

Stem cell-based multi-tissue platforms to model human autoimmune diabetes



Karla F. Leavens¹, Juan R. Alvarez-Dominguez², Linda T. Vo³, Holger A. Russ⁴, Audrey V. Parent^{5,*}

ABSTRACT

Background: Type 1 diabetes (T1D) is an autoimmune disease in which pancreatic insulin-producing β cells are specifically destroyed by the immune system. Understanding the initiation and progression of human T1D has been hampered by the lack of appropriate models that can reproduce the complexity and heterogeneity of the disease. The development of platforms combining multiple human pluripotent stem cell (hPSC) derived tissues to model distinct aspects of T1D has the potential to provide critical novel insights into the etiology and pathogenesis of the human disease.

Scope of review: In this review, we summarize the state of hPSC differentiation approaches to generate cell types and tissues relevant to T1D, with a particular focus on pancreatic islet cells, T cells, and thymic epithelium. We present current applications as well as limitations of using these hPSC-derived cells for disease modeling and discuss efforts to optimize platforms combining multiple cell types to model human T1D. Finally, we outline remaining challenges and emphasize future improvements needed to accelerate progress in this emerging field of research.

Major conclusions: Recent advances in reprogramming approaches to create patient-specific induced pluripotent stem cell lines (iPSCs), genome engineering technologies to efficiently modify DNA of hPSCs, and protocols to direct their differentiation into mature cell types have empowered the use of stem cell derivatives to accurately model human disease. While challenges remain before complex interactions occurring in human T1D can be modeled with these derivatives, experiments combining hPSC-derived β cells and immune cells are already providing exciting insight into how these cells interact in the context of T1D, supporting the viability of this approach.

© 2022 The Authors. Published by Elsevier GmbH. This is an open access article under the CC BY-NC-ND license (<http://creativecommons.org/licenses/by-nc-nd/4.0/>).

Keywords Type 1 diabetes; Autoimmunity; Disease modeling; Pluripotent stem cells; Direct differentiation; T cells; Thymus; Pancreatic β cells; Genome engineering

1. INTRODUCTION

Autoimmune diabetes, commonly known as type 1 diabetes (T1D), is characterized by the erroneous destruction of pancreatic insulin-producing β cells by an individual's own immune system. Decades of research have demonstrated that T1D is a progressive disease initiated by a break in immune tolerance that leads to infiltration of pancreatic islets by islet antigen-specific autoreactive T cells [1–4]. The disease then progresses towards overt diabetes, likely through combined action of innate immune cells, autoreactive CD4⁺ and CD8⁺ T cells, and autoantibody-producing B cells working synergistically to destroy most of the β cells in the pancreas. More recent work has provided increasing evidence for a previously underappreciated role for the pancreatic β cell in triggering its own demise, arguing against a bystander role for the cells targeted by the immune system (reviewed in [5,6]). The contribution from other pancreatic cell types to disease initiation and progression is also the subject of active investigation. The accumulating evidence has thus led to the emerging concept that T1D might not be accurately classified as a single disease, but comprises

many different endotypes that involve different biological systems and mechanisms that contribute to disease manifestation [7].

1.1. The need for stem cell-based models of human T1D

Animal models of spontaneous autoimmune diabetes have provided considerable insights, leading to the formulation of interventions to prevent, halt, and even reverse disease in these animals (see [8] for a detailed review of current animal models of T1D). However, translating these approaches to humans has fallen short. This is likely due to species differences in biological responses [9], as well as failure to understand which patient population would benefit the most from a specific treatment based on their disease endotype. Better preclinical research models are thus needed to gain specific insights into the initiation and progression of the human disease. Critical information can be obtained by studying primary immune cells and pancreatic islets isolated from control donors and patients with T1D. However, primary cells are difficult to obtain, and their availability is unpredictable. They also exhibit considerable variability between donors and tend to lose their functional characteristics when cultured *in vitro*.

¹Department of Pediatrics, Perelman School of Medicine, University of Pennsylvania and Division of Endocrinology and Diabetes, The Children's Hospital of Philadelphia, Philadelphia, PA, USA ²Institute for Diabetes, Obesity and Metabolism, University of Pennsylvania Perelman School of Medicine, Philadelphia, PA, USA ³Innovative Genomics Institute, University of California, Berkeley, Berkeley, CA, USA ⁴Barbara Davis Center for Diabetes, University of Colorado Anschutz Medical Campus, Aurora, CO, USA ⁵Diabetes Center, University of California San Francisco, San Francisco, CA, USA

*Corresponding author. E-mail: audrey.parent@ucsf.edu (A.V. Parent).

Received July 27, 2022 • Revision received September 20, 2022 • Accepted October 4, 2022 • Available online 6 October 2022

<https://doi.org/10.1016/j.molmet.2022.101610>

Abbreviations

T1D	Type 1 diabetes
HIRN	Human Islet Research Network
hPSC	Human pluripotent stem cell
iPSC	Induced pluripotent stem cell
HLA	Human leukocyte antigen
HSC	Hematopoietic stem cell
TEC	Thymic epithelial cell
SC	Stem cell-derived
NOD	Non-obese diabetic
HE	Hemogenic endothelial cell

TCR	T cell receptor
ATO	Artificial thymic organoid
CAR	Chimeric antigen receptor
Treg	Regulatory T cell
TEP	Thymic epithelial progenitor
hESC	Human embryonic stem cell
SCID	Severe combined immunodeficiency
FTOC	Fetal thymus organ culture
RTOC	Reaggregated thymic organ culture
MHC	Major histocompatibility complex
PBMC	Peripheral blood mononuclear cells

These limitations reduce the speed and confidence with which knowledge on specific biological processes can be obtained from primary tissue. Additionally, modeling autoimmune interactions requires appropriate matching of human leukocyte antigens (HLAs) between the different cell types to allow faithful communication between cells while avoiding alloimmune responses. These restrictions hinder the building of complex models that can provide critical insights into autoimmune processes using primary cells.

Human pluripotent stem cells (hPSCs) offer an alternative source for functional cell types to overcome some of these challenges. The ability of hPSCs to self-renew while maintaining the capacity to differentiate into virtually any cell type in the body provides the opportunity to generate unlimited amounts of genetically identical disease-relevant cells. Advances in reprogramming methods to create iPSCs, including viral integration-free approaches, have also facilitated modelling genetically complex diseases involving multiple loci by enabling the generation of patient-specific cell lines. In addition, genome engineering approaches such as CRISPR/Cas9 and TALEN technologies make it possible to effectively conduct genetic modification in hPSCs. These technologies have facilitated the generation of gene knockouts and overexpression models, fluorescence gene reporters, and implementation of distinct SNP configurations, providing an unprecedented ability to interrogate the role of specific human genes in disease onset and progression in an isogenic manner [10]. Such emerging human and patient-specific model systems are especially powerful when married to ever-improving strategies to direct hPSC differentiation toward functional cell types relevant to T1D. For example, it is now possible to perform detailed phenotypical analysis of pancreatic β cells generated by directed differentiation of iPSCs from patients with T1D [11,12]. Thus, advances in iPSC reprogramming, genome engineering, and directed differentiation offer the T1D research field the unique opportunity to investigate distinct aspects of the human disease, including interactions between genetically matched cell types.

1.2. Modeling different stages of T1D

Given the complexity of the disease, optimizing platforms to model human T1D demands combining different cell types to recapitulate their intricate interactions. In this review, we will focus on hematopoietic stem cells (HSCs), which give rise to T lymphocytes and other immune cells, thymic epithelial cells (TECs), which are required to support T cell development, and pancreatic islet cells (Figure 1A). While reproducing all disease features is not currently possible, we envision modeling specific disease stages by integrating different stem cell-derived (SC-) cell types in the same model platform (Figure 1B). For example, modeling defects in central immune tolerance, likely involving deficiencies in thymic selection [13], requires combining HSCs or T cell progenitors with TECs to assess T cell

development. Alternatively, modeling the initial destruction of β cells likely necessitates combining pancreatic β cells with a subset of immune cells (T cells and/or innate cells such as macrophages, dendritic cells, NK cells), while modeling disease progression might require other pancreatic cell types combined with a broader range of immune cells (Figure 1B).

These different cell types can then be combined in the appropriate platform to model distinct disease aspects. For example, hPSC-derived islets (SC-islets) can be cultured *in vitro* with cytokines to assess the effect of inflammation on β cells, or they can be combined with immune cells to measure T cell activation in different conditions (Figure 1C). SC-islets can also be incorporated into microfluidic devices, designed to recreate a normal flow of nutrients and cells through the islet-like clusters, to assess cell migration and immune cell activation (Figure 1C). Alternatively, these different cell types can be combined *in vivo* within humanized mice. For example, SC-islets can be transplanted into immunodeficient mice that have been injected with human peripheral blood mononuclear cells (PBMCs) to assess T cell migration and selective destruction of human β cells within SC-islet grafts (Figure 1D). HSCs, TECs, and pancreatic cells can also be engrafted in irradiated and thymectomized immunodeficient mice to study thymic selection and subsequent immune attack of SC-islet grafts by human T cells and other immune cells (Figure 1D). More details on these *in vitro* and *in vivo* models are provided in a separate issue of the Human Islet Research Network (HIRN) review series [8].

Here, we summarize the current state of hPSC differentiation approaches to generate T1D relevant cell types with a particular focus on pancreatic islet cells, HSCs, T cells, and TECs. The progress in differentiation methods for other relevant cell types, such as innate immune cells, is reviewed elsewhere [14]. We also discuss applications and limitations of using each cell type for disease modeling and review current and emergent multi-tissue *in vitro* and *in vivo* models. Finally, we outline outstanding challenges in the field and highlight future improvements needed to advance this exciting research frontier.

2. PANCREATIC ISLET CELLS

As both the targets of the autoimmune attack and potential key instigators of the immune response leading to their demise [6], β cells are an integral part of T1D models. Over the past two decades, extensive effort has been put into developing and optimizing protocols to generate an abundant source of functional human SC- β cells for cell replacement therapy (see [15–18] for review). Importantly, this work has considerably accelerated our ability to model aspects of human β cell physiology and T1D disease development. While knowledge of some forms of diabetes will advance using only SC- β cells, other discoveries, especially in autoimmune T1D, may require SC-islets or

even whole-pancreas models in conjunction with immune cells. The role of α cells in the development of multiple forms of diabetes has been appreciated in recent years, including an emerging emphasis on the role of α cell dysfunction in T1D (reviewed in [19]). However, their impact on human β cell function and immunogenicity in models of T1D is still unknown. Similarly, more work is needed to determine how non-endocrine islet niche cells, including vascular and extracellular matrix components (see [20,21] for review), would impact the utility of SC-islets for T1D modeling.

2.1. Current state of pancreatic islet cell differentiation

2.1.1. β cells

The pathways driving pancreas development and endogenous β cell generation have been carefully delineated through decades of mostly model organism research (reviewed in [22–24]). This foundation

enabled developing *in vitro* differentiation protocols that generated immature but glucose responsive SC- β cells [25–27]. These early SC- β cells selectively responded to glucose challenges with increased insulin secretion, but also presented phenotypical features akin to immature fetal-like cells. Transcriptional [28–32], epigenomic [33–35], proteomic [36], and metabolomic [37,38] profiling of early SC- β cells indeed revealed that they more closely resemble fetal rather than adult human β cells, indicating a need for further optimization of the differentiation process. Multiple approaches have since been taken to improve SC- β cell physiology with the goal of achieving insulin secretion in response to fuel, typically glucose, with the precision, magnitude, and kinetics of native mature β cells. The generation of SC- β cells and their maturation has been improved by refining the timing of chemical manipulation of known pathways such as TGF β [39,40], ROCK [41], and non-canonical WNT [42]. Alternatively, modulating the circadian clock or cell cycle can also impact β cell functional maturation [33,43]. Finally, dissociation and

