrs FU • ↑ survival rate • ↓ Tbil and MELD • 24 wks FU	
Shi et al. [101]	RCT	HBV-related ACLF	humans (43 pts)	disease therapy	allogenic UC-MSCs	intravenous; 3 times at 4-wk intervals	• ↑ survival rate • ↓ Tbil, ALT and MELD • 48 wks of FU	Single centre study

(*continued on next page*)

Table 1 (*continued*)

Author	Study design	Clinical setting	Experimental setting	Aim	Cell Source	Route and timing of administration	Results	Limitations
Schacher et al. [102]	RCT	ACLF of different aetiologies	humans (9 pts)	disease therapy	allogenic BM-MSCs	intravenous; 5 infusions over 3 wks	• safe and feasible • no improvement in survival • 90 days FU	• small sample size • severe disease • infusion protocol not completed due to high early mortality
Xue et al. [103]	meta-analysis of 4 RCT and 6 non-RCT	ACLF	humans (628 pts)	disease therapy	BM-MSCs; BM-MNCs; UC-MSCs PBSCs	intravenous or through hepatic artery	• ↓ Tbil and MELD • ↑ ALB • MSCs more effective than BM-MNCs • 3–24 mo FU	• different stem cell types used • high heterogeneity between studies
Suk et al. [104]	phase 2 RCT	alcoholic cirrhosis	humans (72 pts)	disease therapy	autologous BM-MSCs	1 or 2-time hepatic arterial infusion	• ↓ collagen area • ↓ Child-Pugh score • 12 mo FU	unknown mechanism of action
Shi et al. [105]	RCT	HBV-related DLC ($n = 219$)	humans (219 pts)	disease therapy	allogenic UC-MSCs	intravenous; 3 times at 4-wk intervals	• ↑ survival rate • ↓ Tbil • ↑ ALB • 75 mo FU	• single centre trial • infused MSCs not tracked in pts in vivo
Salama et al. [106]	RCT	HCV-related ESLD	humans (120 pts)	disease therapy	autologous HSCs CD34+ and CD133+	portal vein; single infusion	• near normalization of liver enzymes • ↑ synthetic liver function • 48 wks FU	single centre trial
Newsome et al. [107]	RCT	compensated liver cirrhosis	humans (81 pts)	disease therapy	Autologous HSCs CD133+	Intravenous; three times at 4-wks intervals	• no improvement in MELD • ↑ frequency of adverse events • 1 yr FU	• absence of a true placebo • no histological endpoints
Zhou et al. [108]	meta-analysis of 24 RCT	liver fibrosis, liver cirrhosis and liver failure	humans (1359 pts)	disease therapy	BM-MSCs, BM- MNCs, UC-MSCs, PBSCs	peripheral vein or portal vein or hepatic artery or multiple routes; single cells injection in 11 studies, multiple cells injection in 11 studies; both in 2 studies	• ↓ all-cause mortality • ↓ Tbil and MELD • ↑ ALB • BM-MSCs more effective than UC-MSCs • hepatic artery infusion more effective than other routes	• high risk of bias • heterogeneity • different endpoints
Hallett et al. [109]	in vivo	biliary disease	immunodeficient mice model	disease therapy	hBECs	single intrasplenic injection	• successful engraftment • ↓ Tbil • resolution of biliary strictures • ↓ of hepatic fibrosis • ↓ overall mortality	• need to define the optimal route of injection • only limited studies on hBECs bipotential state
Cardinale et al. [110]	case series	advanced liver cirrhosis	humans (2 pts)	disease therapy	allogenic hBTSCs	single hepatic artery infusion	• ↓ Child Pugh score MELD and INR at 6 mo • ↑ ALB at 6 mo • one pt maintained a stable improvement for 12 mo	• lack of cell tracing • small sample size

Abbreviations: pts, patients; h, hours; Tbil, total bilirubin; UGT1A1, uridine diphosphate-glucuronosyltransferase 1A1; mo, months; NH3, ammonia; PHA, phenylalanine hydroxylase activity; AAT, alpha-1 antitrypsin deficiency; D, dimensional; HLCs; hepatocyte like cells; hiPSCs, human induced pluripotent stem cells; ER, endothelial reticulum; FVII, coagulation factor VII; DHCA, dihydroxycoprostanoic acids; FU, follow-up; SERPINA1, serine protease inhibitor 1; ATP7b, ATPase copper transporting beta**;** Rag 2, recombination activating gene 2 protein**;** Il2rg**,** interleukin 2 receptor subunit gamma; WD, Wilson disease; wks, weeks; FVIII, coagulation factor VIII; HA, haemophilia A; ECs, endothelial cells; vWF, von Willebrand Factor; CD34, cluster differentiation 34; BA, biliary atresia; KO, knockout;**,** CRISPR/Cas9, clustered regularly interspaced short palindromic repeats/CRISPR-associated nuclease 9; CK, cytokeratin; EpCAM, Epithelial cell adhesion molecule; SOX9, SRY-box transcription factor 9**;** AE2, anion exchange 2**;** CFTR, cystic fibrosis transmembrane conductance regulator**;** YAP, yes-associated protein**;** Ho-FH, homozygous familial hypercholesterolaemia; LDLR, low density lipoprotein receptor; LDL, low density lipoprotein;ALF, acute liver failure; ACLF, acute-on-chronic liver failure; AST, aspartate aminotransferase; LT, liver transplantation;; yrs, years; RCT, randomized controlled trial; HBV, hepatitis B virus; BM, bone marrow; MSCs, mesenchymal stem cells; MELD, model for end-stage liver disease; UC, umbilical cord; ALT, alanine aminotransferase; MNCs, mononuclear stem cells; PBSCs, peripheral blood stem cell; ALB, albumin; DLC, decompensated liver cirrhosis; HCV, hepatitis C virus; ESLD, end-stage liver disease; HSCs, hematopoietic stem cells; hBECs**,** human biliary epithelial cells; hBTSCs, human biliary tree stem/progenitor cells; INR, international normalized ratio.

