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Targeting Type 1 Diabetes: Selective Approaches for New Therapies

Daniel Sheehy, Sean Quinnell, Arturo J. Vegas*

Department of Chemistry, Boston University, Boston, Massachusetts 02215, United States

Abstract

The clinical onset of type 1 diabetes is characterized by the destruction of the insulin-producing β cells of the pancreas, and is caused by autoantigen-induced inflammation (insulinitis) of the islets of Langerhans. The current standard of care for type 1 diabetes mellitus (T1DM) patients allows for management of the disease with exogenous insulin, but patients eventually succumb to many chronic complications such as limb amputation, blindness and kidney failure. New therapeutic approaches now on the horizon are looking beyond glycemic management and are evaluating new strategies from protecting and regenerating endogenous islets to treating the underlying autoimmunity through selective modulation of key immune cell populations. Currently, there are no effective treatments for the autoimmunity that causes the disease, and strategies that aim to delay or prevent the onset of the disease will play an important role in the future of diabetes research. In this review, we summarize many of the key efforts underway that utilize molecular approaches to selectively modulate this disease and look at new therapeutic paradigms that can transform clinical treatment.

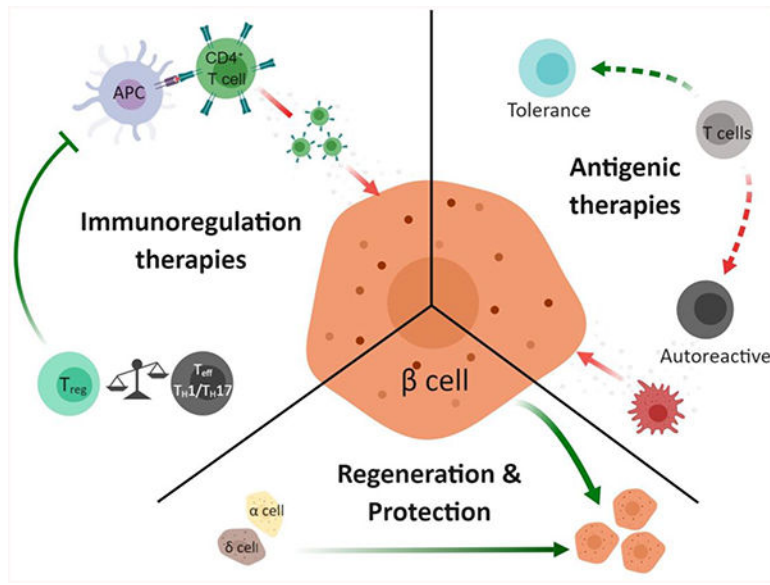
Graphical Abstract

*Corresponding Author: ajvegas@bu.edu.

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INTRODUCTION

Type 1 diabetes mellitus (T1DM) is a global epidemic affecting over 30 million people, and is one of the most common endocrine and metabolic conditions occurring in childhood.¹ The incidence of T1DM has increased 5.3% annually and the economic cost is estimated between \$14.4–14.9 billion in the US alone.^{2–4} T1DM is characterized by the autoimmune destruction of the insulin secreting β cells of the pancreatic islets of Langerhans, leading to insulin deficiency and unregulated blood glucose levels. The current standard of care entails a rigorous routine of blood glucose monitoring coupled to daily exogenous insulin injections. Despite advances in insulin therapies, these individuals still suffer chronic diabetic complications including cardiovascular disease, retinopathy, nephropathy, ketoacidosis, nonketotic hyperosmolar coma, or death.⁵ Whole organ pancreas transplantation has been explored, however it requires patients to receive systemic immunosuppressants and after 5 years 90% of patients are once again dependent on exogenous insulin.⁶ Polymeric encapsulation of donor insulin-producing tissue to overcome the need for systemic immunosuppression has gained momentum with the recent development of new materials and formulations.^{7–10} This therapeutic approach to tissue replacement promises to restore glycemic control for fully symptomatic patients with little to no remaining β cells. To complement this strategy, there is growing interest in interventional strategies that aim to tackle the underlying autoimmunity of the disease and preserve as much endogenous β cells as possible. Currently there are no clinically-approved interventional therapies to treat the underlying autoimmunity, but new therapeutic agents are being clinically tested and numerous new approaches are on the horizon.

Pathogenesis.

Development of an interventional therapy for T1DM has proven challenging owing to its polygenic and heterogeneous nature. There are a plethora of purported environmental triggers whose role in pathogenic processes are poorly understood, while genetic, and

phenotypic characteristics show marked variation.¹ Over 40 loci play a role in T1DM susceptibility, with the major histocompatibility (MHC) class II HLA-DR and HLA-DQ genotypes providing an estimated half of the genetic susceptibility.^{11,12} While these genetic risk factors are necessary for T1DM development, they are not sufficient. Recent attention has turned to a variety of environmental factors including infant diet, vitamin D and the vitamin D pathway constituents, enteroviruses, the hygiene hypothesis, and the gut microbiome.^{1,13} However, no evident influence on pathogenesis has been identified and the exact triggering mechanism remains unknown.

The thymus plays a paramount role in eliminating self-reactive T cell populations through positive and negative selection, termed central tolerance.¹⁴ The transcription factor autoimmune regulator AIRE promotes the expression of self-antigens on the surface of medullary thymic epithelial cells (mTECs). The self-antigens are presented through MHC complexes to allow for targeted removal of potentially autoreactive T cell clones from the repertoire.¹⁵ Such regulation fails in T1DM, leading to escape of autoreactive T cell populations to the periphery. Diabetic MHC class II proteins presenting peptides recognized by these autoreactive T cells form a trimolecular complex with the T cell receptor (TCR) that leads to T cell activation and expansion. This is followed by pancreatic infiltration by T cells, macrophages, B lymphocytes and plasma cells, and subsequent autoimmune destruction of insulin secreting β cells.¹⁶ Symptoms and diagnosis typically occur well after two-thirds of β cells are lost (Figure 1).

Interventional Treatments under Clinical Evaluation.

Several clinical trials evaluating immunomodulatory agents in the past 40 years are discussed and summarized in Table 1. These trials include the systemic immunosuppressants cyclosporine, azathioprine, and mofetil, and immune interfering antibodies against CD20, cytotoxic T lymphocyte antigen-4 (CTLA-4), Interleukin 2 (IL-2), and CD3.¹ The ladder case involving anti-CD3 monoclonal antibodies (mAb) suggested a reversal of hyperglycemia in preclinical studies and phase I trials through inactivation of effector T cells (T_{eff}) and an expansion of the regulatory $CD4^+CD25^+$ T cell (T_{reg}) populations.¹⁷ However, two different anti-CD3 mAb, Otelixizumab and Teplizumab, showed disappointing results in maintaining C-peptide levels in phase III clinical trials.^{18,19} Likewise, all other interventional trials have failed to meet phase III endpoints. This highlights the dire need for both new targets and methods for selectively modulating the immune system, and for mechanistic biomarkers to aid in selecting both appropriate treatments and therapeutic windows.

Here, we highlight emerging methods for targeting the underlying mechanisms of T1DM through the view of recent advances in immunomodulatory therapies, protective and regenerative strategies, and new targeting modalities. A brief perspective is offered into the future direction of treatment for T1DM and how next generation therapies can achieve improved clinical outcomes.

Cytokine Blockade.

T1DM is characterized by an imbalance in pro-inflammatory and anti-inflammatory cytokines which manifest as the disease progresses.^{20,21} Elevated serum levels of pro-inflammatory cytokines, such as IL-1 β , tumor necrosis factor α (TNF- α), IL-6, and IL-7 have spurred clinical investigation into the therapeutic utility of blocking these signaling pathways. IL-1 is an inflammatory cytokine involved in the differentiation of T helper 1 (T_H1) and T_H17 T cells that has profound pro-apoptotic effects on β cells.^{22,23} In non-obese diabetic (NOD) mice, knockout (KO) of the IL-1 receptor (IL-1R) slowed the progression of T1DM, but did not prevent disease onset.²⁴ Initial clinical trials with the IL-1 antagonist anakinra showed promising but mixed results with treated participants having similar hemoglobin A1c (HbA1c) levels requiring significantly less insulin than placebo cohort after 1 month of treatment.²⁵ Separate phase IIa clinical trials failed to find efficacy with two different IL-1 antagonists, canakinumab and anakinra.²⁶ Both treatments were found to be safe, but failed to find any significant difference in C-peptide levels. In T1DM patients, monocyte expression of IL-1 β is high at time of diagnosis but normalizes within 1 month.²⁷ This suggests anti-IL1 treatment may only be effective at early time points in disease progression.