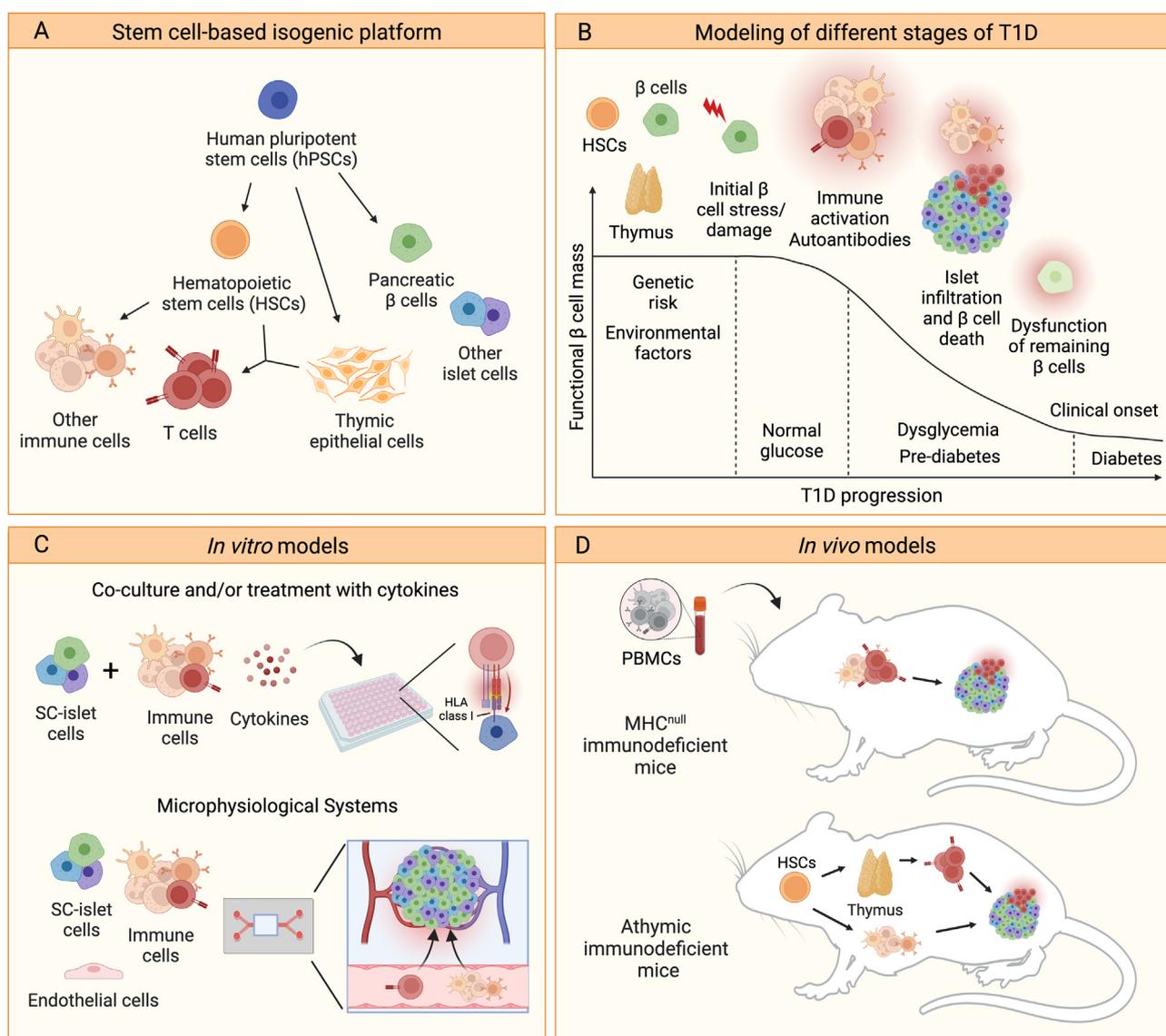


Figure 1: Stem cell-based platforms to model different stages of human T1D. (A) Human pluripotent stem cells (hPSCs) can be used to generate isogenic cells relevant to the initiation and progression of T1D pathogenesis. (B) Modeling of specific stages of disease requires combining different SC-derivatives. (C–D) Relevant SC-derivatives can be combined *in vitro* using co-culture platforms or microphysiological systems (C), or *in vivo* using humanized mouse models (D).

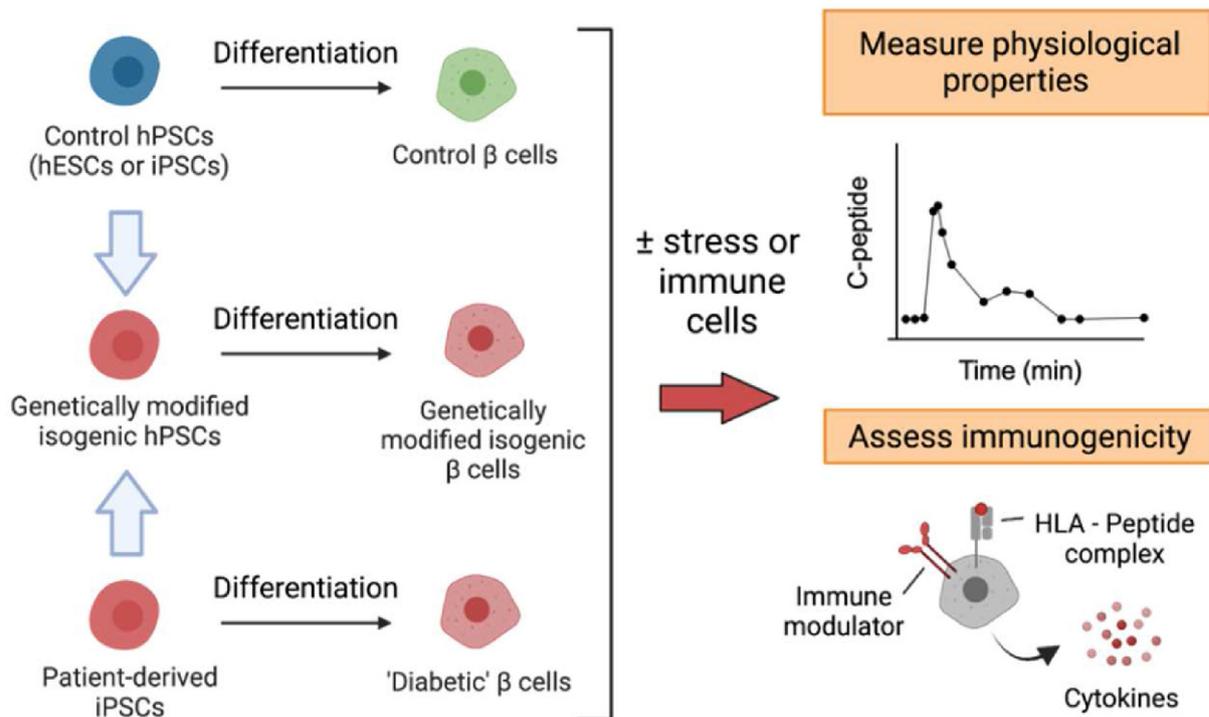


Figure 2: SC-islets can be used to model different forms of diabetes. Reprogramming of somatic cells from healthy donors and patients with diabetes or genetic engineering of control hPSCs enables the generation of disease-relevant SC-β cells. Comparing the physiological and immunogenic properties between control and genetically modified isogenic or diabetic SC-β cells has the potential to reveal new insights into different forms of human diabetes. In some cases, exposure of SC-β cells to stress and/or immune cells might be required to reveal alterations in the phenotype of disease-relevant cells.

cell sorting strategies to enrich for SC-β cells can improve specification and subsequent maturation, while allowing simultaneous resizing to more physiologic islet-like structures [30,39,40,44–51]. Despite these advantages, reaggregation techniques also have disadvantages such as cell loss during the labor process and requirements for complex workflows, which may decrease reproducibility across different cell lines or laboratories. Taken together, these decades-long efforts to optimize differentiation and maturation steps have laid the foundations for using SC-β cells to model diabetes. While there is still ongoing work focused on further refining SC-β function, it is indeed now possible to produce vast numbers of glucose responsive human β cells for disease modeling, or for genetic/drug screens to identify and validate new therapeutic targets.

2.1.2. Other endocrine cell types

While other endocrine cells such as α and δ cells are induced with current β cell differentiation protocols, it remains to be seen if *in vitro* differentiation strategies can be adapted to selectively generate these hormone-expressing cell types independently. A few protocols exist for generating SC- α cells capable of secreting glucagon in response to secretagogues [52–55]. These protocols involve inhibiting BMP and activating retinoic acid signaling at early stages of endodermal patterning, before NKX6.1 is expressed, to drive pancreatic cells to an α -like fate. Alternatively, controlled generation of multiple endocrine cells under the same culture conditions may also be possible, as peninsular structures budding during endocrine differentiation from stem cells contain insulin-, glucagon-, and somatostatin-expressing cells, mimicking the formation of native islets [56].

2.2. Applications of SC-islets in disease modeling

Recent advances in SC-β cell differentiation, combined with reprogramming and genome editing tools, have enabled the development of a first generation of diabetes disease models (Figure 2).

Genome engineering has been used to model monogenic diabetes by knocking out genes critical for pancreatic and β cell development in hPSCs (PDX1, NEUROG3, ARX, GLIS3, NEUROD1) (reviewed in [57,58]). Correcting mutations in patient-derived iPSCs has led to a better understanding of disease mechanisms in rare cases of neonatal diabetes [59–61]. Multiple iPSC lines have also been successfully generated from patients with T1D and their ability to differentiate into β cells has been validated [11,12,62–65]. The function of SC-β cells derived from T1D iPSCs is not significantly different from that of cells derived from control lines, as indicated by *in vitro* and *in vivo* characterization available so far. Since T1D is a heterogeneous disease, it is unclear if these results are representative of most patients, or if cells from more donors need to be assessed to uncover significant differences. Alternatively, assays currently employed might not be able to detect minor differences between SC-β cells from control and T1D patients. Exposing T1D SC-β cells to stress and/or immune cells might also be required to reveal alterations in their phenotype (Figure 2).

2.3. Limitations of SC-islets in disease modeling

Despite rapid progress in differentiating pancreatic cells from hPSCs, challenges remain that affect our ability to broadly use these cells for T1D modeling. In this section, we summarize outstanding bio-processing and physiologic challenges, and discuss how they may be addressed to optimize the use of SC-β cells for disease modeling.

2.3.1. Consistency of differentiation between cell lines

Modeling an heterogeneous disease like T1D requires differentiation of various hPSC lines, including many patient-derived iPSC lines. However, the variability in gene expression, insulin production, and glucose responsiveness in SC-islets derived from distinct lines makes it challenging to compare across different genetic backgrounds [66,67]. In addition, heterogeneity of materials (chemicals, small molecules, growth factors, and biologics such as antibodies) used by researchers may yield differential results in different laboratories across the globe. Finally, differentiation protocols involve specialized techniques that are difficult to define accurately in publications, contributing to a lack of reproducibility between groups. Rigorous characterization of SC- β cells generated from different cells lines is thus recommended, particularly for β cell functional studies (see [68] for a detailed discussion on characteristics that should be met to qualify as a functionally mature β cell). However, how functionality of SC- β cells impacts their immunogenicity is incompletely understood, highlighting the importance of characterizing expression, processing, and presentation of T1D-relevant antigens when studying interactions between SC- β cells and immune cells. As we gain more information on immunogenic properties of healthy and diseased β cells, establishing a more rigorous set of immunological characteristics will be important to increase consistency among SC-based models of T1D.

2.3.2. SC-islet cellular composition and organization

In vitro differentiation of hPSCs into pancreatic β cells yields an heterogeneous mixture of pancreatic endocrine cells (β , α and δ cells) and, to varying degrees, other cell types such as pancreatic progenitors, ductal-, acinar-, and enteroendocrine-like cells [39,56]. As efforts to optimize differentiation protocols continue, better control over the types, proportions, and arrangement of cells formed is likely to be achieved by refining the timing, efficacy, or types of signaling cues directing differentiation, most of which emerged from studies in mice rather than in humans. Islet architecture, in fact, varies greatly between humans and mice, with notable differences in the type and distribution of endocrine cells and their electrophysiology [69]. Deeper knowledge of human embryonic islet development will thus be necessary to further improve the efficiency of differentiation, and may be greatly informed by recent progress in single-cell profiling technologies [70–72]. Although the presence of a non-physiological proportion or arrangement of β cells may hinder β cell functional fine-tuning, it is not clear if it affects immunogenicity in the context of T1D models. If cell purification becomes necessary to form islet-like clusters with defined compositions, endocrine cells could be enriched using different methods such as simple dissociation and reaggregation [39,40], dyes for insulin or zinc content [51], purification using cell surface markers [33,50,65,73], or fluorescent reporters [49,74]. The optimization of enrichment methods opens the door for assembling “designer” islets with defined α to β cell ratios [73]. In the future, it may also be possible to integrate marker-free purification into designer islet assembly [75]. Refining the different cell ratios and parameters for the reproducible generation of SC-islets may also improve their cellular architecture to resemble native islets of Langerhans more closely. Such efforts may also be enhanced by the addition of other cell types typically found within primary islets, including endothelial and mesenchymal cells, that are currently largely absent from SC-islets clusters.

2.3.3. Maturity of SC- β cells

Immature physiology is a common challenge for SC-derived products [76]. Despite recent progress, SC-islets produced *in vitro* still resemble fetal or neonatal rather than adult islets. Although this immature

phenotype affects the ability of β cells to properly secrete insulin in response to glucose, it is unknown how this functional impairment affects the antigen repertoire presented to immune cells, and how this compares to mature native islets. Interactions with immune cells recreated using immature SC-islets may indeed reflect distinct immunomodulatory and survival properties of immature endocrine cells when compared to mature cells. The immaturity of *in vitro* islets may nevertheless offer beneficial applications. Immature β cells engraft better upon transplant [77,78], which is desirable for *in vivo* models. They might also enable modeling of immune–islet interactions that occur during the juvenile maturation period, when early-arising autoimmunity strongly predisposes towards overt T1D [79]. Alternatively, a better knowledge of the maturational cues leading to *in vitro* maturation of β cells could enable longitudinal studies of T1D pathogenesis. For example, comparing immunogenicity of immature and adult β cells could give us insight into whether islet autoimmunity and susceptibility to inflammation is linked to physiologic maturation.