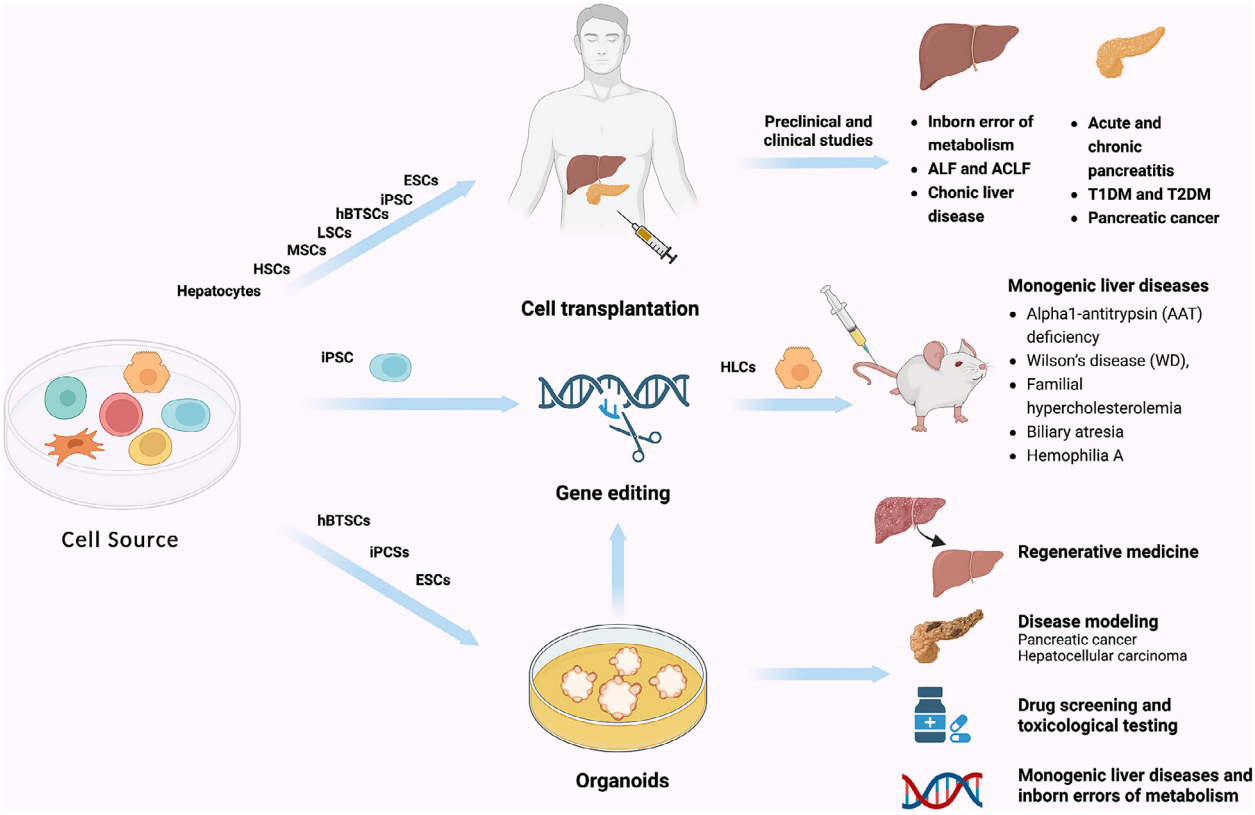

Fig. 1. Overview of the main cell source and applications of cell therapy in hepatobiliary and pancreatic diseases. Different types of SCs have been used in preclinical and clinical research. iPSCs, reprogrammed by gene editing and differentiated into HLCs, are used for the study and correction of monogenic liver diseases in animal models. SCs can be assembled into organoids, 3D cell structures whose main applications are regenerative medicine, disease modelling, drug sensitivity testing, and toxicology testing. In addition, iPSCs-derived organoids allow the recapitulation of monogenic liver diseases and inborn errors of metabolism, which is useful for studying their pathophysiology and investigating the efficacy of measures that could potentially correct the disease.
Abbreviations: *ESCs, embryonic stem cells; iPSCs, induced pluripotent stem cells; hBTSCs, human biliary tree stem/progenitor cells; HLCs, hepatocyte-like cells; HSCs, hematopoietic stem cells; LSCs, liver stem cells; MSCs, mesenchymal stem cells; ALF, acute liver failure; ACLF, acute-on-chronic liver failure; T1DM, type 1 diabetes mellitus; T2DM, type 2 diabetes mellitus.*

