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Targeted animal models for preclinical assessment of cellular and gene therapies in pancreatic and liver diseases: regulatory and practical insights

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ABSTRACT

Cellular and gene therapy (CGT) products have emerged as a popular approach in regenerative medicine, showing promise in treating various pancreatic and liver diseases in numerous clinical trials. Before these therapies can be tested in human clinical trials, it is essential to evaluate their safety and efficacy in relevant animal models. Such preclinical testing is often required to obtain regulatory approval for investigational new drugs. However, there is a lack of detailed guidance on selecting appropriate animal models for CGT therapies targeting specific pancreatic and liver conditions, such as pancreatitis and chronic liver diseases. In this review, the gastrointestinal committee for the International Society for Cell and Gene Therapy provides a summary of current recommendations for animal species and disease model selection, as outlined by the US Food and Drug Administration, with references to EU EMA and Japan PMDA. We discuss a range of small and large animal models, as well as humanized models, that are suitable for preclinical testing of CGT products aimed at treating pancreatic and liver diseases. For each model, we cover the associated pathophysiology, commonly used metrics for assessing disease status, the pros and limitations of the models, and the relevance of these models to human conditions. We also summarize the use and application of humanized mouse and other animal models in evaluating the safety and efficacy of CGT products. This review aims to provide comprehensive guidance for selecting appropriate animal species and models to help bridge the gap between the preclinical research and clinical trials using CGT therapies for specific pancreatic and liver diseases.
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Introduction

Cellular and gene therapy (CGT) has become popular in regenerative medicine and is being attempted in clinical trials to treat various diseases [1]. Before advancing to clinical trials, it is crucial to conduct preclinical assessments of CGT products to evaluate their safety, toxicity, pharmacokinetics and efficacy, typically *in vitro* and/or in animals. These preclinical studies are often essential for obtaining approval from regulatory agencies for investigational new drugs or biological products [1,2]. Regulatory agencies from different countries or regions provide guidelines and recommendations to aid sponsors and individuals in selecting appropriate animal species and disease models necessary to bolster clinical trial support [3,4]. Here, we discuss such recommendations from the Food and Drug Administration (FDA) in the United States. Similar regulations from the European Union and Japan, two other founding members of the International Conference of Harmonization (ICH), are listed in Tables 1 and 2. Moreover, Japan has unique regulatory pathways tailored to regenerative medicines, including gene therapy. Below are the general FDA recommendations for selecting animal species and disease models.

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Table 1
Individual regulations that regulate CGT products in the United States, EMA and Japan.

Regulatory agency	Relative guidelines	Year	Published by	References
FDA, United States	Cell and gene therapy, tissue engineering • Guidance for **industry: preclinical assessment** of investigational cellular and gene therapy products (FDA-2021-D-1038) • Interpreting sameness of **gene therapy products under the orphan drug regulations**; guidance for industry (FDA-2019-D-5392) • Guidance for industry: considerations for **allogeneic pancreatic islet cell products** (FDA-2008-D-0293) • Eligibility determination for **donors of human cells, tissues and cellular and tissue-based products**; guidance for industry (2004d-0193) • Regulatory considerations for **human cells, tissues and cellular and tissue-based products**: minimal manipulation and homologous use (FDA-2017-D-6146)	2007, 2009, 2020, 2013, 2021	Center for Biologics Evaluation and Research (CBER) Center for Devices and Radiological Health (CDRH)	[3,5–9]
EMA, Europe	Cell and gene therapy, tissue engineering • Reflection paper on **stem cell-based medicinal products** (EMA/CAT/571134/2009) • Reflection paper on in-vitro cultured **chondrocyte-containing products** for cartilage repair of the knee (EMA/CAT/CPWP/568181/2009) • Guideline on human cell-based medicinal products (EMEA/CHMP/CPWP/83508/2009) • Guideline on potency testing of **cell-based immunotherapy medicinal products** for the treatment of cancer (CHMP/BWP/271475/06) • Reflection paper **on clinical aspects related to tissue-engineered products** (EMA/CAT/573420/2009) • Guideline on **safety and efficacy follow-up and risk management of advanced therapy medicinal products** (EMEA/149995/2008) • Guidance on **the quality, nonclinical and clinical aspects of gene therapy medicinal products**, and animal species/model selection (EMA/CAT/80183/2014)	2008, 2011, 2018	Committee for Advanced Therapies (CAT) Committee for Medicinal Product for Human Use (CHMP)	[10,5,11–13]
MHLW, Japan	Cell-therapy and gene-therapy • Guidelines on **clinical research using human stem cells**; Provisional Translation (as of July 2020)[a] (PSEHB/MDED Notification No.0709-2)	2006, 2019	Ministry of Health, Labour and Welfare	[5,14,15]

[a] The purpose of this English translation of the Japanese Notification is to serve as a convenient reference material for users.

Selection of animal species

FDA requests that the chosen animal species for evaluating bioactivity and safety must exhibit a biological response to the investigational CGT product akin to what is anticipated in humans. This requirement aims to produce data that inform the design of clinical trials. FDA regulations relevant to this include the "Good Laboratory Practice for Nonclinical Laboratory Studies" (21 CFR Part 58) [16], EMA provides guidelines in the form of ICH S6, R1 [10] and Japan MHLW guides cell and gene therapy products (The Act on Securing Quality, Efficacy and Safety of Products Including Pharmaceuticals and Medical Devices, 2013 [Act No. 145, 1960]) [17,18] (Table 1).

Four major factors should be carefully considered when selecting the relevant animal species for a CGT product evaluation. First, the animal's physiology and anatomy should closely resemble that of humans to ensure accurate prediction of responses to CGT products. Second, the animal's susceptibility to viral or microbial vectors utilized in CGT therapy must be considered. Third, immune tolerance to a CGT product is crucial, as immune responses from healthy, immune-competent animals may reject the administered human cells, complicating the safety and efficacy evaluation. Cross-species immunogenicity may require modifying the animal model to create an immune-tolerant environment. Lastly, the feasibility of the clinical delivery system must be considered to ensure proper access to the administration site, precise dosing and the availability of immunodeficient animals for long-term safety assessments. Furthermore, unconventional test species, such as genetically modified rodents (eg, transgenics or knockouts), or large animals (eg, sheep, pigs, goats and horses), may be used with sufficient rationale. Additionally, while safety and efficacy of CGT products can be assessed in a single species, factors such as product origin and administration method may require testing in multiple species.

Selection of animal models in disease/injury studies

Disease or injury animal models utilized in the basic research or discovery phases of product development have the potential to yield data that support clinical trials for CGT products. Given the unique characteristics of CGT products –such as their potent, long-lasting effects, persistence in vivo and complex mechanisms of action involving interactions with the disease environment, the following animal models should be considered: (i) immune-competent animals treated with immunosuppressive agents, (ii) genetically immunodeficient animals, (iii) humanized animals (which carry functional human genes, cells, tissues and/or organs), (iv) administration into an immune-privileged site and (v) a combination of these scenarios. Each model offers insights into different aspects of CGT behavior to help address the challenges posed by these therapies.

In an investigational new drug application, the FDA requires detailed information on the limitations of potential animal models, addressing factors such as inherent variability, the availability of baseline data, technical constraints related to physiological and anatomical fidelity, animal care consideration and the limited capacity to

Table 2
Specific recommendations of animal species and models in the United States, EMA and Japan for preclinical study.

	Animal species selection	Animal model selection
FDA, cell and gene therapy product	• Physiology and anatomy compared to those of humans • Permitting/susceptibility to infection by, and replication of, viral vectors or microbial vectors for gene therapy • Immune tolerance to a human CGT product or human transgene expressed by a GT product • The feasibility of using the planned clinical delivery system/procedure	• Immunocompetent animals considering administering immunosuppressive agents • Study includes genetically modifying animals to be immunodeficient • Crucial aspect of the research humanizing animals to mimic human physiology • Exploring administering treatments into immune-privileged sites, along with combinations of these scenarios
EMA, cell therapy product	• Local tolerance, tissue compatibility and tolerance to excreted substances • Expression levels of biologically active molecules, route of cell administration and injected cell dosages • Selection of appropriate number of animals, consideration of gender differences and frequency and duration • Assessing long-term tissue regeneration and repair, and ensuring comprehensive safety monitoring • Use of large animal models when the size, physiology or immune system of the chosen animal model is crucial for accurately studying clinical effects, such as tissue regeneration • Persistence and functionality of the administered cells	• The selected animal model immunocompromised, knockout or transgenic animals • Homologous animal models for establishing proof-of-concept • The use of genetically immunocompromised animal model or a humanized animal model (eg, an animal model with a humanized immune system) for assessing the potential of a stem cell product in forming teratomas or tumors • The use of immunosuppressants for tumor formation due to their inherent properties
EMA, gene therapy product	• Investigating the expression and tissue distribution of cellular receptors for a virus/virion/bacteria in the animal model • Examining the activity of regulatory elements and their control to drive tissue-specific expression, as well as the expression level of the transgene • Analyzing the biological response to the transgene, including target expression, distribution, binding, occupancy, functional consequences such as cell signaling and regulation of associated genes if applicable • Evaluating the immune status of the animal, its immune response, and potential pre-existing immunity • Identifying the presence of animal genes/gene products homologous to the therapeutic gene/transgene products • Considering metabolism and other pharmacological aspects, if necessary • Using large or diseased animal models to simulate particular clinical scenarios or the distribution of the GTMP, based on the product's characteristics, administration route, and optionally, the delivery system utilized (eg, intra-cerebral administration) • Considering the biological traits of product components concerning dosage and safe administration volume to test animals • Investigating the active and/or passive distribution of virus/vector in the model organism and the potential for recombination of the GTMP (or part of the GTMP) with the host's endogenous virus	• The choose of animal models may include wild type, immunocompromised, knockout, knock-in, humanized or transgenic animals • Multiple models should be used if a single one is insufficient for the study's objectives • Utilizing transgenic animals to model various human diseases
Japan, cell product	• To validate the efficacy of human stem cells for transplantation, animal species studies are crucial for comprehending their mechanism of action • Cell therapy studies should not be limited to small animals exclusively but should also include investigations on large animals • Nonclinical safety studies should be conducted with an awareness of the limitation that cellular products of human origin may trigger a xenogeneic immune response in animals	• When there is a concern about the potential for tumorigenicity, it is advisable to address this risk by conducting appropriate animal model studies
Japan, gene therapy product	• Whether the transgene loaded on the expression vector express in target cells • Whether the nucleic acid or protein derived from the transgene exert the pharmacological action expected in humans • If using a viral vector, whether the corresponding wild-type virus infects the animal and targets specific tissues/cells in the animal in a manner similar to that in humans • Whether the same administration method proposed for the clinical use can be applied	• Testing toxicity and safety in normal animals • When a gene therapy product targets specific tissues/cells, developers must confirm tissue specificity, duration of gene expression and biological activities in appropriate animal models, in addition to conducting biodistribution studies • Assessment gene therapy products using a single suitable animal species • The use of small animals like rodents for safety studies and the clinical application route • If traditional nonclinical species are not suitable, alternative approaches involving genetically modified or transgenic animals may be considered

model human pathophysiology relevant to the target disease or injury. Furthermore, the FDA mandates that data be provided to demonstrate the relevance and effectiveness of the chosen animal model (s) in replicating the target disease population and facilitating the assessment of investigational CGT product safety.