TNF- α is a cytokine involved in regulating the maturation of dendritic cells (DCs) and has been affiliated with the activation of islet-specific T cells in pancreatic lymph nodes (PLNs).²⁸ T1DM patients have significantly higher levels of plasma TNF- α compared to healthy subjects.²⁹ In NOD mice, the systemic administration of TNF- α led to a 5 week earlier onset of disease and a higher incidence rate compared to control mice,³⁰ indicating the therapeutic value in modulating this target. However, efficacy in treatments targeting TNF- α may depend strongly on time of administration during disease progression. Administration of anti-TNF- α mAb in 2-week-old NOD mice protected against T1DM development compared to control mice, whereas 8-week-old treated mice showed only 50% incidence of the disease.³⁰ A small clinical trial had promising results with treated patients having lower HbA1c levels and increased C-peptide levels, but there have been no subsequent studies.³¹

IL-7 is a pro-inflammatory cytokine that impacts proliferation and survival of naïve T cells.^{32,33} It is implicated in multiple autoimmune diseases and represses the suppressive functions of T_{regs}.^{34,35} Two studies showed blocking the IL-7R in NOD mice resulted in a significant reduction of T1DM incidence and even remission of established T1DM.^{36,37} In addition, both studies found that IL-7 suppression resulted in increased expression of the Programmed Death-1 (PD-1) receptor on the surface of T_{eff} cells. Strikingly, NOD mice that received anti-IL-7 treatment followed by a PD-1 antagonist show increased incidence of T1DM, suggesting that PD-1 expression is required for anti-T1DM effects (discussed further below).³⁶ Additionally, blocking IL-7R also resulted in an increase in T_{regs} without affecting their suppressive activity.³⁷

IL-6 is a cytokine involved in host defense against pathogens, but is also involved in autoimmune diseases with increased T_H17 cell development and inhibited T_{reg} development.³⁸ In patients with T1DM, IL-6 sensitivity correlated with increased expression of genes involved in T cell migration and inflammatory responses, including CD4⁺ cells.³⁹ These results suggest IL-6 may be a target for modulating T1DM and other autoimmune diseases.

NOD/Wehi mice treated with an anti-IL-6 antibody resulted in a three-fold reduction in T1DM incidence compared to treatment with a control antibody.⁴⁰ Inhibition of IL-6 can be a promising therapeutic strategy and further validation and studies elucidating the mechanism of action should prove insightful.

Inducing Antigenic Tolerance.

T1DM pathogenesis is characterized by a loss of central tolerance and the production of autoreactive T cells that have escaped thymic negative selection. Antigen-specific immunotherapy seeks to provide a targeted approach towards inducing peripheral immune tolerance without leading to systemic immune inhibition and associated complications that are typically seen in current non-antigenic therapies.⁴¹ Antigenic therapies seek to achieve tolerance through induction of T_{regs} (active tolerance) that inhibit T_H1/T_H17 populations or removal of autoreactive cell populations (passive tolerance) by eliminating autoantigen expression or the cell populations that propagate autoantigen sensitivity. Here we focus on promising targets involving antigenic responses that may lead to re-establishing immune tolerance.

The T1DM autoantigen GAD65, encoded by the *GAD2* gene, is responsible for production of the inhibitory neurotransmitter γ -aminobutyric acid (GABA)^{42,43} which impairs insulin secretion.⁴⁴ Administration of human recombinant GAD65 formulated with aluminum hydroxide (GAD65-alum) induces a favorable immune response, with decreased GAD-specific CD4⁺ and CD8⁺ T_{eff} cells and increased GAD65-specific T_{regs}. However, after 4 years of treatment there was no detectable change in T cell populations over placebo.⁴⁵ Thus, while antigenic treatment with GAD65 does activate immune tolerance pathways, a combinatorial approach may be necessary for long-term efficacy.

Heat-shock protein 60 (HSP60) is an implicated autoantigen in T1DM, yet is involved in preventing stress-induced damage to proteins and functioning as a chaperone protein. This duality has been explored by administration of a peptide derived from HSP60 (p277), which protected NOD mice from both induced and spontaneous diabetes.⁴⁶ Additionally, a stable version of p277 (DiaPep277) promotes anti-inflammatory effects, cell adhesion, and inhibits migration through interaction with Toll-like receptor 2 (TLR2).^{45,47} DiaPep277 in a phase II T1DM trial ultimately failed to meet endpoints despite previous encouraging results of a shift in T cell populations to a T_H2 phenotype.^{48–50} Combining DiaPep277 with therapies that inhibit inflammation may be needed to enable stronger T_{reg} induction.⁵¹

Peptide immunotherapy seeks to achieve immune tolerance by expanding T_{regs} and forcing pathogenic T cells through anergy and/or deletion. Recent clinical peptide immunotherapy efforts have involved administration of either an altered peptide ligand of Insulin B:9–23 or natural peptide sequences from proinsulin.^{52–54} The former failed to show efficacy and a single peptide sequence of proinsulin trial did not look at efficacy. One promising preclinical peptide therapy involves generating “navacims” through coating peptide-MHC complexes onto nanoparticles followed by administration to induce immune tolerance. Using this method, several autoimmune-disease-relevant peptide-MHC class II complexes triggered expansion of Type 1 regulatory-like CD4⁺ T cells and restored normoglycemia in the NOD mouse model.⁵⁵ Importantly, these peptide-MHC coated nanoparticles did not compromise

systemic immunity, which has been a major challenge in antigen-specific peptide immunotherapy.

Taken together, antigenic induced immune tolerance shows efficacy in diabetic mouse models but have so far failed to replicate in human populations when administered as monotherapies. Inadequate dosages and the inherent challenges in attempting to reverse symptomatic T1DM rather than delay the onset of the disease may be contributors to these results. T1DM prevention studies in humans also lack robust early biomarkers. Preclinical studies have suggested that combinatorial administration of antigenic therapies with immune modulators may be required for efficacy in T1DM patients.^{56–58} Future clinical studies with this approach are needed.

Lymphocyte Modulation.

T cells.—The persistence and proliferation of autoreactive T cells are at the root of the autoimmune reaction in T1DM. CD3 is a component of the TCR complex and is required for T cell activation, and multiple anti-CD3 therapies have emerged and are comprehensively reviewed.^{59–61} Briefly, blockade of CD3 in NOD mice resulted in prevention of T1DM incidence and remission of established T1DM in 60–80% of mice.⁶² In the first clinical study of anti-CD3 treatment, 9 of 12 treated patients maintained or reduced their insulin requirements compared to 2 of 12 in the control cohort.⁶³ These results were promising and sparked extensive clinical trials of anti-CD3 therapies, with mixed outcomes. In a phase III clinical trial with teplizumab, effects were only seen at the highest dosing regimens and the trial failed to reach primary outcomes of patients using less than 0.5 U/kg of insulin per day and HbA1C reduction of less than 6.5% after 1 year.¹⁸ A subsequent trial identifying drug responders and nonresponders showed no significant difference in C-peptide levels between drug responders and nonresponders, however they had significant differences in their baseline insulin requirements and lower levels of CD8⁺ effector memory cells.⁶⁴

There is rapidly growing interest in targeting T cell metabolism in autoimmune diseases. Mitochondrial metabolic activity plays a key role in modulating T cell activation, proliferation, and programmed cell death.⁶⁵ Additionally, upon antigen recognition the T cell mitochondria modulates processes important for IL-2 production.^{66–68} A recent study shows that T1DM patients have an altered mitochondrial and cytokine response following TCR stimulation linked to persistent T cell mitochondrial inner membrane hyperpolarization.⁶⁹ Of particular interest are metabolic pathways that inhibit proliferation and effector functions of both CD4⁺ and CD8⁺ T cells through mechanisms such as mTOR inhibition and selective reduction of glucose uptake. Blockage of glucose metabolism in the pre-diabetic NOD mouse model using 2-deoxy-D-glucose (2DG), a competitive inhibitor of phosphoglucose isomerase, reduces insulinitis and the frequency of a subset of diabetogenic CD8⁺ T cells.⁷⁰ While promising, a key challenge for metabolic therapies is identifying the optimal period for inhibition, as currently there are no predictive biomarkers for autoreactive T cell activation.

A major suppressor of autoimmunity are T_{regs}, which make up less than 10% of CD4⁺ T cells in peripheral circulation.⁷¹ T_{regs} play a primary role for maintaining immune tolerance

by downregulating the induction of other immune cells including CD4⁺ and CD8⁺ T cells, B cells, NK cells, macrophages, and DCs.^{72,73} T1DM patients have been found to possess an imbalance between T_{eff} and T_{reg},⁵¹ leading to an inability to properly inhibit T_{eff} responses.^{72,74,75} In NOD mice, T_{reg} populations initially increase in the inflamed pancreas but decrease as insulinitis progresses.⁷⁶ Numerous studies have attempted to restore T_{reg} populations and are reviewed elsewhere in detail,^{71,77} with more recent progress mentioned here.

