# World Journal of

# *Diabetes*

# World Journal of Diabetes

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## ABOUT COVER

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## AIMS AND SCOPE

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   *WJD* mainly publishes articles reporting research results and findings obtained in the field of diabetes and covering a wide range of topics including risk factors for diabetes, diabetes complications, experimental diabetes mellitus, type 1 diabetes mellitus, type 2 diabetes mellitus, gestational diabetes, diabetic angiopathies, diabetic cardiomyopathies, diabetic coma, diabetic ketoacidosis, diabetic nephropathies, diabetic neuropathies, Donohue syndrome, fetal macrosomia, and prediabetic state.

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# New therapeutic approaches for type 1 diabetes: Disease-modifying therapies

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## Abstract

It has been 100 years since the first successful clinical use of insulin, yet it remains the only treatment option for type 1 diabetes mellitus (T1DM) patients. Advances in diabetes care, such as insulin analogue therapies and new devices, including continuous glucose monitoring with continuous subcutaneous insulin infusion have improved the quality of life of patients but have no impact on the pathogenesis of the disease. They do not eliminate long-term complications and require several lifestyle sacrifices. A more ideal future therapy for T1DM, instead of supplementing the insufficient hormone production (a consequence of β-cell destruction), would also aim to stop or slow down the destructive autoimmune process. The discovery of the autoimmune nature of type 1 diabetes mellitus has presented several targets by which disease progression may be altered. The goal of disease-modifying therapies is to target autoimmune mechanisms and prevent β-cell destruction. T1DM patients with better β-cell function have better glycemic control, reduced incidence of long-term complications and hypoglycemic episodes. Unfortunately, at the time symptomatic T1DM is diagnosed, most of the insulin secreting β cells are usually lost. Therefore, to maximize the salvageable β-cell mass by disease-modifying therapies, detecting autoimmune markers in an early, optimally presymptomatic phase of T1DM is of great importance. Disease-modifying therapies, such as immuno- and regenerative therapies are expected to take a relevant place in diabetology. The aim of this article was to provide a brief insight into the pathogenesis and course of T1DM and present the current state of disease-modifying therapeutic interventions that may impact future diabetes treatment.

**Key Words:** Type 1 diabetes; Mesenchymal stem cell; Immunotherapy; Islet cells; Auto-immunity; Regenerative medicine

**Core Tip:** Our knowledge is rapidly growing about the pathomechanism of type 1 diabetes mellitus, and new and improved therapies have emerged. However, the long-term complications and the required lifestyle changes cannot be eliminated. There is a growing number of research that aims to find specific immunological markers/targets that have a role in disease development. The ultimate goal is finding new therapeutic ways to treat the disease and to delay or even prevent its development. The aim of this review was to provide a brief insight into the current state of disease-modifying therapeutic interventions that may impact future diabetes care.

## INTRODUCTION

For many years it was accepted that type 1 diabetes mellitus (T1DM) starts with the classical triad of polyuria, polydipsia and polyphagia. However, it became clear that T1DM is a long standing, progressive disease with a preclinical phase without symptoms and with the appearance of multiple T1DM-associated autoantibodies. The preclinical phase is followed by a symptomatic clinical phase[1]. The burden of living with the chronic disease is considerable for the patient, the family and the society. This minireview focused on the clinical applications of novel disease-modifying therapeutic intervention options in early stages of T1DM that may prevent or reverse clinically overt symptomatic T1DM. The presentation of the latest improvements available for middle and late stage disease and diabetic complications is out of the scope of the current review.

### Immunopathogenesis of T1DM

The pathogenesis of T1DM involves a complex interaction between pancreatic β cells and the innate and natural immune systems. The precise mechanism that leads to the loss of immune tolerance is still unclear. However, viral infections, nutritional factors and the perinatal environment have been associated with the disease[2-4]. It is assumed that the stability of the trimolecular complex (T cell receptor/human leukocyte antigen/peptide) during thymic selection plays a major role in the escape of autoimmune T cells[5].