The FDA advocates for a tiered approach when selecting an appropriate animal model. This approach includes conducting preliminary studies to evaluate the suitability of a specific animal species or model for use in definitive preclinical studies evaluating the intended CGT product. Additionally, it may be necessary to utilize multiple animal species and models, to comprehensively identify functional aspects and potential toxicities of a single product under investigation. In such scenarios, the preclinical testing strategy may involve (i) both small and large animal models, (ii) various small animal models or (iii) exclusively large animal models.

Special considerations during animal species and model selection

Although regulatory agencies advocate for replacing *in vivo* animal studies with in vitro models, the limited complexity of current *in*

vitro systems often restricts the ability to conduct comprehensive analyses and draw definitive conclusions. Nonetheless, the principle of the 3Rs Replacement, Reduction and Refinement- recommended by the ICH must be adhered to. This means that when animal testing cannot be fully replaced, efforts should be made to reduce the number of animals used and refine experimental approaches to minimize animal suffering when evaluating CGT products [19]. Another critical consideration during study design is to use clinical-grade materials during the animal study when possible. This can help generate more reliable preclinical data that better predict human outcomes, ensure compliance with regulatory expectations and facilitate transition to clinical trials.

In addition, it is critical to seek guidance from the FDA or other regulatory agencies during a design of a preclinical study. The early dialogue may help the decision to choose the right animal models, align them more closely with human physiology, reduce the risks of later-stage study failures, save time and resources, improve the relevance of the data and help streamline the approval process. This can be achieved by having presubmission meetings with a regulatory agency during the development process to discuss the study design, manufacturing and clinical plans. Before the meeting, prepare comprehensive documentation, outline the research, be open to feedback and be willing to adjust the plan based on regulatory input. Utilize programs like the FDA's Fast Track or EMA's scientific advice to receive guidance on specific scientific and regulatory questions relevant to the CGT product as well as stay informed with new regulations and guidance on CGT.

The following sections will provide a summary of commonly used animal species and disease models for various pancreatic diseases (Section II), as well as chronic liver diseases (Section III), with a focus on humanized mouse models (Section IV). Additionally, Section V will discuss the application of these models used in CGT therapy studies (Figure 1).

Section II: Animal Model for Pancreatitis and Type 1 and Type 3c Diabetes (T3CD)

Pancreatic diseases include pancreatitis, pancreatic cancer, cystic fibrosis, diabetes and many others. This review will only focus on animal models for acute, chronic, autoimmune, genetic pancreatitis and type 1 and 3c diabetes (Figure 1). Animal models for other pancreatic diseases such as pancreatic cancer, cystic fibrosis and others have been comprehensively addressed in other literature and will not be included in this paper.

Pancreatitis

Pancreatitis is a common digestive disorder that primarily affects adult and elderly subjects and can significantly reduce the quality of life and life expectancy. Over the past century, several preclinical models have been developed that have largely contributed to understanding the pathophysiology of these conditions. Although many animal species have been applied, such as rabbits, cats, dogs, pigs, opossum and zebrafish, the most frequently used and cost-efficient model is rodents [20]. Of course, anatomic differences exist between the pancreas of human and experimental models. For instance, rats do not have a gallbladder, their pancreas is composed of multiple segments, and they have both a common bile and pancreatic duct. In contrast, humans have two separate ducts that converge into a short common duct just before reaching the duodenum. However, rats serve as an excellent and reliable experimental animal model as their pancreas shares the same cellular components, having both exocrine and endocrine functions, and their pancreatic duct has the ability to drain pancreatic juices into the duodenum [21]. Below, we introduce animal models for acute, chronic, autoimmune and genetic models of pancreatitis.

Acute pancreatitis

Acute pancreatitis (AP) is an inflammatory disease characterized by acinar cell necrosis caused by intracellular digestive enzyme activation due to several etiologies, leading to interstitial edema and acute inflammatory cell infiltration. Usually, it is self-limited, and the tissue damage is reversible. However, in 10–15% of cases, extensive necrosis can occur with organ failure or multi-organ failure [22]. Gallstone and alcohol abuse account for 70–80% of AP causes in the US, whereas hypertriglyceridemia, genetic mutation, post-endoscopic retrograde cholangiopancreatography, drugs and infections are other possible etiologies [23]. In preclinical models of AP, many driver mechanisms have been reproduced (Table 3), with the most common resumed here below:

Cerulein-induced AP model

Cerulein-induced AP model is the most widely used AP model, as it is highly reproducible and cost-effective. Cerulein is a hormone analogous to cholecystokinin and induces pancreatic enzyme secretion. A repeated intravenous or intraperitoneal injection of an overdose of cerulein in mice or rats leads to hyperamylasemia, inflammatory cell infiltration of the pancreas, interstitial edema,

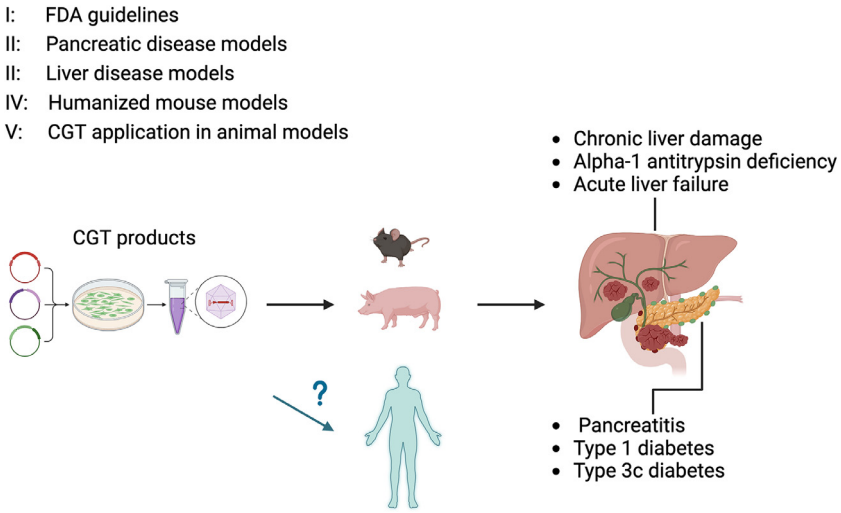

Fig. 1. Summary of the main content of this review. (Color version of figure is available online.)

Table 3
Commonly used animal models of acute pancreatitis (A), chronic pancreatitis (B) and autoimmune pancreatitis (C).

Pancreas disease	Experimental model	Administration	Species	Injury type	Advantage	Disadvantage
A: Acute pancreatitis (AP)	Cerulein-induced AP model	Intraperitoneal injection of cerulean	Mice or rats	◦ Hyperamylasemia ◦ Pancreatic infiltration by inflammatory cells, ◦ Interstitial edema ◦ Acinar cell vacuolization ◦ Intrapancreatic enzyme activation	◦ Highly reproducible model ◦ Mimics mild form of human AP ◦ Suitable for investigating pathogenesis and cellular changes in early phases of AP	Does not mimic severe forms of human AP
	Bile salt-induced AP model	Direct insertion of sodium taurocholate into pancreas	Mice or rats	Severe hemorrhagic necrosis	◦ Short period needed to induce severe necrosis ◦ Suitable for studying multiorgan failure	Requires abdominal incision and expertise in cannula insertion
	CDE diet-induced pancreatitis model	Choline-deficient diet enriched with ethionine	Mice	Severe necrotizing pancreatitis	◦ Mimics severe human pancreatitis ◦ Suitable for testing therapies	◦ Costly diet ◦ Requires on-site protocol standardization and careful monitoring of dietary intake
	Basic amino acid-induced AP model	intraperitoneal injection of L-Arginine, L-lysine or L-ornithine	Rats	Severe necrotizing AP	◦ Highly reproducible ◦ Severity can be controlled	Limited to rats
	Pancreatic duct ligation model	Duct obstruction	Dogs, rabbits, opossums, rats and mice	Mimicking gallstone-induced AP	Applicable to different species	Requires manual expertise and knowledge of animal anatomy
	Alcohol-related AP model	Continuous intragastric infusion of ethanol	Rats	Mimics ethanol-induced liver injury but not alcohol-induced pancreatitis	Allows study of alcohol's role in predisposing to AP	Does not directly induce AP
	Coxsackie B virus-induced AP model	Infecting mice with Coxsackie B virus subtypes B1, B3, B4 or B5	Mice	Severe pancreatitis	Allows study of viral-induced AP	Limited to specific viral subtypes
B: Chronic pancreatitis (CP)	Duct obstructive model	Prolonged pancreatic duct ligation	Mice or rats	Pancreatic fibrosis	Mimics pancreatic fibrosis seen in CP	Technically challenging to perform
	Repeated cerulein-induced CP model	Repeated bouts of cerulein-induced AP over several weeks	Mice or rats	◦ Chronic injury ◦ Collagen deposits ◦ Fibrosis	Mimics progression from AP to CP	May not fully replicate human CP progression
	Alcohol-induced CP model	Alcohol combined with other factors like cerulein or lipopolysaccharide	Mice or rats	Chronic damage	Allows study of alcohol's role in CP development	Alcohol alone may not induce CP
	Toxic chemical-induced CP model	Intravenous injection of toxic substances like Dibutyltin dichloride	Mice	AP followed by fibrosis due to direct toxic effects on pancreas and bile duct epithelium	Allows study of chemical-induced CP	Limited to specific toxic substances
	L-Arginine-induced CP model	Long-term intraperitoneal administration of L-arginine	Rats	◦ Progressive degeneration of pancreas ◦ Accumulation of extracellular matrix ◦ Replacement by adipose tissue	Allows study of progressive CP	Limited to rats
C: Autoimmune pancreatitis (AIP)	MRL/Mp mice treated with poly(I:C)	Repeated intra-peritoneal injections of polyinosinic:polycytidylic acid	Mice	◦ Pancreatic damage resembling AIP ◦ Serum auto-antibodies observed in mice	Mimics histopathological findings of human AIP.	Limited to specific mouse strain

CDE, choline-deficient, ethionine-supplement.

acinar cell vacuolization and intrapancreatic enzyme activation comparable to what happens in humans. This process is usually mild and resolves entirely after cerulein is withdrawn, thus reproducing a mild form of AP. It is, therefore, suitable for investigating the pathogenesis of the disease and the cellular changes observed in the early phases of the process [21].