Perturbation in IL-2R α (CD25) expression in T_{regs} has shown T1DM susceptibility by a reduction in STAT5 phosphorylation.⁷⁸ Additionally, IL-2 deprivation in T1DM patients results in low levels of anti-apoptotic protein Bcl-2, leaving T_{regs} prone to apoptosis.^{79–81} Administration of a low dose IL-2 therapy overcomes IL-2 signaling defects in patients and was effective at increasing T_{reg} populations.⁸² However, other CD25 expressing populations are also activated with low dose IL-2 administration and when combined with rapamycin an increase in both CD56^{high} NK cells and CD4⁺ memory cell populations are observed and negatively impacts β cell function.^{83,84} A clinical trial addressing dosing has completed, however no results are reported.⁸⁵

These results from IL-2 therapies led to interest in using T_{regs} themselves as a therapeutic approach. Indeed, adoptive transfer of T_{regs} have been investigated in two clinical trials and are safe, long-lived, and stable.^{73,86,87} Follow-up phase II studies and an interesting study of T_{regs} in combination with low-dose IL-2 are still ongoing.⁷⁷ Antigen-specific T_{regs} may be more effective at preventing insulinitis,⁸⁸ and chimeric antigen receptor (CAR) engineered T_{regs} show promising results in various pre-clinical models of autoimmunity.^{89–92} Other avenues to expand T_{regs} are being explored by targeting memory T cells.⁹³ Of note are T_{eff}-depleting agents teplizumab and alefacept, which show promise for sustaining remission and tolerance.^{64,94} Alternatively, polarizing T_H17 pro-inflammatory cells towards a T_{reg} phenotype has been achieved using small molecule glutamate receptor enhancers.⁹⁵

A major mechanism of maintaining tolerance is through T cell anergy, the inactivation of autoreactive T cells. T cell anergy can manifest through MHC-peptide-TCR complex recognition in the absence of co-stimulation through CD28 (T cell) and B7 (APC) where the T cell cannot produce IL-2, thus preventing proliferation.⁹⁶ Similar effects happen with CTLA-4 (related to CD28) which blocks the CD28-dependent T cell activation. Abatacept, a CTLA4-Ig approved for rheumatoid arthritis, prevents disease progression in T1DM animal models. However, T1DM clinical trials involving CTLA-4 have failed to meet endpoints.⁹⁷ Encouragingly, recent work with a selective CD28 antagonist in combination with rapamycin (mTOR pathway inhibition) inhibits T cell activation and migration into the pancreas in NOD mice.⁹⁸ Indeed, tolerogenic APCs display low numbers of co-stimulatory molecules like CD80, CD86, and CD40, and anergy can be achieved using rapamycin, corticosteroids, IL-10, and transforming growth factor β 1 (TGF β -1).^{99,100} These studies provide additional options to rapamycin in combination trials with CTLA4-Ig.

T_{regs} can also play a similar role in T cell anergy through ligation of CTLA4 to CD80 and CD86, which induces APCs to express Indoleamine 2,3-dioxygenase (IDO) resulting in abolished T cell activation.^{101–103} Additionally, T_{regs} can inhibit APC maturation resulting

in T cell anergy.¹⁰⁴ Increased expression and binding of lymphocyte activation gene 3 (LAG-3) to MHC II induces the ITAM-mediated inhibitory signaling pathway, resulting in suppression of DC maturation and immunostimulatory capacity. Additionally, CTLA4-Ig therapies may be beneficial to modifying DCs present in inflammatory microenvironments through CTLA4-Ig binding to B7 while simultaneously inducing DC expression of IDO which leads to T_{reg} expansion.¹⁰⁵ Subsequently, T_{regs} are reported to downregulate CD80/86 expression on APCs and DCs, which modifies them towards a tolerogenic state.^{106,107}

A complementary approach to T cell anergy in restoring immune tolerance is inducing apoptosis of autoreactive cell populations. Activated periphery T cells express death receptors from the TNF family (Fas/FasL), making them susceptible to activated-induced cell death (AICD).^{108,109} Specifically, T_H1 cells are most susceptible to AICD while T_H2/T_{reg} cells are considered protective against AICD.^{110,111} A potential therapy that induces Fas would have to be administered pre-insulinitis due to inflammation induced upregulated expression of Fas in β cells leading to Fas-mediated β cell apoptosis during insulinitis.^{112,113} The therapeutic implications of inhibiting the Fas/FasL interaction alone after early insulinitis have been debated elsewhere and is thought to have low efficacy due to redundant pro-inflammatory mechanisms.¹¹⁴ However, recently the combination of engineered microgels displaying FasL with a short course of rapamycin achieved localized immunomodulation of islet allografts for over 200 days.¹¹⁵ Since there are still no predictive early biomarkers for T1DM, overactivation of the Fas/Fas-ligand pathway must be avoided since it is associated with cancer and can lead to liver toxicity.^{109,116}

Antigen Presenting Cells.—An ideal therapy for targeting APCs will mask the presentation of self-antigens known to activate T cells. One of the major autoantigens is proinsulin, with 90% of CD4⁺ insulin-reactive T cell clones from prediabetic NOD mice targeting the insulin B:9–23 peptide.^{117,118} Preventing autoreactive T cells from recognizing insulin bound to MHC class II could delay or attenuate T1DM autoimmunity. The therapeutic potential of this approach was explored by Kappler and coworkers through generation of mAb287 that specifically recognizes NOD I-A^{g7} with insulin B:9–23 bond in register 3.¹¹⁹ Administration of mAb287 blocked CD4⁺ TCR interaction with the complex resulting in a delayed disease onset and the majority of 30-week-old mice had intact islets with only mild pre-insulinitis. This work established that blocking TCR recognition of the MHC-insulin complex gives a beneficial physiological response in NOD mice, and human validation is a next step for mAb287.

Recently, inhibiting autoantigen presentation was expanded to using small molecules in humans. *In silico* screening methods of FDA approved drugs against DQ8 identified methyl dopa, and treatment in NOD mice showed a reduction in primary antigen-specific T cell responses to insulin.¹²⁰ Additionally, a phase Ib dose-escalation study with methyl dopa in patients with recent-onset T1DM showed reduced inflammatory T cell responses to insulin. These results further highlight the potential of this approach and that small molecules, not just antibodies, can disrupt autoantigen presentation. However, because methyl dopa is a clinically approved anti-hypertension drug,¹²¹ repurposing methyl dopa for immunomodulation may have its own challenges.

CD40 is a member of the TNF receptor family and is expressed by professional APCs, B cells, and T cells.^{122–124} The natural ligand for CD40 is CD154, in either soluble or membrane-bound form, and the CD40-CD154 interaction is important for the initiation of insulinitis.^{124–129} Initial therapeutic attempts to block this interaction in lupus patients using anti-CD154 resulted in risk of life-threatening thrombotic events.¹³⁰ Current efforts to avoid these antibody-induced thrombotic events utilize a small peptide, KGY_Y₁₅, which uses the primary CD154 domain that interacts with CD40.¹³¹ In NOD mice, KGY_Y₁₅ administration was shown to prevent diabetes onset, and human validation is a next step.

B cells.—In recent years, it has become clear that B lymphocytes contribute to autoimmunity and secrete autoantibodies prior to insulinitis.^{132–139} In NOD mice B cells support the expansion of pathogenic T cell responses, and these pathogenic populations contribute to T1DM progression in humans.^{140–142} Abnormalities in the thymus B cell compartment prior to T1DM presentation have also been found in the NOD mouse.¹⁴³ This suggests thymic B cells in T1DM have a potentially harmful impact on the effectiveness of negative selection on autoreactive T cell precursors, thus making thymus B cells a potential target for T1DM therapies.

The generation of pathogenic B cell populations is thought to take place during affinity maturation through aberrant selection leading to expansion of autoreactive B lymphocytes.^{144,145} Clinical trials targeting B cells with a depleting P-specific therapy, rituximab, failed to preserve C-peptide production past two years and a follow-up study showed an increase of asymptomatic viremia.^{146,147} This highlights the challenge that systemic non-antigenic therapies face. Promising preclinical approaches have targeted B cells undergoing affinity maturation by disrupting the activated-induced cytidine deaminase protein (AID), a protein involved in the dsDNA break repair mechanism mediated by the RAD51 complex.¹⁴⁸ Inhibition of RAD51 showed subsequent inhibition of T1DM development in NOD mice partially through expansion of CD73⁺ B lymphocyte populations that regulate pathogenic T cell responses. Interestingly, these results were mirrored through CRISPR-Cas9 directed ablation of the gene encoding AID.¹⁴⁸

Immunoregulatory.—In recent years, therapies targeting critical junction points controlling immune inhibition/activation has revolutionized how cancer is treated. Recognition that many types of cancer modulate the immune system led to the development of several immune checkpoint inhibitors (CPIs) that has drastically improved patient outcomes in several cancers.¹⁴⁹ Drawing from this success, approaches aimed at attenuating autoimmunity through checkpoint modulation are now actively being explored.