2.3.4. Stability of SC- β cells

Like primary human islets, SC- β cells intrinsically lose their ability to properly respond to glucose during long-term culture [38–40]. Why this occurs is unclear, but these studies suggest that it is not due to de-differentiation or cell death. It is possible that support from vasculature, nerves, extracellular matrix, or other tissues is needed for phenotypic durability. While this functional instability is a major challenge for physiological assays, the impact on modeling studies is unclear since changes in β cell immunogenicity during long-term culture have not been characterized. For example, how does processing and antigen presentation of autoimmune targets such as insulin change over time, and how does it compare to native and T1D human islets? As an alternative to *in vitro* culture, *in vivo* transplantation of SC- β cells might be better suited for modeling aspects of T1D that require more time and more mature cells. However, phenotypic stability, namely glucose responsiveness, has only been assessed within months of transplant *in vivo*, and informative courses of diabetic autoimmunity may demand longer windows of observation. Information on how immunogenicity of the cells evolves after transplantation is also lacking.

2.3.5. β cell heterogeneity

Another challenge of using SC- β cells to model T1D will be to determine if *in vitro* differentiation can replicate β cell heterogeneity observed in human islets. Many groups have reported different β cell subpopulations with distinct gene expression profiles, proliferative capacity, and functional characteristics (reviewed in [80]). A subpopulation of β cells with an immature phenotype that evades immune-mediated damage in non-obese diabetic (NOD) mice has also been reported [81]. This is credited to a combination of lower gene expression of T1D relevant antigens (insulin, IGRP, ZnT8, IA-2, and GAD1), and increased expression of the immunomodulatory protein PD-L1 in this β cell subpopulation. Whether a similar hypoinmunogenic β cell subpopulation exists in humans remains to be determined and will be of particular interest when generating SC- β cells for modeling T1D. Assuming that some of these subtypes reflect an adaptive response to the environment, heterogeneity may be fostered in a controlled manner by recreating the right chemical or physical triggers in SC-islet cultures. The proportions of each subtype might also be adjusted using cell surface markers or traits like size and granularity to more faithfully recreate the ratio found in human islets. Finally, while β cell subtypes have been mainly defined by transcriptional or functional differences, it will be important to assess differences in expression of immunomodulatory molecules, as well as in the

antigen repertoire, to understand the contribution of each subtype to disease.

3. HSCS AND T CELLS

A major component to modeling human autoimmune disease is the hematopoietic system. Efforts are currently ongoing to establish *bona fide* HSCs from hPSCs that can give rise to all differentiated blood cell types, especially immune cells relevant to T1D (T and B cells, innate immune cells) to study their interactions with the target tissue, eg. the pancreas and its insulin-producing β cells. Given that T cells have been shown to play a central role in the development of autoimmune diabetes [1], the ability to generate these lymphoid cells efficiently from hPSCs is of paramount importance to model immune dysfunction during T1D pathogenesis.

3.1. Current state of HSC and T cell differentiation

In vitro differentiation protocols for HSCs from hPSCs (SC-HSCs) have largely been biased towards primitive hematopoiesis (the first developmental wave of hematopoiesis, which gives rise mostly to erythromyeloid innate cells and few lymphoid cells) [82–85]. This restricted hematopoietic potential was found to be constrained in part by the Polycomb group protein EZH1, and downregulation of this epigenetic regulator promotes multilineage potential from restricted progenitors [86]. However, the discovery that definitive HSCs, which can give rise to all mature blood cells, are born from hemogenic endothelial cells (HE) during embryogenesis has prompted groups to recapitulate this development *in vitro* [87]. Wnt/ β -catenin signaling has been used to promote HE development during mesoderm specification *in vitro*, and defined a resulting population of $CD34^+CD43^-$ HE progenitors from hPSCs that can give rise to T lymphoid cells, in addition to myeloid and erythroid lineages [88].

As an alternative to SC-HSC generation, it is also possible to differentiate hPSCs directly into T cells *in vitro* [89–93]. Current protocols progress through intermediate $CD34^+$ hematopoietic progenitors, and invariably transfer $CD34^+$ progenitors to mouse stromal lines that overexpress signals critical for T cell specification, such as the NOTCH ligands Delta-like 1 (DLL1) or Delta-like 4 (DLL4) [85–89] (Figure 3). In two-dimensional (2D) co-culture methods using these stromal cells, there is limited positive selection of thymocytes and the cells generated are usually immature $CD4^+CD8^+$ T cell progenitors or mature $CD8^+$ effector T cells with very few $CD4^+$ T cells [94–96]. Furthermore, T cell development is biased towards unconventional T cells that express the $CD8\alpha\alpha^+$ homodimer, an inefficient T cell receptor (TCR)

coreceptor, instead of the HLA class I-restricted $CD8\alpha\beta^+$ that is expressed on conventional T cells [86,92,97]. To circumvent this issue, a three-dimensional (3D) artificial thymic organoid (ATO) system has been developed, which promotes positive selection and can support the development of functional $CD8\alpha\beta^+$ T cells [94–96]. High efficiency differentiation of $CD4^+$ T cells as well as negative selection, the intra-thymic process of eliminating thymocytes that express T cell receptors with high affinity for self-antigens, is nonetheless still lacking in this xenogeneic culture system.

3.2. Applications of SC-HSCs and SC-T cells in disease modeling

SC-HSCs are currently used to model myeloid or erythroid disorders (reviewed in [98]), while modeling diseases involving T cells is impeded by a lack of robust lymphoid development from SC-HSCs *in vivo*. SC-T cells differentiated *in vitro* are therefore currently better suited to model diseases driven by T lymphocytes. For example, iPSC-based models have led to a better understanding of the mechanisms behind some primary immunodeficiencies, enabling testing of strategies to correct them [99–103]. In addition to modeling primary immunodeficiencies, SC-T cells have been used to explore antigen-specific interactions in the context of cancer immunotherapy (Figure 3). For example, SC-T cells have been engineered to target human tumor cells by introducing a chimeric antigen receptor (CAR) specific for the CD19 antigen in iPSCs. $CD34^+$ hematopoietic progenitors were generated from these modified iPSCs using a serum- and feeder-free protocol, followed by transfer of $CD34^+$ cells to OP9-DL1 feeder cells to induce T cell differentiation [92]. While these SC-T cells possessed an innate-like $\gamma\delta$ T cell signature, they effectively lysed $CD19^+$ target tumor cells *in vitro* and controlled tumor growth *in vivo*. These experiments confirmed the functionality and ability of SC-T cells to interact with target cells expressing a specific antigen. In addition, iPSC-derived $\alpha\beta$ T cells generated using the 3D ATO culture system can target tumor cells in an antigen-specific manner after introducing a TCR or a CAR [95,96]. Most recently, knockdown of the epigenetic regulator EZH1 enabled the generation of mature $\alpha\beta$ CAR T cells using a stroma-free system that exhibited potent antitumor activity *in vivo* [104]. While functionality of SC-T cells has been tracked up to 7 weeks, it remains to be determined how long SC-T cells persist *in vivo* [104]. Taken together, these cancer immunotherapy models provide important proof-of-concept data showing that SC-T cells can recapitulate antigen-specific interactions between cytotoxic T cells and target cells, paving the way for the development of platforms to model autoimmune diseases like T1D.

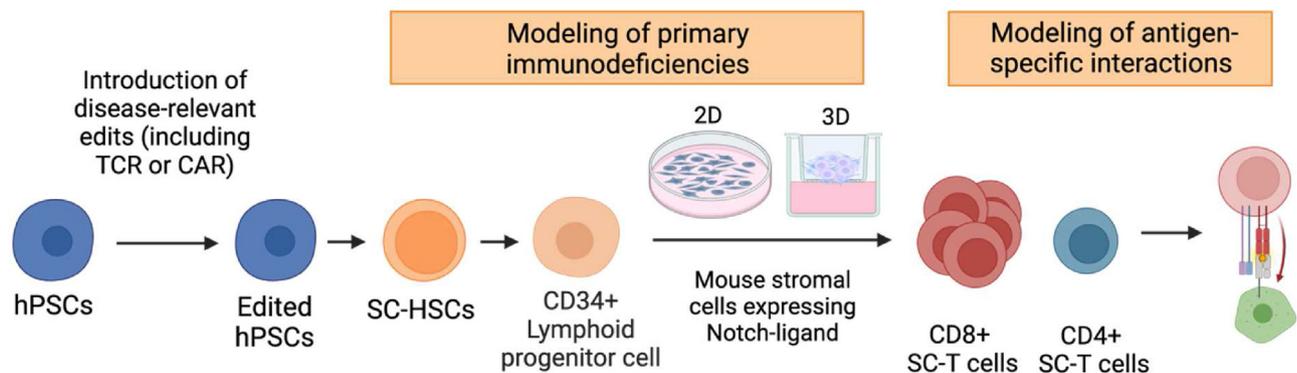


Figure 3: Applications of SC-HSCs and SC-T cells in disease modeling. Differentiation of SC-HSCs into SC-T cells can be used to model primary immunodeficiencies while introduction of CARs or TCRs enables modeling of antigen-specific interactions in cancer immunotherapies and autoimmune settings.

3.3. Limitations of SC-HSCs and SC-T cells in disease modeling

3.3.1. In vivo engraftment of SC-HSCs

A major limitation of SC-HSCs for disease modeling is the lack of robust long-term, multilineage engraftment *in vivo*. HE cells lack engraftment capability in irradiated mice, a common bottle neck for the HSC differentiation field [88,90]. Enforced expression of seven transcription factors imparts low-level engraftment with multilineage potential in iPSC-derived HE cells [105]. However, a robust method to generate SC-HSCs with long-term, multilineage potential *in vitro* and/or *in vivo* remains to be established. For this reason, *in vivo* disease modeling of hematological diseases have been limited to myeloid disorders (reviewed in [93]) or *in vitro* modeling of immunodeficiencies [94–98]. Robust engraftment from SC-HSCs would further enable modeling of diseases affecting multiple blood lineages.

3.3.2. Bioprocessing challenges

Similar to what has been discussed for SC-islets, there are bioprocessing challenges limiting the widespread use of SC-HSCs and SC-T cells in disease modeling. For example, the use of serum as well as the choice of cell of origin for reprogramming (e.g. T cells, which have a pre-arranged TCR, vs other cell types) can cause variability in T cell differentiation potential [106,107]. The yield of T cells produced from pluripotent stem cells is also lower than when using cord blood cells as starting material [107] while requirement for stromal feeders complicates our ability to produce large amounts of SC-T cells. However, recent studies have described alternative approaches using microbeads or immobilized Notch ligands that supports the development of SC-T cells, avoiding the need for stromal cells [106,108].

3.3.3. Positive and negative selection of SC-T cells

A few key challenges related to *in vitro* differentiation of SC-T cells remain. For example, it is still difficult to generate robust populations of functional CD4⁺ T cell subsets, including regulatory T cells (Tregs). This limitation is likely due to co-cultures of SC-T cells and stromal cells being deficient in signals such as HLA class II, which are required for endogenous positive selection of CD4⁺ T cells. To address this issue, T cell differentiation platforms could incorporate additional cell types such as TECs, which are critical for the formation of both CD8 and CD4 T cells in the thymus, or cells engineered to express HLA class II, to support the development of CD4 T cells. Alternatively, a better understanding of the cell intrinsic and extrinsic mechanisms that control T cell subset differentiation might enable derivation of CD4⁺ T cells using small molecules or other approaches, independently of interaction with HLA class II. However, while these alternative approaches might facilitate positive selection, proper negative selection of autoreactive T cells will likely require the presence of functional TECs, which have the unique ability to express a vast array of tissue-specific antigens, such as insulin [109]. Finally, while the functionality of SC-T cells has been shown in the context of tumor cells, it remains to be seen if SC-CD8⁺ T cells engineered to express an autoreactive TCR can target SC-β cells in an antigen-specific manner, to mimic how primary T cells and β cells interact in T1D patients.

4. THYMIC EPITHELIAL CELLS (TECS)

Given the critical role of the thymus in supporting the development of naive T cells, generating functional TECs from hPSCs has the potential to alleviate two key bottlenecks in modeling autoimmune diseases like T1D. First, distinct subpopulations of native TECs express HLA class II

molecules on their surface, thereby facilitating positive selection of developing CD4⁺ T cells. Second, TECs can mediate negative selection of developing T cells by presenting self-antigens normally found only in specialized tissues. Negative selection, which is paramount for establishing a diverse, functional, and self-tolerant T cell repertoire, is central to the development of autoimmune disease.

4.1. Current state of TEC differentiation

In vitro differentiation methods have been developed to produce thymic epithelial progenitors (TEPs) from human embryonic stem cells (hESCs) [110–114] and iPSCs [113,115–117] (SC-TEPs). These protocols recapitulate the multi-step development of the thymus and can generate immature SC-TEPs within ~2 weeks (reviewed in [118,119]). However, these protocols have variable and relatively low efficiency, likely due to low expression of the key thymic transcription factor FOXP1. SC-TEPs also lack expression of important late-stage TEC markers associated with functional maturation. Notably, some groups have shown a modest improvement in efficiency of TEP induction, as well as an increase in the expression of functional molecules such as CCL25 and DLL4, following overexpression of FOXP1 using recombinant proteins [112] or a lentivirus [120]. However, these strategies did not lead to upregulation of other functional proteins, like HLA class II, which are likely induced *in vivo* by crosstalk with thymocytes or with other stromal cells present in the thymus. A better understanding of signals regulating FOXP1 expression and activity is thus needed to improve *in vitro* differentiation methods. Recent studies analyzing pharyngeal endoderm development [121] and thymic tissue composition at different stages of life [122,123] may provide critical information on the regulatory programs driving human thymic differentiation. This information should also help expand the limited toolbox currently available to assess differentiation efficiencies into the thymic lineage. The lack of robust antibodies against critical transcription factors, such as PAX1 and FOXP1, has indeed made it challenging to optimize SC-TEP differentiation. Reporter lines tracking expression of PAX9 [124] and FOXP1 [113] have been developed, but require targeting of the reporter to be repeated for each new cell line and can lead to haploinsufficiency of the gene of interest. Cell surface markers have also been used to quantify differentiation efficiency [113,117], although they are not entirely specific to TEPs. Identifying and characterizing additional markers, including benchmarks that should be met to qualify as functional SC-TECs, would accelerate optimization of differentiation for multiple hPSC lines.