Bile salt-induced AP model

First reported by Aho et al. [24,25] almost 40 years ago, this model involves the direct insertion of sodium taurocholate from the duodenum into the pancreas using a cannula through the pancreatic duct orifice. This approach leads to rapid onset of hemorrhagic necrosis, probably due to the deterrent effect of the bile salt, realizing a model of severe AP. The advantages of this model are the short period needed to induce severe hemorrhagic necrosis of the pancreas and the possibility of better understanding the consequent multiorgan failure. However, the model also has notable disadvantages, including the requirement for an abdominal incision and the technical expertise needed to cannulate the pancreatic duct [21,24,25].

Choline-deficient, ethionine-supplement diet-induced pancreatitis model

Choline is a component of the vitamin B complex and of cell membranes. In 1938, Griffith et al. [26] reported that a choline-deficient diet damaged the pancreas. Later, it was found that mice fed a choline-deficient diet enriched with ethionine (a derivative of methionine) developed severe necrotizing pancreatitis with a mortality rate of 100% within 5 days of treatment [27]. The choline-deficient, ethionine-supplement diet-induced pancreatitis shares many characteristics with severe human pancreatitis and has been used to test novel therapies. However, the diet is costly, requires on-site protocol standardization, careful monitoring of dietary intake [20].

Basic amino acid-induced AP model

L-Arginine is an essential amino acid first applied to induce severe necrotizing AP in rats through intraperitoneal injection of high concentration in 1984 by Mizunuma et al. [28]. It has shown that up to 100% of acinar cells underwent dose-dependent necrosis upon a single injection, with maximal damage after 72 hours. After this period, acinar cells will begin regenerating, and the pancreas will be fully recovered 2 weeks postinjection [29]. This is a highly reproducible model, and the severity of pancreatic necrosis can be controlled by regulating the dose and the timing of L-arginine injection [30]. Recently, further essential amino acids have been reported to effectively induce AP similarly to L-arginine, such as L-lysine and L-ornithine [31,32].

Pancreatic duct ligation model

This model is based on the theory that duct obstruction blocks pancreatic juice flow leading to AP, thus mimicking gallstone-induced AP. This technique has been applied to several animal species, including dogs, rabbits, opossums, rats and mice, and requires manual expertise and essential animal anatomy knowledge [30].

Alcohol-related AP model

A continuous intragastric infusion of ethanol over several weeks in rats produces ethanol-induced liver injury but not alcohol-induced pancreatitis [33], thus suggesting that ethanol may only increase the predisposition to acute and chronic pancreatitis (CP).

Coxsackie B virus-induced AP model

Various infectious agents have been associated with AP, including viruses, bacteria, fungi and parasites [34]. The most well-studied agent is the Coxsackie B virus infection with six identified serotypes as possible triggers of AP. However, only subtypes B1, B3, B4 and B5 produce severe pancreatitis when infecting mice [35].

Large animal and primate models for AP

Due to similar pancreatic anatomy and physiology in comparison to humans, pigs are a good model for AP that can be triggered by using several methods, such as closure of the duodenal loop, intraductal infusion of bile acids, and ischemia-reperfusion injury [36]. Dogs are another large animal model that has been extensively used to study AP, whose induction methods include ligation of the pancreatic duct, intraductal infusion of agents like bile acids or enzymes and diet-induced pancreatitis [37]. Nonhuman primates, like macaques, can spontaneously develop AP, especially in diabetic animals [38]. However, despite the resemblance of large animal and primate models to human AP, their use is limited by technical difficulty, costs and reproducibility for rodent models [30,37].

Chronic pancreatitis

Persistent or recurrent damage of the pancreas causes progressive tissue destruction leading to CP. This condition is characterized by infiltration of chronic inflammatory cells, formation of intraductal protein plugs, calcium salt deposition and interstitial fibrosis, resulting in loss of both endocrine and exocrine cell mass and parenchymal atrophy. Clinical manifestations include abdominal pain, weight loss and malabsorption syndrome due to maldigestion and diabetes [39]. Alcohol, smoking habits, obstructive lesions, other toxic agents and genetic factors are the leading causes of CP [39].

Several experimental CP models have been developed based on different pathogenetic mechanisms. Their common histopathological feature is pancreatic fibrosis, which is mediated by pancreatic stellate cells, a resident cell population that is usually quiescent and regulate extracellular matrix production but may be activated by inflammatory stimuli [40,41]. Because acute and recurrent AP in humans can progress to CP in a sort of biological continuum [42], preclinical models of CP are derived from minor modifications of those protocols for AP. The main models used are as follows:

Duct obstructive model

Prolonged pancreatic duct ligation has been found to induce pancreatic fibrosis, suggesting that pancreatic duct hypertension plays an important role in the initiation and development of CP [43]. However, this model is technically challenging.

Repeated cerulein-induced CP model

Repeated injections of cerulein-induced AP over several weeks cause chronic injury, leading to collagen deposits and fibrosis, thus mimicking recurrent episodes of AP that lead to CP in humans [43].

Alcohol-induced CP model

Alcohol is one of the major etiologic factors of CP. However, it has been demonstrated that alcohol alone does not induce CP [44], suggesting that it may predispose the gland to develop chronic damage in combination with genetic, environmental or dietary factors, so other factors have been applied together with ethanol to develop CP, such as cerulein or lipopolysaccharide (LPS) injection [40,45].

Toxic chemical-induced CP

Intravenous injection of toxic substances such as dibutyltin dichloride, an industrial chemical, in mice, has been shown to cause AP in an early phase, followed by the development of fibrosis in a late phase due to its direct toxic effect on the pancreas and the bile duct epithelium that induces duct stenosis [46].

L-Arginine-induced CP model

Long-term intraperitoneal administration of L-arginine in rats for 4 weeks causes progressive degeneration of the pancreas with accumulation of the extracellular matrix surrounding acinar units, blood

vessels and islets, and gradual replacement of pancreatic tissue by adipose tissue [47].

Large animal and primate models for CP

Several animal models have been developed to study CP, including large animals like dogs, pigs and nonhuman primates. Dogs can develop spontaneous CP, making them a valuable tool for studying disease pathophysiology and potential treatments [30,48]. Swine models share similar digestive systems and pancreatic functions with humans, thus allowing researchers to study new treatment responses that are more comparable to those of humans [30,49]. Finally, nonhuman primates may be relevant because of their genetic and anatomical similarities to humans, however, ethical considerations and costs limit their widespread use [50,51].

Autoimmune pancreatitis (AIP)

A peculiar form of CP is AIP, which is characterized by fibro-inflammatory degeneration [52]. Two subtypes can be identified based on its histological features: type 1 and type 2. Type 1 AIP (lymphoplasmacytic sclerosing pancreatitis) displays dense lymphoplasmacytic infiltration, storiform fibrosis, obliterative phlebitis and immunoglobulin (Ig)G4$^+$ plasma cells at immunochemistry. Type 2 AIP (idiopathic duct–centric pancreatitis) is less common and is characterized by a typical neutrophil-rich infiltrate that gives rise to the so-called granulocytic epithelial lesion [53]. Despite the similar clinical and imaging presentations of both conditions, only type 1 AIP is a pancreatic manifestation of the IgG4-related disease (IgG4-RD), with the possible involvement of multiple organs such as the biliary tree, kidneys, lymph nodes, lungs and salivary glands [54].

MRL/Mp mice treated with poly(I:C)

The immune pathogenesis of IgG4-RD still needs to be better understood. Initial studies focused on adaptive immune responses, suggesting an antigen-triggered immune response that eventually drives B lymphocytes to IgG4 production and T lymphocytes to secrete profibrotic cytokines. A subset of T helper-type 2/regulatory T cells has been proposed to favor collagen deposition by producing profibrotic cytokines [55–58]. By contrast, recent evidence based on the results obtained by Murphy Roths Large (MRL/Mp) mice, although lacking the IgG4 subtype, suggests that the innate response plays a pivotal role [59]. Briefly, MRL/Mp mice are known to have an autoimmune disease-prone genetic background that leads to spontaneous development of AIP, with an incidence of 74% in 34–38 week-old females and a lower incidence of about 40% in males at a later age, around 45–50 week-old [60]. When these mice undergo repeated intra-peritoneal injections of polyinosinic:polycytidylic acid [poly(I:C)], pancreatic damage resembling AIP develops. Poly(I:C) acts as a Toll-like receptor three ligand that can induce systemic type 1 interferon responses, so repeated injections result in the destruction of pancreatic acinar architecture, massive immune cell infiltration, and fibrosis, similar to human AIP histopathological findings [59]. Moreover, this experimental model frequently observes the elevation of serum levels of autoantibodies against pancreatic secretory trypsin inhibitor, carbonic anhydrase-II and lactoferrin, like human IgG4-RD [61–63]. Profound immunological studies of this model showed that the pancreatic chronic fibro-inflammatory response depends on a large amount of interferon-α and interleukin (IL)-33 produced by activated plasmacytoid dendritic cells. Remarkably, these cells are also found in IgG4-RD patients and have been showed to promote IgG4 production by B cells [64,65]. These findings suggest that the MRL/Mp mice treated with poly(I:C) represent a valuable model for testing new therapeutic tools for this condition, whose present treatment relies only on systemic steroids.

Transgenic and knockout animal models of pancreatitis

Hereditary pancreatitis depends on gain-of-function missense mutations of trypsinogen (PRSS1) [66], loss-of-function mutations of the serine protease inhibitor Kazal type (SPINK) type 1 [67] or chymotrypsin-C (CTRC) genes [68] that are associated with regulation of intrapancreatic trypsin activity. Another inherited cause of pancreatitis is cystic fibrosis due to the presence of mutations in the transmembrane conductance regulator (CFTR) gene that plays a central role in the pancreatic ductal secretory function by transporting $Cl^-$and $HCO3^-$ions across the apical membrane [69]. Studies have been carried out to identify the underlying disease mechanisms depending on these genetic mutations. Remarkably, no spontaneous pancreatitis developed in transgenic animals expressing human PRSS1 in either wild-type form or with a p.R122H mutation, while they created a severe form of pancreatitis following external stimuli [70], thus explaining the absence of pancreatitis in many human mutation carriers. A genetic model of CP (X-SPINK1 mice) was used to investigate the role of the regenerative gene (Reg) family proteins, recently implicated in the repair and regeneration of inflamed pancreatic tissue, by knocking out Reg1-3 genes [71]. The authors found a reduction of pancreatic parenchymal loss and a decrease in both proinflammatory cytokine levels and collagen deposition, thus suggesting that blocking Reg1-3 can slow the progression of CP. As far as the CFTR gene in animal models is concerned, Geisz et al. [72] were able to generate a CTRC$^+$ mouse in which the severity of cerulein-induced acute and CP was significantly improved, thus providing evidence for a protective role of CTRC in pancreatitis.

Confirmation of the role of intracellular activation of trypsinogen in triggering local and systemic inflammation in AP comes from a study where wild-type and cathepsin B knockout mice underwent induction of experimental pancreatitis in which activation of trypsinogen arose not only from pancreatic acinar cells but also from infiltrating macrophages that ingest zymogen-containing vesicles from damaged acinar cells [73]. Intra-macrophage activation of proteases was found to depend on pH and cathepsin B and to be associated with NF-κB translocation and the release of high concentrations of pro-inflammatory cytokines, which, in turn, contribute to systemic inflammation and the severity of pancreatitis.