IDO is inducible in APCs and is associated with inhibition of T cell proliferation, prevention of memory T cell formation, and induction of T_{reg} differentiation.¹⁵⁰ Although the precise mechanism of activation and IDO's role is still being explored, that IDO could have a therapeutic role in diabetes is becoming more apparent.¹⁵¹ Notably, treatment with IDO inhibitor 1-methyl-D-tryptopan (1MT) accelerated T1DM progression in NOD mice.¹⁵² Conversely, increased IDO expression from bystander fibroblasts can inhibit macrophage and infiltration of CD3⁺ cells into islet xenogeneic grafts, thus impairing pro-inflammatory responses.¹⁵³ Recently, IDO expression from encapsulated Sertoli cells attenuated T1DM in

experimental autoimmune encephalomyelitis (EAE) induced T1DM in C57BL/6 mice.¹⁵⁴ However, no clinical trials have commenced for increasing IDO in T1DM.

The programmed death ligand 1 (PD-L1) is a ligand for PD-1, an inhibitory receptor expressed on activated lymphocytes that serves as an immune checkpoint to regulate tolerance.^{155,156} Malignant cancers hijack this checkpoint to locally suppress anti-tumor immunity, and clinically successful CPIs have been developed to inhibit formation of the PD-1/PD-L1 complex.¹⁵⁷ Interestingly, both *CD274* encoding (PD-L1) and *PDCD1LG2* (encoding PD-L2) are up-regulated in newly onset T1DM,¹⁵⁸ suggesting that PD-L1 might have a protective effect on T1DM. Additionally, cancer patients on anti-PD-1 therapy can develop T1DM.¹⁵⁹ The NOD mouse model recapitulates these results, with anti-PD-L1 therapies accelerating disease progression.¹⁶⁰

Overexpression of PD-L1 may prove more useful for T1DM treatment. PD-L1 expressing autologous hematopoietic stem and progenitor cells (HSPCs) transplanted into NOD revert diabetes in recent onset mice.¹⁶¹ Migration of these PD-L1.Tg HSPCs to the pancreas appears to be driven by the chemokine profile of the inflamed islets through high expression of C-X-C chemokine receptor type 4 (CXCR4) in these HSPCs. PD-L1 also plays a significant role in HSPC immunobiology and there is a strong link between PD-L1 defects and T1DM.^{160,162,163} Taken together, HSPC expression of PD-L1 plays an important role in maintenance of immunocompetence and a cell based therapy using HSPCs to correct PD-L1 expression deficiency in T1DM is a promising approach.

Targeting Innate Immunity.

The innate immune system is involved in the detection and removal of pathogens.¹⁶⁴ Innate immune receptors, including pattern recognition receptors, such as TLRs, have been shown to play a role in the development of T1DM.¹⁶⁵ Modulation of TLRs and other proteins involved in their signaling pathways have been explored for modulating the incidence of T1DM. The role of innate immunity in T1DM has been reviewed elsewhere,^{166,167} and here we highlight some of the important molecular findings from recent studies.

MyD88.—The adapter protein myeloid differentiation primary response gene 88 (MyD88) is involved in signaling pathways for multiple TLRs and IL-1R.¹⁶⁸ NOD MyD88 KO mice are protected from T1DM when housed in normal specific pathogen-free (SPF) conditions, but had high incidence of T1DM when housed in germ-free conditions.¹⁶⁹ Interestingly, MyD88 KO mice showed tolerization of T cells in the PLNs under SPF conditions, whereas T cells in the spleen and mesenteric lymph nodes were still autoreactive to diabetes-associated peptides. Additionally, MyD88 KO mice in SPF conditions have changes in gut microbiota composition and further studies on the interplay between the innate immune system and gut microbiome have yielded promising results.¹⁶⁹ In a related study, MyD88 inhibition utilizing a small molecule probe, TJ-M2010-6, in NOD mice had two-fold lower T1DM incidence, an increase in T_{regs} , and a reduction in $CD4^+/CD8^+$ T cell proliferation.¹⁷⁰ More work is underway to confirm the role of the microbiome in T1DM and targeted approaches to altering it.

TRIF.—The TIR-domain-containing adapter-inducing interferon- β (TRIF) is an adapter molecule involved in TLR3 and TLR4 signaling. NOD TRIF KO mice were protected from T1DM when housed with other TRIF $^{-/-}$ mice, but not when cohoused with WT mice.¹⁷¹ Similar to MyD88 KO mice, TRIF KO lead to a different microbiota compared to WT mice, along with DCs that had reduced levels of inflammatory cytokines, such as TNF- α , and increased TGF- β .¹⁷² In addition, TRIF KO mice APCs had impaired antigen presentation and ultimately reduced T cell activation and proliferation.

TLRs.—Patients with T1DM have increased expression levels of TLR2 and TLR4 in monocytes.¹⁷³ Studies with TLR4 KO mouse models have yielded mixed results from inflammation reduction, no effect, to an increase in T1DM incidence.^{169,174,175} Antibody inhibition of the TLR4/MD-2 interaction, mediated by both MyD88 and TRIF, resulted in a significant reduction of T1DM incidence accompanied by a significant increase in Fox3p⁺ T_{regs}.¹⁷⁶ A decrease in APC-mediated T cell proliferation is one potential mechanism, as NOD scid mice treated with TLR4-Ab followed by addition of NOD CD4⁺ and CD8⁺ T cells were protected from T1DM incidence. Underscoring its clinical potential, treatment in NOD mice with established T1DM led to a permanent reversal in 71% of treated mice.¹⁷⁶

NETs.—Neutrophils are the most abundant leukocyte in mammals, play important roles in both immunosurveillance and inflammation.¹⁷⁷ To fight bacterial infection, neutrophils eject nuclear chromatin and bactericidal proteins to form structures called neutrophil extracellular traps (NETs).¹⁷⁷ Patients with T1DM have been shown to have increased levels of neutrophil elastase and proteinase 3, two neutrophil serine proteases, and corresponding higher levels of NET formation.¹⁷⁸ In NOD mice, neutrophil infiltration and NET formation appears as early as 3 weeks of age. Upon further investigation, depleting NOD mice of neutrophils resulted in a three-fold decrease in T1DM incidence.¹⁷⁹ In a separate study, *Lactococcus lactis*, a food grade microorganism, was used to deliver staphylococcal nuclease (SNase) to disrupt NETs. NOD mice treated with *L. lactis* expressing SNase had a two-fold lower T1DM incidence, enhanced glucose tolerance, and a three-fold reduction of severe neutrophil infiltration of the islets,¹⁸⁰ indicating clinical potential.

Islet Protection.

A complementary therapeutic approach to immunomodulation is to inhibit pathways involved in cytokine-mediated cell death of β cells. Cytokine-induced stress is known to induce autoimmune-mediated destruction of islets cells through endoplasmic reticulum (ER) dysfunction, altering cellular metabolism, and deregulating calcium homeostasis. Here we discuss progress made in modulating these pathways to preserve β cell mass and function.

Cellular Stress.—ER stress and the affiliated unfolded protein response (UPR) has an adverse effect in many autoimmune diseases, including T1DM.¹⁸¹ During ER stress, the ER transmembrane kinase/endoribonuclease (RNase), IRE1 α , degrades mRNA to promote apoptosis. Treatment of NOD mice with the tyrosine kinase inhibitor Imatinib led to T1DM remission in 80% of mice in an ABL protein kinase-dependent manner, leading to inactivation of IRE1 α .^{182,183} Accordingly, direct inhibition of IRE1 α with the small molecule KIRA8 also led to 90% reversal of T1DM in NOD mice.¹⁸⁴ Imatinib is currently

being investigated in a phase II trial for treatment of T1DM (Table 1), highlighting that inhibition of proteins affiliated with ER stress and the UPR are promising targets to preserve β cell mass.

T1DM patients display islet cells with hyperexpression of HLA Class I proteins along with elevated expression of signal transducer and activator of transcription 1 (STAT1).¹⁸⁵ A recent analysis indicated JAK-STAT and IFN signaling being highly enriched in multiple autoimmune diseases.¹⁸⁶ Suppression of STAT1 in transgenic NOD mice can also reduce incidence of T1DM.¹⁸⁷ Inhibition of JAK1/JAK2, enzymes that phosphorylate and activate STAT1, with the small molecule inhibitor AZD1480 in NOD mice reduced MHC Class I expression on β cells and results in three-fold lower incidence of T1DM.¹⁸⁸ Directly inhibiting STAT1 to reduce MHC Class I expression is a promising therapeutic strategy, but an inhibitor of STAT1 has yet to be developed.

Cellular Metabolism.—Sphingolipid metabolism and sphingosine 1-phosphate (S1P) play an important role in the development of several inflammatory and autoimmune disorders, and their role has been thoroughly reviewed elsewhere.¹⁸⁹ Briefly, S1P is a bioactive signaling molecule that is degraded by the ER protein S1P lyase (SPL) and is involved in the activation of innate and adaptive immune pathways, including the T_H1/T_{reg} balance.¹⁹⁰ Treatment with fingolimod, an antagonist for the S1P receptor, increases survival and reduces insulinitis scoring in NOD mice.^{191,192} In INS-1 cells, overexpression of SPL protects against cytokine-related cell death and caspase-3 activation, likely through prevention of Ca^{2+} leakage from the ER into the cytosol.¹⁹³ Disruption of Ca^{2+} homeostasis in the β cell from cytokine exposure has been linked to ER stress and contributes to β cell death.^{194,195} Modulating Ca^{2+} homeostasis in INS-1 cells with the small molecules dantrolene and sitagliptin displayed less cytokine-related cell death and lower expression of thioredoxin-interacting protein (TXNIP). Knockdown of TXNIP resulted in a similar protection from cytokine-mediated cell death,¹⁹⁶ suggesting that TXNIP is a mediator in Ca^{2+} homeostasis and a potential target for reducing β cell death.