4.3. Chronic liver disease (liver fibrosis, cirrhosis)

Many studies have demonstrated SCs' ability to improve survival and liver function in patients with liver cirrhosis (Table 1). In fact, there have been reported significant amelioration in histological fibrosis quantification [104], Child-Pugh score, and liver synthetic function, as well as normalization of liver enzymes and increase in survival compared to the standard of care [105,106]. However, in a phase 2 randomized controlled trial (RCT) involving 81 patients with compensated liver cirrhosis of different aetiologies from three hospitals in the United Kingdom, Newsome et al. failed to demonstrate any improvement in liver function or liver fibrosis after the infusion of G-CSF and CD133+ HSCs. Conversely, G-CSF plus HSCs therapy was associated with an increased rate of adverse events such as ascites, sepsis, and hepatic encephalopathy [107]. A subsequent meta-analysis taking into account 24 RCT involving patients with liver fibrosis, cirrhosis, and liver failure demonstrated that therapy with SCs such as BM-MNCs, bone marrow-derived mesenchymal stem cells (BM-MSCs), umbilical cord-derived mesenchymal stromal cells (UC-MSCs) and peripheral blood stem cells (PBSCs) was associated with a significantly lower all-cause mortality and improved liver function compared to the standard of care [108]. In addition, BM-MSCs were found to be more effective than UC-MSCs, and the same was demonstrated for the infusion via the hepatic artery. No severe adverse events were recorded; however, the risk of bias was high, due to the heterogeneity in cell isolation, administration route, dosage, and injection frequency adopted in the analysed studies.

4.4. Bile duct damage

In preclinical studies, the infusion of human biliary tree stem cells (hBTSCs), isolated from the gallbladder, in a model of liver cirrhosis lead to the formation of adult hepatocytes and cholangiocytes and produced consistent amelioration of liver tests [64]. In another study, cholangiocytes isolated from discarded human livers were transplanted into an immunodeficient mice model of biliary disease, obtaining successful engraftment, reduction in overall mortality, resolution of biliary strictures and regression of hepatic fibrosis [109].

In one study including patients with advanced cirrhosis, the transplantation of foetal hBTSCs via hepatic artery infusion confirmed the effects already displayed by pre-clinical studies such as the improvement in liver function tests, Child-Pugh score, and MELD score [110].

However, there is still a need for more stringent clinical trials attesting the efficacy and the long-term safety of SCs therapy for the treatment of chronic liver disease.

5. Clinical application of cellular therapies in pancreatology

Reconstruction of pancreatic islets is a critical therapeutic need, since it could have a remarkable impact on patients' morbidity and quality of life.

SCs therapy has been studied in both exocrine and endocrine pancreatic diseases such as acute and chronic pancreatitis, pancreatic cancer, and diabetes mellitus. The most frequently used type

of SCs is MSCs, HSCs, ESCs, and iPSCs, the characteristics of which have been already described for hepatobiliary diseases (Fig. 1). They can be administered via intravenous infusion, portal vein injection, or directly into the pancreatic parenchyma. However, as with liver disease, there is no consensus regarding resources, route of administration, timing, and dosage of SCs infusions. Moreover, ethical issues and carcinogenic risk should be taken into account as well.

5.1. Disorders of the exocrine pancreas

5.1.1. Acute and chronic pancreatitis

Current treatments for acute and chronic pancreatitis mainly target the symptoms rather than the cause of the disease and, apart from alcohol and smoking cessation, there are no effective approaches to control disease progression or induce remission [117,118]. For these reasons, the interest in cell-based therapy has increased, and a recent systematic review of pre-clinical studies reported on the use of MSCs in the setting of either acute or chronic pancreatitis [119]. Eighteen papers were included; in animal studies, SCs therapy was applied more frequently for acute pancreatitis (fifteen articles) than for chronic pancreatitis (three articles); no randomized clinical trial was found. MSCs therapy reduced pancreatic inflammation in acute pancreatitis and pancreatic fibrosis in chronic pancreatitis. The Authors also concluded that, in both types of pancreatitis, the main mechanisms of action were related to the immunomodulatory effects mediated by the secretion of pro- and anti-inflammatory cytokines by these cells. In addition, MSCs can reduce the damage induced by oxidative stress in acute pancreatitis, inhibiting apoptosis, promoting the regeneration of the injured pancreatic tissue, and limiting the damage to the other organs involved in the systemic inflammatory response syndrome. In chronic pancreatitis, MSCs can reduce pancreatic fibrosis and restore the exocrine compartment by differentiating into acinar cells.

Beyond these speculations, the precise mechanism of action of MSCs is still unknown [120,121].

5.2. Disorders of the endocrine pancreas

5.2.1. Type 1 diabetes mellitus

SCs therapy as a potential cure for type 1 diabetes mellitus is a field of great interest. Patients on insulin therapy who do not obtain a satisfactory glycaemic control or present treatment-related complications can benefit from islet transplantation, which, however, is a procedure that requires immunosuppression, is limited by the shortage of donors, and may not be completely effective [122,123]. Therefore, considerable efforts have been focused on protocols to generate functional and glucose-responsive β cells [124]. One of the most challenging issues regards the best type and source of cells to be employed. Several studies demonstrated that human ESCs can be used to reproduce functional insulin-producing cells able to revert diabetes in mice models [125–129]. However, ESCs immunologically unmatched with the host may be destroyed by autoimmune reactions and rejection [130]. Although the use of patient-specific nuclear transfer ESCs can overcome this problem [131], ethical issues and the risk of teratoma development are still obstacles to ESCs' clinical application [132]. MSCs infusion can improve glycaemic control and insulin levels, supporting the hypothesis that these cells can facilitate the regeneration of endogenous islets [133,134]. Co-transplantation with MSCs allows reduction of the number of cells required for islet transplantation in diabetic rats, achieving similar metabolic results [135,136]. Various groups of researchers reported that MSCs from different tissues could be successfully induced to differentiate into insulin-producing cells, and even reverse diabetes in animal models [135,137]. However, the differentiation efficiency is lower than that of iPSCs even using the most recent protocols [135,138].