In this regard, the prominent role of the NF-κB pathway was highlighted by the finding of a significant attenuation of inflammation, necrosis and severity of pancreatitis in a mouse model (referred to as PKD3Δpanc mice) carrying pancreas-specific deletion of protein kinase (PK)D3, whose activation is required for key pathological features of AP, including acinar cell necrosis and zymogen activation [74].

Another critical mechanism for triggering AP is a sustained calcium influx in the cytosol and mitochondria of pancreatic acinar cells. Since the mitochondrial calcium uniporter (MCU) mediates mitochondrial Ca^{++} uptake that regulates cell bioenergetics, an MCU knockout mouse was applied in three different experimental models of AP (induced by caerulein, taurolithocholic acid 3-sulfate or palmitoleic acid plus ethanol) [75]. Surprisingly, deletion of the MCU gene failed to reduce the severity of the local and systemic injury, probably because of the redundancy of damaging mechanisms [75]. By contrast, stimulation of the mechanically activated, calcium-permeable ion channel Piezo1 in the pancreatic acinar cells caused sustained increase of intracellular calcium through the transient receptor potential vanilloid subfamily 4 channel opening, thus triggering mitochondrial depolarization, intracellular trypsin activation and cell death that in turn lead to pressure-induced pancreatitis; as proof of this, transient receptor potential vanilloid subfamily 4 gene–knockout mice were protected from Piezo1 agonist–and pressure-induced pancreatitis [76]. Another pathway leading to increased intracellular calcium levels in pancreatic acinar cells is activating cholinergic receptor muscarinic 3 (M3R), a G protein-coupled receptor. To test

the role of the M3R activation in pancreatitis, a transgenic mouse was developed that carried mutant M3R on pancreatic acinar cells that lacked responsiveness to its native ligand, but was activated by an inert small molecule, clozapine-N-oxide (CNO) [77]. Compared with cerulein-induced AP, a single injection of CNO caused more widespread acinar cell death and inflammation by elevating intracellular calcium and amylase. Furthermore, CP developed at 4 weeks after only three injections of CNO.

Another crucial factor in preventing pancreatitis relies on the homeostasis of the autophagy/lysosomal pathway in secretory pancreatic acinar cells. Indeed, spontaneous pancreatitis develops not only in mice with pancreas-specific deletion of autophagy-related genes [atg5 [78], atg7 [79], atg8 [80]], and double knockout of genes (tfeb and tfe3) encoding for the transcription factor EB, a master regulator of lysosomal biogenesis [81], but also in lamp2 knockout mice [82] that encodes key enzyme controlling the delivery of acid hydrolases to the lysosome.

Even the maintenance of the endoplasmic reticulum (ER) proteostasis in pancreatic acinar cells plays a pivotal function in preventing pancreatitis. In an original study, Cooley and coworkers investigated the role of the ER acetyl-CoA transporter AT-1 in an AT-1 knockout mouse model. They showed that chronic ER stress causes spontaneous mild/moderate CP characterized by accumulation of intracellular trypsin, immune cell infiltration and fibrosis [83]. Similarly, genetic blocking of the lipocalin 2 gene, involved in iron-induced oxidative damage through ferroptosis, causes the worsening of L-arginine-induced pancreatitis [84].

At variance with previous studies highlighting dangerous pathways, the development of a pancreas-specific, conditional miR-29a/b1−knockout mouse model discovered a protective role of this microRNA in caerulein-induced AP [85] since its loss results in TGFβ1-mediated pancreatic stellate cell activation that, in turn, causes extracellular matrix deposition and disease progression. All these animal models helped us understand the complex pathogenesis of AP and its progression toward the chronic form while providing powerful tools for developing and testing novel therapeutic tools for human pancreatitis.

Type 1 Diabetes (T1D)

Diabetes mellitus, a multifaceted metabolic disorder, has become prevalent in the past decades [86]. Among the many forms of diabetes, T1D is a chronic autoimmune disorder in which the immune cells attack and destroy insulin-secreting pancreatic β cells which leads to an absolute insulin deficiency and hyperglycemia. Several rodent models have been developed to mimic certain features of T1D. This includes the spontaneous nonobese diabetic (NOD) mice, the BioBreeding rats, the humanized mouse models, transgenic mouse models, chemical-induced mouse models and others. Each model described below has its unique characteristics, which allow researchers to study the autoimmune responses, pancreatic β cell function, and other features of T1D. In this section, we only focus on NOD mice and models of chemically or surgically-induced T1D mice as they are used extensively in the literature. Humanized T1D mice will be discussed separately in Section IV.

The NOD mice

Since becoming available, the NOD mouse has been the most widely used model to assess the many facets of human T1D and has been the preferred model for invasive, preclinical/translational studies as they share several genetic and immunologic traits with the human form of the disease [87]. It is an essential component of a comprehensive approach to understanding T1D. The NOD mice are genetically predisposed to T1D [88–93], and like humans, develop the disease spontaneously. T1D occurs when the insulin-secreting pancreatic islet β-cells are destroyed by the immune cells [89,94]. A progressive loss of cell function is present in humans and NOD mice. These similarities with human autoimmune diabetes have led to the use of NOD mice as the mainstay of preclinical diabetes research [95,96]. The timeline of diabetes development starts at 5 weeks of age, and by 30 weeks of age, most females will develop diabetes. The pathogenesis of the disease may be classified into three major stages. The insulitis stage is characterized by an influx of inflammatory immune cells into the islet that reaches a significant level around 8 weeks of age [97]. During this stage, the blood glucose level remains normal (below 140 mg/dL) but the mice may test positive for insulin autoantibodies (such as IAA) [98]. The prediabetic stage manifests as a slow progressive loss of β cell mass that leads to a sudden rise in blood glucose levels above physiologic concentrations (160–250 mg/dL). The diabetic stage begins around 14 weeks of age with blood glucose equal to or higher than 300 mg/dL and will eventually lead to clinical diabetes. Spontaneous diabetes in the NOD mouse is 60–80% in females and 40–50% in males by 30 weeks of age. The onset of diabetes also varies between males and females: commonly, onset is delayed in males by several weeks.

Like human T1D, NOD mice develop spontaneous T1D with genetic and environmental components relevant to human disease. Polymorphisms in a major histocompatibility complex class (MHC) II molecule usually confer the most disease risk in both species. Disease is caused by perturbations by the same gene or different genes in the same biological pathways. Diabetes onset is preceded by circulating autoreactive T cells and autoantibodies that recognize many of the same islet antigens [99]. A recent study demonstrated that modifying single base pairs of NOD mice can generate loci that contain alleles that are orthologous to humans [100]. Therefore, the NOD mice are a powerful tool and valuable implement for T1D research.

One dissimilarity of diabetes between the NOD mice and the human T1D is the appearance of insulitis. The human insulitis is less severe and less frequent than what can be observed in NOD mice [101]. This may result from the fact that the autoimmunity in parental mice is very aggressive, and disease onset occurs over an abbreviated timeline (weeks) compared to the decidedly more attenuated onset in humans (years after the appearance of the autoantibodies).

While the NOD mice have proved helpful in many preclinical research studies, significant tension has arisen in attempts to translate therapies developed in the NOD mice to humans as less than 50% of animal studies accurately predict human outcome [99]. In the NOD mice, investigators apply three approaches: early prevention (treatment at 3–4 weeks of age), late prevention (treatment at 10–12 weeks of age) or intervention after the onset of T1D (reversal). Most preclinical success in NOD has come in early prevention. Later prevention represents a modality-like trial established in humans where autoantibody-positive individuals are identified and enrolled [102–104]. Few therapies have resulted in T1D reversal in new-onset NOD mice and fewer in NOD mice with established disease. For example, anti-CD3 therapy and a combination of antithymocyte globulin and granulocyte-colony stimulating factor (G-CSF) have been used in clinical trials with similar efficacy as in NOD mice. Like other models in rodent species, such as the BioBreeding rat, this model fails to translate to humans in many aspects [105].

Chemically induced T1D mouse model

Chemical induction of T1D by destroying endogenous beta cells using chemicals such as streptozotocin (STZ) or alloxan are also commonly used in T1D research. STZ is a potential alkylating agent that selectively destroys pancreatic beta cells by interfering with glucose transport and glucokinase function and by DNA strand damage [106]. As a result of this action, the animals experience insulin deficiency, hyperglycemia, polydipsia and polyuria, all of which are characteristic of human T1D mellitus [107]. STZ can be used to induce diabetes

in mice, rats and monkeys. Glut2 transports, such as Alloxan, can causes β cell death by forming reactive oxidative species and producing hydrogen peroxide and hydroxyl radicals. The oxidation and inhibition of glucokinase impedes insulin secretion [108]. These mice often have little endogenous insulin production after treatment, leading to hyperglycemia and weight loss [109]. In general, hyperglycemia can occur at around 5–7 days post-treatment.

Chemically induced diabetic mice may experience insulin deficiency, hyperglycemia, polydipsia and polyuria, all of which are characteristics of human T1D mellitus [108]. Mice are commonly used in testing drugs or therapies where the major mechanism of action is lowering blood glucose in a non-β cell-dependent fashion [38], such as to evaluate new insulin formulations. Chemically induced diabetic mice are also often utilized in islet/beta cell transplantation studies, in which islet grafts can be removed to see if the glucose-lowering effects are from the endogenous cells or from transplanted grafts.

STZ can be given at a single high dose or at multiple low doses. High doses (100–200 mg/kg) cause quick destruction of β cells and hyperglycemia. Multiple low doses can be given at multiple times over 4–5 days to induce insulitis in mice [110,111]. This can cause a reduction in islet numbers in addition to macrophage infiltration. Unlike human T1D situations, diabetes can develop without T cells and B cells in the STZ-treated mice. Therefore, this model does not mimic human autoimmunity, although it is frequently used in islet transplantation.

Another disadvantage of the chemically induced diabetes model is that the chemicals can be toxic to other organs of the body. For example, STZ can cause damage to the liver, kidney, lung, intestine and other tissues. Adverse reactions following STZ use included weight loss, respiratory failure, rapid and sudden changes in glycemia leading to life-threatening hypoglycemia and generalized poor condition of the animals. In addition, cells may recover after treatment. Therefore, proper controls should be used to exclude this possibility. Lastly, injection of a dose of STZ that is too low does not completely damage the β cells of the pancreatic islets [112]. Nevertheless, STZ-treatment rodent models represent a simple and inexpensive model for T1D-related research.

Large animal models for diabetes studies

Large animal models for diabetes studies include chemical induced dogs, pigs and nonhuman primates, surgically induced large models of diabetes and virus-induced diabetic monkeys as described in detail below.