While SC-TEPs have not yet been reported to differentiate into functional TECs *in vitro*, they can further mature *in vivo* to support the development of murine T cells in athymic nude mice [110–112,115,117], or of human T cells in humanized mice [111,112,114] (Figure 4). These *in vivo* models provide hematopoietic cells and other stromal cells important for further TEC maturation. Although nude mice do not have a functional thymus due to a mutation in the *Foxn1* gene, they maintain a pool of hematopoietic stem and progenitor cells, which can give rise to T cells after transplantation of exogenous functional thymic tissue [125]. Importantly, thymopoiesis has been observed upon transplantation of human thymic tissue, demonstrating that human TECs can partially support the development of mouse thymocytes. This model is easier to establish since nude mice do not require irradiation and engraftment of HSCs prior to transplantation of SC-TEPs, but has limitations due to cross-species barriers. The limited ability of some murine cytokines and growth factors to signal through their human cognate receptors can indeed impair the crosstalk with thymocytes that is required for generating functionally mature TECs. Humanized mouse models (where human

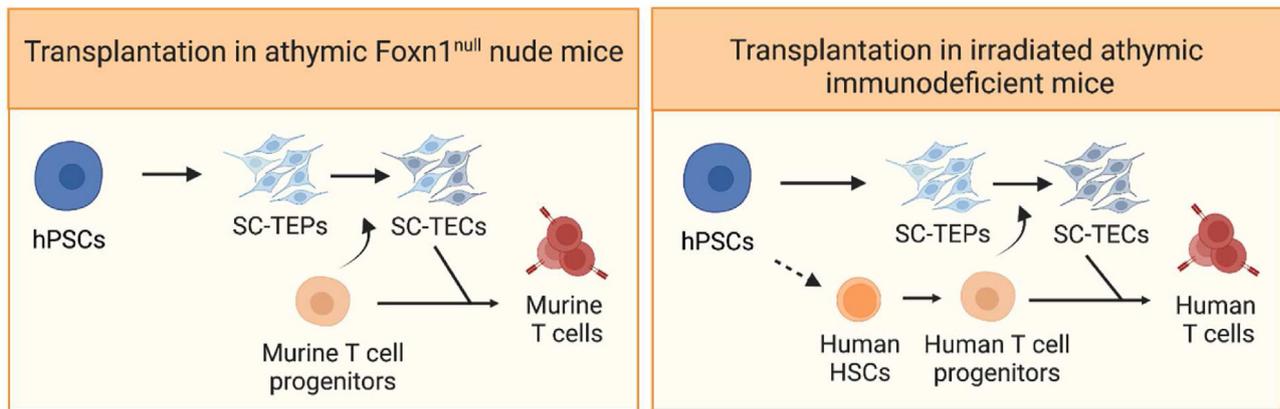


Figure 4: *In vivo* models to generate mature SC-TECs. SC-TEPs can currently be matured into functional SC-TECs that support the development of murine T cells using athymic nude mice or human T cells in irradiated athymic immunodeficient humanized mice. These established models will further knowledge of TEC maturation, which can be applied to improve *in vitro* differentiation of TECs to be incorporated in T1D disease platforms.

HSCs are engrafted in irradiated immunodeficient hosts) address some of these issues by incorporating human T cell progenitors. In these models (discussed in greater detail in a separate article of the HIRN review series [8]), mice are first thymectomized to prevent formation of T cells in the endogenous thymus, followed by irradiation and injection of allogeneic HSCs for long-term engraftment of human immune cells. Mice are then transplanted with SC-TEPs and assessed for engraftment of human immune cells and the formation of human T cells over months. While these humanized models are useful to study SC-TEP function, one caveat is that they do not generate isogenic T cells since the HSCs and TEPs are from different sources. Further optimization of differentiation methods to produce SC-HSCs capable of stable engraftment *in vivo* is thus required to generate isogenic T cells in such *in vivo* models.

4.2. Applications of SC-TECs in disease modeling

SC-TECs derived from iPSCs have been used to model disease in patients with severe combined immunodeficiencies (SCID). For example, Yamazaki and colleagues used iPSC-TEPs to show that loss-of-function mutations in the *PAX1* gene can cause a form of SCID due to altered thymus development [116]. A recent study also demonstrated that SC-TEPs can improve thymocyte development in humanized mice after integration in a supportive swine thymic structure [114]. While this study focused on control SC-TEPs, efforts are underway to generate SC-TEPs from T1D iPSCs or genetically modified hPSCs to gain insight into potential thymic defects in patients with T1D.

4.3. Limitations of SC-TECs in disease modeling

Generating isogenic systems using SC-TECs and SC-HSCs from the same cell line remains a highly sought-after goal. However, considerable challenges need to be addressed before these systems can be widely used to model human T1D.

4.3.1. SC-TECs maturation

One major issue is the complexity and timeline of current *in vivo* models for generating functional TECs. Nude mice are inherently limited due to cross-species barriers, while humanized mice are technically complex, requiring specialized techniques and reagents. Both nude and humanized mice are also impractical due to the extended timeline required to detect T cells. It is thus critical to

optimize *in vitro* models to alleviate some of these issues. It has, however, been challenging to identify culture conditions that can promote and maintain TECs in a functional state *in vitro*. Primary TECs grown in monolayer culture lose expression of functional molecules important to support T cell development, such as Notch ligands, FOXP1, and AIRE during *in vitro* expansion [126]. Alternatively, primary TECs and other stromal and lymphoid components assembled to form 3D organ cultures retain the ability to support T cell development *in vitro* [127], suggesting that a 3D environment is critical for TEC functionality. Directly exposing TECs to air, such as via air-liquid interface culture systems, also seems to be critical since fetal thymus lobes placed in low oxygen submersion FTOCs fail to support thymocyte development [128]. It is thus likely that a 3D air-liquid interface platform (similar to what has been described for primary TECs) will be necessary to support the generation and long-term culture of functional SC-TECs.

4.3.2. SC-TECs heterogeneity and organization

Another open question is whether reconstructing the complex structure of the thymus will be necessary to properly support the development and function of SC-TECs. The thymus is indeed organized into areas in which distinct TEC subsets support specific stages of T cell development [129]. In addition to TECs and immune cells, this complex 3D architecture includes other stromal cell types, which are likely important for the development and function of TECs [130]. Notably, platforms that can successfully support T cell maturation include a combination of stromal cells and hematopoietic cells [127]. The maturation and function of SC-TEPs could thus potentially be improved by reaggregation with support cells such as mesenchymal cells, endothelial cells, and pericytes, which are normally found in the human thymus [122]. Alternatively, SC-TEPs could be seeded in thymic scaffolds to enable their differentiation into functional TECs [114,131,132].

Finally, another limitation is the paucity of information on the repertoire of self-antigens expressed by SC-TECs. Given the critical role of AIRE-dependent antigens in shaping the T cell repertoire [109] as well as the more recent concept that TECs can modulate immune tolerance through the generation of heterogenous mimetic cell types [122,133], it will be important to characterize how expression of these antigens differs between SC-TECs and primary TECs.

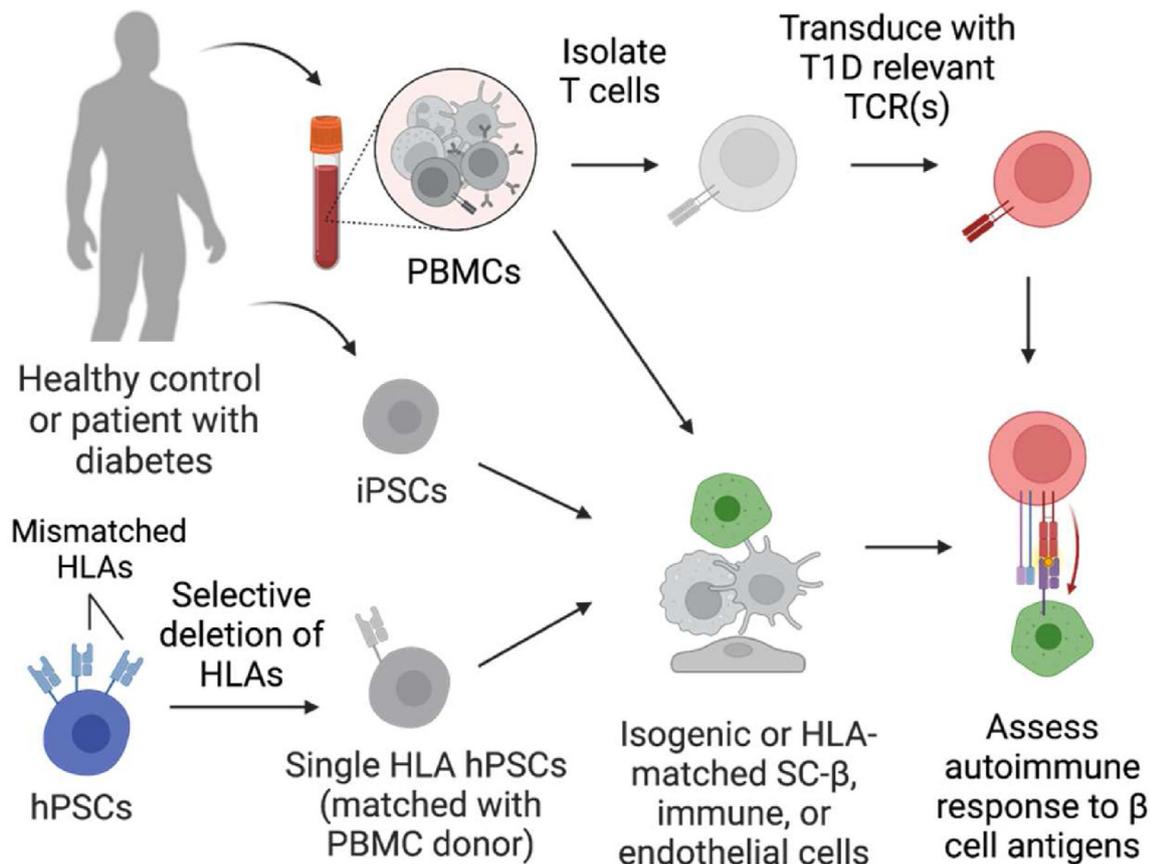


Figure 5: Development of stem cell-based multi-tissue platforms to model interactions between human β cells and immune cells. Different strategies combining isogenic or HLA-matched stem cell derivatives and primary immune cells have enabled assessment of autoimmune interactions between human SC- β cells and human immune cells.

5. DEVELOPMENT OF STEM CELL-BASED MULTI-TISSUE PLATFORMS

Given the complexity of T1D, next-generation models need to combine multiple cell types to gain deeper insight into the human disease. T1D is polygenic, with about 50% of disease heritability linked to HLA genes involved in antigen presentation. Genome-wide disease association studies have also identified >60 additional (non-HLA) genetic loci conferring T1D risk. Most of these map to DNA regulatory regions, but how they contribute mechanistically to the development of disease remains unknown (reviewed in [134]). Genes controlled by these loci could in principle act in β cells, T cells, innate immune cells, TECs, or in a combination of multiple cell types. Dissecting the role of each of these loci will thus require complex models wherein different combinations of control and genetically modified or patient-derived cells can be assessed in detail, with each specific question likely necessitating a discrete experimental set-up.

Models incorporating patient-derived and gene-modified SC derivatives *in vitro* and *in vivo* have started to emerge. For example, Castro-Gutierrez and colleagues demonstrated that genetically manipulating HLA or PD-L1 expression in SC- β cells reduced *in vitro* stimulation of autoreactive CD8 T cell transductants in a HLA-peptide-TCR dependent manner [135]. Genetically modified SC-islets have also been transplanted together with human primary T cells to enable assessment of allogeneic interactions between human SC- β cells and immune cells. Yoshihara et al. used NSG-SGM3 mice (NSG mice

expressing human IL3, GM-CSF, and SCF to support the stable engraftment of myeloid lineages and regulatory T cell populations) engrafted with human PBMCs to demonstrate that increased expression of the immune checkpoint protein PD-L1 can protect SC-islets from allogeneic rejection in this model [136]. Parent and colleagues also showed reduced allogeneic rejection of SC- β cells modified to express only one HLA allele in humanized NSG-MHC^{null} mice (NSG mice lacking major histocompatibility complex class I and II engrafted with human PBMCs) [137]. Although these multi-cellular systems have successfully been used to study allogeneic interactions, they are still limited in their ability to model autoimmune interactions due to the need to HLA-match immune cells with their target cells.