In the development of T1DM, the initial step of the destructive process is considered to be the uptake and presentation of β-cell-derived peptides, autoantigens, by the antigen-presenting cells. Next, antigen-presenting cells, which can be both macrophages and dendritic cells, migrate to lymph nodes around the pancreas and activate CD4+ helper T cells (Th)[6]. Th cells differentiate into Th1, which have a proinflammatory phenotype. Th1 cells are the key effector cells in the pathogenesis of T1DM and are capable of producing interferon-γ, tumor necrosis factor α, interleukin 1 (IL-1) and IL-2. These cytokines inhibit Th2 polarization, the cells responsible for the protection of islets[7]. Th1 cells are necessary for the activation and recruitment of other autoreactive cells, such as CD8+ cytotoxic T lymphocytes (CTL), which are responsible for the lysis/apoptosis of β cells presenting the major histocompatibility complex I autoantigen complex. The cell-destructive effect of activated CTLs is due to macromolecules stored in granules (*e.g.*, perforin, granzyme), to the cytokines and to caspase-dependent apoptosis[8].

B cells are stimulated by Th1 cells and produce autoantibodies against β cells [islet cell antibody, glutamic-acid-decarboxylase antibody (GADA), islet tyrosine phosphatase 2 antibody, insulin autoantibody and zinc transporter 8 antibodies]. These antibodies have become the biomarkers of T1DM [9]. Furthermore, Th1 cells enhance antigen presenting, costimulatory and effector functions of macrophages and dendritic cells. Natural killer cells also contribute to β-cell destruction through their cytolytic effects and antibody-dependent cellular cytotoxicity. Th17, with strong inflammatory effects, is also involved in the inflammatory process: It secretes IL-17 family cytokines and plays an important role in neutrophil granulocyte recruitment and activation[10].

Under ideal conditions, regulatory T cells (Treg) inhibit the autoreactive lymphocytes. If Treg cells are deficient, the rate of T1DM progression is increased[11]. The above-mentioned immune cells infiltrate the islets (insulitis) and eventually cause β-cell death and reduced insulin levels (Figure 1).

### New staging classification system of T1DM

T1DM is the result of a destructive autoimmune-mediated process in which insulin-producing β cells in the islets of Langerhans are damaged. According to the novel staging classification system proposed by
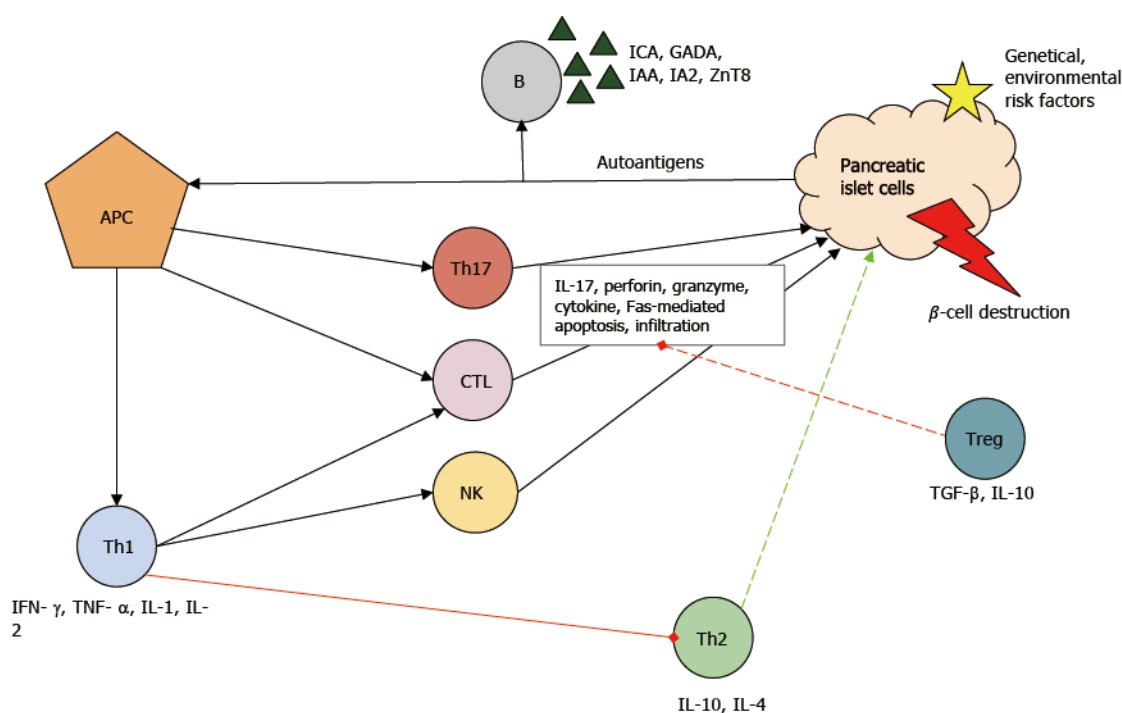
**Figure 1 Immunopathogenesis of type 1 diabetes mellitus.** Autoantigens from pancreatic islet β-cells are presented by antigen-presenting cells (APCs), thereby activating T cells including helper T (Th) cells type 1 and type 17 and cytotoxic T lymphocytes (CTLs). Th type 1 cells play a key role in the development of the autoimmune response. They stimulate the activity of inflammatory T cells, macrophages and natural killer (NK) cells by producing proinflammatory cytokines and stimulate B cells (B), which produces autoantibodies and inhibits the protective Th type 2 cell function. Together, these immune cells contribute to the destruction of pancreatic β-cells. Red line: Inhibition; Green line: Stimulation. GADA: Glutamic-acid-decarboxylase antibody; IA2: Islet tyrosine phosphatase 2 antibody; IAA: Insulin autoantibody; ICA: Islet cell antibody; IL: Interleukin; IFN-γ: Interferon γ; TGF-β: Transforming growth factor β; TNF-α: Tumor necrosis factor α; Treg: Regulatory T cell; ZnT8: Zinc transporter 8 antibody.