5.2.2. Type 2 diabetes mellitus

MSCs therapy has demonstrated promising therapeutic bnefits in glycaemic control in type 2 diabetes mellitus both in vivo and in vitro. Thirteen papers have already been published, although only four of them were randomized, placebo-controlled studies [139]. Overall, MSCs injection significantly reduced haemoglobin A1c (HbA1c) serum levels and insulin requirements in type 2 diabetes mellitus patients [140–142], but in some studies, this effect was not sustained in the long-term follow-up [143,144]. Improvement in islet function was regarded as the primary mechanism of action; however not all studies have reported a significant increase in fasting C-peptide, and its levels had the tendency to decline over time [139,143–146]. Some studies showed that even if serum C-peptide remained low, insulin requirements reduced; the most probable explanation could be a rapid improvement in general insulin resistance induced by MSCs, leading to a reduction in the endogenous insulin secretion and in the need for exogenous insulin injection [145,147]. The procedure was generally safe, and no acute allergic and immunologic adverse events occurred.

5.3. Pancreatic cancer

SCs therapy can also be useful to modulate tumour inflammatory microenvironment in pancreatic ductal adenocarcinoma [148]. The available studies on this subject report a downregulation of pro-inflammatory cytokines and chemokines, and a decrease in tumour burden [149–151] (Table 2). MSCs have also been used as vehicles for chemotherapy [152].

Although the literature provides substantial evidence in vitro and in animal models, human studies are scarce, and include small numbers of patients, with either resectable or unresectable diseases [148]. A variable reduction in tumour burden, blood tumour markers, and pain relief has been reported, with some patients experiencing graft-versus-host disease [153–155]. In solid tumours, the effect of HSCs transplantation is dependent on the graft-versus-tumour effect rather than on an anti-tumour cytotoxic effect; the mechanism, despite being poorly understood, might be similar to GVHD, that is, donor T cells react against tumour-associated antigens and elicit an immune response. SCs therapy has also been tested as adjuvant treatment after Whipple procedure, resulting in improved recurrence-free survival [156].

6. Organoids in hepatobiliary and pancreatic diseases

Organoids are three-dimensional (3D) structures generated in vitro from pluripotent SCs (e.g. ESCs, iPSCs or multipotent progenitors), or adult cells, which aggregate via cell-cell and cell-matrix interaction in an organotypic manner. Therefore, compared to two-dimensional (2D) cell cultures, organoids reflect the spatial and temporal characteristics of a specific tissue. According to the consensus on hepatic, pancreatic, and biliary (HPB) organoids, they can be classified into a) epithelial organoids, derived from a single germ layer from a single organ; b) multi-tissue organoids, derived from multiple germ layers from a single organ; and c) multi-organ organoids, derived from many germ layers of different organs [158].

Over the past two decades, many attempts have been made to obtain organoids with the morphological and functional characteristics of the human liver, able to self-replicate in vitro [159,160] (Table 3). In 2013, Takebe et al. aimed to recapitulate early organogenesis cultivating human iPSCs obtained from immature endodermal cells (iPSCs-HEs) with umbilical vein endothelial cells and MSCs. After 48 h, iPSCs organized into a 3D model resembling liver

Table 2
Available studies reporting on the use of cellular therapy in pancreatic cancer.

Author	Clinical setting	Experimental setting	Aim	Type of SCs	Route and timing of administration	Results	Limitations
Kidd et al. [149]	–	Mice xenograft model	disease therapy	IFN-β engineered hBM-MSCs	Intraperitoneal; weekly for 3 wks	• selective homing • ↓ tumour growth by ↓ proinflammatory cytokines/chemokines	anti-inflammatory agents ↓ the beneficial effects of therapy
Cousin et al. [150]	–	Planar culture Mice model	disease therapy	UC-MSCs	Intraperitoneal; on days 2 and 4 after cancer cells inoculation	• G0/G1 arrest • ↓ proliferation of tumour cells • ↓ peritoneal tumour burden • ↑ survival	Unknown mechanism of action
Zischek et al. [151]	–	Orthotopic syngeneic mouse model	disease therapy	Thymidine kinase-engineered BM-MSCs	Intravenous; once a week for 3 wks	• ↓ primary tumour growth by 50% • ↓ liver metastases	need ganciclovir for therapeutic effect
Brini et al. [152]	–	Planar culture	disease therapy	hMSCs from gingival tissue	–	able to uptake and release paclitaxel	–
Kanda et al. [153]	Unresectable pancreatic cancer	Human case-control study (7 pts)	disease therapy	Human HSCs from HLA-matched donors	Single Intravenous infusion	• minor tumour response in two pts • partial tumour markers response in 1 pt • stable disease in 3 pts GVT effect involved in tumour response	• small sample size • no effect on survival need to control GVT effect
Takahashi et al. [154]	Unresectable pancreatic cancer	Human study case series (5 pts)	disease therapy	Human HSCs from HLA-matched donors	Single Intravenous infusion	• ↓ tumour size in 2 pts • GVT effect involved in tumour response • no effect on survival	• small sample size • need to control GVT effect
Abe et al. [155]	Chemotherapy-resistant unresectable pancreatic cancer	Human case series (5 pts)	disease therapy	Human HSCs from HLA-matched donors	Single intravenous infusion	• ↓ tumour size in 2 pts with one of them showing tumour disappearance • no effect on survival • short duration of response	• small sample size • need to control GVT effect
Omazic et al. [156].	Resected pancreatic cancer after adjuvant chemotherapy	Human case-control study (8 pts)	disease therapy	Human HSCs from HLA-matched donors	Single Intravenous infusion at 1.5 or 2 yrs after surgery	• ↑ tumour free survival	• small sample size
Huang et al. [157]		3D culture Mice xenograft model	disease modelling	hPSCs-derived pancreatic progenitors	–	creation of a disease model useful for precision therapy strategies	only late phase of tumorigenesis

Abbreviations: IFN-β, interferon-beta; hBM, human bone marrow; MSCs, mesenchymal stem cells; wks, weeks; UC, umbilical cord; G0, gap 0 phase; G1, gap 1 phase; pts, patients; HSCs, hematopoietic stem cells; HLA, human leucocyte antigen; GVT, graft versus tumour; yrs, years; hPSCs, human pluripotent stem cells.

buds, which were able to self-renewal and expressed markers of hepatic differentiation such as AFP and albumin [161].