Oxidative stress also contributes to T1DM pathogenesis.¹⁹⁷ Of note, reactive oxygen species (ROS) act on the innate immune system to induce the production of inflammatory cytokines, such as TNF- α and IL-1 β that ultimately led to the activation of CD4⁺ and CD8⁺ T cells.^{197,198} The high levels of ROS in T1DM has prompted studies utilizing antioxidants for their ability to prevent β cell destruction in T1DM. The antioxidant quercetin preserves β cell mass compared in streptozotocin (STZ) treated mice.¹⁹⁹ Quercetin treatment of INS-1 cells protects against oxidative death from H_2O_2 and increased glucose-induced insulin secretion.²⁰⁰ Unfortunately, clinical investigations utilizing antioxidants in children with recently onset T1DM have failed to find any effects on C-peptide levels.²⁰¹

Modulating specific pathways involved in ROS production also affect T1DM development. The enzyme 12/15-Lipoxygenase (12/15-LOX) catalyzes the oxygenation of arachidonic acid to form 12- and 15-hydroxyeicosatetraenoic acid (HETE), a pro-inflammatory mediator of insulinitis.^{202,203} 12-HETE also decreases insulin secretion and increases β cell death in human islets.^{203,204} In both STZ and NOD models, 12/15-LOX KO mice had a lower incidence of T1DM.^{205,206} Inhibition of the 12-LOX pathway protects mouse and human

islets against cytokine-mediated cell death/disregulation.^{207–210} A follow-up study in STZ-induced T1DM mice showed treatment with the 12/15-LOX inhibitor ML351 displayed similar glucose tolerance and identical β cell mass to control C57BL/6 mice,²¹¹ making the 12-LOX pathway a promising target for β cells preservation.

IL-35 is a newly discovered cytokine believed to have immunosuppressive effects and an important role in autoimmunity.^{212,213} Systemic administration of recombinant IL-35 reverses established T1DM in a NOD mouse model through tolerization by T_{regs} .²¹⁴ Transgenic expression and viral transfection of IL-35 in the pancreatic β cells of NOD mice displayed a four-fold lower T1DM incidence compared to control NOD mice and had reduced insulinitis.²¹⁵ While these preliminary results are promising, more work is needed to confirm the β cell protective effects of IL-35.

Epigenetic histone modifications have been shown to play a role in T1DM.^{216,217} Histone deacetylases (HDACs) are enzymes that catalyze the removal of acetyl groups from the lysine residues of histone proteins, generally reducing gene expression and play a role in transcriptional regulation.²¹⁸ HDAC inhibitors have anti-inflammatory properties and are actively being explored as therapeutics for cancer, rheumatoid arthritis, and T1DM.²¹⁹ The HDAC inhibitor ITF2357 protected islet cells from STZ-mediated destruction and INS-1 cells from cytokine-mediated cell death through inhibition of HDAC3.^{220–222} Furthermore, NOD mice treated with the specific HDAC3 inhibitor BRD3308 showed a significantly lower incidence in T1DM and significantly less mononuclear islet infiltration.²²³ Thus far, β cell protective strategies have produced promising results in animal models and may be candidates for combination therapies with immunomodulatory agents.

Regeneration.

β Cell Protection and Proliferation.—The restoration of lost or at-risk β cells is a major therapeutic focus in both T1DM and T2DM. These efforts focus on either selectively expanding or protecting β cells and reprogramming non- β cells into functional β cells. Pancreas protection and regeneration studies were initially spurred by signs of β cell regeneration in adolescence and during times of stress such as pregnancy and obesity.²²⁴ However, adult endocrine islets have limited regenerative capacity. Recent studies have shown that α cells (glucagon producing) and δ cells (somatostatin producing) can undergo transdifferentiation to functional β cells or β -like cells.^{225,226} Although the precise mechanism and protection of β cells is unclear, potential *in vivo* therapeutic targets have been identified.

Inducing β cells to undergo mitosis through increases in relevant cyclins, cyclin-dependent kinases (CDKs), and with reductions in cell cycle inhibitors can protect against losses in β cell mass.²²⁷ A high-throughput small molecule screen identified harmine as a cell mitogenic compound and two relevant protein targets: tyrosine-regulated kinase-1a (DYRK1A) and the Nuclear Factors of activated T cells (NFAT).²²⁷ Harmine inhibits the ATP binding pocket of DYRK1A, inducing c-MYC activation, and driving β cell proliferation. Harmine also causes translocation of NFAT from the cytoplasm to the nucleus and increases expression of the transcription factors involved in β cell differentiation (NKx6.1, PDX1, and MAFA).²²⁷ Ultimately, harmine induced human β cell proliferation to

levels comparable in early adolescence. This work established DYRK1A and NFAT as attractive clinical targets, but harmine has limited therapeutic utility due to off-target effects against other DYRK, CDC-like kinases (CLK), and inhibition of monoamine oxidases (MAOs).

Physiological glycemic regulation is modulated by the thyroid hormones triiodothyronine (T3) and the T3 precursor, levothyroxine (T4). T3 supplementation attenuates hyperglycemia in STZ-treated mice,²²⁸ while T4 supplementation in wild-type C57BL/6 mice enhanced glucose clearance and simultaneously increased the proliferation and apoptosis of pancreatic β cells, while increasing insulin production.²²⁹ T4 supplementation was also shown to reduce the onset of T1DM in the RIP-B7.1 model of early diabetes and increase survival of STZ-treated mice through increased expression of Akt and glucokinase. Clinical application will need to avoid the detrimental effects of high circulating levels of these hormones (thyrotoxicosis).^{230,231}

Maintaining a stable rate of β cell turnover would be beneficial, yet an ideal therapy would also keep β cell mass within a normal range and prevent future insulinitis. Substance P (SP) is a neuropeptide that acts as a pain-sensing neurotransmitter in the central nervous system and as an immune modulator.²³² SP directly induces proliferation of β cells through the PI3K/Akt pathway, affects the preservation of β cells at early time points after STZ treatment, and inhibits further insulinitis.²³³ This suggests early intervention of T1DM can benefit from both β cell and neural components in the pancreatic islets.

Novel agents that can shift a pro-inflammatory autoimmune islet environment towards an anti-inflammatory milieu are also desirable for islet protection and regeneration therapies. Recently, liver receptor homologue-1 (LRH-1) was shown to modulate the expression of genes involved in glucose homeostasis and is protective against both inflammation and ER stress.^{234–236} Small molecule BL001 activates LRH-1 in several T1DM mouse models resulting in a shift towards the anti-inflammatory macrophage phenotype (M2), secretion of IL-10, T_{reg} expansion, and a reduction in T_H1 cells with increasing T_H2 phenotype.²³⁷ This resulted in reduced insulinitis and LRH-1 stimulated regeneration of β cells through a non-proliferation/apoptosis manner, suggesting LRH-1 activation may promote α to β cell transdifferentiation.

α to β Cell Transdifferentiation.— β cell regeneration can be achieved through transdifferentiation of developmentally related pancreatic cell types into functional β cells. Notable work in elucidating the mechanism of either α or δ to β cell transdifferentiation highlights the activation of transcription factors *pdx1* and *pax4*, the downregulation of *arx* and *foxo1*, and the influence of epigenetic modulation.^{225,226,238–241} Direct inhibitors to transcription factors and epigenetic modulators has traditionally been considered “undruggable”, however recent progress has been made and more specific inhibitors have been developed.²⁴² Here we focus on extracellular and extranuclear targets that stimulate islet cell transdifferentiation to β cells which act, in part, through transcription factor modulation.