Chemical-induced diabetic dogs, pigs and nonhuman primates

These chemically induced models share numerous pathophysiological and pathological features with humans and can be used to study T1D. They mimic symptoms and pathophysiology like those observed in humans. However, these models are expensive, and it can be challenging to produce irreversible diabetes. Additionally, dogs have loose skin, which may impact the application of certain experimental procedures [113,114]. Anesthesia administration requires a skilled veterinarian, and achieving diabetic conditions often necessitates higher doses of the inducing agents. Furthermore, it can be difficult to produce irreversible diabetes in large animals. They may develop moderate hyperglycemia without significant changes in body weight or blood insulin levels [109,113,114].

Surgically induced large models of diabetes

Pancreatectomy or thymectomy in dogs effectively induces hyperglycemia and leverages the advantages of dogs' large size and longer lifespan. However, they involve invasive procedures and have a higher risk of hypoglycemia. The models also come with high costs and demand advanced technical skills, which can be challenging [109,114]. Surgical primate models also offer the benefit of longer lifespans and closely mimic human physiology. Islet/β cell transplantation in primates is a valuable model for translating research into clinical applications. This is due to their evolutionary proximity to humans, with approximately 95% nucleotide homology, which enhances the relevance and safety of translating findings into tangible clinical outcomes. However, their use in preclinical studies is constrained by ethical considerations, limited availability, high cost and the requirement of high levels of technical expertise [114,115]. A recent study developed a diabetes model in marmosets using a combination of partial pancreatectomy and intravenous STZ injection. The marmosets manifested a hyperglycemic state with impaired glucose tolerance that was persistent and irreversible for over 3 months. The condition was effectively managed with daily insulin administration [116].

Virus-induced diabetic monkeys

Diabetes can be induced in monkeys using viruses, such as encephalomyocarditis, Coxsackie virus and others [117]. Specifically, Coxsackie B virus, can reside in these cells before the onset of infection. Despite its effectiveness in modeling diabetes, the precise mechanism by which the virus triggers the disease is not fully understood. Additionally, this approach lacks specificity and may inadvertently affect other organs [114,117].

Type 3c Diabetes

T3cD, or pancreatogenic diabetes, develops secondary to pancreatic diseases, most notably CP, cystic fibrosis, hemochromatosis or pancreatic cancer. CP alone accounts for 70–90% of T3cD cases, with other causes including pancreatic surgery and cancer. Although T3cD may affect 5–10% of diabetes cases in Western populations, it is frequently underdiagnosed and often misclassified as type 1 or type 2 diabetes, especially in regions with high pancreatitis prevalence, such as Southeast Asia [118]. Distinct from other diabetes types, T3cD is marked by a unique interplay of endocrine and exocrine dysfunctions, including insulin deficiency, hepatic insulin resistance and impaired glucagon and incretin responses, which lead to severe glycemic variability or "brittle diabetes." These issues stem from progressive inflammation and β cell loss in the pancreas, alongside pancreatic polypeptide and incretin deficiencies. Exocrine pancreatic insufficiency is also common, causing fat malabsorption and vitamin D deficiency, further complicating metabolic management and adding challenges to glycemic control [119,120]. One of the challenges of T3c diabetes research is that while insulin secretion is relatively straightforward to study in animal models, exocrine pancreatic function (involving enzyme secretion for digestion) is more complex to measure and model. Some animal models focus on the broader impact of exocrine insufficiency in T3c diabetes.

Although studies rarely focus specifically on T3cD, some animal models show promise for advancing research in this area. Genetically modified rodents commonly used in T1D research, such as mice and rats with acute or chronic pancreatic damage induced by chemical agents or surgical techniques, can probably be adapted to investigate T3cD. For example, rat models of AP typically involve inducing pancreatitis via injections of chemicals, like cerulein, or by surgical methods. This resulting damage to pancreas tissue mimics the destruction observed in human pancreatitis, and the development of insulin insufficiency and diabetes-like symptoms can be observed. In the CP models, pancreatitis is induced and allowed to progress over weeks to months, closely mimicking the chronic form of the disease that leads to T3cD.

Pancreatitis insult models such as the STZ-induced mouse model have been used to study both the endocrine and exocrine of the pancreas. This can stimulate conditions where the pancreas is unable to produce insulin due to extensive damage, as seen in T3cD. In addition, the partial pancreatectomy model that removes part of the

pancreas can stimulate loss of pancreatic function seen in T3cD. The remaining pancreatic tissues is typically unable to compensate fully for insulin production, leading to the development of diabetes.

These models help illuminate the disease's underlying mechanisms and provide platforms for testing novel therapies, including CGT therapies. For instance, a 2009 study produced transgenic cloned pigs carrying a mutated human hepatocyte nuclear factor 1-alpha gene, associated with T3cD of the young, using intracytoplasmic sperm injection and somatic cell nuclear transfer. While most of the 22 cloned piglets did not survive past weaning, four lived between 20 and 196 days and exhibited elevated blood glucose levels, indicative of diabetes-like symptoms [121].

CGT therapy presents promising solutions for both endocrine and exocrine dysfunctions characteristic of T3cDM. CGTs targeting β cell regeneration could restore insulin production, while gene therapies might alleviate exocrine insufficiency and reduce malnutrition-related complications. Immunomodulatory CGTs could also address pancreatic inflammation, preserving residual β cell function and enhancing metabolic regulation. In this complex disease context, CGT approaches offer targeted strategies to improve glucose control, manage pancreatic insufficiency and ultimately improve patient outcomes [120].

Section III: Animal Models for Liver Diseases

The lack of solid therapeutic benefits determined so far by cell transplantation-based liver regenerative medicine is mainly due to the lack of preclinical scientific evidence concerning the therapeutic targets of such therapies. The main approaches are replacing the tissue by using epithelial cell sources or modulation of tissue repair by using mesenchymal-derived cells capable of modifying the microenvironment [122]. Data on each liver disease and the assessment of the liver tissue before and after treatment are expected in preclinical and clinical settings to characterize regenerative pathways and microenvironment changes responsible for the therapeutic benefits [122]. In this context, accurate investigation in animal models is essential for understanding human liver pathogenesis and identifying therapeutic targets. Several animal models have been developed to study different liver conditions. Rodents represent the preclinical model mostly used to provide informative data for hepatic, metabolic and immunological liver functions.

Recently, animal models of liver injury have been frequently used to examine specific gene functions, pathological conditions and the therapeutic effects of drugs and cells [123]. Rodents such as mice and rats are mainly used, but fish such as zebrafish and medaka, and large animals such as pigs and monkeys are also utilized as needed [124–127]. Animal models have also been used to examine the dynamics of hepatocytes, mesenchymal stem cells (MSCs) and extracellular vesicles and their therapeutic effects. In this section, we describe animal models of chronic and acute liver damage that can be used as targets when considering CGT therapy as a treatment modality (Figure 1).

Acute Liver Failure (ALF)

ALF is a very rare heterogeneous liver disease characterized by a worse prognosis and is the key clinical indication for urgent liver transplantation. In this condition, the acute massive hepatocyte injury has been treated by hepatocyte transplantation (HT) to replace and support liver function until the host liver recovers, driven by self-regeneration [128,129]. A total of 41 subjects have been treated with human hepatocytes and 8 with unsorted fetal hepatocytes [129,130]. The safety and efficacy of HT have not been proven yet.

An accurate model of ALF should reflect and mimic the etiology and leading features of the human counterpart. ALF is mainly based on acute liver injury caused by several different etiologies and with defined pathobiology features: acute hepatic necrosis (acetaminophen/APAP, ischemia) and acute hepatitis (viruses, idiosyncratic DILI, autoimmune hepatitis). The animal models resembling human ALF are reported in Table 4.

ALF due to acute hepatocellular toxicity

The administration of APAP to mice is the most common ALF model since the APAP doses that cause toxicity are similar in mice and humans (≥150 mg/kg) [131,134]. The overdose of APAP (>150 mg/kg) in rodents induces hepatocyte necrosis/apoptosis through the generation of a toxic metabolite (*N*-acetyl-p-benzoquinone imine), mitochondrial dysfunction and alteration of innate immunity [131,135]. Injury type varies based on the amount of APAP administered. APAP 750 mg/kg by i.p. injection induces ALF, while 600 mg/kg of APAP induces acute intoxication.

The most common animal model of direct hepatotoxicity mimicking DILI is based on administering high hepatotoxic doses of carbon tetrachloride (CCl_4) to rodents. The single intraperitoneal injection of CCl_4 (1 or 1.5 mL/kg, corresponding to 1.58 and 2.38 g/kg) causes acute hepatotoxicity by oxidative stress and mitochondrial dysfunction. Overall, the liver rescue treatment of APAP and CCl_4 ALF models is monitored by histology examinations to assess necrotic/apoptotic cells and proliferative cells. Serum tests are aimed at evaluating plasma levels of ALT, AST, bilirubin, coagulation and pro-inflammatory cytokines, such as IL-6 and TNF-α [132].

Idiosyncratic drug-induced liver injury

Idiosyncratic drug-induced liver injury consists of liver damage associated with the adverse effects of drugs, which can be exploited to reproduce an in vivo model of ALF. Idiosyncratic DILI is reported to be unpredictable and the related liver damage is caused by a drug mechanism of action that is nondose dependent [136]. While the exact mechanisms of hepatotoxicity remain uncertain, strategies have been developed targeting specific endpoints, such as immune system involvement and oxidative stress [137].

Immune-mediated liver injury

The inflammatory liver microenvironment plays a crucial role in the development and establishment of acute liver injury. The inflammatory-mediated liver injury is focused on the apoptosis triggered by TNF-α signaling. Animal models of inflammatory-mediated liver injury are based on the administration of D-galactosamine/endotoxin (Gal/ET) [138]. The treatment with D-galactosamine and LPS in rodents can induce the production of reactive oxygen species and TNF-α, resulting in liver cell apoptosis [138,139]. The liver rescue treatment is evaluated through histological and functional features using liver biopsies and a measure of serum components, respectively. Serum alanine aminotransferase, aspartate aminotransferase and bilirubin are detected. Histologically, NLRP3 and caspase-1 staining were investigated in liver biopsies.

Chronic Liver Damage Model

Most chronic liver disease models are nonalcoholic steatohepatitis (NASH)/nonalcoholic fatty liver disease (NAFLD) or alcoholic liver disease models (Table 5A) [140]; in most cases, mice and rats have been used. The NASH/NAFLD model can be divided into two main types, depending on the nature of the diet. One model excludes certain nutrients, such as a methionine- and choline-deficient diet. Deficiencies of methionine, an essential amino acid that cannot be synthesized de novo, and choline, a precursor of acetylcholine, a component of cell and mitochondrial membranes and a known neurotransmitter, inhibit phosphatidylcholine synthesis, resulting in diminished very low-density lipoprotein assembly and secretion. The fact that this process differs from NASH/NAFLD, which is usually seen in humans

Table 4
Animal models resembling human acute liver failure.