Human hepatobiliary organoids can be obtained from bipotent cells, and are able to differentiate into hepatocytes or cholangiocytes depending on the growth factors used in the culture [32,162,163,188,189]. Another strategy is the commitment of iPSCs into hepatobiliary progenitors, which can further generate hepatocytes, cholangiocytes and endothelial cells under specific stimulation. The resulting hepatobiliary organoids have been transplanted under the splenic capsule of immunodeficient mice; after four weeks, it was possible to detect the presence of both bile duct-like structures positive for human cytokeratin 19 (CK19), and clusters of hepatocytes expressing human albumin [189].

6.1. Potential applications of organoids in hepatobiliary and pancreatic diseases

Large-scale development of liver buds from human iPSCs may be potentially used to reduce the need for LT [164,190]. Animal studies have shown that intra-splenic injection of organoids is associated with partial repopulation of the original liver, positively influencing its functions [160,161,188,189]. Transplanted liver buds can connect with host vasculature within 48 h, and express markers and functions of adult human hepatocytes [161], being able to replicate for almost 11 months [163]. Similarly, cholangiocytes-derived organoids can replicate the morphological and functional characteristics of the extrahepatic biliary tree and, if transplanted into the kidney capsule of mice, achieve a duct–like aspect and express markers of biliary commitment; furthermore, when cultured into biodegradable scaffolds, they form a tissue that could repair the gallbladder wall or the biliary tree [165]. Sampaziotis et al. confirmed the plasticity of cells obtained from the biliary tree both in vitro and in vivo [166]. They first transplanted cultures of biliary organoids derived from cells of the gallbladder in immunodeficient mice with cholangiopathy; results demonstrated that these cells rapidly lost the expression of SRY-box transcription factor (SOX17), a marker of the extrahepatic biliary tree, and upregulate intrahepatic biliary tree markers. Then, an inverse experiment was performed using organoids derived from the bile ducts to regenerate gallbladder tissue, with positive results. Organoids derived from gallbladder cells were finally transplanted into intrahepatic ducts of deceased human liver donors with signs of ischaemic cholangiopathy; the engraftment was successful, recovering 40–85% of the injected intrahepatic bile ducts. An extensive contribution of transplanted hepatocytes or gallbladder organoids in intrahepatic bile ducts regeneration was also

Table 3
Organoids application in hepatobiliary and pancreatic diseases.

Author	Clinical setting	Experimental setting	Aim	Type of Organoid	Route and timing of administration	Results	Limitations
Michalopoulos et al. [159]	liver organogenesis and development	in vitro	disease modelling	HOs cultured on collagen support +HGF, EGF; dexamethasone	–	generation of a 3D structure composed of epithelial, hepatocytes and endothelial cells	• in vitro • rapid loss of replicative potential
Huch et al. [160]	DILI	• in vitro • in vivo (FAH -/- mice)	disease modelling	mice HBOs from LRG5+ HBSCs+ Matrigel	intrasplenic injection	FAH+ nodules were detected in liver mice at 3 wks after transplantation (1% of total parenchyma)	cells obtained from animals
Takebe et al. [161]	liver organogenesis	• in vitro • in vivo (FAH-/- mice)	disease modelling	LBs obtained from hiPSCs committed into HLCs cultivated with MSCs, ESCs and HUVECs	intraperitoneal injection	• LBs appeared after 48 h with high stability and ability to self-replicate. after transplantation in mice, engraftment and formation of new capillaries was observed	need for different type of cells for obtain the complexity of the liver
Sampaziotis et al. [32]	ChOs generation	• in vitro	disease modelling	ChOs from hiPSCs + activin, retinoid acid, FGF expression of SOX9+ to induce adult cholangiocytes	–	cholangiocyte organoids form cystic-like structures expressing CK7, CK18, CK19, GGT, CFTR, JAGGED1, Notch	in vitro
Huch et al. [162]	AAT deficiency physiopathology	• in vitro • in vivo (SCID mice)	disease modelling	human HBOs derived from HBSCs	intrasplenic injection	organoids from AAT deficiency pts can be expanded and mimic in vivo AAT deficiency phenotype	• cells obtained from animals poor replicative potential
Hu et al. [163]	HO regenerative potential after a stressor event (partial hepatectomy)	• in vitro • in vivo (SCID mice)	disease modelling	HOs obtained from human HBSCs and FLCs + Matrigel	–	HOs express hepatocytes markers and cholangiocytes/progenitor marker, LDL uptake, glycogen storage abilities, and bile canaliculi formation	lack of in vivo model
Takebe et al. [164]	liver organogenesis and development	• in vitro	disease modelling	LBs from hiPSCs committed into MSCs, HUVECs and HLCs, cultivation in microplates for large scale production	–	• hiPSCs entirely recreate LBs Production of large-scale organoids on microplates, able to cover the activities of a fully human baby liver in vitro	• risk of tumorigenesis for hiPSCs reduced replicative ability
Sampaziotis et al. [165]	plasticity of ChOs	• in vitro • in vivo (NOD mice)	disease modelling	ChOs obtained from hiPSCs originated from extrahepatic bile ducts cells.	kidney capsule	• able to rebuild intrahepatic bile ducts in mice • in vitro, organoids recapitulate the structure and functions of a gallbladder on a scaffold	• rapid loss of replicative potential in vivo • risk of differentiation into non biliary cell types
Sampaziotis et al. [166]	regenerative medicine	• in vitro • in vivo (mice and humans)	disease therapy	human gallbladder-derived ChOs	intraductal delivery in mice and human liver donor	• transplanted ChOs rescued mice from cholangiopathy • in human livers, ChOs successfully engrafted into intrahepatic bile ducts and recovered 40–85% of them from ischaemic cholangiopathy • survival up to 3 mo in mice	• rapid loss of replicative potential in vivo • lack of niche stimulation
Andersson et al. [167]	Alagille Syndrome	• in vitro	disease modelling	HOs from murine hepatocytes with a missense mutation (H268Q) in Jag1	–	• receptor-selective missense mutation in mouse JAG1 (H268Q) causes Alagille Syndrome • apical polarity of bile ducts severely disrupted	• mouse model based on homozygous mutation of JAG1, while human pts present heterozygous for JAG1 mutations