The insulin-like growth factor (IGF) signaling pathway plays a role in cell proliferation and differentiation.²⁴³ Overexpression of the IGF-binding protein 1 (IGFBP1) in zebrafish results in inhibition of IGF signaling and promotes β cell regeneration through α to β transdifferentiation.²⁴⁴ Importantly, IGF receptor inhibitor picropodophyllin replicates this result and increases α cell expression of PDX1, an important marker for α to β transdifferentiation. The role of IGFBP1 was further explored in transgenic mice overexpressing the protein and found to restore normoglycemia when administered exogenous IGF-I.²⁴⁵ Enhancing IGFBP1's potential role in human diabetes, high serum levels of IGFBP1 was associated with a reduced risk of T2DM development.^{246,247}

Aristaless related homeobox (ARX) is a master regulator of α cell fate and is required for maintaining glucagon production.²⁴⁸ Targeting ARX can modulate the pancreatic ratio of α , β , δ , and pancreatic polypeptide cells. Direct inactivation of ARX alone is insufficient to induce α to β cell conversion,^{241,249} and inhibition of DNA methyltransferase 1 (DNM1) is also required for conversion of α cells to functional β -like insulin secreting cells.²⁵⁰ Heterogeneity of expressed β cell regulators was observed, showing incomplete conversion to a full β cell morphology, but the observed response to glucose is encouraging. Indirect functional repression of ARX also shows therapeutic promise. The anti-malarial drug Artemisinin induces ARX translocation to the cytoplasm and stimulates GABA_A receptor signaling, resulting in increased β cell mass in zebrafish and rodent models.²⁵¹ Interestingly, GABA signaling is known to modulate the immune system and promote β cell proliferation.^{252,253}

Taken together, β cell regeneration either through protection, proliferation, or transdifferentiation offers hope as a successful regeneration therapy and need only restore normoglycemia to greatly impact treatment for T1DM patients.

ISLET TARGETING

In addition to modulating specific pathogenic pathways, targeted technologies for therapeutic and diagnostic purposes are under investigation for T1DM. Targeted β cell/islet probes by themselves do not have therapeutic effects, but are used to image and monitor disease progression, serve as a biomarker for disease incidence, or play a role in targeting drug delivery systems. Indeed, drug-loaded targeted constructs are now being investigated to better localize existing drugs/therapies to the islet microenvironment and improve treatment outcomes. Here we discuss advances in β cell imaging probes and islet delivery technologies.

β Cell Imaging.

Current clinical tests for T1DM diagnosis, such as insulin/C-peptide measurements after a glucose challenge, serve mainly as indicators of β cell function, but not β cell mass. In addition, changes in these markers are typically only detected when nearly 80% of β cells have already lost function. Therefore, development of probes to measure/monitor β cells is urgently needed to accurately monitor T1DM progression.

β cells are scattered in the pancreas in the islets of Langerhans and constitute only 1–3% of total pancreas mass, thus imaging requires a probe with high specificity and high signal-to-noise ratio. While there are a number of imaging probes used to monitor β cell mass, only a small number of probes have been tested clinically, including exendin-4/exenatide, tetrabenazine, 5-hydroxy-tryptophan (HTP), sulfonylurea, and (+)-4-propyl-9-hydroxynaphthoxazine (PHNO) based probes.^{254–257}

Exendin-4, also known as exenatide, is a 39 amino acid peptide isolated from *Heloderma horridum* that shares 50% homology with endogenous glucagon-like peptide 1 (GLP-1), but has much slower clearance and high stability *in vivo*.²⁵⁸ Clinically, exendin-4 is typically prescribed with metformin for treatment of T2DM to increase insulin secretion and reduce body weight.²⁵⁹ GLP-1R expression is restricted to β cells in the pancreas, with low expression in exocrine tissue. Exendin-4 has been extensively utilized as a targeting ligand for β cell imaging probes.²⁶⁰ An exendin-4 based probe conjugated to diethylenetriaminepentaacetic acid (DTPA) chelated with Indium-111 was successfully used to image transplanted islets in humans.²⁶¹ Additional clinical studies with a ¹¹¹In-DTPA-Exendin probe found a linear relationship between β cell mass and pancreatic uptake of the probe, but failed to find any statistical significance in uptake between healthy and T1DM human subjects.²⁶²

Vesicular monoamine transporter 2 (VMAT-2) is involved in the storage and release of amines, such as dopamine and serotonin, and is expressed both in neuroendocrine cells and β cells.^{263,264} Tetrabenazine and its various analogs, such as dihydrotetrabenazine (DTBZ) and fluoropropyl-dihydrotetrabenazine (FP-DTBZ), are the most widely used VMAT2 antagonists.^{265,266} The analog [¹⁸F]-FP-DTBZ successfully correlates β cell function and mass, after accounting for pancreatic volume,²⁶⁷ with T1DM subjects showing an average 59% loss in specific binding of the probe compared to healthy subjects. Currently, there is some disagreement about using VMAT2 as a biomarker for β cell mass, as binding in the pancreas may be mainly nonspecific,²⁶⁸ and further studies are needed.

Although they come from different germinal layers, pancreatic β cells and neurons have shared functions, including serotonin production.²⁶⁹ In many mammals, including humans, serotonin production is exclusive to the islets with no expression in the exocrine pancreas.²⁷⁰ [¹¹C]5-hydroxy-tryptophan ([¹¹C]5-HTP) is a probe used to visualize serotonin biosynthesis in disease and is used to monitor neuroendocrine tumors.^{271,272} Initial studies with [¹¹C]5-HTP in exocrine PANC-1 cells and endocrine human insulinoma cell lines (CM) suggested signal may not be representative of β cell mass, but PANC-1 cells rapidly lost signal whereas CM cells maintained signal.²⁷³ [¹¹C]5-HTP was further studied in non-human primates, where it had two-fold higher pancreatic uptake compared to the liver. STZ-induced diabetic mice showed 66% lower signal compared to wild-type mice, suggesting a correlation to functional β cell mass.²⁷⁴ Clinical studies show a correlation between plasma C-peptide levels and the pancreatic uptake of [¹¹C]5-HTP, suggesting that the probe may be used as a marker for β cell mass.²⁷⁵

Sulfonylureas, such as glibenclamide, bind to the sulfonylurea receptor 1 (SUR1) on β cells and are used clinically as insulin secretagogues for treatment of T2DM.²⁷⁶ Glibenclamide

analogues for β cell imaging have been synthesized with high affinity to SUR1 and variable lipophilicities.^{276,277} A promising glipizide analogue conjugated to ^{99m}Tc -DTPA was developed and displays a thirty-fold higher pancreatic uptake with reduced kidney and liver uptake.²⁷⁸ To complement this effort, multivalency using glibenclamide-polyamidoamine (PAMAM) probes have been evaluated. PAMAM dendrimers with 15 glibenclamide molecules/dendrimer have higher affinity for SUR1 than the unmodified glibenclamide and successfully labeled human islets *in vitro* and murine islets *ex vivo*.²⁷⁹

The dopamine receptors, D2 and D3, also have overlap between neural and pancreatic cells. D2/D3 receptors colocalize with insulin-containing granules suggesting that probes used to image D2/D3 receptors in the brain can also image the receptors on β cells.²⁸⁰ Iodobenzamide, a molecule that is used clinically to image D2 receptors in neurodegenerative diseases, was recently shown to be effective for imaging islet grafts. Unfortunately, [^{123}I]-Iodobenzamide also had high uptake in spleen, liver, and kidney, where grafts are often placed.²⁸¹ Fallypride, another D2/D3 antagonist used for clinically imaging D2 receptors in the brain, has been investigated for imaging transplanted islet grafts. [^{18}F]Fallypride had 0.05% injected dose uptake in pancreas, but encouragingly successfully visualized transplanted islets.²⁸² A later study confirmed that [^{18}F]fallypride uptake was 77% lower in STZ-treated mice compared to control, indicating potential utility in monitoring β cell mass.²⁸³ A currently used compound for positron emission tomography (PET) brain radioligands, [^{11}C](1)-4-propyl-9-hydroxynaphthoxazine (PHNO), was recently identified as a D2/D3 antagonist and used to differentiate between a healthy, T1DM C-peptide positive, and a T1DM C-peptide negative subject based on PET imaging.²⁸⁴

A number of other probes have been developed and tested in multiple animal models, but have failed to garner any data in humans. Of note is the G-coupled protein receptor 44 (GPR44), which binds Prostaglandin D2 (PGD_2) and was identified as a β cell-specific biomarker through a proteomic screen.²⁸⁵ Initial investigations *in vitro* with human islets and EndoC- β H1 cells confirmed the surface expression of GPR44 and identified the antagonist AZD3825 as having nanomolar affinity and high endocrine-exocrine binding.²⁸⁶ Biodistribution studies in non-human primates and pigs with a related GPR44 antagonist indicated pancreatic binding, but the probe also accumulated in the small intestines and spleen.²⁸⁷

Islet Delivery.

Several targeting ligands are under investigation for their ability to selectively deliver therapeutics to the islet microenvironment. Using phage display and a library of small cyclic peptides, the peptide CHVLWSTRC was found to specifically home to islets where it binds to the EphrinA4 receptor on islet capillary cells.²⁸⁸ This cyclic targeting ligand has been utilized twice in attempts to deliver therapeutics specifically to the islet microenvironment. Conjugation of this peptide to a PLGA-PEG polymer and nanoformulation with the anti-inflammatory drug genistein yielded nanoparticles targeted to the islet microenvironment. In rat islet capillary endothelial cells that endogenously express EphA4, the targeted nanoparticles had three-fold higher uptake compared to untargeted/scrambled nanoparticles.²⁸⁹ This peptide targeting ligand was also used to deliver plasmid DNA encoding retinoic

acid early inducible gene-1 (RAE-1) to islet capillary cells. The targeted polyplex displayed 2.5-fold higher uptake compared to untargeted control in MS-1 cells that express EphA4.²⁹⁰ Targeted constructs utilizing the CHVLWSTRC peptide have shown promising results *in vitro*, but will need to be validated in animal models for their ability to bind islet vasculature.