Whilst platforms combining isogenic SC-islets and SC-HSCs or SC-T cells are still in development, alternative isogenic models for autoimmune interactions use primary T cells together with iPSC-derived β cells generated from the same donor (Figure 5). For example, Leite and colleagues co-cultured enriched iPSC-derived α and β cells with donor-matched PBMCs, demonstrating *in vitro* T cell activation by autologous β cells when exposed to an ER stress inducer. However, activation of T cells occurred with cells from both healthy and T1D patients, suggesting additional steps are warranted to accurately model autoimmunity using this approach [64]. Genetic manipulation of stress- and immune-interaction-related genes can then protect the SC- β cells from autologous T cell-mediated apoptosis [138]. This illustrates the key advantage of amenability to genetic manipulation of SC- β cells, which is less tractable in primary cadaveric β cells.

Armitage and colleagues have also co-cultured genetically engineered primary T cells transduced with specific TCRs with donor-matched dendritic, macrophage, endothelial, and β cells generated from the same iPSC line [139]. Using this system, they showed that CD8⁺ T cells can be expanded by isogenic DC and macrophages. They also successfully modeled antigen-specific binding to endothelial cells, and lysis of isogenic SC- β cells by the transduced CD8⁺ T cells. The development of these isogenic models combining SC- β cells and primary T cells is critical, since it lays the foundation for assessing autoimmune interactions between T cells and SC- β cells. However, one limitation of this approach is the requirement to regularly obtain blood from the same patient who supplied somatic cells for iPSC reprogramming. This limits the number of immune cells available for each experiment and can complicate coordination when generating multiple cell types. The rarity of autoreactive T cells in the blood of patients is also problematic, but this caveat can be partially addressed by engineering T cells to express autoreactive TCRs to increase their frequency.

As an alternative to using iPSCs and PBMCs from the same donor, selective deletion of HLA genes using genome engineering can also enable assessment of autoimmune interactions (Figure 5). In this approach, HLA genes causing allogeneic responses in hPSCs are inactivated while antigen presentation capability is conserved through retention of HLA alleles relevant to T1D [137,140]. This strategy facilitates matching between SC- β cells and primary T cells from unrelated donors, since SC- β cells only express one HLA molecule that needs to be matched with PBMC donors. It also enables incorporating HLA-edited SC- β cells into humanized mice reconstituted with a combination of non-isogenic primary HSCs and thymus, which only need to be matched for the single HLA allele retained in SC- β cells (reviewed in detail in [8]) (Figure 1D). Taken together, different approaches have opened multiple paths to developing stem cell-based multi-tissue models to assess human autoimmune interactions.

6. REMAINING CHALLENGES AND FUTURE DIRECTIONS

Developing isogenic multi-tissue platforms to model human T1D that are fully stem cell-based entails differentiating a single hPSC line into multiple cell types relevant to the disease. This approach is not currently available, as it faces the limitations regarding differentiation into HSCs, T cells, and TECs described above. Some of these challenges are shared between different cell types, including issues with consistency of differentiation between cell lines and maturation *in vitro*. Whether directed differentiation can reproduce the heterogeneity of functional subsets seen with primary cells also remains to be seen. Overcoming these limitations will require synchronized efforts from many laboratories. Given that differentiation protocols currently need to be optimized for each cell line, the development of multicellular platforms would be accelerated by coordinating the choice of cell lines used by each group early in the process. This choice should take into consideration the ability of the cells to differentiate into the relevant lineages, the relevance of their HLA typing for T1D, and intellectual property matters. Additionally, methods for shipping and storing the cells that are compatible with long-distance collaborations need to be established, similar to what has recently been described for pancreatic islets [141]. There are indeed very few institutions that can generate all relevant cell types in the same location. While primary human islets and immune cells can be shipped across different locations, it remains to be determined if the same shipping conditions can be applied to stem cell derivatives.

When building models of human autoimmune diabetes, it is also critical to establish appropriate controls, including defining what is a physiologically relevant number of immune cells to include. It is indeed important to keep in mind that islet-reactive T cells are already present in healthy individuals, although they do not accumulate in islets to the same extent as in T1D patients [142,143]. In addition to defining T1D relevant T cell specificities, models should thus focus on understanding what attracts T cells to pancreatic islets, and what drives their local activation and proliferation in T1D but not healthy individuals. Finally, as the field continues to optimize methods to produce and characterize mature SC- β cells, SC-HSCs, SC-T cells, and SC-TECs, assays need to be developed to better understand the factors driving pathogenic interactions between β cells and the immune system. For SC- β cells, most studies have compared transcriptome or functional properties to their native counterparts, but very little attention has been focused on characterizing the expression of molecules critical for the immune response. For example, quantifying expression of immunomodulatory molecules, such as PD-L1, or developing assays to define the antigen repertoire associated with different HLAs in SC- β cells, will be critical to better understand how β cell-intrinsic factors influence autoimmune responses.

7. CONCLUSION

Recent technological advancements in hPSC differentiation, reprogramming, and genome engineering have unlocked new opportunities to model complex human diseases like T1D. While challenges remain to be overcome before entirely stem cell-based multi-tissue platforms are widely available, the value of developing them is undeniable. Efforts to model monogenic diabetes using SC- β cells, as well as recent progress in the development of multi-tissue platforms to assess allogeneic and autoimmune interactions, are already providing exciting insights that support the feasibility of this approach. New platforms combining SC- β cells with immune cells have the potential to provide critical information on the initiation and progression of human T1D and will also enable testing the effects of new therapeutics on multiple cell types relevant to the disease. Stem cell-based multi-tissue platforms are thus poised to accelerate the development and preclinical testing of immune-protective strategies that can be readily translated for the benefits of patients with diabetes.

FUNDING AND ACKNOWLEDGMENTS

This work was written using resources and/or funding provided by the NIDDK-supported Human Islet Research Network (HIRN, RRID: SCR_014393; <https://hirnetwork.org>). The following grants currently support (or have supported) the development and use of *in vitro* and/or *in vivo* models by the authors to study human T1D-associated autoimmune processes: K12DK94723 and K08DK131253 to KFL, U24-DK104162 to KFL and JRA-D, 1K01DK129442-01 to JRA-D, UC24 DK1041162 to HAR, U01DK123559 to AVP, Thomas J. Beatson, Jr. Foundation (grant 2019-019) to AVP, Juvenile Diabetes Research Foundation grant 5-COE-2019-860-S-B to AVP, UCSF Diabetes Center Innovation Award to AVP, and UCSF DRC P&F award P30DK063720 to AVP. Work in the Russ lab is also supported by NIH/NIDDK grant R01DK12044, R01DK132387, P30DK116073, the Culshaw Junior Investigator Award in Diabetes, the Children's Diabetes Foundation, the JDRF and the Gate's Grubstake Fund. Figures were created with BioRender (<http://www.biorender.com>).

CONFLICT OF INTEREST

The authors declare no conflict of interest in connection with this manuscript. HAR is a SAB member at Sigilon Therapeutics and Prellis Biologics and is a consultant for Eli Lilly and Minutia. AVP is a consultant for Minutia and is on the SAB of Thymune Therapeutics and holds stock options in the company.

DATA AVAILABILITY

No data was used for the research described in the article.

REFERENCES

- [1] Bluestone, J.A., Herold, K., Eisenbarth, G., 2010. Genetics, pathogenesis and clinical interventions in type 1 diabetes. *Nature* 464(7293):1293–1300. <https://doi.org/10.1038/nature08933>.
- [2] Atkinson, M.A., Eisenbarth, G.S., Michels, A.W., 2014. Type 1 diabetes. *The Lancet* 383(9911):69–82. [https://doi.org/10.1016/S0140-6736\(13\)60591-7](https://doi.org/10.1016/S0140-6736(13)60591-7).
- [3] Warshauer, J.T., Bluestone, J.A., Anderson, M.S., 2020. New frontiers in the treatment of type 1 diabetes. *Cell Metabolism* 31(1):46–61. <https://doi.org/10.1016/j.cmet.2019.11.017>.
- [4] Michels, A.W., Redondo, M.J., Atkinson, M.A., 2022. The pathogenesis, natural history, and treatment of type 1 diabetes: time (thankfully) does not stand still. *The Lancet. Diabetes & Endocrinology* 10(2):90–92. [https://doi.org/10.1016/S2213-8587\(21\)00344-2](https://doi.org/10.1016/S2213-8587(21)00344-2).
- [5] Mallone, R., Eizirik, D.L., 2020. Presumption of innocence for beta cells: why are they vulnerable autoimmune targets in type 1 diabetes? *Diabetologia* 63(10):1999–2006 <https://doi.org/10.1007/s00125-020-05176-7>.
- [6] Roep, B.O., Thomaidou, S., van Tienhoven, R., Zaldumbide, A., 2021. Type 1 diabetes mellitus as a disease of the β -cell (do not blame the immune system?). *Nature Reviews Endocrinology* 17(3):150–161. <https://doi.org/10.1038/s41574-020-00443-4>.
- [7] Battaglia, M., Ahmed, S., Anderson, M.S., Atkinson, M.A., Becker, D., Bingley, P.J., et al., 2020. Introducing the endotype concept to address the challenge of disease heterogeneity in type 1 diabetes. *Diabetes Care* 43(1): 5–12. <https://doi.org/10.2337/dc19-0880>.
- [8] Khosravi-Maharlooie, M., Madley, R., Borsotti, C., Ferreira, L.M.R., Sharp, R.C., Brehm, M.A., et al., 2022. Modeling human T1D-associated autoimmune processes. *Molecular Metabolism* 56:101417. <https://doi.org/10.1016/j.molmet.2021.101417>.
- [9] Roep, B.O., Atkinson, M., von Herrath, M., 2004. Satisfaction (not) guaranteed: re-evaluating the use of animal models of type 1 diabetes. *Nature Reviews Immunology* 4(12):989–997. <https://doi.org/10.1038/nri1502>.
- [10] Hockemeyer, D., Jaenisch, R., 2016. Induced pluripotent stem cells meet genome editing. *Cell Stem Cell* 18(5):573–586. <https://doi.org/10.1016/j.stem.2016.04.013>.
- [11] Maehr, R., Chen, S., Snitow, M., Ludwig, T., Yagasaki, L., Goland, R., et al., 2009. Generation of pluripotent stem cells from patients with type 1 diabetes. *Proceedings of the National Academy of Sciences of the United States of America* 106(37):15768–15773. <https://doi.org/10.1073/pnas.0906894106>.
- [12] Millman, J.R., Xie, C., Van Dervort, A., Gürtler, M., Pagliuca, F.W., Melton, D.A., 2016. Generation of stem cell-derived β -cells from patients with type 1 diabetes. *Nature Communications* 7:11463. <https://doi.org/10.1038/ncomms11463>.
- [13] Kissler, S., 2022. Genetic modifiers of thymic selection and central tolerance in type 1 diabetes. *Frontiers in Immunology* 13:889856. <https://doi.org/10.3389/fimmu.2022.889856>.
- [14] Joshi, K., Cameron, F., Tiwari, S., Mannering, S.I., Elefanty, A.G., Stanley, E.G., 2021. Modeling type 1 diabetes using pluripotent stem cell technology. *Frontiers in Endocrinology* 12:635662. <https://doi.org/10.3389/fendo.2021.635662>.
- [15] Migliorini, A., Nostro, M.C., Sneddon, J.B., 2021. Human pluripotent stem cell-derived insulin-producing cells: a regenerative medicine perspective. *Cell Metabolism* 33(4):721–731. <https://doi.org/10.1016/j.cmet.2021.03.021>.
- [16] Velazco-Cruz, L., Goedegebuure, M.M., Millman, J.R., 2020. Advances toward engineering functionally mature human pluripotent stem cell-derived β cells. *Frontiers in Bioengineering and Biotechnology* 8:786. <https://doi.org/10.3389/fbioe.2020.00786>.
- [17] Bourgeois, S., Sawatani, T., Van Mulders, A., De Leu, N., Heremans, Y., Heimberg, H., et al., 2021. Towards a functional cure for diabetes using stem cell-derived beta cells: are we there yet? *Cells* 10(1) <https://doi.org/10.3390/cells10010191>.
- [18] Siehler, J., Blöchliger, A.K., Meier, M., Lickert, H., 2021. Engineering islets from stem cells for advanced therapies of diabetes. *Nature Reviews Drug Discovery* 20(12):920–940. <https://doi.org/10.1038/s41573-021-00262-w>.
- [19] Gromada, J., Chabosseau, P., Rutter, G.A., 2018. The α -cell in diabetes mellitus. *Nature Reviews Endocrinology* 14(12):694–704. <https://doi.org/10.1038/s41574-018-0097-y>.
- [20] Salvatori, M., Katari, R., Patel, T., Peloso, A., Mugweru, J., Owusu, K., et al., 2014. Extracellular matrix scaffold technology for bioartificial pancreas engineering: state of the art and future challenges. *Journal of Diabetes Science and Technology* 8(1):159–169. <https://doi.org/10.1177/1932296813519558>.
- [21] Sneddon, J.B., Tang, Q., Stock, P., Bluestone, J.A., Roy, S., Desai, T., et al., 2018. Stem cell therapies for treating diabetes: progress and remaining challenges. *Cell Stem Cell* 22(6):810–823. <https://doi.org/10.1016/j.stem.2018.05.016>.
- [22] Arda, H.E., Benitez, C.M., Kim, S.K., 2013. Gene regulatory networks governing pancreas development. *Developmental Cell* 25(1):5–13. <https://doi.org/10.1016/j.devcel.2013.03.016>.
- [23] Zaret, K.S., 2008. Genetic programming of liver and pancreas progenitors: lessons for stem-cell differentiation. *Nature Reviews Genetics* 9(5):329–340. <https://doi.org/10.1038/nrg2318>.
- [24] Zorn, A.M., Wells, J.M., 2009. Vertebrate endoderm development and organ formation. *Annual Review of Cell and Developmental Biology* 25:221–251. <https://doi.org/10.1146/annurev.cellbio.042308.113344>.
- [25] Pagliuca, F.W., Millman, J.R., Gürtler, M., Segel, M., Van Dervort, A., Ryu, J.H., et al., 2014. Generation of functional human pancreatic β cells in vitro. *Cell* 159(2):428–439. <https://doi.org/10.1016/j.cell.2014.09.040>.
- [26] Rezanian, A., Bruin, J.E., Arora, P., Rubin, A., Batushansky, I., Asadi, A., et al., 2014. Reversal of diabetes with insulin-producing cells derived in vitro from human pluripotent stem cells. *Nature Biotechnology* 32(11):1121–1133. <https://doi.org/10.1038/nbt.3033>.
- [27] Russ, H.A., Parent, A.V., Ringler, J.J., Hennings, T.G., Nair, G.G., Shveygert, M., et al., 2015. Controlled induction of human pancreatic progenitors produces functional beta-like cells in vitro. *The EMBO Journal* 34(13):1759–1772. <https://doi.org/10.15252/emboj.2015191058>.
- [28] Hrvatin, S., O'Donnell, C.W., Deng, F., Millman, J.R., Pagliuca, F.W., Dilorio, P., et al., 2014. Differentiated human stem cells resemble fetal, not adult, β cells. *Proceedings of the National Academy of Sciences of the United States of America* 111(8):3038–3043. <https://doi.org/10.1073/pnas.1400709111>.
- [29] Wesolowska-Andersen, A., Jensen, R.R., Alcántara, M.P., Beer, N.L., Duff, C., Nylander, V., et al., 2020. Analysis of differentiation protocols defines a common pancreatic progenitor molecular signature and guides refinement of endocrine differentiation. *Stem Cell Reports* 14(1):138–153. <https://doi.org/10.1016/j.stemcr.2019.11.010>.

