



# New Horizons in the Treatment of Type 1 Diabetes: More Intense Immunosuppression and Beta Cell Replacement

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Since the discovery of autoimmunity as the main pathophysiologic process involved in type 1 diabetes, many attempts have tried to delay or stop beta cell destruction. Most research protocols in humans have investigated the effects of therapeutic agents targeting specific steps of the autoimmune response. In spite of safety and some degree of beta cell preservation, the clinical impact of such approaches was similar to placebo. Recently, research groups have analyzed the effects of a more intense and wider immunologic approach in newly diagnosed type 1 diabetic individuals with the “immunologic reset,” i.e., high-dose immunosuppression followed by autologous hematopoietic stem cell transplantation. This more aggressive approach has enabled the majority of patients to experience periods of insulin independence in parallel with relevant increments in C-peptide levels during mixed meal tolerance test. However, on long-term follow-up, almost all patients resumed exogenous insulin use, with subsequent decrease in C-peptide levels. This has been at least in part explained by persistence of islet-specific T-cell auto-reactivity. Here, we discuss future steps to induce immune tolerance in individuals with type 1 diabetes, with emphasis on risks and possible benefits of a more intense transplant immunosuppressive regimen, as well as strategies of beta cell replacement not requiring immunomodulation.

**Keywords:** type 1 diabetes, immunotherapy, autologous hematopoietic stem cell transplantation, immunologic reset, autoimmunity, beta cell preservation

## THE COMPLEX AUTOIMMUNE REPERTOIRE OF TYPE 1 DIABETES (T1D)

Type 1 diabetes is an autoimmune disease characterized by a silent phase of progressive beta cell destruction, followed by a symptomatic phase of hyperglycemia, when great amount (not well defined) of beta cell mass and function has been reduced. This phenomenon occurs in individuals with genetic background exposed to still undefined immunologic triggers (1). The rate of beta cell destruction is not absolutely linear as previously described. The pattern of temporal beta cell loss is typical of relapsing-remitting diseases, as periods of exacerbated beta cell destruction alternate with those of inactivity (1). In addition, subjects who are older at diagnosis present a slower process of autoimmune destruction, and, as a consequence, larger residual beta cell mass (2, 3).

Recent data from the TEDDY (The Environmental Determinants of Diabetes in the Young) trial showed that autoantibodies against islet antigens may occur early in life of a subset of patients. In fact, the age of patients at diagnosis of overt diabetes and the order of antibody positivity depend

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both on HLA and non-HLA genotypes (4, 5). The exact triggers of autoimmunity against pancreatic islet beta cells have not yet been identified, and much of the immunologic mechanisms involved in this process are still under study. There is a pathological cross talk involving B and T lymphocytes, regulatory cells (B and T reg cells), autoantibodies, monocytes, natural killer cells, cytokines, and the beta cells themselves (1, 6–8). Although there is evidence that CD8+ effector T cells may be directly involved in beta cell death, other cell types most certainly participate in this autoimmune response (8, 9). In this scenario, which are the effects of immune interventions in humans with T1D?

## THE ROLE OF IMMUNOINTERVENTIONS IN THE PRESERVATION OF BETA CELL MASS

Since the discovery of autoimmunity as the key phenomenon leading to T1D, many immunologic interventions have been investigated as strategies to stop beta cell destruction. The Diabetes Control and Complication Trial showed that higher C-peptide levels are associated with lower incidence of diabetic nephropathy, retinopathy, and less episodes of hypoglycemia (10). Preservation of beta cell mass is also associated with less exogenous insulin requirement (3). Autoimmunity in T1D is complex and involves different pathways, connections, organs, and cells. Nevertheless, most research protocols have attempted to hinder beta cell destruction targeting specific molecules or pathways, instead of wider immunosuppressive approaches. The argument is that systemic immunosuppression may expose the patient to undesirable

adverse effects. **Table 1** summarizes the main outcomes of recent clinical trials on beta cell preservation in patients with T1D (secondary prevention trials).

Effector T cells are directly related to beta cell death, and secondary prevention trials with teplizumab (anti-CD3 monoclonal antibody) (11) and oteplizumab (anti-CD3 monoclonal antibody) (12–15) have used these cells as targets to preserve pancreatic function. In these studies, treated patients presented a less accelerated rate of decline in C-peptide levels and also some reduction in daily insulin doses, when compared to non-treated patients, indicating a beneficial effect of these immunomodulatory agents on beta cell preservation. However, less than 5% of individuals experienced periods free from insulin.