Several antibodies have been developed to specifically home to the islet microenvironment. An *in vitro* phage display experiment with INS-1 cells was used to identify antibody SCA B5 binding specifically to β cells with low exocrine tissue affinity.²⁹¹ *In vivo*, 5.12% of injected dose of ¹²⁵I-labeled SCA B5 localized to the pancreas with low (<0.70%) accumulation in other tissues. The putative molecular target of SCA B5 is currently unknown.²⁹¹ As previously mentioned, GAD-65 is an autoantigen affiliated with T1DM, so it may be used as a unique feature in the pancreatic microenvironment to target. An anti-GAD antibody fragment (Fab) has been developed as a β cell specific nucleic acid delivery system and showed a ten-fold higher transfection in GAD-expressing MIN-6 cells compared to an untargeted construct with low transfection in GAD-negative HEK-293 cells.²⁹² These antibodies had promising *in vitro* results, but need to be validated *in vivo*.

β cell selective imaging probes are of vital need to monitor loss of β cell mass as the disease progresses. These probes could enable β cell mass to be a new biomarker for disease incidence. Finally, these targeting ligands have potential for islet-specific delivery of highly potent therapeutics that otherwise may give detrimental effects when administered systemically.

FUTURE PERSPECTIVE

Significant progress has been made developing new therapeutic modalities that attack T1DM on multiple fronts: from immunomodulatory agents, β cell protective and regenerative strategies, to new diagnostic probes. All of these approaches take advantage of key molecular mechanisms and targets in T1DM, and while pre-clinical results have been promising, clinical translation of these approaches has remained elusive. Many of these therapeutic modalities are more effective when administered in pre-symptomatic stages of the disease, which is currently challenging in humans due to a lack of predictive and reliable diagnostic readouts. Clinical evaluation of some of these therapies have revealed adverse effects through the distribution and systemic action of these agents at unrelated tissue sites. While many monotherapies are effective in pre-clinical models, the added complexity of human biology may require combinatorial approaches to achieve clinical efficacy.

The next generation of therapeutic modalities (Figure 3) will benefit from current efforts at developing predictive biomarkers and probes in T1DM-susceptible individuals. Improved diagnostics will enable many of the therapies discussed here to be interventional and expand the temporal window for many immunomodulatory and β cell protection/regeneration therapies. Equally important will be the emergence of drug delivery systems that can bias the systemic distribution of these agents to relevant tissue sites, enabling localized therapeutic modulation and avoiding adverse effects in healthy tissues. T1DM is a complex disease where our understanding of the pathogenesis is evolving. The diversity and richness of the

approaches reviewed here reveal the direction of future treatment paradigms for this disease that could dramatically improve clinical outcomes and the quality of life for these patients.

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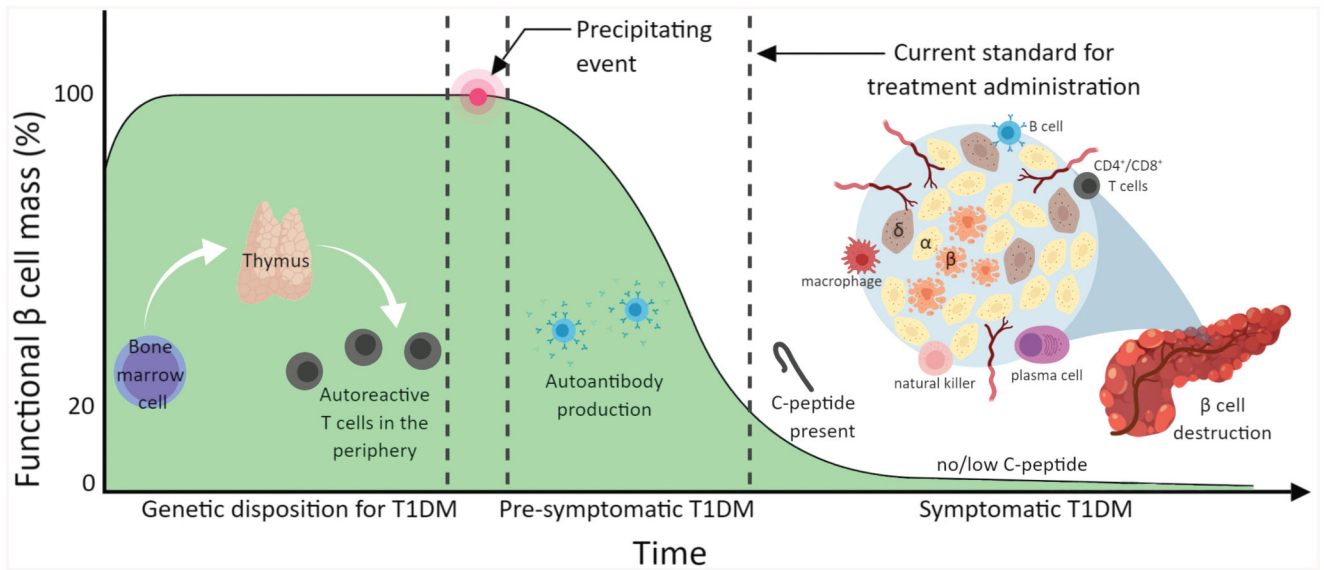


Figure 1.

Progression of β cell loss and primary cells involved in the pathogenesis of T1DM. Predisposition from bone marrow, thymus, and immune populations followed by a precipitating event lead to β cell mass loss prior to clinical diagnosis and therapeutic intervention.

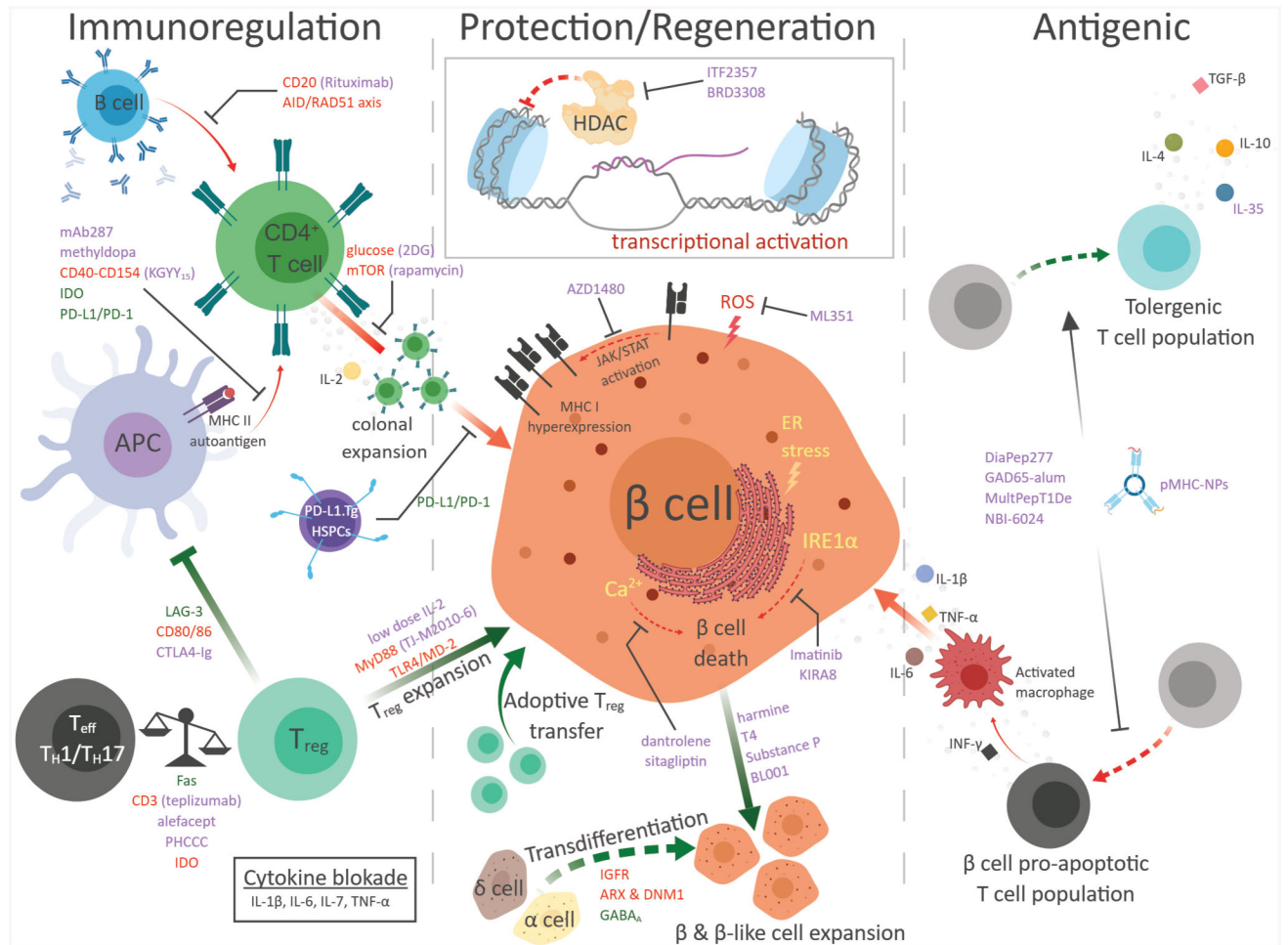


Figure 2. Selective T1DM pre-clinical and clinical therapeutic intervention strategies through immunoregulation, β cell protection and regeneration, and antigenic therapy. Solid arrows denote a causal event. Dashed arrows denote cell population shift. Red arrows indicate the pathway leads to T1DM autoimmunity and green arrows indicated protective and immune tolerance pathways. Green text indicates increase secretion or strengthening of indicated molecular interaction. Red text indicates suppression of indicated molecular interaction. Purple text indicates additional therapies discussed herein.