Related human disorder	Animal model	Injury type	Animal phenotype	Serum alterations	Histological examinations	References
ALF due to hepatotoxicity	BALB/c mice	APAP 750 mg/kg by i.p. injection and Intoxication with 600 mg/kg of APAP	Liver weight loss	ALT, TBIL, MDA, SOD, AST, GSH-PX, GSH, T-AOC Inflammatory cytokines and factors: IL-1β, IL-6, TNF-α, HGF	H&E staining, TUNEL staining, PCNA+ cells	[131]
DILI	Swiss CD1 mice	Single intraperitoneal injection of CCl4 (1 or 1.5 mL/kg)	n/a	ALT, AST, IL-6, TNF-α, GSH	H&E staining, Hepatocytes morphology (TEM), IHC for specific markers (MDA, TBARs)	[132]
Immune-mediated liver injury	C57Bl/6 mice	Single injection of LPS (5 mg/kg) and D-GalN (150 mg/kg) i.p.	n/a	ALT, AST, bilirubin	H&E and IHC for NLRP3 and caspase-1	[133]

ALT, alanine aminotransferase; AST, aspartate aminotransferase; TP, total protein; DBIL, ALB, direct bilirubin; LD, lactic dehydrogenase; GGT, gamma-glutamyl transpeptidase; ALP, alkaline phosphatase; TBA, total bile acid; A/G, albumin/globulin ration; PA, prealbumin; 4-OH-DEB, 4-hydroxydebrisoquine; n/a, not available.

as a part of the metabolic syndrome, is important to evaluate using this model.

Models using high-fat diets are commonly used to induce obesity and fatty liver, especially in mice and rats. In addition, the characteristics of this diet make it difficult to conduct experiments as it takes a long time for NASH-like or hepatocellular carcinoma (HCC) to develop. Notably, each mouse and rat strain react differently to high-fat diet. However, rats and males are more strongly affected. Melanocortin 4 receptor-knockout mouse models do not lose their appetite. Carbon tetrachloride (CCl_4) and thioacetamide are regularly administered in models of chronic hepatocellular damage, and common bile duct ligation and administration of 3,5-diethoxycarbonyl-1,4-dihydrocollidine are performed to induce bile duct damage. The Stelic Animal Model mice, in which pancreatic β cells are damaged by streptozotocin in the fetal stage, induce the early onset of diabetes, NASH and carcinogenesis. Additionally, diethyl nitrosamine has been administered to a mouse model to induce carcinogenesis [175]. Therefore, selecting these mice according to their target pathology is necessary.

Alcohol-related liver disease models

Several alcoholic liver disease models have been developed, although none of these models closely resemble the human condition. However, improvements are being made to better understand the pathophysiology of alcohol-induced diseases. Currently, it is thought that it is challenging to create a completely human-like condition in animal models for several reasons, including high basal metabolism, natural aversion to alcohol, spontaneous cessation of drinking when blood alcohol concentration increases, short-term modeling as opposed to long-term development in humans, and differences in the innate immune system. To overcome these problems, the Lieber-DeCarli liquid diet, NIAAA and Tsukamoto-French models have been developed [123].

Animal models for alpha-1 antitrypsin (AAT) deficiency

Alpha-1 antitrypsin deficiency (AATD) is the leading genetic cause of liver disease among children and a common condition in older adults. In AATD, protein misfolding, particularly with the Glu342Lys mutation that creates the "Z" (ATZ) protein, leads to the accumulation of insoluble misfolded mutant proteins that can cause inflammation, fibrosis, cirrhosis and increased risk of HCC, particularly when polymerized to other misfolded Z variants. The only treatment option for AATD is liver transplantation [176], although RNA inhibition therapy is in human FDA phase 3 clinical trials [177]. Several small and large animal models for the study liver disease in AATD exist. Each model has its own strengths and limitations depending on the specific aspects of AATD being studied.

The PiZ transgenic mice. The PiZ mice express a human AAT transgene that contains the AATD-associated Glu342Lys mutation while being deficient in native AAT production and display a range of AATD-related symptoms, such as the accumulation of AAT protein aggregates, elevated hepatocyte death and the development of liver fibrosis [178]. So far, the PiZ mouse model is the most extensively studied model for liver disease associated with AATD and has been used to test the therapeutic effects of new treatments including using CGT products [179–186].

The pallid mice. The pallid mice have genetic errors that lead to the abnormal production of AAT and spontaneously occurring emphysema. These mice have markedly lower levels of serum AAT, which is associated with a severe deficiency in serum elastase inhibitory capacity, but they have normal AAT mRNA in the liver and, therefore, are mostly used in lung disease research [187].

Table 5
Commonly used animal models of chronic liver diseases (A), acute liver diseases (B) and acute-on-chronic liver disease (C).

Liver disease	Experimental model	Administration route	Species	Induction time	Patterns of liver injury	References
A: Chronic liver disease						
NASH/NAFLD	Methionine- and choline-deficient diet	Oral	Rat or mouse	4–10 weeks	Steatosis, inflammation, fibrosis	[141]
	High-fat diet (HFD)	Oral	Rat or mouse	16 weeks/1 year	High inter-individual variability in steatosis, inflammation and fibrosis	[123]
	Choline-deficient L-amino-defined diet	Oral	Rat or mouse	12–84 weeks	Steatosis, inflammation, fibrosis	[142]
	Melanocortin 4 receptor-deficient mouse, HFD	Oral	Mouse	8 weeks to 1 year	Steatosis, inflammation, fibrosis	[143]
	HFD+ choline deficiency	Oral	Rat or mouse	24 weeks	Steatosis, inflammation, fibrosis	[144]
	HFD+ fructose	Oral	Mouse	16–30 weeks	Steatosis, inflammation, fibrosis	[145]
	HFD+ cholesterol	Oral	Mouse	16–20 weeks	Steatosis, inflammation, fibrosis	[146]
	HFD+ cholesterol+ fructose	Oral	Mouse	12–24 weeks	Steatosis, inflammation, fibrosis	[147]
	STAM mouse	-	Mouse	8–12 weeks	Steatosis, inflammation, fibrosis	[148]
Alcohol liver disease	Lieber-DeCarli liquid diet	Oral, chronic	Rat or mouse	4–12 weeks	Hepatocellular	[140]
	Lieber-DeCarli liquid diet	Oral, chronic & single/multiple	Rat or mouse	4–6 weeks	Hepatocellular	[140]
	Lieber-DeCarli liquid diet	Oral, chronic, DEN/LPS/CCl4/APAP	Rat or mouse	4–12 weeks	Hepatocellular	[140]
	Ethanol ad libitum feeding	Oral	Mouse	10 days/1–2 weeks	Hepatocellular	[140]
	The Tsukamoto-French model	Intragastric infusion	Rat or mouse	2–3 months	Hepatocellular	[140]
	The NIAAA model	LDE, single ethanol bridge	Mouse	Not described	Hepatocellular	[140]
	The NIAAA model	LDE, 3 ethanol bridges	Rat or mouse	Not described	Hepatocellular	[140]
	Ethanol,CCl4 treatment	Oral, i.p.	Mouse	8 weeks	Hepatocellular	[140]
Others	Carbon tetrachloride (CCl4)	Inhalation, i.p., oral (gavage)	Rat or mouse	Inhalation: 13–16 weeks, i.p., oral: 6–12 weeks	Steatosis, inflammation, fibrosis	[149]
	Common bile duct ligation (cBDL)	-	Rat or mouse	3–4 weeks	Steatosis, inflammation, fibrosis	[150]
	3,5-diethoxycarbonyl-1,4-dihydrocollidine (DDC)	Oral	Mouse	4–8 weeks	Inflammation, fibrosis	[151]
	Thioacetamide (TAA)	In drinking water or i.p.	Rat or mouse	10–12 weeks	Steatosis, inflammation, fibrosis	[152]
	Dimethylnitrosamine (DMN)	Oral (gavage) or i.p.	Rat or mouse	4 weeks	Inflammation, fibrosis	[153]
	Mdr2$^{-/-}$ mouse	-	Mouse	12–24 weeks	Inflammation, fibrosis	[154]
B: Acute liver disease	CCl4	i.p.	Mouse	2–48 hours	Inflammation, necrosis	[155]
	Common bile duct ligation	-	Rat	8 days	Inflammation, fibrosis (BDL for 22 days)	[156]
	90% partial hepatectomy	-	Rat	6 hours to 10 days	Steatosis, necrosis, congestion	[157]
C: Acute-on-chronic liver failure	has (subctaneal and i.v.) & LPS/D-GalN	i.p.	Rat	5 days to 10 weeks	Hepatocellular	[158]
	CCl4 (in oil i.p.) & Not described	Not described	Rabbit	10–11 weeks	Hepatocellular	[159]
	HAS (subctaneal and i.v.) & LPS/D-GalN	i.p.	Rat	5 days to 10 weeks	Hepatocellular	[160]
	CCl4 (in oil i.p.) & LPS/D-GalN	i.p.	Rat	10–11 weeks	Hepatocellular	[161]
	BD ligature & 70% liver reduction	Liver reduction	Rat	2 weeks	Mixed	[162]
	CCl4 (in oil i.p.) & LPS/D-GalN	i.p.	Rat	8–9 weeks	Hepatocellular	[163]
	BD ligature & Not described	Not described	Rat	2–10 weeks	Mixed	[164]
	PS (i.p.) & LPS/D-GalN	i.p.	Rat	11–12 weeks	Hepatocellular	[36]
	CCL4 or TAA or BD ligature & LPS	i.p.	Rat	4–16 weeks	Mixed	[165]
	CCl4 (in olive oil i.p.) & Ethyl alcohol	oral	Rat	6–9 weeks	Hepatocellular	[166]
	CCl4 (in oil i.p.) & LPS/D-GalN	i.p.	Rat	10–11 weeks	Hepatocellular	[167]
	CCl4 (in oil i.p.) & LPS/D-GalN	i.p.	Mouse	6–7 weeks	Hepatocellular	[168]
	CCl4 (in oil i.p.) & *Klebsiella pneumoniae*	i.p.	Rat	8–16 weeks	Hepatocellular	[169]
	BD ligation & LPS	i.p.	Rat	1–7 weeks	Mixed	[170]
	CCl4 (in oil i.p.) & APAP/LPS	i.p.	Mouse	10–11 weeks	Hepatocellular	[171]
	CCl4 (in oil i.p.) & LPS/D-GalN	i.p.	Rat	8–9 weeks	Hepatocellular	[163]
	PS (i.p.) & LPS/D-GalN	i.p.	Rat	12–13 weeks	Hepatocellular	[172]
	PS (i.p.) & LPS/D-GalN	i.p.	Rat	11–12 weeks	Hepatocellular	[173]
	HSA (subctaneal and i.v.) & LPS/D-GalN	i.p.	Rat	6–7 weeks	Hepatocellular	[174]

ALB, albumin; ALP, alkaline phosphatase; ALT, alanine aminotransferase; ANOVA, analysis of variance; αSMA, α-smooth muscle actin, AST, aspartate aminotransferase; BMDMs, bone marrow-derived macrophages; BW, body weight; C-P, Child-Pugh; CAGE, cap analysis of gene expression; CAID, cirrhosis-associated immune dysfunction; CCl4, carbon tetrachloride; ELISA, enzyme-linked immunosorbent assay; EVs, extracellular vesicles; FBS, fetal bovine serum; HSCs, hepatic stellate cells; LDH, lactate dehydrogenase; LPS, lipopolysaccharide; APAP, acetaminophen; BD, bile duct; BDL, bule duct ligation; cBDL, common bile duct ligation; CCl4, carbon tetrachloride; DDC, 3,5-diethoxycarbonyl-1,4-dihydrocollidine; DEN, diethylnitrosamine; D-GalN, D-galactosamine Hydrochloride; D-gal, galactosamine; DMN, dimethylnitrosamine; HSA, human serum albumen; HFD, high fat diet; i.p., intraperitoneal injection; i.v., intravenous injection, LDE, Lieber-De Carli ethanol diet; LPS, lipopolysaccharide; NAFLD, nonalcoholic fatty liver disease; NASH, nonalcoholic steatohepatitis; NIAAA, national institute on alcohol abuse and alcoholism; PS, porcine serum; TAA, thioacetamide administration.