- [30] Augsornworawat, P., Maxwell, K.G., Velazco-Cruz, L., Millman, J.R., 2020. Single-cell transcriptome profiling reveals β cell maturation in stem cell-derived islets after transplantation. *Cell Reports* 32(8):108067. <https://doi.org/10.1016/j.celrep.2020.108067>.
- [31] Arda, H.E., Li, L., Tsai, J., Torre, E.A., Rosli, Y., Peiris, H., et al., 2016. Age-dependent pancreatic gene regulation reveals mechanisms governing human β cell function. *Cell Metabolism* 23(5):909–920. <https://doi.org/10.1016/j.cmet.2016.04.002>.
- [32] Petersen, M.B.K., Azad, A., Ingvorsen, C., Hess, K., Hansson, M., Grapin-Botton, A., et al., 2017. Single-cell gene expression analysis of a human ESC model of pancreatic endocrine development reveals different paths to β -cell differentiation. *Stem Cell Reports* 9(4):1246–1261. <https://doi.org/10.1016/j.stemcr.2017.08.009>.
- [33] Alvarez-Dominguez, J.R., Donaghey, J., Rasouli, N., Kenty, J.H.R., Helman, A., Charlton, J., et al., 2020. Circadian entrainment triggers maturation of human in vitro islets. *Cell Stem Cell* 26(1):108–122.e10. <https://doi.org/10.1016/j.stem.2019.11.011>.
- [34] Wang, A., Yue, F., Li, Y., Xie, R., Harper, T., Patel, N.A., et al., 2015. Epigenetic priming of enhancers predicts developmental competence of hESC-derived endodermal lineage intermediates. *Cell Stem Cell* 16(4):386–399. <https://doi.org/10.1016/j.stem.2015.02.013>.
- [35] Xie, R., Everett, L.J., Lim, H.-W., Patel, N.A., Schug, J., Kroon, E., et al., 2013. Dynamic chromatin remodeling mediated by polycomb proteins orchestrates pancreatic differentiation of human embryonic stem cells. *Cell Stem Cell* 12(2):224–237. <https://doi.org/10.1016/j.stem.2012.11.023>.
- [36] Vethe, H., Bjørlykke, Y., Ghila, L.M., Paulo, J.A., Scholz, H., Gygi, S.P., et al., 2017. Probing the missing mature β -cell proteomic landscape in differentiating patient iPSC-derived cells. *Scientific Reports* 7(1):4780. <https://doi.org/10.1038/s41598-017-04979-w>.
- [37] Davis, J.C., Alves, T.C., Helman, A., Chen, J.C., Kenty, J.H., Cardone, R.L., et al., 2020. Glucose response by stem cell-derived β cells in vitro is inhibited by a bottleneck in glycolysis. *Cell Reports* 31(6):107623. <https://doi.org/10.1016/j.celrep.2020.107623>.
- [38] Balboa, D., Barsby, T., Lithovius, V., Saarimäki-Vire, J., Omar-Hmeadi, M., Dyachok, O., et al., 2022. Functional, metabolic and transcriptional maturation of human pancreatic islets derived from stem cells. *Nature Biotechnology* 40(7):1042–1055. <https://doi.org/10.1038/s41587-022-01219-z>.
- [39] Veres, A., Faust, A.L., Bushnell, H.L., Engquist, E.N., Kenty, J.H.-R., Harb, G., et al., 2019. Charting cellular identity during human in vitro β -cell differentiation. *Nature* 569(7756):368–373. <https://doi.org/10.1038/s41586-019-1168-5>.
- [40] Velazco-Cruz, L., Song, J., Maxwell, K.G., Goedegebuure, M.M., Augsornworawat, P., Hogrebe, N.J., et al., 2019. Acquisition of dynamic function in human stem cell-derived β cells. *Stem Cell Reports* 12(2):351–365. <https://doi.org/10.1016/j.stemcr.2018.12.012>.
- [41] Ghazizadeh, Z., Kao, D.-I., Amin, S., Cook, B., Rao, S., Zhou, T., et al., 2017. ROCKII inhibition promotes the maturation of human pancreatic beta-like cells. *Nature Communications* 8(1):298. <https://doi.org/10.1038/s41467-017-00129-y>.
- [42] Yoshihara, E., Wei, Z., Lin, C.S., Fang, S., Ahmadian, M., Kida, Y., et al., 2016. ERR γ is required for the metabolic maturation of therapeutically functional glucose-responsive β cells. *Cell Metabolism* 23(4):622–634. <https://doi.org/10.1016/j.cmet.2016.03.005>.
- [43] Sui, L., Xin, Y., Du, Q., Georgieva, D., Diedenhofen, G., Haataja, L., et al., 2021. Reduced replication fork speed promotes pancreatic endocrine differentiation and controls graft size. *Journal of Clinical Investigation* 131(5):141553. <https://doi.org/10.1172/jci.insight.141553>.
- [44] Cogger, K.F., Sinha, A., Sarangi, F., McGaugh, E.C., Saunders, D., Dorrell, C., et al., 2017. Glycoprotein 2 is a specific cell surface marker of human pancreatic progenitors. *Nature Communications* 8(1):331. <https://doi.org/10.1038/s41467-017-00561-0>.
- [45] Ameri, J., Borup, R., Prawiro, C., Ramond, C., Schachter, K.A., Scharfmann, R., et al., 2017. Efficient generation of glucose-responsive beta cells from isolated GP2+ human pancreatic progenitors. *Cell Reports* 19(1):36–49. <https://doi.org/10.1016/j.celrep.2017.03.032>.
- [46] Li, X., Yang, K.Y., Chan, V.W., Leung, K.T., Zhang, X.-B., Wong, A.S., et al., 2020. Single-cell RNA-Seq reveals that CD9 is a negative marker of glucose-responsive pancreatic β -like cells derived from human pluripotent stem cells. *Stem Cell Reports* 15(5):1111–1126. <https://doi.org/10.1016/j.stemcr.2020.09.009>.
- [47] Mahaddalkar, P.U., Scheibner, K., Pfluger, S., Ansarullah, Sterr, M., Beckenbauer, J., et al., 2020. Generation of pancreatic β cells from CD177+ anterior definitive endoderm. *Nature Biotechnology* 38(9):1061–1072. <https://doi.org/10.1038/s41587-020-0492-5>.
- [48] Zhu, S., Russ, H.A., Wang, X., Zhang, M., Ma, T., Xu, T., et al., 2016. Human pancreatic beta-like cells converted from fibroblasts. *Nature Communications* 7:10080. <https://doi.org/10.1038/ncomms10080>.
- [49] Nair, G.G., Liu, J.S., Russ, H.A., Tran, S., Saxton, M.S., Chen, R., et al., 2019. Recapitulating endocrine cell clustering in culture promotes maturation of human stem-cell-derived β cells. *Nature Cell Biology* 21(2):263–274. <https://doi.org/10.1038/s41556-018-0271-4>.
- [50] Parent, A.V., Ashe, S., Nair, G.G., Li, M.-L., Chavez, J., Liu, J.S., et al., 2022. Development of a scalable method to isolate subsets of stem cell-derived pancreatic islet cells. *Stem Cell Reports*. <https://doi.org/10.1016/j.stemcr.2022.02.001>.
- [51] Davis, J.C., Helman, A., Rivera-Feliciano, J., Langston, C.M., Engquist, E.N., Melton, D.A., 2019. Live cell monitoring and enrichment of stem cell-derived β cells using intracellular zinc content as a population marker. *Current Protocols in Stem Cell Biology* 51(1):e99. <https://doi.org/10.1002/cpsc.99>.
- [52] Rezaia, A., Riedel, M.J., Wideman, R.D., Karanu, F., Ao, Z., Warnock, G.L., et al., 2011. Production of functional glucagon-secreting α -cells from human embryonic stem cells. *Diabetes* 60(1):239–247. <https://doi.org/10.2337/db10-0573>.
- [53] Peterson, Q.P., Veres, A., Chen, L., Slama, M.Q., Kenty, J.H.R., Hassoun, S., et al., 2020. A method for the generation of human stem cell-derived alpha cells. *Nature Communications* 11(1):2241. <https://doi.org/10.1038/s41467-020-16049-3>.
- [54] Russell, R., Carnese, P.P., Hennings, T.G., Walker, E.M., Russ, H.A., Liu, J.S., et al., 2020. Loss of the transcription factor MAFB limits β -cell derivation from human PSCs. *Nature Communications* 11(1):2742. <https://doi.org/10.1038/s41467-020-16550-9>.
- [55] Yabe, S.G., Fukuda, S., Nishida, J., Takeda, F., Nashiro, K., Okochi, H., 2021. Efficient induction of pancreatic alpha cells from human induced pluripotent stem cells by controlling the timing for BMP antagonism and activation of retinoic acid signaling. *PLoS One* 16(1):e0245204. <https://doi.org/10.1371/journal.pone.0245204>.
- [56] Sharon, N., Chawla, R., Mueller, J., Vanderhooft, J., Whitehorn, L.J., Rosenthal, B., et al., 2019. A peninsular structure coordinates asynchronous differentiation with morphogenesis to generate pancreatic islets. *Cell* 176(4):790–804.e13. <https://doi.org/10.1016/j.cell.2018.12.003>.
- [57] Balboa, D., Prasad, R.B., Groop, L., Otonkoski, T., 2019. Genome editing of human pancreatic beta cell models: problems, possibilities and outlook. *Diabetologia* 62(8):1329–1336. <https://doi.org/10.1007/s00125-019-4908-z>.
- [58] George, M.N., Leavens, K.F., Gadue, P., 2021. Genome editing human pluripotent stem cells to model β -cell disease and unmask novel genetic modifiers. *Frontiers in Endocrinology* 12:682625. <https://doi.org/10.3389/fendo.2021.682625>.
- [59] Balboa, D., Saarimäki-Vire, J., Borshagovski, D., Survila, M., Lindholm, P., Galli, E., et al., 2018. Insulin mutations impair beta-cell development in a patient-derived iPSC model of neonatal diabetes. *Elife* 7. <https://doi.org/10.7554/eLife.38519>.