The combination of antithymocyte globulin plus granulocyte colony-stimulating factor (G-CSF) has also been investigated, as another attempt to stop T cell auto-reactivity. Individuals up to 2 years after diagnosis of T1D (different from other secondary prevention trials) were included. Along 1 year, the decline in C-peptide levels was slower in treated patients, when compared to the control group, but there was no difference in insulin requirements (16). In 2014, Peskovitz and colleagues turned the focus on B cells, treating newly diagnosed T1D individuals with rituximab (anti-CD20 monoclonal antibody). The treatment was considered safe and able to induce slower decline in C-peptide levels; however, no significant difference in insulin requirements was detected between treated and placebo groups (17).

Later, T cells were again targeted by immunomodulatory approaches with alefacept (LFA3-IgG1 fusion protein that binds CD2) (18) and abatacept (CTLA-4-IgG1 fusion protein that binds CD80/CD86) (19) that induce apoptosis and inhibit activation of

**TABLE 1** | Recent secondary prevention trials in individuals with type 1 diabetes and their effect on beta cell preservation.

Immunomodulatory approach	Main target of medication	Follow-up	Effect on C-peptide on time	Comments
Teplizumab (11)	T cell (CD3+)	2 years	Slower decline compared with placebo	At 1 year, 5.3% (11/207) of patients in the full-dose group were free from insulin. In year 2, three of these 11 were still insulin-free
Otelixizumab (12–15)	T cell (CD3+)	4 years	Slower decline in C-peptide with higher doses of oteplizumab. No effect with lower doses	The higher the dose of oteplizumab, the better the clinical response, but the more the risk of side effects. No patient became insulin-free
ATG + granulokine (16)	T cell (CD4+, CD8+), B cell, T reg	1 year	Slower decline compared with placebo	No differences in insulin dose compared with placebo. No patient insulin-free. Time of disease at randomization: 4 and 24 months
Rituximab (17)	B cell	2 years	Slower decline compared with placebo	Lower insulin requirements in the treated group. No patient insulin-free
Alefacept (18)	T cell (CD4+, CD8+)	2 years	Slower decline compared with placebo	Reduced insulin dose in treated group. No patient insulin-free
Abatacept (19)	T cell (CD80+, CD86+, CD28+)	3 years	Slower decline compared with placebo	No differences in insulin dose compared with placebo
Autologous mesenchymal stem cell (21)	T cell, T reg	1 year	No change compared with placebo	No differences in insulin dose compared with placebo. Cells were harvested from bone marrow
Autologous T regs (23)	T reg	2 years	No changes in C-peptide along the time compared to baseline	No differences in insulin dose along the time
Chemotherapy followed by autologous hematopoietic stem cell transplantation (26–35)	“Immunologic reset”	Up to 7 years	Increase in C-peptide > 3 y post-transplantation and then returned to baseline levels after 6 years and (compared to baseline)	Around 80% of patients became insulin-free for variable periods. Lack of randomized, parallel, double-blind, placebo-controlled trials. One death occurred in the Polish group. Potential risk of severe side effects

T-cells, respectively. Both drugs promoted beta cell preservation, with slower rate of reduction of C-peptide levels and were considered safe. Nevertheless, clinical effects were disappointing, as alefacept promoted only slight reduction of insulin requirements, while abatacept did not change them at all.

Cell-based therapies were also investigated in secondary prevention trials. Multipotent mesenchymal stromal cells (MSCs) are considered safe promising tools to change the natural history of T1D and other immune-mediated diseases. They exhibit immunomodulatory potential, migratory capacity to injured and inflamed areas and can contribute to tissue regeneration [directly or by the secretion of bioactive factors (20)]. In 2015, Carlsson and colleagues analyzed the effect of autologous MSCs in adults with recent-onset T1D. In contrast with non-treated T1D patients, who presented a significant decline in serum C-peptide levels, beta cells were still preserved in the MSC-treated group at 1-year follow-up, with stable levels of C-peptide. The beneficial effect on pancreatic function, however, did not reflect on insulin requirements of the MSC-treated group. No relevant side effects were observed (21).