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Table 3 (*continued*)

Author	Clinical setting	Experimental setting	Aim	Type of Organoid	Route and timing of administration	Results	Limitations
Gomez-Mariano et al. [168]	AAT deficiency	• in vitro	disease modelling	LOs from hepatocytes of pts with homozygous (ZZ) and heterozygous (MZ) deficiency and normal (MM) genotypes of AAT	in vitro	• MZ and ZZ derived organoids showed intracellular aggregation and lower secretion of AAT, ALB and APOB	• in vitro study • gene correction in vivo not performed
Ouchi et al. [169]	NASH; Wolman Disease	in vitro	disease modelling	HOs (Hepatocytes+ MCs and HSCs) derived from hiPSCs exposed to FFA	–	• FFA exposure induces inflammatory and fibrotic changes in HOs • In Wolman organoid, FGF19 alleviates the NASH phenotype	• in vitro • rapid loss of replicative potential • risk of tumorigenesis in hiPSCs
Hohwieler et al. [170]	CF	In vitro	Disease modelling	POs from clonal iPSCs of 2 pts with CF and healthy donors	In vitro	• CF-POs displayed CF phenotype with impaired intraluminal chloride secretion • commitment step towards acinar-like/ duct-like cells unaltered in CF	• in vitro study
Kruitwagen et al. [171]	WD	in vitro in vivo (dogs)	disease therapy	LOs derived from dog's HBSCs with COMMD1 gene correction before transplantation	repeated portal vein injection	• liver function restored • repeated portal vein injections were safe • 1–10% engraftment efficiency • survival up to 2 yrs	• Low engraftment and repopulation • Transplanted cells did not fully integrate in vivo
Wang et al. [166]	alcohol liver injury; alcoholic fatty liver disease	• in vitro • in vivo SCID mice	disease therapy	HOs from hESCs and hybrid of hESCs + hFLMCs + serum free medium	epididymal fat pads of diabetic mice	• HOs hBSCs restricted to hepatic lineage in vivo • mice liver function recovered after transplantation • significant difference in terms of survival between transplanted mice and controls • 20% of liver parenchyma engrafted • In vitro ethanol induces AdH and CYP21E activity	• diabetic phenotype could favour inflammation and fibrosis development
Elbadawy et al. [172]	NASH	• in vitro • in vivo immunodeficient mice cohort case control	disease modelling	mouse NASH–HOs cultured on a Matrigel support.	3 cohorts of C57BL/6 mice fed with MCD diet for 4, 8 and 12 wks and 1 cohort of unexposed C57BL/6 mice as controls	according to the grade of exposition to MCD diet, NASH organoids showed activation of HSCs and deposition of collagen *I* + EMT	in vitro study
Ramli et al. [173]	NASH	in vitro; comparison with NASH liver biopsies	disease modelling	hiPSCs and ESCs + Matrigel, palmitic for NASH induction	–	HOs exposed to FFA had gene expression signatures similar to NASH pts	• in vitro model • absence of other cell types in the liver
Broutier et al. [174]	primary liver cancer (HCC; CC, HCC/CC)	• in vitro • in vivo (SCID mice)	disease modelling	LOs from healthy donors, HCC, CC and HCC/CC pts	subcutaneous and renal capsule injection	• PLCOs induce tumorigenesis in vivo • metastatic potential in vitro • possible identification of novel drugs and biomarkers	lack of immune system and stromal components
Takai et al. [175]	HCC	• in vitro • in vivo SCID mice	disease modelling	HCC organoids from Huhs + alginate matrix	hepatic vein injection (1×106 cells/50 μl) mice sacrificed 4 wks	• HCC organoid recapitulates HCC features • mice developed peritoneal metastases	lack of primary HCC cells in this 3D culture model

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Table 3 (*continued*)