the Juvenile Diabetes Research Foundation, the Endocrine Society and the American Diabetes Association, there are three distinct stages in T1DM[12]. Genetic predisposing factors are present from birth. The autoimmune reaction may be initiated in genetically susceptible individuals by environmental risk factors, which are not well understood[13]. People with a first- or second-degree relative with T1DM have a 15 times greater risk of developing the disease compared to the general population [14] (Figure 2).

Stage 1 is the critical point of no return since eventually the affected individuals will develop clinical diabetes. It is characterized by the presence of immune markers, two or more of the T1DM-associated islet antibodies, such as islet cell antibodies, GADA, Islet tyrosine phosphatase 2 antibodies and zinc transporter 8 antibodies, normoglycemia and absence of diabetic symptoms. In stage 2, the β-cell volume is critically decreased, and metabolic markers become detectable in asymptomatic patients. These individuals, besides being antibody positive, display impaired fasting glycemia, impaired glucose tolerance, abnormal oral glucose tolerance test or glycated hemoglobin (HbA$_{1C}$) ≥ 5.7%. Stage 3 represents the phase of clinical diagnosis and the manifestation of typical diabetic symptoms such as polyuria, polydipsia, weight loss, fatigue, diabetic ketoacidosis, *etc*[12]. Over time, most of the residual β cells are lost. However, sensitive C-peptide measurements have shown that 30%-80% of patients with long–standing T1DM are insulin microsecretors. This means that these patients have detectable stimulated C-peptide value of < 30 pmol/L (< 0.09 ng/mL)[15], an important consideration in therapeutic approaches targeting β-cell survival[16]. Shield *et al*[17] identified two clear phases of C-peptide decline after the diagnosis of T1DM: an exponential fall in the first 7 years (-47%/year) followed by a stable phase (-0.1%/year) (Figure 2).