The pathophysiology of T1D also involves defective function of regulatory T cells (Tregs), and perhaps T effector cells that may be refractory to Treg suppression (22). In 2015, Bluestone and colleagues evaluated the effects of intravenous infusions of expanded autologous polyclonal Tregs in recent-onset T1D patients (22). In this phase I open label study, autologous Treg infusions were safe, but did not change the temporal secretion of C-peptide and exogenous insulin use along 2 years of follow-up. Phase 1/2 trials have also investigated the effects of low-dose exogenous IL-2 on Treg function (23, 24). However, although the studies have detected increments in Treg numbers, the effects on glycemic control have yet to be established.

Most of the research protocols in humans used approaches that aimed to target only some steps of autoimmunity repertoire. Despite safety and some degrees of beta cell preservation, the clinical impact of such agents was similar to the placebo groups. Moreover, most of these immunomodulatory agents were originally developed and/or tested to prevent progression of other autoimmune diseases, and they may not necessarily share similar pathophysiological mechanisms of T1D.

Under the rationale of having a wider approach to the complex immune dysfunctions linked to T1D, in 2007 Voltarelli and colleagues published the first trial analyzing the effect of the “immunologic reset” in newly diagnosed T1D patients. The strategy included ablation of the autoreactive immune system, followed by generation of a new and tolerant system through infusion of autologous stem cells. Hematopoietic stem cells were mobilized with cyclophosphamide (2.0 g/m<sup>2</sup>) and G-CSF (10 µg/kg per day) and then were harvested from the peripheral blood by leukapheresis and cryopreserved. Subsequently, hematopoietic stem cells were injected intravenously after conditioning with cyclophosphamide (200 mg/kg) and rabbit antithymocyte globulin (4.5 mg/kg). In this prospective non-randomized trial, most patients (21 out of 25) became insulin-free after transplant. At 4 years posttransplantation, C-peptide levels were significantly higher than pretransplant levels (25–30). Furthermore, at 7 years, most patients had already resumed exogenous insulin use, but C-peptide levels were still similar to those pretransplantation.

Independent research groups have reproduced the Brazilian transplant protocol with slight modifications to increase efficacy of the procedure (31–34). In the Polish protocol, patients underwent 2 or 3 plasmapheresis sessions before transplant and acarbose was used as maintenance drug (31, 32). In the Mexican protocol, the transplant conditioning regimen included cyclophosphamide plus fludarabine (34). Nevertheless, results regarding duration of insulin independence and transient increase in C-peptide levels were similar to those shown by Voltarelli and colleagues.

Recently, to compare long-term effects of the “immunologic reset” with the real world scenario, a cross-sectional analysis was made with BrazDiab1 (the largest multicenter observational study in T1D in Brazil) data. During the long-term follow-up of 8 years, none of the transplanted patients had developed microvascular complications, while 21.5% of the non-transplanted BrazDiab1 patients had presented at least one microvascular complication (30). Despite limitations, this study suggests that hematopoietic stem cell transplantation may promote long-term beneficial metabolic effects beyond insulin freedom.

Ethical and safety issues are key points of research protocols that involve high-dose systemic immunosuppression. Since there are potential short-term risks of infection, acute organ dysfunction and death, and theoretical long-term risks of malignancies and secondary autoimmune diseases, the inclusion of young children with T1D has been restricted in these trials. The majority of patients included in the studies presented only nausea, vomiting, alopecia, and fever as transplant-related adverse events. In the Polish study, however, there was one death due to *Pseudomonas aeruginosa* sepsis (32). To date, no severe long-term side effects have been described.

In 2017, Malmegrim and colleagues analyzed the effects of autologous hematopoietic stem cell transplantation on the immune system (27). Although CD8<sup>+</sup> T-cells reconstituted early after transplant, CD4<sup>+</sup> T-cell remained lower than baseline for several months, resulting in a prolonged inversion of the CD4/CD8 ratio. B cells reconstituted to baseline levels at 2–3 months posttransplantation and regulatory T cell (CD4<sup>+</sup>CD25<sup>hi</sup>FoxP3<sup>+</sup> and CD8<sup>+</sup>CD28<sup>-</sup>CD57<sup>+</sup>) counts increased. In the overall population, memory cells comprised most of T cells detected on follow-up of patients after transplantation; however, in patients that remained insulin-free for longer periods after transplant, there was slower reconstitution of effector memory cells. When analyzed separately, islet-specific autoreactive CD8<sup>+</sup> T cells were still present after high-dose immunosuppression, indicating insufficient ablation of these cells.