- [60] Saarimäki-Vire, J., Balboa, D., Russell, M.A., Saarikettu, J., Kinnunen, M., Keskitalo, S., et al., 2017. An activating STAT3 mutation causes neonatal diabetes through premature induction of pancreatic differentiation. *Cell Reports* 19(2):281–294. <https://doi.org/10.1016/j.celrep.2017.03.055>.
- [61] Cardenas-Diaz, F.L., Osorio-Quintero, C., Diaz-Miranda, M.A., Kishore, S., Leavens, K., Jobaliya, C., et al., 2019. Modeling monogenic diabetes using human ESCs reveals developmental and metabolic deficiencies caused by mutations in HNF1A. *Cell Stem Cell* 25(2):273–289.e5. <https://doi.org/10.1016/j.stem.2019.07.007>.
- [62] Thatava, T., Kudva, Y.C., Edukulla, R., Squillace, K., De Lamo, J.G., Khan, Y.K., et al., 2013. Inpatient variations in type 1 diabetes-specific IPS cell differentiation into insulin-producing cells. *Molecular Therapy* 21(1): 228–239. <https://doi.org/10.1038/mt.2012.245>.
- [63] Sui, L., Leibel, R.L., Egli, D., 2018. Pancreatic beta cell differentiation from human pluripotent stem cells. *Current Protocols in Human Genetics* 99(1): e68. <https://doi.org/10.1002/cphg.68>.
- [64] Leite, N.C., Sintov, E., Meissner, T.B., Brehm, M.A., Greiner, D.L., Harlan, D.M., et al., 2020. Modeling type 1 diabetes in vitro using human pluripotent stem cells. *Cell Reports* 32(2):107894. <https://doi.org/10.1016/j.celrep.2020.107894>.
- [65] Docherty, F.M., Riemondy, K.A., Castro-Gutierrez, R., Dwulet, J.M., Shilleh, A.H., Hansen, M.S., et al., 2021. ENTPD3 marks mature stem cell-derived β -cells formed by self-aggregation in vitro. *Diabetes* 70(11):2554–2567. <https://doi.org/10.2337/db20-0873>.
- [66] Hogrebe, N.J., Maxwell, K.G., Augsornworawat, P., Millman, J.R., 2021. Generation of insulin-producing pancreatic β cells from multiple human stem cell lines. *Nature Protocols* 16(9):4109–4143. <https://doi.org/10.1038/s41596-021-00560-y>.
- [67] Sui, L., Danzl, N., Campbell, S.R., Viola, R., Williams, D., Xing, Y., et al., 2018. β -Cell replacement in mice using human type 1 diabetes nuclear transfer embryonic stem cells. *Diabetes* 67(1):26–35. <https://doi.org/10.2337/db17-0120>.
- [68] Kaestner, K.H., Campbell-Thompson, M., Dor, Y., Gill, R.G., Glaser, B., Kim, S.K., et al., 2021. What is a β cell? - chapter I in the human islet research Network (HIRN) review series. *Molecular Metabolism* 53:101323. <https://doi.org/10.1016/j.molmet.2021.101323>.
- [69] Cabrera, O., Berman, D.M., Kenyon, N.S., Ricordi, C., Berggren, P.-O., Caicedo, A., 2006. The unique cytoarchitecture of human pancreatic islets has implications for islet cell function. *Proceedings of the National Academy of Sciences of the United States of America* 103(7):2334–2339. <https://doi.org/10.1073/pnas.0510790103>.
- [70] Kelsey, G., Stegle, O., Reik, W., 2017. Single-cell epigenomics: recording the past and predicting the future. *Science* 358(6359):69–75. <https://doi.org/10.1126/science.aan6826>.
- [71] Slavov, N., 2020. Unpicking the proteome in single cells. *Science* 367(6477): 512–513. <https://doi.org/10.1126/science.aaz6695>.
- [72] Tanay, A., Regev, A., 2017. Scaling single-cell genomics from phenomenology to mechanism. *Nature* 541(7637):331–338. <https://doi.org/10.1038/nature21350>.
- [73] Rasouli, N., Melton, D.A., Alvarez-Dominguez, J.R., 2020. Purification of live stem-cell-derived islet lineage intermediates. *Current Protocols in Stem Cell Biology* 53(1):e111. <https://doi.org/10.1002/cpsc.111>.
- [74] Leavens, K.F., Liao, C.-M., Gagne, A.L., Kishore, S., Cardenas-Diaz, F.L., French, D.L., et al., 2020. Generation of a double insulin and somatostatin reporter line, SCSe001-A-3, for the advancement of stem cell-derived pancreatic islets. *Stem Cell Research* 50:102112. <https://doi.org/10.1016/j.scr.2020.102112>.
- [75] Baron, C.S., Barve, A., Muraro, M.J., van der Linden, R., Dharmadhikari, G., Lyubimova, A., et al., 2019. Cell type purification by single-cell transcriptome-trained sorting. *Cell* 179(2):527–542.e19. <https://doi.org/10.1016/j.cell.2019.08.006>.
- [76] Alvarez-Dominguez, J.R., Melton, D.A., 2022. Cell maturation: hallmarks, triggers, and manipulation. *Cell* 185(2):235–249. <https://doi.org/10.1016/j.cell.2021.12.012>.
- [77] Faleo, G., Russ, H.A., Wisel, S., Parent, A.V., Nguyen, V., Nair, G.G., et al., 2017. Mitigating ischemic injury of stem cell-derived insulin-producing cells after transplant. *Stem Cell Reports* 9(3):807–819. <https://doi.org/10.1016/j.stemcr.2017.07.012>.
- [78] Hayek, A., Beattie, G.M., 1997. Experimental transplantation of human fetal and adult pancreatic islets. *The Journal of Clinical Endocrinology and Metabolism* 82(8):2471–2475. <https://doi.org/10.1210/jcem.82.8.4151>.
- [79] Insel, R.A., Dunne, J.L., Atkinson, M.A., Chiang, J.L., Dabelea, D., Gottlieb, P.A., et al., 2015. Staging presymptomatic type 1 diabetes: a scientific statement of JDRF, the Endocrine Society, and the American Diabetes Association. *Diabetes Care* 38(10):1964–1974. <https://doi.org/10.2337/dc15-1419>.
- [80] Benninger, R.K.P., Kravets, V., 2022. The physiological role of β -cell heterogeneity in pancreatic islet function. *Nature Reviews Endocrinology* 18(1): 9–22. <https://doi.org/10.1038/s41574-021-00568-0>.
- [81] Rui, J., Deng, S., Arazi, A., Perdigoto, A.L., Liu, Z., Herold, K.C., 2017. β cells that resist immunological attack develop during progression of autoimmune diabetes in NOD mice. *Cell Metabolism* 25(3):727–738. <https://doi.org/10.1016/j.cmet.2017.01.005>.
- [82] Chadwick, K., Wang, L., Li, L., Menendez, P., Murdoch, B., Rouleau, A., et al., 2003. Cytokines and BMP-4 promote hematopoietic differentiation of human embryonic stem cells. *Blood* 102(3):906–915. <https://doi.org/10.1182/blood-2003-03-0832>.
- [83] Kaufman, D.S., Hanson, E.T., Lewis, R.L., Auerbach, R., Thomson, J.A., 2001. Hematopoietic colony-forming cells derived from human embryonic stem cells. *Proceedings of the National Academy of Sciences of the United States of America* 98(19):10716–10721. <https://doi.org/10.1073/pnas.191362598>.
- [84] Vodyanik, M.A., Bork, J.A., Thomson, J.A., Slukvin, I.I., 2005. Human embryonic stem cell-derived CD34+ cells: efficient production in the coculture with OP9 stromal cells and analysis of lymphohematopoietic potential. *Blood* 105(2):617–626. <https://doi.org/10.1182/blood-2004-04-1649>.
- [85] Doulatov, S., Vo, L.T., Chou, S.S., Kim, P.G., Arora, N., Li, H., et al., 2013. Induction of multipotential hematopoietic progenitors from human pluripotent stem cells via respecification of lineage-restricted precursors. *Cell Stem Cell* 13(4):459–470. <https://doi.org/10.1016/j.stem.2013.09.002>.
- [86] Vo, L.T., Kinney, M.A., Liu, X., Zhang, Y., Barragan, J., Sousa, P.M., et al., 2018. Regulation of embryonic haematopoietic multipotency by EZH1. *Nature* 553(7689):506–510. <https://doi.org/10.1038/nature25435>.
- [87] Dzierzak, E., Speck, N.A., 2008. Of lineage and legacy: the development of mammalian hematopoietic stem cells. *Nature Immunology* 9(2):129–136. <https://doi.org/10.1038/ni1560>.
- [88] Sturgeon, C.M., Ditadi, A., Awong, G., Kennedy, M., Keller, G., 2014. Wnt signaling controls the specification of definitive and primitive hematopoiesis from human pluripotent stem cells. *Nature Biotechnology* 32(6):554–561. <https://doi.org/10.1038/nbt.2915>.
- [89] Timmermans, F., Velghe, I., Vanwalleghem, L., De Smedt, M., Van Coppennolle, S., Taghon, T., et al., 2009. Generation of T cells from human embryonic stem cell-derived hematopoietic zones. *Journal of Immunology* 182(11):6879–6888. <https://doi.org/10.4049/jimmunol.0803670>.
- [90] Kennedy, M., Awong, G., Sturgeon, C.M., Ditadi, A., LaMotte-Mohs, R., Zúñiga-Pflücker, J.C., et al., 2012. T lymphocyte potential marks the emergence of definitive hematopoietic progenitors in human pluripotent stem cell differentiation cultures. *Cell Reports* 2(6):1722–1735. <https://doi.org/10.1016/j.celrep.2012.11.003>.
- [91] Nishimura, T., Kaneko, S., Kawana-Tachikawa, A., Tajima, Y., Goto, H., Zhu, D., et al., 2013. Generation of rejuvenated antigen-specific T cells by