The PiZ rats. The PiZ rat is another model replicating many AATD features, including the hepatic accumulation of misfolded AAT and the development of liver injury, fibrosis and inflammation. While less commonly used than the PiZ mouse, it is an alternative for studying the disease in a larger mammal.

The PiZZ ferrets. For large animals, AAT-knockout and PiZZ knock-in (E342K, the most common mutation in humans) ferrets have been generated. The PiZZ mutation leads to altered AAT protein folding in the liver hepatic injury and reduced plasma concentrations of AAT, and PiZZ ferrets develop obstructive lung disease [188]. This model develops progressive obstructive pulmonary disease seen in AAT-deficient patients and may serve as a platform for preclinical testing of therapeutics including CGT products.

Acute-on-chronic liver failure (ACLF) model

Compared with the chronic liver damage model described above, in both the acute liver damage and the acute-on-chronic liver failure models, the liver is damaged from the beginning or after chronic liver injury due to a very short period of intense stress, as shown in Table 5B and 5C, respectively. In the acute liver damage model, CCl4, common bile duct ligation, and 90% partial hepatectomy are used to induce acute injury, which is analyzed within a few days. In contrast, in an acute-on-chronic liver failure model, chronic liver injury is induced using CCl_4, followed by LPS/D-galactosamine. The degree of injury is defined according to the objectives of each researcher and the animal species.

Inborn Errors of Metabolism

Liver-based metabolic disorder (LBMD) is a Mendelian disease caused by genetic alterations that impact the function of liver metabolic enzymes. In this context, the hepatocytes are not injured in the early phase and the liver is not structurally altered, thus functioning as the backbone of the cell engraftment. In LBMD, cell therapy aims to allow a sufficient repopulation of the liver with transplanted cells, estimated at ~5–10% of the theoretical liver mass, to rescue the deficit or absence of a single enzyme function [128]. So far, HT in LBMD has been reported in less than 40 cases, with initial biochemical and clinical benefits. However, a decline in cell function has been observed after approximately 9–12 months. Thus, in the context of the cell therapy approach, choosing the appropriate animal model resembling the human metabolic error is crucial (Table 6). Particularly, we focused on the LBMDs whose therapeutic approach comprises regenerative medicine, namely hereditary tyrosinemia type 1 (HT-1), Crigler–Najjar syndrome type 1 (CN-1) and Wilson's disease (WD).

Hereditary tyrosinemia type 1

HT-1 is a severe, autosomal recessive inborn error of metabolism caused by deficiency of fumarylacetoacetate hydrolase (FAH), a metabolic enzyme that catalyzes the last step of tyrosine metabolism, leading to the accumulation of toxic metabolites. The first preclinical model for this human liver disease was developed in mice by deletion of the FAH gene, which led to the elucidation of disease pathophysiology and therapeutic development. Null mice transplanted with WT hepatocytes resulted in the replacement of FAH+ cells with improved clinical outcomes and an increase in animals' survival rate. To study the effects of transplanted human hepatocytes, immunodeficient mice triple mutant for *Fah, Rag2* and *IL2rg* (FRG mice) were developed as a xenograft model [192]. As reported by Azuma et al., [192] injected human FAH+ hepatocytes isolated from brain-dead organ donors and surgical liver resection successfully repopulate the liver of immunodeficient FRG null mice. However, the shortage of organ donors has motivated the development of alternative strategies by generating hepatorganoids (HOs). As demonstrated by Yang et al., 3D

Table 6
Animal models resembling metabolic-related human disorders.

Related human disorder	Genetic alteration	Animal model	Injury type	Animal phenotype	Serum alterations	Histological examinations	References
Tyrosinemia type I (HT1)	Deficiency of fumarylacetoacetate hydrolase (FAH)	Mice	Knockout of FAH and RAG2	Survival and body weight	ALT, AST, TP, ALB, DBIL, LD, GGT, ALP, TBA, A/G, PA, Specific Amino acids, alpha-1 antitrypsin, factor VII and factor IX, 4-OH-DEB*	H&E, IF for specific cell markers (ie, CD31, ALB)	[189]
Crigler–Najjar syndrome 1 (CN1)	Lack of UGT1A1	Gunn rats	Knockout of UGT1A1 gene	n/a	HSA and human transferrin, bil, bilirubin glucuronides, hepatic UGT1A1 activity	IHC for hUGT1A1 and HAS	[190]
Human Wilson's disease	Abnormality in copper metabolism	Long–Evans cinnamon rat	Mutation causing the deficiency in serum ceruloplasmin activity	Hepatic copper accumulation	Copper content and Ceruloplasmin activity	n/a	[191]

ALT, alanine aminotransferase; AST, aspartate aminotransferase; TP, total protein; DBIL, ALB, direct bilirubin; LD, lactic dehydrogenase; GGT, gamma-glutamyl transpeptidase; ALP, alkaline phosphatase; TBA, total bile acid; A/G, albumin/globulin ration; PA, prealbumin; 4-OH-DEB, 4-hydroxydebrisoquine; n/a, not available.

bioprinted HOs were transplanted into abdominal cavities of mice of the Fah$^{-/-}$Rag2$^{-/-}$liver injury mouse model after 7 days of in vitro differentiation. In vivo, the 3DP-HOs further matured and displayed an increased synthesis of liver-specific proteins, resulting in an improved survival rate in treated mice. Despite the advantages shown by Fah$^{-/-}$ mice, they did not develop cirrhosis, a critical disease phenotype. Therefore, Fah$^{-/-}$ rat, rabbit and pig models were developed [189].

Crigler–Najjar syndrome type 1

CN-1 is an autosomal recessive, ultra-rare disease characterized by the lack of the uridine diphosphate glucuronosyltransferase 1A1 (UGT1A1) activity due to a genetic defect in the UGT1A1 gene. This enzyme is required for the conjugation of glucuronic acid to bilirubin, increasing its solubility and thus its excretion into bile. Abnormalities in this process unequivocally lead to hyperbilirubinemia causing severe jaundice and toxicity, especially in the brain with kernicterus [193]. Gunn rats represent the first animal model of CN-1. Similar to humans, these animal models congenitally lack all glucuronidation activity catalyzed by the UGT1A1, owing to a premature stop codon in the gene [194]. Since serum bilirubin levels, as well as the excretion of bilirubin glucuronides in bile can be easily measured to assess the efficacy of different therapeutic approaches, this animal model was found to be convenient for evaluating the effects of HT. Although a null Ugt$^{-/-}$ mouse model was developed using the same point mutation of Gunn rat, they are mainly used to support the gene therapy approach [195].

Wilson's disease

The Long–Evans cinnamon (LEC) rat represents the first genetic model of WD, a rare autosomal recessive disorder affecting copper metabolism and resulting in the buildup of copper toxic levels in several organs, including the liver, brain and eyes. WD is ascribed to pathogenic mutations affecting the ATPase copper-transporting beta (ATP7B) gene, whose protein product regulates biliary copper secretion and copper loading of ceruloplasmin. The LEC rats are characterized by a partial deletion in the 3′ end of rat *Atp7b* spanning regions of the coding sequence and the untranslated region and is based on the accumulation of copper in the liver, low levels of serum ceruloplasmin, and low excretion of copper into the bile [134,191]. Some animals exhibit the progression to severe hepatitis and necrosis with early death while others progress to fibrosis and eventual HCC [196], a rare condition detected in WD human patients. As demonstrated by Park et al., [197] the intrasplenic injection of healthy hepatocytes into 8-week-old LEC rats resulted in restored biliary copper excretion and reduced hepatic copper concentration 16 weeks post-transplantation, with ameliorated 6-month survival rates. Sauer et al. [198] confirmed these results and found that multiple HT led to better outcomes. Other variants of LEC rats have been developed, including inbred mouse models (toxic milk (tx), the Jackson Laboratory toxic milk (tx-j) and the genetically engineered ATP7B$^{-/-}$(knockout) mouse with different extents of liver damage. However, only LEC and ATP7B$^{-/-}$are susceptible to liver tumors [199].

Other Animal Models

There are other uses of animal models in addition to those described above. When human hepatocytes are transplanted into uPA/SCID mice [200], which are immunocompromised and develop liver failure, they are almost completely replaced by human hepatocytes. These mice are used in experiments involving hepatitis virus infection. Medaka and zebrafish are excellent for creating transgenic lines of model fish, and recently, medaka with fatty livers have been reported [126,127]. These models have the potential to be used efficiently for drug evaluation, but the lack of sufficient analytical systems is a limitation. However, for safety evaluation, the use of pigs and monkeys is advantageous due to their size in comparison to humans though their use is limited by the difficulty in obtaining sufficient individuals for efficacy evaluation. Thus, they must be used with caution [124,125].

Section IV: Humanized Mouse Models

Humanized animal models, which enable the study of human tissue (such as islets or hepatocytes) and immune system interactions, have become crucial for translating therapeutic strategies into human clinical trials [201]. These models are useful for predicting the efficacy, drug toxicity and drug interaction related to human disease [202]. Specifically, these mice are a robust system for studying the safety and efficacy of CGT products for the immune system [203]. This section will focus on the humanized model using the T1D model as an example. Similar models can be established by reconstituting recipient mice with other tissues, such as hepatocytes [202].

Humanized T1D models allow researchers to (i) explore adaptive immune responses to human islets, better understand T1D pathology and evaluate the safety and efficacy of human-specific therapies. These models often replicate human immune interactions and disease mechanisms more closely than other T1D mouse models [105,204,205]. Using these models can improve the likelihood of success in clinical trials. There are several ways to generate humanized mice for T1D studies. The most common method is engrafting the NOD-scid IL2 gamma null (NSG) mice with the hematopoietic stem cells (HSCs) or the peripheral mononuclear cells (PBMCs). The PBMC model is easy to establish and widely used in the study of the mechanism of xenograft rejection and immunosuppressive agents as preclinical therapeutics [61–63,206].