The lack of knowledge of the exact mechanisms of disease, genetics, and environmental triggers may be one of the reasons for not restoring immunological balance in secondary prevention trials. On the other hand, the organ-specific autoreactivity may be too intense and persistent to be controlled, even by systemic ablation of the immune system.

## THE FUTURE: BETA CELL REPLACEMENT OR MORE INTENSE IMMUNOSUPPRESSION?

As secondary prevention trials did not achieve complete restoration of immune balance, development of new strategies to preserve

and/or increase beta cell mass are still required. If even the most intense immune-based approach of “immunologic reset” with autologous hematopoietic stem cell transplantation was not able to change the natural history of T1D, it is less probable that low intensity or target-specific immunomodulatory approaches would achieve clinical success in this field.

**Figure 1** presents some of the strategies that can be investigated in the near future to manage T1D autoreactivity.

## Increasing the Intensity of the Conditioning Regimen for Autologous Hematopoietic Stem Cell Transplantation

As previously shown, clinical trials with autologous hematopoietic stem cell transplantation for T1D included similar drugs as conditioning regimen. The Brazilian protocol used high dose cyclophosphamide plus rabbit ATG, while the Polish protocol added plasmapheresis to the procedure and the Mexican protocol used fludarabine plus cyclophosphamide. All provided similar outcomes, indicating insufficient control of islet-specific autoreactivity. Therefore, new protocols of autologous hematopoietic stem cell transplantation should be developed, aiming to increase effectiveness of the immunosuppressive approach. One possibility is to increase the intensity of transplant conditioning regimens. In this context, a three-drug immunosuppressive regimen (cyclophosphamide + fludarabine + rabbit antithymocyte globulin) regimen has been proposed, aiming to more efficiently destroy the memory T and B cell compartment and possibly improve treatment outcomes (28).

Graft manipulation with CD34<sup>+</sup> may also be investigated as a strategy to be added to future transplant protocols. Although there is no consensus and still a matter of debate in transplantation for other autoimmune diseases, CD34<sup>+</sup> selection has not

been investigated in T1D. The rationale for this approach is that during unselected infusions, memory T cells are reinfused, perpetuating the autoimmune process after transplant. Importantly, graft manipulation is associated with higher incidence of post-transplant viral infections.

For every newly proposed intervention, safety and long-term toxicity must be considered, especially when higher immunosuppression is expected. Despite lifetime insulin-dependence and poor quality of life of patients, T1D is a non-malignant disease, and new strategies to improve glycemic control are constantly under investigation.