Author	Clinical setting	Experimental setting	Aim	Type of Organoid	Route and timing of administration	Results	Limitations
Wang et al. [176]	HCC	• in vitro	disease modelling	HCC organoid from HCC human cells + non parenchymal human cells + Matrigel	–	• non parenchymal cells influence HCC aggressiveness and invasiveness • recapitulation of TME interaction with HCC cells	in vitro
Nie et al. [177]	HBV infection; drug sensitivity test	in vitro comparison of hiPSCs-LO, hiPSCs-HLC, HepG2-organoids, and PHHs	disease modelling	hiPSCs-derived LO infected with HBV-DNA with a 3D microwell system	–	• viral load causes rise in inflammatory and epithelial to mesenchymal transition markers • myrcludex downregulates viral replication and reduces inflammation and hepatic dysfunction • HBV-DNA levels drop after IFN alpha therapy	• in vitro • some characteristics different from adult hepatocytes
Baktash et al. [178]	HCV infection	in vitro creation of 3D hepatoma model	disease modelling	Huh-7.5 cells on ECM support infected with HCV	–	• recapitulation of the pathogenetic mechanisms that lead to HCV infection in human cells	• in vitro • no evaluation of immune system response or drug sensitivity test
Soroka et al. [179]	PSC	in vitro	disease modelling	ChOs derived from biliary ducts of PSC pts on Matrigel support	–	• ↑ serpin peptidase inhibitor E2 and p21, markers of senescence • ↑ CCL20, HLADMA, and CD74, markers of autoimmune phenotype	• in vitro model • rapid loss of replicative potential
Nie et al. [180]	DILI	• In vitro In vivo SCID mice	disease therapy	LOs from hiPSCs endoderm, UC-ECs, and UC-MCs derived from UCs cultured in Matrigel	Renal subcapsular inoculation (~1 × 106 hepatocytes)	• LOs improved survival in 70% of transplanted ALF mice vs controls • 5–10% over the total liver	poor engraftment
Vorrink et al., [181]	DILI	• in vitro	disease modelling	HOs model derived from PHH to test hepatotoxicity of 123 drugs with or without direct implication in DILI	–	• ATP quantifications as endpoint • the model distinguished between hepatotoxic and non-toxic structural analogues with higher sensitivity and specificity than all previously published in vitro assay	• in vitro • costly • need for specific laboratories
Shinozawa et al. [182]	DILI	• in vitro	disease modelling	hiPSCs derived LOs	Drug sensitivity assay with multiplexed readouts measuring viability, cholestatic + mito-chondrial toxicity in vitro	• high predictive values for 238 marketed drugs at 4 different concentrations (sensitivity: 88.7%, specificity: 88.9%)	• costs • low replicability
Lim et al. [183]	HCC-TME interaction model	In vitro	disease modelling	HCC culture or HCC pt derived organoid +liquid biofilm + ECs		recapitulation of angiocrine crosstalk and TME influence on HCC maintenance	in vitro
Huch et al. [184]	pancreas development	• in vitro • in vivo SCID mice	disease modelling	mouse bipotent pancreatic progenitors expressing LRG5+	Kidney capsule injection after 1 mo mice are sacrificed	Pancreatic duct ligation induces LRG5+ progenitors that could produce both endocrine cells and ducts	need for foetal bovine serum (risk of immune reaction)
Li et al. [185]	pancreas and PDAC development	in vitro	disease modelling	murine pancreatic organoid cultured in an air-liquid generation of tumour organoids (K ras and p53 mutation)	–	• pancreatic organoids express markers and features of pancreatic ducts • dysplasia rapidly developed in presence of both KRAS and p53 mutation	lack of immune cells and TME

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Table 3 (*continued*)

Author	Clinical setting	Experimental setting	Aim	Type of Organoid	Route and timing of administration	Results	Limitations
Huang et al. [157]	PDAC development	in vitro	disease modelling	ductal pancreatic cells from mice+ collagen matrix; 3 models with KRAS, p53 or both mutations	–	Dysplasia in vitro appeared after 1 mo in tumour models	• lack of stromal cells interaction • in vitro
Li et al. [186]	CC, HCC; drug sensitivity tests	in vitro	disease modelling	pts derived organoids (27 different lines	–	• possibility to perform screening of more than 132 drugs at the same time • high sensitivity and specificity	• high costs • low reproducibility in vivo lack of immune system
Boi et al. [187]	PDAC development	• in vitro • in vivo SCID mice	disease modelling	human pancreatic ductal cells pancreatic progenitors from mice organoids derived from PDAC cells and from metastases	anterior abdomen incision, tail region of the pancreas	• murine and human PDAC organoids generate lesions similar to PanIN and progress to invasive PDAC • metastases-derived organoid progress to PDAC in 1 mo in murine models	Lack of in vivo studies