The humanized CD34+ mice (Hu-CD34)

Hu-CD34 mice are a robust system for studying the safety and efficacy of CGT products for the immune system. The NOD-scid IL2 gamma null (NSG) mice engrafted with cord blood-derived HSCs develop multilineage engraftment and display robust T-cell maturation and T-cell dependent inflammatory responses and have been used in many studies. In this model, 5–7-week-old NSG mice are irradiated with 240–250 cGy and then injected with 4×10^4 human CD34$^+$ cells via the tail vein within 24 hours postirradiation. The presence of human CD45$^+$ cells in the mice is later confirmed by measuring the human CD45$^+$ cells in peripheral blood 12–16 weeks postengraftment and just before transplantation. The hu-CD34 NSG model helps study long-term graft survival and rejection. The disadvantage is that mice have to be irradiated and reconstitution takes a long time (>16 weeks) [207].

The humanized PBMC (hu-PBMC) mice

Hu-PBMC mice feature quick engraftment of adult PBMCs and enable short-term studies requiring mature human T cells. NSG mice engrafted with human PBMCs develop an acute xenogeneic graft versus host disease (GVHD)-like disease upon recognizing the murine cells and tissues by mature human T cells. The hu-PBMC-NSG MHC I/II double knockout mice improved upon the limited brief window available in the hu-PBMC model to conduct experiments before the PBMC engrafted mice become affected by GVHD. The latter model showed delayed GVHD and supported long-term studies of human immunity requiring human T cell engraftment and human tumor xenograft models, enabling the evaluation of immune modulators targeting human T cells [61–63,206,208,209]. In addition, the hu-PBMC-NSG MHC I/II double knockout mice engineered with MHC class I and II knockouts help to mitigate the rapid onset of GVHD seen in the standard hu-PBMC model. It supports longer-term research by

prolonging the time to study human immunity and evaluate immune modulators [208].

The NOD-Rag1^{null} Prf1^{null} Ins2^{Akita}

The AKITA mouse model, which has a spontaneous mutation in the insulin-2 gene (Ins2Akita), develops insulin-dependent diabetes similar to Permanent Neonatal/Infancy-Onset Diabetes Mellitus in humans. This mutation, characterized by replacing cysteine at position 96 with tyrosine, disrupts a crucial disulfide bond necessary for proper insulin folding. The misfolding triggers the unfolded protein response, leading to β-cell apoptosis and ultimately causing diabetes and permanent hyperglycemia [201,210].

Humanized peripheral blood lymphocytes mice (Hu-PBL-SCID Model)

This model, which transfers PBL from individuals with T1D into SCID mice, has been historically employed to investigate various autoimmune diseases. However, while autoantibodies against islet components were detected, there was no evidence of β-cell destruction or significant islet infiltration in early studies. Recent advancements, including human T cell clones targeting specific islet autoantigens, have shown islet infiltration but still no cell destruction [201,210].

The Hu-SRC-SCID mice

While the Hu-PBL-SCID model primarily supports the engraftment of mature human T cells, it does not efficiently engraft other critical immune cells implicated in T1D pathogenesis, such as macrophages, NK cells, NKT cells, dendritic cells and B cells. To overcome this, human HSCs can be engrafted into immunodeficient mice to generate these additional cell lineages. However, obtaining HSCs from donors with a genetic predisposition to T1D typically involves invasive procedures like bone marrow biopsies or mobilizing HSCs into the peripheral blood through G-CSF treatment [201,210].

The NSG HLA-A2 mice

These transgenic mice are a specialized model developed on the NSG (NOD scid gamma) strain background, incorporating HLA class I and class II alleles, representing up to 80% of HLA-locus genes associated with T1D. These transgenic mice are designed to advance the study of human immune responses by supporting the development of HLA-A2-restricted CD8$^+$ T cells. This capability is facilitated through engraftment with umbilical cord blood HLA-A2+ HSCs, which allows the study of human immune interactions and responses in a controlled, relevant model system [210].

The YES mice

In research by Luce et al., a new mouse model, known as YES mice, was generated by crossing strains lacking murine MHC class I and II as well as insulin genes with those expressing human HLA-A*02:01, HLA-DQ8, and insulin genes as transgenes. The YES mice maintained metabolic and immune profiles similar to their parental strains. They remained free of insulitis and diabetes throughout a 1-year study period, demonstrated stable blood glucose levels in response to an intraperitoneal glucose challenge, maintained average β cell mass and showed standard immune responses to conventional antigens [205]. A subsequent study by the same research group introduced a novel humanized mouse model known as YES-RIP-hB7.1. This model is unique in that it lacks murine MHC class I and II molecules, and native murine insulin genes. Instead, it is engineered to express transgenes for the high-risk diabetes-associated alleles HLA-A*02:01, HLA-DQ8A and HLA-DQ8B, along with the human insulin gene and the costimulatory molecule B7.1 specifically within insulin-producing cells. The YES-RIP-hB7.1 mouse model spontaneously develops T1D, characterized by CD4+ and CD8+ T-cell responses against human preproinsulin epitopes. Significantly, many of the immune responses observed in this model have been validated in patients with T1D, underscoring its value as a pertinent preclinical tool. This model presents substantial potential for detailed investigation of human preproinsulin-specific epitopes in T1D and helps identify factors that may trigger autoimmune responses targeting insulin-secreting cells [211].

In a different study, humanized mice were developed by transplanting HLA-DQ8+ human fetal thymus and CD34+ cells into immunodeficient, HLA−DQ8−transgenic mice. These mice developed hyperglycemia and diabetes following the introduction of autologous human CD4+ T cells, which were specific to the HLA-DQ8/insulin-B:9-23 epitope. This research provided key evidence of the pathogenic role of islet autoreactive CD4+ T-cell responses in triggering T1D in humans. Additionally, it highlighted the crucial involvement of target β cells in their immune-mediated destruction, demonstrating that T cells recognizing the InsB:9-23 epitope can initiate disease when islet inflammation is present [212].

Section V: APPLICATIONS of Animal Models Used for CGT Studies for Pancreatic and Liver Diseases

1. Application of animal models in cell therapy for pancreas and liver diseases

Recent advances in regenerative medicine and stem cell research, including the use of MSCs, inducible pluripotential stem cells, hepatocytes, ESCs, biliary tree stem cells and HSCs, have led to an increase in preclinical and clinical studies exploring their diverse applications in pancreatitis and T1D [213,214]. These cells are classified into totipotent, multipotent and specialized types, each capable of developing into complete organisms, various tissues or specific cell lineages under appropriate conditions. Cell therapy, either by transplanting islet cells or stem cells-derived insulin-secreting cells, has been tested in numerous animal models of T1D and is now being used for treating diabetes in clinical trials [215]. Recent advancements in whole pancreas and islet allotransplantation are attributed to improved donor and recipient selection criteria, refined surgical techniques and new immunosuppressive therapies. These developments have significantly improved the survival rates of both the transplanted organs and the patient [215,216]. Additionally, stem cell therapies, such as those using MSCs and inducible pluripotential stem cells, have shown promising results in experimental murine models of chronic, AP and inflammatory bowel disease [217–221]. These therapies help inhibit disease progression, reduce pancreatic damage and minimize fibrosis and cell death [222–225].

In the context of liver diseases, stem cells and hepatocytes derived from the liver have been tested in animal models for regenerative therapies [213,226,227]. The progress in stem cell therapies and transplantation techniques holds promise for overcoming current treatment limitations. Continued research and refinement of animal models are essential for improving therapeutic outcomes and translating these advancements into effective patient treatments.

2. Application of animal models in gene therapy for pancreas and liver diseases

Gene therapy aims to treat diseases by altering genetic material in cells. It involves introducing new genes, replacing faulty ones or inactivating defective genes. There are two main types of gene therapy: somatic gene therapy, which targets existing cells, and germline gene therapy, which targets reproductive cells. This method shows promise for treating various conditions, including autoimmune diseases, diabetes, cancers and heart diseases [228].

Numerous studies have explored gene therapy of T1D using various animal models [229]. For example, overexpression of key genes

such as insulin-like growth factor 1 [230], Reg3g (a regenerating islet-derived protein 3 gamma) in the NOD mice [231], and hepatocyte growth factor delivered via adenoviral vectors to rodent islets [232] have been explored. These gene therapy approaches have shown potential in improving disease outcomes and reducing the number of islets needed for transplantation. Additionally, gene therapy with modifying neurogenin-3 (Ngn3), epidermal growth factors and pancreas duodenal homeobox-1, molecules that play critical roles in islet cell development, glucose metabolism and inflammation, and are being tested in both T1D and T2D animal models [228].

In the context of pancreatitis, several genes that are crucial in regulating trypsin activity and preventing pancreatic damage have been tested in animal models for treating pancreatitis. For example, mutations in PRSS1 gene can lead to increased trypsin activity by enhancing the conversion of trypsinogen to trypsin or making trypsin more resistant to degradation, contributing to hereditary pancreatitis. Similarly, CTRC gene encodes an enzyme that helps degrade active trypsin, and variants in this gene can impair its function, resulting in elevated trypsin levels and a higher risk of pancreatitis. These genetic variations can weaken the body's defenses against premature trypsin activation and subsequent pancreatic injury [233]. Given the potential benefits of exogenous trypsin inhibitors for the prevention and treatment of pancreatitis, SPINK1, which encodes a trypsin inhibitor, has demonstrated the ability to safely target the pancreas with high transduction efficiency, effectively ameliorating pancreatitis phenotypes in cerulein-induced mice [234].

For hepatocyte therapy, CRISPR-Cas9-mediated targeted integration is effectively used to correct pathogenic mutations in FAH or Ornithine transcarbamylase [235]. These edited hepatocytes not only achieved high repopulation levels in the injured mouse liver but also underwent maturation, successfully treating tyrosinemia in mice following transplantation [236]. Additionally, liver-directed gene therapy ensures stable expression of ABCB4-dependent phospholipid transport in the liver, leading to long-term correction of the phenotype in a murine model of Progressive Familial Intrahepatic Cholestasis Type 3 (PFIC3) [237]. These advancements underscore the potential of gene therapy to effectively address complex liver and pancreas disorders. Future research should focus on optimizing these therapies and translating them into clinical practice for broader patient benefit.

Summary

Various animal models are available for evaluating CGT products in certain pancreatic and liver diseases and should be carefully selected based on specific research needs. To ensure the most relevant and reliable outcomes, the strengths and limitations of each model, the treatment dynamics, and the underlying mechanisms of the CGT product's therapeutic effects should be carefully considered. Additionally, it is essential to seek guidance from the FDA or other regulatory agencies when designing preclinical studies involving animal models to ensure compliance with regulatory standards and best practices.

Declaration of competing interest

The authors declare no conflicts of interest.

Data Availability

All data needed to evaluate the conclusions of this study are provided in the main text of the manuscript and/or the Supplementary information. This study includes no data deposited in external repositories, and additional data related to this study may be requested from the authors.

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