Age has a major influence on the rate of disease progression. In children, the clinical stage develops more rapidly, and β-cell loss is more pronounced compared to adults. About 6 years to 9 years after the diagnosis of T1DM, 20% of those diagnosed in childhood and 60% of those diagnosed in adulthood had detectable C-peptide secretion, an indicator of endogenous insulin production[18]. In addition, the autoantibody titer and profile are also a determinant of β-cell loss. Most people will not develop diabetes if they have a single autoantibody. In contrast, the more autoantibodies a person has and the higher their serum concentration is, the rate of disease progression is greater[19]. Lately, stage-specific therapies have been the focus of clinical trials for modifying disease progression[13,19].
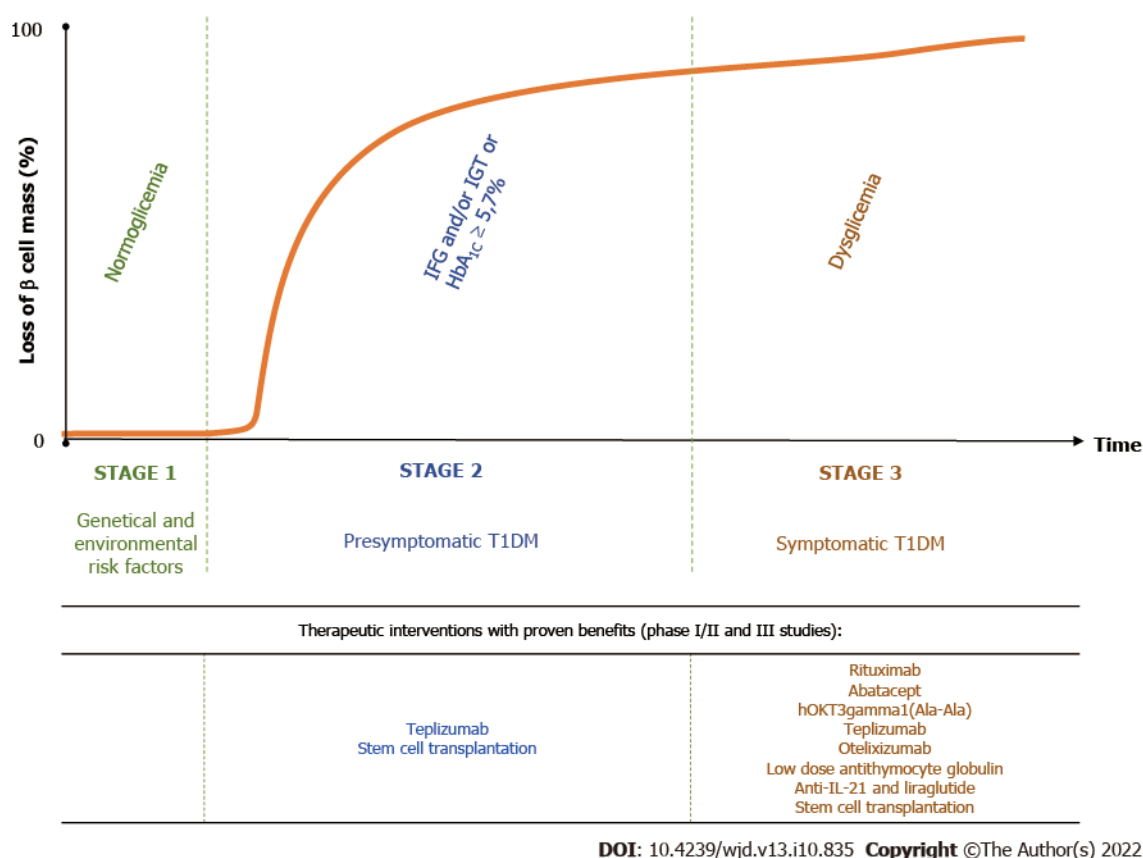
**Figure 2 Stages of type 1 diabetes mellitus and options of therapeutic interventions with proven therapeutic benefits in phase II and III clinical trials.** The interaction between genetic predisposing factors, which are present from birth, and environmental factors trigger the autoreactive process. Stage 1 is characterized by individuals who exhibit at least two of the type 1 diabetes mellitus (T1DM)-associated antibodies (glutamic-acid-decarboxylase-, islet tyrosine phosphatase 2-, islet cell- and zinc transporter 8 antibodies). Stage 2 is characterized by dysglycemia due to reduced β-cell function. Stage 3 represents the onset of clinical T1DM and usually but not always the manifestation of typical symptoms. The application of the previously proposed staging system[12] in clinical trials, which even recognizes the earliest stages of T1DM, can improve the research and development of novel therapies that might delay/prevent the onset of the disease. IL: Interleukin; IFG: Impaired fasting glycemia; IGT: Impaired glucose tolerance; HBA$_{1c}$: Glycated hemoglobin. Figure adapted from Insel *et al*[12].