- reprogramming to pluripotency and redifferentiation. *Cell Stem Cell* 12(1): 114–126. <https://doi.org/10.1016/j.stem.2012.11.002>.
- [92] Themeli, M., Kloss, C.C., Ciriello, G., Fedorov, V.D., Perna, F., Gonen, M., et al., 2013. Generation of tumor-targeted human T lymphocytes from induced pluripotent stem cells for cancer therapy. *Nature Biotechnology* 31(10):928–933. <https://doi.org/10.1038/nbt.2678>.
- [93] Vizcardo, R., Masuda, K., Yamada, D., Ikawa, T., Shimizu, K., Fujii, S.-I., et al., 2013. Regeneration of human tumor antigen-specific T cells from iPSCs derived from mature CD8(+) T cells. *Cell Stem Cell* 12(1):31–36. <https://doi.org/10.1016/j.stem.2012.12.006>.
- [94] Seet, C.S., He, C., Bethune, M.T., Li, S., Chick, B., Gschwend, E.H., et al., 2017. Generation of mature T cells from human hematopoietic stem and progenitor cells in artificial thymic organoids. *Nature Methods* 14(5):521–530. <https://doi.org/10.1038/nmeth.4237>.
- [95] Montel-Hagen, A., Seet, C.S., Li, S., Chick, B., Zhu, Y., Chang, P., et al., 2019. Organoid-induced differentiation of conventional T cells from human pluripotent stem cells. *Cell Stem Cell* 24(3):376–389.e8. <https://doi.org/10.1016/j.stem.2018.12.011>.
- [96] Wang, Z., McWilliams-Koeppen, H.P., Reza, H., Ostberg, J.R., Chen, W., Wang, X., et al., 2022. 3D-organoid culture supports differentiation of human CAR+ iPSCs into highly functional CAR T cells. *Cell Stem Cell* 29(4):515–527.e8. <https://doi.org/10.1016/j.stem.2022.02.009>.
- [97] Cheroutre, H., Lambolez, F., 2008. Doubting the TCR coreceptor function of CD8alpha. *Immunity* 28(2):149–159. <https://doi.org/10.1016/j.immuni.2008.01.005>.
- [98] Georgomanoli, M., Papapetrou, E.P., 2019. Modeling blood diseases with human induced pluripotent stem cells. *Disease Models & Mechanisms* 12(6). <https://doi.org/10.1242/dmm.039321>.
- [99] Chang, C.-W., Lai, Y.-S., Westin, E., Khodadadi-Jamayran, A., Pawlik, K.M., Lamb, L.S., et al., 2015. Modeling human severe combined immunodeficiency and correction by CRISPR/Cas9-Enhanced gene targeting. *Cell Reports* 12(10):1668–1677. <https://doi.org/10.1016/j.celrep.2015.08.013>.
- [100] Menon, T., Firth, A.L., Scripture-Adams, D.D., Galic, Z., Qualls, S.J., Gilmore, W.B., et al., 2015. Lymphoid regeneration from gene-corrected SCID-X1 subject-derived iPSCs. *Cell Stem Cell* 16(4):367–372. <https://doi.org/10.1016/j.stem.2015.02.005>.
- [101] Brauer, P.M., Pessach, I.M., Clarke, E., Rowe, J.H., Ott de Bruin, L., Lee, Y.N., et al., 2016. Modeling altered T-cell development with induced pluripotent stem cells from patients with RAG1-dependent immune deficiencies. *Blood* 128(6):783–793. <https://doi.org/10.1182/blood-2015-10-676304>.
- [102] Themeli, M., Chhatta, A., Boersma, H., Prins, H.J., Cordes, M., de Wilt, E., et al., 2020. iPSC-based modeling of RAG2 severe combined immunodeficiency reveals multiple T cell developmental arrests. *Stem Cell Reports* 14(2): 300–311. <https://doi.org/10.1016/j.stemcr.2019.12.010>.
- [103] Laskowski, T.J., Van Caenegem, Y., Pourebrahim, R., Ma, C., Ni, Z., Garate, Z., et al., 2016. Gene correction of iPSCs from a wiskott-aldrich syndrome patient normalizes the lymphoid developmental and functional defects. *Stem Cell Reports* 7(2):139–148. <https://doi.org/10.1016/j.stemcr.2016.06.003>.
- [104] Jing, R., Scarfo, I., Najja, M.A., Lummertz da Rocha, E., Han, A., Sanborn, M., et al., 2022. EZH1 repression generates mature iPSC-derived CAR T cells with enhanced antitumor activity. *Cell Stem Cell* 29(8):1181–1196.e6. <https://doi.org/10.1016/j.stem.2022.06.014>.
- [105] Sugimura, R., Jha, D.K., Han, A., Soria-Valles, C., da Rocha, E.L., Lu, Y.-F., et al., 2017. Haematopoietic stem and progenitor cells from human pluripotent stem cells. *Nature* 545(7655):432–438. <https://doi.org/10.1038/nature22370>.
- [106] Trotman-Grant, A.C., Mohtashami, M., De Sousa Casal, J., Martinez, E.C., Lee, D., Teichman, S., et al., 2021. DL4-μbeads induce T cell lineage differentiation from stem cells in a stromal cell-free system. *Nature Communications* 12(1):5023. <https://doi.org/10.1038/s41467-021-25245-8>.
- [107] Michaels, Y.S., Edgar, J.M., Major, M.C., Castle, E.L., Zimmerman, C., Yin, T., et al., 2022. DLL4 and VCAM1 enhance the emergence of T cell-competent hematopoietic progenitors from human pluripotent stem cells. *Science Advances* 8(34):eabn5522. <https://doi.org/10.1126/sciadv.abn5522>.
- [108] Iriguchi, S., Yasui, Y., Kawai, Y., Arima, S., Kunitomo, M., Sato, T., et al., 2021. A clinically applicable and scalable method to regenerate T-cells from iPSCs for off-the-shelf T-cell immunotherapy. *Nature Communications* 12(1): 430. <https://doi.org/10.1038/s41467-020-20658-3>.
- [109] Anderson, M.S., Venanzi, E.S., Klein, L., Chen, Z., Berzins, S.P., Turley, S.J., et al., 2002. Projection of an immunological self shadow within the thymus by the aire protein. *Science* 298(5597):1395–1401. <https://doi.org/10.1126/science.1075958>.
- [110] Parent, A.V., Russ, H.A., Khan, I.S., LaFlam, T.N., Metzger, T.C., Anderson, M.S., et al., 2013. Generation of functional thymic epithelium from human embryonic stem cells that supports host T cell development. *Cell Stem Cell* 13(2):219–229. <https://doi.org/10.1016/j.stem.2013.04.004>.
- [111] Sun, X., Xu, J., Lu, H., Liu, W., Miao, Z., Sui, X., et al., 2013. Directed differentiation of human embryonic stem cells into thymic epithelial progenitor-like cells reconstitutes the thymic microenvironment in vivo. *Cell Stem Cell* 13(2):230–236. <https://doi.org/10.1016/j.stem.2013.06.014>.
- [112] Su, M., Hu, R., Jin, J., Yan, Y., Song, Y., Sullivan, R., et al., 2015. Efficient in vitro generation of functional thymic epithelial progenitors from human embryonic stem cells. *Scientific Reports* 5:9882. <https://doi.org/10.1038/srep09882>.
- [113] Soh, C.-L., Giudice, A., Jenny, R.A., Elliott, D.A., Hatzistavrou, T., Micallef, S.J., et al., 2014. FOXP1 (GFP/w) reporter hESCs enable identification of integrin-β4, HLA-DR, and EpCAM as markers of human PSC-derived FOXP1(+) thymic epithelial progenitors. *Stem Cell Reports* 2(6):925–937. <https://doi.org/10.1016/j.stemcr.2014.04.009>.
- [114] Gras-Peña, R., Danzl, N.M., Khosravi-Maharlooei, M., Campbell, S.R., Ruiz, A.E., Parks, C.A., et al., 2021. Human stem cell-derived thymic epithelial cells enhance human T-cell development in a xenogeneic thymus. *The Journal of Allergy and Clinical Immunology*. <https://doi.org/10.1016/j.jaci.2021.09.038>.
- [115] Chhatta, A.R., Cordes, M., Hanegraaf, M.A.J., Vloemans, S., Cupedo, T., Cornelissen, J.J., et al., 2019. De novo generation of a functional human thymus from induced pluripotent stem cells. *The Journal of Allergy and Clinical Immunology* 144(5):1416–1419.e7. <https://doi.org/10.1016/j.jaci.2019.05.042>.
- [116] Yamazaki, Y., Urrutia, R., Franco, L.M., Giliani, S., Zhang, K., Alazami, A.M., et al., 2020. PAX1 is essential for development and function of the human thymus. *Science Immunology* 5(44). <https://doi.org/10.1126/sciimmunol.aax1036>.
- [117] Ramos, S.A., Morton, J.J., Yadav, P., Reed, B., Alizadeh, S.I., Shilleh, A.H., et al., 2022. Generation of functional human thymic cells from induced pluripotent stem cells. *The Journal of Allergy and Clinical Immunology* 149(2): 767–781.e6. <https://doi.org/10.1016/j.jaci.2021.07.021>.
- [118] Magaletta, M.E., Siller, R., Maehr, R., 2020. Differentiation of human pluripotent stem cells toward pharyngeal endoderm derivatives: current status and potential. *Current Topics in Developmental Biology* 138:175–208. <https://doi.org/10.1016/bs.ctdb.2020.01.004>.
- [119] Provin, N., Giraud, M., 2022. Differentiation of pluripotent stem cells into thymic epithelial cells and generation of thymic organoids: applications for therapeutic strategies against APECED. *Frontiers in Immunology* 13:930963. <https://doi.org/10.3389/fimmu.2022.930963>.
- [120] Otsuka, R., Wada, H., Tsuji, H., Sasaki, A., Murata, T., Itoh, M., et al., 2020. Efficient generation of thymic epithelium from induced pluripotent stem cells that prolongs allograft survival. *Scientific Reports* 10(1):224. <https://doi.org/10.1038/s41598-019-57088-1>.
- [121] Magaletta, M.E., Lobo, M., Kernfeld, E.M., Aliee, H., Huey, J.D., Parsons, T.J., et al., 2022. Integration of single-cell transcriptomes and chromatin

- landscapes reveals regulatory programs driving pharyngeal organ development. *Nature Communications* 13(1):457. <https://doi.org/10.1038/s41467-022-28067-4>.
- [122] Bautista, J.L., Cramer, N.T., Miller, C.N., Chavez, J., Berrios, D.I., Byrnes, L.E., et al., 2021. Single-cell transcriptional profiling of human thymic stroma uncovers novel cellular heterogeneity in the thymic medulla. *Nature Communications* 12(1):1096. <https://doi.org/10.1038/s41467-021-21346-6>.
- [123] Park, J.-E., Botting, R.A., Domínguez Conde, C., Popescu, D.-M., Lavaert, M., Kunz, D.J., et al., 2020. A cell atlas of human thymic development defines T cell repertoire formation. *Science* 367(6480). <https://doi.org/10.1126/science.aay3224>.
- [124] Kearns, N.A., Genga, R.M.J., Ziller, M., Kapinas, K., Peters, H., Brehm, M.A., et al., 2013. Generation of organized anterior foregut epithelia from pluripotent stem cells using small molecules. *Stem Cell Research* 11(3):1003–1012. <https://doi.org/10.1016/j.scr.2013.06.007>.
- [125] Kollmann, T.R., Goldstein, M.M., Goldstein, H., 1993. The concurrent maturation of mouse and human thymocytes in human fetal thymus implanted in NIH-beige-nude-xid mice is associated with the reconstitution of the murine immune system. *Journal of Experimental Medicine* 177(3):821–832. <https://doi.org/10.1084/jem.177.3.821>.
- [126] Mohtashami, M., Zúñiga-Pflücker, J.C., 2006. Three-dimensional architecture of the thymus is required to maintain delta-like expression necessary for inducing T cell development. *Journal of Immunology* 176(2):730–734. <https://doi.org/10.4049/jimmunol.176.2.730>.
- [127] Anderson, G., Jenkinson, E.J., 2000. Review article: thymus organ cultures and T-cell receptor repertoire development. *Immunology* 100(4):405–410.
- [128] Han, J., Zúñiga-Pflücker, J.C., 2021. High-oxygen submersion fetal thymus organ cultures enable FOXP1-dependent and -independent support of T lymphopoiesis. *Frontiers in Immunology* 12:652665. <https://doi.org/10.3389/fimmu.2021.652665>.
- [129] Petrie, H.T., Zúñiga-Pflücker, J.C., 2007. Zoned out: functional mapping of stromal signaling microenvironments in the thymus. *Annual Review of Immunology* 25:649–679. <https://doi.org/10.1146/annurev.immunol.23.021704.115715>.
- [130] Nitta, T., Takayanagi, H., 2020. Non-epithelial thymic stromal cells: unsung heroes in thymus organogenesis and T cell development. *Frontiers in Immunology* 11:620894. <https://doi.org/10.3389/fimmu.2020.620894>.
- [131] Hun, M., Barsanti, M., Wong, K., Ramshaw, J., Werkmeister, J., Chidgey, A.P., 2017. Native thymic extracellular matrix improves in vivo thymic organoid T cell output, and drives in vitro thymic epithelial cell differentiation. *Biomaterials* 118:1–15. <https://doi.org/10.1016/j.biomaterials.2016.11.054>.
- [132] Fan, Y., Tajima, A., Goh, S.K., Geng, X., Gualtierotti, G., Grupillo, M., et al., 2015. Bioengineering thymus organoids to restore thymic function and induce donor-specific immune tolerance to allografts. *Molecular Therapy* 23(7):1262–1277. <https://doi.org/10.1038/mt.2015.77>.
- [133] Michelson, D.A., Hase, K., Kaisho, T., Benoist, C., Mathis, D., 2022. Thymic epithelial cells co-opt lineage-defining transcription factors to eliminate autoreactive T cells. *Cell* 185(14):2542–2558.e18. <https://doi.org/10.1016/j.cell.2022.05.018>.
- [134] Bakay, M., Pandey, R., Grant, S.F.A., Hakonarson, H., 2019. The genetic contribution to type 1 diabetes. *Current Diabetes Reports* 19(11):116. <https://doi.org/10.1007/s11892-019-1235-1>.
- [135] Castro-Gutierrez, R., Alkanani, A., Mathews, C.E., Michels, A., Russ, H.A., 2021. Protecting stem cell derived pancreatic beta-like cells from diabetogenic T cell recognition. *Frontiers in Endocrinology* 12:707881. <https://doi.org/10.3389/fendo.2021.707881>.
- [136] Yoshihara, E., O'Connor, C., Gasser, E., Wei, Z., Oh, T.G., Tseng, T.W., et al., 2020. Immune-evasive human islet-like organoids ameliorate diabetes. *Nature* 586(7830):606–611. <https://doi.org/10.1038/s41586-020-2631-z>.
- [137] Parent, A.V., Faleo, G., Chavez, J., Saxton, M., Berrios, D.I., Kerper, N.R., et al., 2021. Selective deletion of human leukocyte antigens protects stem cell-derived islets from immune rejection. *Cell Reports* 36(7):109538. <https://doi.org/10.1016/j.celrep.2021.109538>.
- [138] Leite, N.C., Pelayo, G.C., Melton, D.A., 2022. Genetic manipulation of stress pathways can protect stem-cell-derived islets from apoptosis in vitro. *Stem Cell Reports* 17(4):766–774. <https://doi.org/10.1016/j.stemcr.2022.01.018>.
- [139] Armitage, L.H., Stimpson, S.E., Santostefano, K.E., Sui, L., Ogundare, S., Newby, B.N., et al., 2021. Use of induced pluripotent stem cells to build isogenic systems and investigate type 1 diabetes. *Frontiers in Endocrinology* 12:737276. <https://doi.org/10.3389/fendo.2021.737276>.
- [140] Han, X., Wang, M., Duan, S., Franco, P.J., Kenty, J.H.-R., Hedrick, P., et al., 2019. Generation of hypoimmunogenic human pluripotent stem cells. *Proceedings of the National Academy of Sciences of the United States of America* 116(21):10441–10446. <https://doi.org/10.1073/pnas.1902566116>.
- [141] Zhan, L., Rao, J.S., Sethia, N., Slama, M.Q., Han, Z., Tobolt, D., et al., 2022. Pancreatic islet cryopreservation by vitrification achieves high viability, function, recovery and clinical scalability for transplantation. *Nature Medicine* 28(4):798–808. <https://doi.org/10.1038/s41591-022-01718-1>.
- [142] Culina, S., Lalanne, A.I., Afonso, G., Cerosaletti, K., Pinto, S., Sebastiani, G., et al., 2018. Islet-reactive CD8+ T cell frequencies in the pancreas, but not in blood, distinguish type 1 diabetic patients from healthy donors. *Science Immunology* 3(20). <https://doi.org/10.1126/sciimmunol.aao4013>.
- [143] Bender, C., Rodriguez-Calvo, T., Amirian, N., Coppieters, K.T., von Herrath, M.G., 2020. The healthy exocrine pancreas contains preproinsulin-specific CD8 T cells that attack islets in type 1 diabetes. *Science Advances* 6(42). <https://doi.org/10.1126/sciadv.abc5586>.