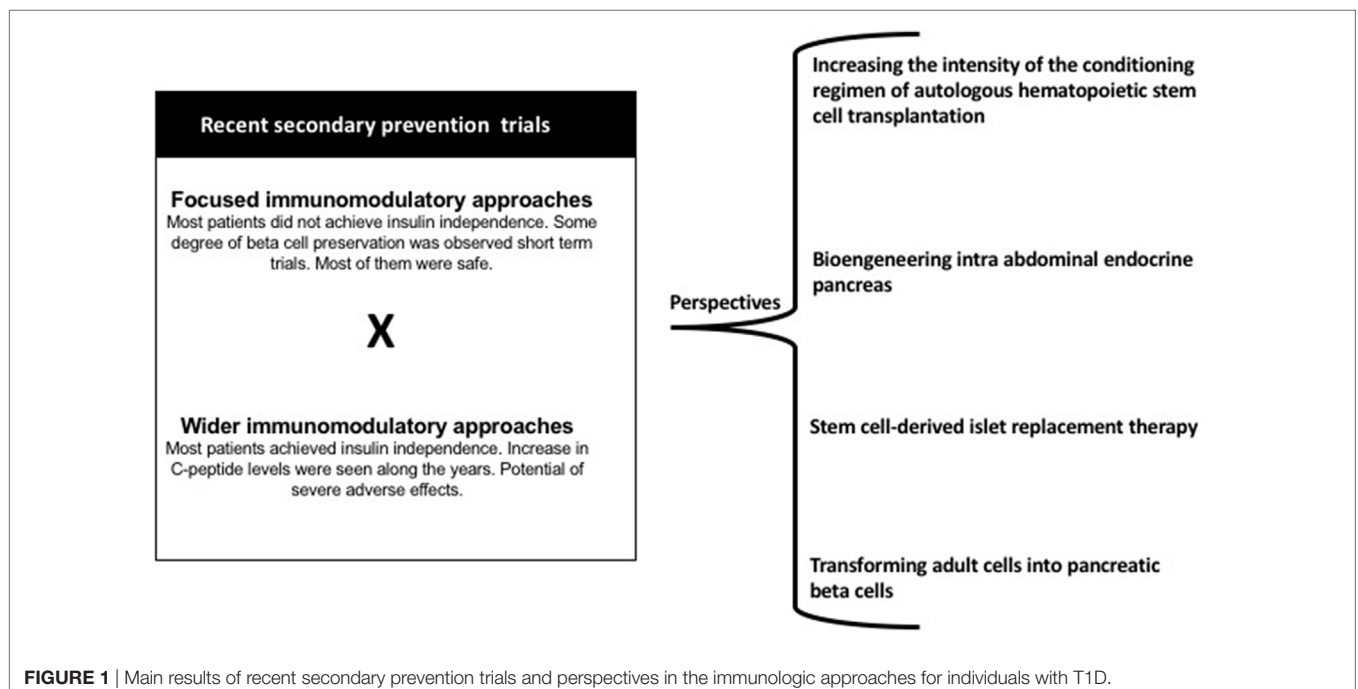
In addition to the immune-based interventions, other ongoing research protocols investigate means to restore insulin secretion. Most of the current effort involves development of technologies for beta cell or stem cell encapsulation.

## Intra-Abdominal Endocrine Pancreas Bioengineering

One of the greatest challenges in islet transplantation is the need for chronic immunosuppression (with or without corticosteroids) to avoid rejection of allogeneic cells. This problem may be circumvented by encapsulation of the islet cells to physically prevent host immune cells from reaching the graft.

Recently, Baidal and colleagues (35) reported the case of a woman with longstanding T1D who received pancreatic islets from a deceased donor and became insulin-free 17 days after the procedure. Islets were combined with host autologous plasma and were laparoscopically layered onto the omentum. A degradable biologic scaffold was created, but immunosuppression regimen still had to be used in this case.

The next step will be the improvement of the technique of islet encapsulation so that immunosuppression will be no longer



needed. In the future, embryonic stem cells or even induced pluripotent stem cells (iPS) may be used as alternative sources for insulin-producing cells.

## Stem Cell-Derived Islet Replacement Therapy

As a further step in the use of allogeneic insulin-producing cells, an ongoing study investigates transplantation of pancreatic endoderm cells derived from human embryonic stem cells to restore insulin and glucose homeostasis (NCT02239354). Immune-mediated rejection is prevented by a surrounding semi-permeable and protective membrane, enabling pancreatic endoderm cells to further differentiate into mature and functional pancreatic cells, and not requiring use of immunosuppressive drugs.

## Transforming Adult Cells Into Pancreatic Beta Cells

The use of autologous sources for beta cell replacement is another strategy to avoid rejection. Doiron and colleagues designed a lentiviral vector construct expressing the glucokinase gene under control of the cytomegalovirus promoter (36). In this study, insulin-secreting cells could be generated, *in vivo*, from adult pancreatic tissue of a mouse model of partial pancreatectomy. Treated animals presented long-term normalization of glucose tolerance and insulin secretion. Despite technical difficulties still to be circumvented, mainly the use of non-viral vectors, this is an attractive approach to restore organ function on humans. As beta cells would be generated from the patient's own tissue, no immunosuppression would be necessary.

## CONCLUSION

Many attempts have been made to modulate or even to reset the immune system in type 1 diabetic individuals, aiming to

avoid pancreatic beta cell destruction. However, even high-dose immunosuppression followed by infusion of autologous hematopoietic stem cells was not able to sustainedly restore immune tolerance. Given these observations, new approaches need to be developed. These would include the use of more intense immunosuppressive protocols to preserve beta cell mass, perhaps coupled with replacement of beta cells protected against immune destruction.

## AUTHOR CONTRIBUTIONS

CEBC, KCRM, and MCO researched data and wrote the manuscript.

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**Conflict of Interest Statement:** The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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