## DISEASE-MODIFYING THERAPEUTIC OPTIONS

Groundbreaking studies with cyclosporin A in the 1980s showed that the disease course of T1DM can be altered with immune therapy[20] and gave rise to research in the field of definitive treatment of T1DM. Despite extensive efforts so far, immune-altering therapies could not reach approval for routine clinical use for several reasons. Some agents such as cyclosporin A have many side effects and lack long-term efficacy[21]. Other options with a more favorable tolerance profile such as the adjuvant-formulated GAD-alum vaccine, which incorporated recombinant human glutamic acid decarboxylase, had no effect on disease progression[22]. The recent development of more targeted immunotherapies, the advances in regenerative therapies and lessons learned about β-cell survival from type 2 diabetes mellitus studies gave rise to more promising therapeutic options. Of the immunotherapy agents, teplizumab has come closest to achieving success. In July 2021, the United States Food and Drug Administration considered the use of teplizumab in high-risk individuals but deemed further studies necessary before granting approval[23].

### B-cell targeting agents

Even though T1DM is mainly a T cell-mediated autoimmune disease, antigen-presenting B lymphocytes can also play a pathogenic role by activating T-lymphocytes and triggering the autoimmune destruction of β cells. In animal models and triggered human studies, T1DM anti-B lymphocyte therapies have been shown to be effective[24].

**Rituximab:** Rituximab is an anti-CD20 monoclonal antibody known to cause B-cell depletion. It is widely used in clinical practice in malignant hematological diseases such as non-Hodgkins lymphomas and chronic lymphocytic leukemias. In a placebo controlled randomized trial, including 87 recent onset T1DM patients, it has been shown that rituximab treatment was associated with slower progression of β-cell dysfunction and better metabolic control during the 12-mo long study period[25]. Insulin dose requirements decreased during the study. However, none of the patients were able to become insulin

free. Extensive follow-up of the patients showed a constant decline in C-peptide production. It suggests that B-cell depletion by its own is not sufficient in restoring β-cell tolerance in the long run and does not fundamentally alter the course of overt T1DM[26].

It has been reported that rituximab can suppress insulin autoantibodies, but no such effect could be found in the case of GADA, islet tyrosine phosphatase 2 antibody and zinc transporter 8 antibody[27]. Compared to placebo controls, rituximab-treated T1DM patients, whose C-peptide response was significant, have shown increased proliferative responses to islet, neuronal and disease-relevant environmental antigens ultimately resulting in increasing insulin secretory function[28]. Moreover, the combination of rituximab with CD4+ CD25high CD127- T regulatory cells[29] or therapy targeting CD4+ T cells[30] can further improve treatment efficacy. One side effect of rituximab, reported by the study of Kroll *et al*[31], might be the reactivation of some asymptomatic polyomavirus infections. Rituximab is currently being tested in earlier stages of T1DM (NCT03929601[32]).

### T-cell targeting agents

**T-cell co-stimulation inhibition:** Abatacept is a cytotoxic T lymphocyte-associated antigen 4 immuno-globulin fusion protein designed to selectively bind to CD80/86 to inhibit the early activation and proliferation of naïve T lymphocytes. Since effector memory T cells are less dependent on CD28 costim-ulation, abatacept is a more selective way to inhibit T cell activation compared to general immunosup-pressants. In a phase II placebo-controlled study, Orban *et al*[33] investigated the effect of abatacept in a population with recent onset T1DM. Patients in the treatment group received a monthly dose of 10 mg/kg intravenous abatacept for 2 years. The authors found that cytotoxic T lymphocyte-associated antigen 4 inhibition slowed the decline of β-cell function during the 2 years of the treatment and had a beneficial effect during the 1-year follow-up period without active treatment[34]. However, the observed positive effect was only temporary and declined over time. Furthermore, it was found that abatacept does not change immunogenicity of other vaccines in T1DM patients[35], but different follicular Th and central memory CD4+ T cell phenotypes might affect the efficacy of the treatment[36, 37]. There are still ongoing clinical trials (*e.g.*, NCT01773707[33], NCT04118153[38] and NCT03929601 [32]) investigating whether abatacept may have a more potent impact on the disease if administered at earlier stages (*e.g.*, at stage 2) of T1DM pathogenesis.