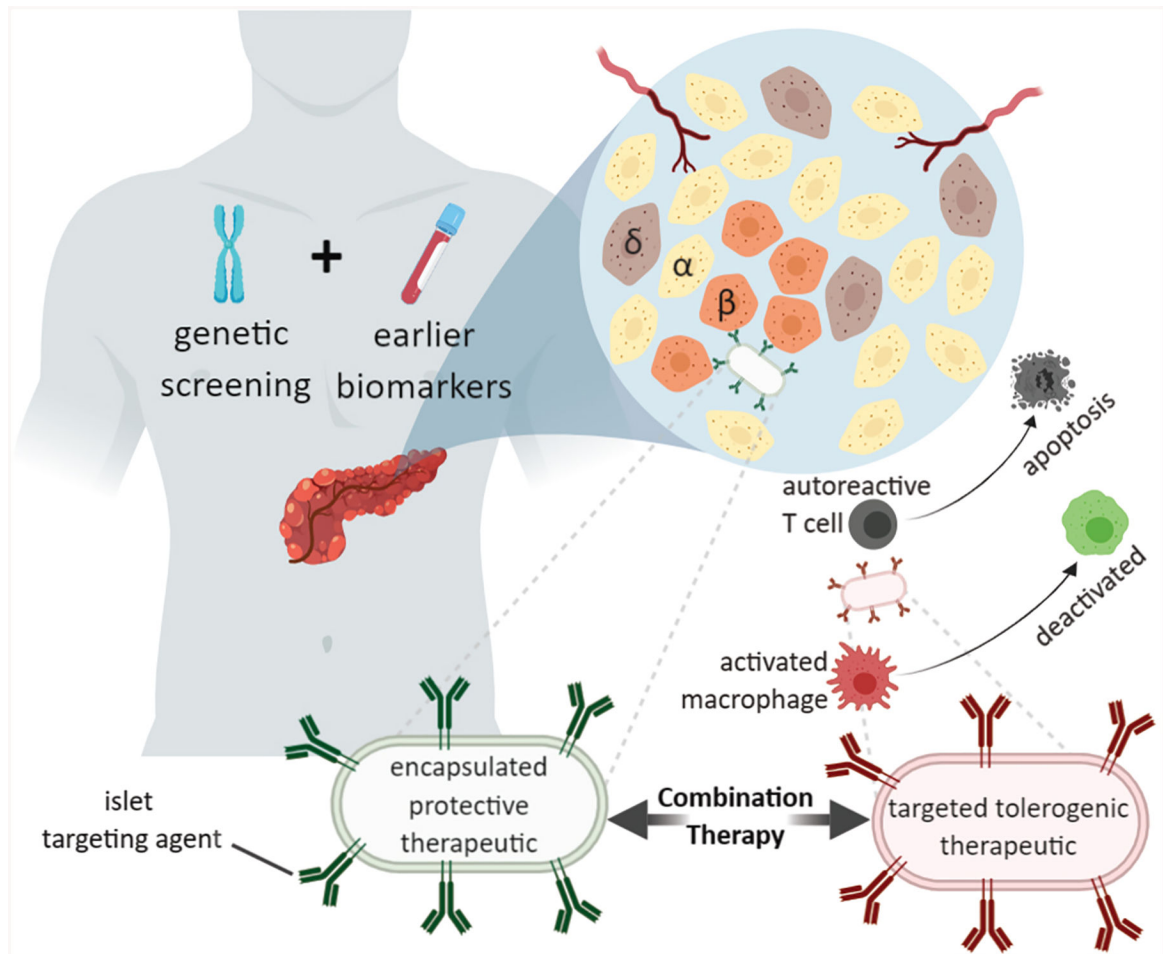


Figure 3.

Future therapeutic paradigm for localized early delivery of therapeutics for T1DM. In combination with genetic screening and early biomarker T1DM indication, formulated nanoparticles with encapsulated therapeutic agent can be conjugated to islet targeting agents that populate the nanoparticle surface. These localized delivery agents will likely need to be used in combination therapies.

Table 1.

T1DM and Immunoregulatory Clinical Trials

Therapy Group	Therapy	Phase	ID	Comments
Cytokine Blockade	Anti-IL-1 β (Canakinumab)	II		No C-peptide level difference. ²⁶
	IL-1R antagonist (Anakinra)	II		No C-peptide level difference. ²⁵
	Soluble TNF- α receptor (Etanercept)	I/II		Significant reduction of HA1c and increase in C-peptide levels. ³¹
Inducing Antigenic Tolerance	Human recombinant GAD-alum	II		Decreased T _{eff} , increased T _{regs} , but no C-peptide difference. ⁴⁵
	HSP60 (DiaPep277)	II	N/A	Delayed decrease in C-peptide levels. ⁴⁹
	HSP60 (DiaPep277)	II	N/A	30 children, no C-peptide, insulin dose, or HA1c level difference. ⁴⁸
	Altered peptide ligand of B9-23 epitope (NBI-6024)	I	N/A	T cell population shift from T _{H1} to T _{H2} . ⁵²
	Altered peptide ligand of B9-23 epitope (NBI-6024)	I		Repeated administration shows no efficacy. ⁵³
	Multiple Islet peptide (MultiPepT1De)	Ib		Recruiting. ⁵⁴
Lymphocyte Modulation: T cell	Anti-CD3 (Otelixizumab)	III		No C-peptide level difference. ¹⁸
	Anti-CD3 (Teplizumab)	II/III		Primary outcome not met, 5% of patients were not taking insulin 1 year after treatment. ¹⁹
	Anti-CD3	II		Reduced insulin dosage requirements. ⁶³
	Anti-CD3 (Teplizumab)	II		No C-peptide level difference. Reduced insulin requirements. ⁶⁴
	IL-2 (Aldesleukin)	I & II		Selectively activates T _{regs} . No efficacy endpoints. ⁸²
	IL-2/Rapamycin combination	I		T _{reg} , NK and T _{eff} populations increased. B cell function impaired. ⁸⁴
	IL-2 (Aldesleukin)	I & II		Dosage testing. No results reported. ⁸⁵
	Ex vivo expanded human autologous polyclonal T _{regs}	I		Safe. Subset were long-lived. C-peptide levels stabilized through 2 years. ⁷³
	Ex vivo expanded CD4+CD25+CD127-T _{regs}	II	ISRCTN06128462	Safe. Increased C-peptide levels. Reduced insulin requirements. ⁸⁷
	Ex vivo expanded human T _{regs}	II		Follow up study. No results reported. ⁷⁷
	Anti-CTLA4 (Abatacept)	II		Slowed β cell reduction for 24 months, but no improved function. ⁹⁷
Lymphocyte Modulation: APC	Methyldopa	Ib		Reduced inflammatory T cell response to insulin. ¹²⁰
	Methyldopa	II		Recruiting.
	Anti-CD40 ligand for Lupus	I	N/A	Terminated due to risk of life-threatening thrombotic events. ¹³⁰
Lymphocyte Modulation: B cell	Anti-CD20 (Rituximab)	II		Inhibits T cell activation and further insulinitis over a 1 year period. Follow-up study showed increase of asymptomatic viremia. ^{146,147}

Therapy Group	Therapy	Phase	ID	Comments
Immuno-regulatory	Anti-PD1 for various cancers	N/A	N/A	Various different trials report development of insulin-dependent diabetes following anti-PD1 therapy. ¹⁵⁹
Islet Protection	Tyrosine Kinase Inhibitor (Imatinib)	II		Preserved β cell function, reduced insulin requirements up to 1 year (unpublished, presented at American Diabetes Association Scientific Sessions 2017).
β Cell Imaging	GLP-1R targeted probe ¹¹¹ In-DTPA-Exendin	N/A		Found difference in pancreatic uptake between healthy and T1D patients. ²⁶²
	VMAT2 targeted probe [¹¹ C]-DTBZ	N/A		Did not correlate β cell function to mass. ²⁶⁶

APPROACHES FOR IMMUNOMODULATION