Abbreviations: HOs, hepatic organoids; HGF, hepatocyte growth factor; EGF, epidermal growth factor; D, dimensional; DILI, drug induced liver injury; FAH, fumaryl acetate hydrolase; HBO, hepatobiliary organoid; LRG5+, leucine-rich repeat-containing G-protein coupled receptor 5; HBSCs, hepatobiliary stem cells; wks, weeks; LBs, liver buds; hiPSCs, human induced pluripotent stem cells; HLCs, hepatocyte like cells; MSCs, mesenchymal stem cells; ESCs, embryonic stem cells; HUVECs, human umbilical vein embryonic cells; h, hours; ChOs, cholangiocytes organoid; FGF, fibroblast growth factor; SOX9, SRY-box transcription factor 9; CK, cytokeratin; GGT, gamma glutamyl transferase; CFTR, cystic fibrosis transmembrane receptor; JAGGED1, jagged canonical notch ligand 1; Notch, neurogenic locus notch homologue protein 1; AAT, alpha-1 antitrypsin deficiency; SCID, severe combined immune deficiency; pts, patients; FLC, foetal liver cells; LDL, low density lipoprotein; NOD, non-obese diabetic mice; mo, months;; LO, liver organoid; ALB, albumin; APOB, apolipoprotein; NASH, nonalcoholic steatohepatitis; MCs, mesenchymal cells; HSCs, hepatic stellate cells; FFA, free fatty acids;;CF, cystic fibrosis; PO, pancreatic organoid; WD, Wilson disease; COMMD1, copper metabolism domain containing 1; yrs, years; hESCs, human embryonic stem cells; hFLMCs, human foetal liver mesenchymal cells; AdH, aldehyde dehydrogenase; CYP21E, cytochrome p21E; MCD, methionine choline diet; EMT, epithelial to mesenchymal transition; HCC, hepatocellular carcinoma; CC, cholangiocarcinoma; PLCOs, patients liver cancer organoid; TME, tumour microenvironment; HBV, hepatitis B virus; HepG2, type of cell line; PHH, patients human hepatocytes; IFN, interferon; HCV, hepatitis C virus; HuH, type of cell line; ECM, extracellular matrix; PSC, primary sclerosing cholangitis; CD, cluster differentiation; CCL20, Chemokine (C–C motif) ligand 20; HLADMA, human leucocyte antigen DM alpha chain; UC, umbilical cord; ECs, endothelial cells; ALF, acute liver failure; ATP, adenosine three phosphate; PDAC, pancreatic ductal adenocarcinoma; K RAS, Kirsten rat sarcoma virus; p53, tumoral protein 53; PanIN, pancreatic intraepithelial neoplasia.

observed in cholangiocyte-deficient mice models of Alagille syndrome [166,191]. iPSCs-derived liver organoids have also been used to explore the pathophysiology and to correct monogenic diseases, including alpha-1 antitrypsin deficiency, Wilson's disease, lysosomal acid lipase deficiency, Alagille Syndrome, biliary atresia, and cystic fibrosis [167–171,192–194] (Table 3). Organoids can also reproduce the multistep process which causes liver dysfunction, cirrhosis, and cancer; the paradigm of alcoholic and non-alcoholic fatty liver disease, steatohepatitis, and liver fibrosis has been recapitulated by several studies in vitro and in vivo [169,172–176,195,196]. Other studies focused on the pathogenesis and therapeutic approach in viral liver diseases, such as those related to hepatitis B or C virus [177,178], as well as on primary sclerosis cholangitis [179,197,198]; the investigation of the mechanisms of drug-induced liver injury (DILI), drug sensitivity testing, and research into new disease-specific pharmacotherapies are other applications currently under development [180–182,199]. Finally, another promising use of organoids is the development of cancer models, such as hepatocellular carcinoma, cholangiocarcinoma, and pancreatic ductal adenocarcinoma, to better understand the role of tumour microenvironment, inflammation and immune response and identify precision therapy strategies based on patient-specific sensitivity to therapeutic agents (Fig. 1) [157,174–176,183–187,200,201].

7. Future application of cell therapies: usefulness and limitations

SCs therapy and organoids are rewriting the history of transplantology and regenerative medicine. The use of iPSCs, able to exhibit the characteristics of any cell and to build liver organoids under proper conditioning, may reduce the risk of rejection, enhancing tissue engraftment. Novel technologies, such as microfluidic and liver-on-a-chip, may allow to better resemble the sophisticated characteristics of a real hepatobiliary or pancreatic unit. Indeed, 3D models are far to reproduce the dimension and complexity of a human liver or pancreas. Liver-on-a-chip technology is based on both 2D and 3D cultures, with or without matrix support, allowing to rapidly perform drug toxicity and sensitivity tests, together with the analysis of the microvascular structure and metabolic processes [202,203]. The ultimate frontier of SCs application is made by 3D bioprinted scaffolds covered with autologous iPSCs-derived organoids, a hybrid technology that allows the construction of miniature livers made of different lineages of cells (hepatocytes-like cells, mesenchymal and endothelial cells), which can be used to determine the fibrotic and metabolic changes after drugs administration. Studies on these models are ongoing, with promising preliminary results [204].

Finally, the future development of biobanks including organoids derived from several tissues, such as the pancreas, represents a resource with enormous potential to explore the personalized response to drugs and perform a rapid genetic evaluation [205].

Despite available data portending a bright future for cellular therapies in hepatobiliary and pancreatic diseases, the possibility to recapitulate a fully functional human organ is still far. Nowadays, these techniques have several relevant limitations. While SCs could guarantee a persistent self-renewal ability, they may not perfectly match the functional and morphological complexity of the adult tissue counterpart; although adult cells are fully comparable to the original tissue and could generate stable liver organoids, they rapidly lose their replicative potential. Moreover, to obtain these cells, invasive procedures are needed [206]. On the contrary, iPSCs are simple to be obtained and able to develop any tissue under commitment, but excessive manipulation may induce gene mutations with the risk of tumorigenesis [207]. In addition, large-scale production of organoids is now unsustainable, because they are cultured under 3D conditions with technologies that are not widely available [208]. Matrigel, which is the scaffold of choice for 3D structures, is obtained by sarcoma mouse cell lines that may potentially lead to tumorigenesis if implanted in immunosuppressed patients, and, considering the animal origin, could cause immune reactions [209]. To overcome these limitations, fully defined biological hydrogels are being developed, and decellularized tissues obtained from living or deceased donors are being studied for use as biological hydrogels [210,211]. In conclusion, cell therapies are innovative tools in regenerative medicine and transplantology. In recent years, progress in this field has been remarkable, with the development of increasingly complex technologies to narrow the gap between translational and clinical applications. Reproducing a fully human-like organ is still a long way off, but preliminary results and advances in biomedicine are promising and will lead to interesting results in the near future.

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