**Anti-CD3 therapy:** So far the most promising therapeutic target in modifying the course of T1DM is the ε chain of the CD3 receptor on the T cell surface, previously known as muromonab-CD3 (trade name: Orthoclone OKT3)[39]. Animal studies have shown that anti-CD3 therapy can induce diabetes remission in the models of T1DM[40]. This main effect is associated by the induction of Treg cells and immu-nosuppressive cytokines such as transforming growth factor β[41]. One of the first human trials reported significantly improved C-peptide response and other clinical parameters after a single shot of hOKT3gamma1(Ala-Ala)[42].

Teplizumab is a humanized anti-CD3 monoclonal antibody, which has been shown to be the most potent agent in slowing the progression of T1DM. In a series of human clinical trials, it was demonstrated that the treatment of teplizumab was a potent way to delay the decline of C-peptide production[43-45] and can help to preserve β-cell function[46], and its effect can be sustained by an average of 15.9 mo in T1DM[47]. Furthermore, the Protegé[48,49] study in which 516 T1DM patients were enrolled and treated with teplizumab has demonstrated that anti-CD3 therapy delayed the decline of insulin secretion and induced disease regression, and 5% of the patients became insulin independent. Even though teplizumab has shown promising results in preventing disease progression, it must be noted that significant metabolic benefits such as a significant reduction in HbA$_{1C}$ could not be demonstrated.

These promising results led to further trials to evaluate the effect of anti-CD3 therapy in T1DM prevention. The recently published results of a phase III follow-up trial including non-diabetic patients at high risk of developing T1DM, defined as having impaired glucose tolerance and at least two diabetes-specific autoantibodies, demonstrated the efficacy of teplizumab in delaying the onset of T1DM by 48.4 mo compared to the placebo group (24.4 mo)[50]. C-peptide levels of those who responded to treatment remained significantly better even after a 7-year follow-up period[51]. Furthermore, clinical responders to teplizumab therapy have shown significant reduction in circulating CD4+ effector memory T cells and decreased activation and regulatory gene expression in circulating CD8+ central memory T cells[52]. Currently, studies are running to investigate teplizumab in at-risk individuals[53] and recent-onset T1DM patients[54,55].

Otelixizumab is another humanized anti-CD3 antibody that has been evaluated both in the treatment of overt[56] and new-onset[57,58] T1DM. Similarly to teplizumab, a 6 d treatment of otelixizumab preserved residual β-cell function for at least 18 mo in 40 patients with recent-onset T1DM. The protective effect of otelixizumab treatment appears to be dose dependent as studies attempting to lower adverse reactions by administering lower doses could not demonstrate a benefit in C-peptide preser-vation[59,60]. The protective effect of otelixizumab showed the highest benefit in insulin autoantibody-positive T1DM patients[61].

Anti-CD3 therapies have been overall well tolerated among patients. Adverse reactions that were significantly more prevalent in the treatment group included: vomiting, rash, chills, cytokine release syndrome, Epstein-Barr virus reactivation[57,62] and headache. Recently, a subcutaneous formulation was also introduced[63], which significantly reduced such undesirable effects. Adverse reactions were mostly mild to moderate and self-limited; 9% of patients were not able to complete all drug doses compared to a 2% dropout rate in the placebo group. The most common cause for treatment cessation was lymphopenia, neutropenia, elevated liver enzymes and reduced platelet counts.

**Low dose antithymocyte globulin:** Antithymocyte globulin (ATG) is a polyclonal immunoglobulin G antibody against multiple human T cell antigens and their precursors. Only a limited number of studies are available, and their results are somewhat controversial: 6.5 mg/kg ATG alone could not preserve β-cell function, but C-peptide secretion was preserved in older participants suggesting a possible age-specific action[64,65]. In contrast, low dose ATG treatment (2.5 mg/kg administered as 0.5 mg/kg on day 1 and 2 mg/kg on day 2) in combination with pegylated granulocyte colony-stimulating factor acts by decreasing the number of activated effector T cells while relatively preserving Treg cells. T1DM patients with a diabetes onset between 4 mo and 2 years receiving low dose ATG + granulocyte colony-stimulating factor have shown a benefit in disease progression[66,67]. Patients in the treatment group have had higher C-peptide production after a mixed meal test, and lower HbA$_{1C}$ after 6 mo was also recorded in the treatment group compared to the placebo group.

A more recent study published by the same group indicated that low-dose ATG monotherapy without granulocyte colony-stimulating factor can delay the decline of C-peptide, can reduce the HbA$_{1C}$ level and affect T cell phenotypes in new-onset T1DM[68,69]. The ongoing follow-up of this study is in progress along with two additional studies[70,71], and their results will help further evaluate the potential benefits of low-dose ATG and its therapeutic potential for preventing T1DM.

**Anti-IL-21 and liraglutide:** A new strategy to modify the disease course in T1DM is using a drug combination that not only halts or delays the progressive autoimmune process but aims at preserving and improving residual β-cell function. This approach may have the advantage over previous monotherapies in achieving disease modification with milder immunomodulation in a safer, more sustainable way. IL-21 plays a key role in the pathomechanism of T1DM by activating and leading CD8$^+$ T lymphocytes from lymph nodes and the exocrine pancreas to the pancreatic islets eventually leading to β-cell destruction[72,73]. Based on these findings, IL-21 inhibition has emerged as a potential disease-modifying target in preventing T1DM. 35-Liraglutide, a glucagon like peptide-1 analog that has routinely been used in type 2 diabetes therapy, has been proven to increase β-cell survival[74] and can improve glucose dependent insulin secretion not only in type 2 diabetes but in T1DM as well[75,76].

In a recent phase II clinical trial the effect of liraglutide and IL-21 inhibition was evaluated in 308 T1DM patients with recent onset disease and residual β-cell function[77]. The combination treatment was effective to preserve both fasting and postprandial endogenous insulin secretion resulting in a nonsignificant decrease in the number of hypoglycemic events and level of HbA$_{1C}$ for 52 wk. During the follow-up period the combination treatment was considered safe, and there were no safety concerns raised. The study included a 26 wk off-drug observation period during which the effect of the treatment deteriorated rapidly, suggesting the need for continued treatment. Overall, this combination treatment seems to be a promising candidate for further evaluations in a phase III clinical trial.

## MESENCHYMAL STEM CELL THERAPY IN T1DM

### Characteristics of mesenchymal stem cells

Over the past two decades, stem cell transplantation has received increased attention in clinical trials as a promising therapy within regenerative medicine for T1DM. While the treatment of T1DM with hematopoietic stem cells was more typical in the 2000s and first half of the 2010s[78], the most recent studies focus more on the treatment with mesenchymal stem cells (MSCs)[13]. This modern approach to treat T1DM has several advantages over previous treatment options. MSC transplantation is hypo-immunogenic because the cells do not express costimulatory antigens (CD80, CD86, CD40, CD40L *etc*) nor major histocompatibility complex II and major histocompatibility complex I. MSCs allow both autologous and allogeneic transplantation, even without conditioning treatment[79].

MSCs can be easily cultured *in vitro* due to their high dividing capacity, and they can be isolated from many adult and perinatal sources (*e.g.*, bone marrow, adipose tissue, peripheral blood, dental pulp, skeletal muscle, liver, lung, umbilical cord blood, Wharton's jelly and placenta)[80]. Of these, the umbilical cord and its derivatives stand out as they can be obtained non-invasively, are considered as 'medical waste' and have an exceptional differentiation capacity towards insulin-secreting cells[81]. Unlike embryonic stem cell therapy, the use of these tissue sources does not raise special ethical issues.

In addition, MSCs have no known tumorigenic effect, whereas embryonic stem cells can form teratomas and teratocarcinomas *in vivo*[82]. However, tumorigenesis cannot be completely ruled out as a possible adverse effect: MSCs may be a direct source of malignant cells, may maintain various cancer processes (*e.g.*, breast and colon cancer) through paracrine factor secretion or may enhance tumor

growth and progression through their immunosuppressive effects[83-85]. However, to the best of our knowledge, no similar side effects have been reported in clinical trials using MSCs[86].

MSCs are capable of generating tissue types of mesodermal origin, such as musculoskeletal, cartilaginous and adipose tissue, and may cross the boundaries of germ layers and transdifferentiate into ectodermal neurons or even endodermal islet cells[87,88]. Low amounts of MSCs in the target tissue do not explain regeneration or even wound healing. However, *in vivo* experiences show that MSCs have other, more pronounced therapeutic effects, such as remodeling of the diabetic microenvironment[89, 90]. It should be noted that in systemic administration, MSCs are entrapped in capillaries, especially in the lungs, reducing the number of migrating cells towards the target tissue, suggesting that better outcome could be obtained through local injection[91].

### Immunoregulatory function of MSCs in T1DM

The strong immunoregulatory function of MSCs plays a key role in the regeneration of β cells. This protective effect in T1DM is due to secretion of soluble factors and cell-cell interactions. Insulin deficiency and irreversible β-cell destruction are the consequences of the autoimmune reaction in T1DM, and MSCs are able to intervene at several points in this process, modulating immune cells. MSC transplantation with its paracrine effects, due to the production of cytokines, chemokines and growth factors, can affect the local environment, inhibit apoptosis and induce proliferation. The identified bioactive factors are: IL-6, IL-8, transforming growth factor β, vascular endothelial growth factor, hepatocyte growth factor and nitric oxide[13].

Two types of MSCs are known: proinflammatory MSCs (MSC1) and anti-inflammatory MSCs (MSC2). The type of polarization depends on the inflammatory milieu. In the absence of an inflammatory environment MSCs adopt a proinflammatory phenotype and amplify T cell responses. Conversely, in an inflammatory environment (high interferon-γ and tumor necrosis factor α levels), MSCs may adopt an immunosuppressive phenotype and suppress T cell proliferation *via* secreted soluble factors[92]. MSCs have a regulatory function against effector T cells. In the pathogenesis of T1DM, Th1 cells are the main effector cells, and Th2 cells have been shown to be protective.

Beneficial effects of MSCs in diabetes can be attributed to: (1) Secreted IL-4; (2) Altered Th1/Th2 ratio with a Th1 to Th2 shift; and (3) Promoted maturation of naïve T cells towards Th2[93]. Furthermore, MSCs can directly and indirectly inhibit through several pathways: (1) Th17 cell development and thus IL-17 production; (2) CTL function and thus Fas-mediated β-cell apoptosis; and (3) Both maturation and activation of antigen-presenting cells, principally dendritic cells, by secreting for example prostaglandin E2, IL-6 and macrophage colony-stimulating factor[13,94,95].

Two types of macrophages are known: M1 and M2 producing proinflammatory and anti-inflammatory cytokines, respectively. MSCs can modulate the phenotype shift, causing an M1 to M2 shift[96]. Treg cells are components of MSC-induced indirect immunosuppression. *In vivo* and *in vitro*, MSCs have been shown to enhance Treg proliferation through cell-cell interaction[13]. By producing IL-10 and transforming growth factor β, Treg cells downregulate Th1- and Th17-mediated inflammatory response and the cytotoxicity of CTLs, thereby leading to immune tolerance in the organism[97]. These mechanisms can contribute to both amelioration of auto-reactivity and of β-cell death (Figure 3).

### Clinical application of MSC therapy for the treatment of T1DM

In recent years, MSCs have attracted the attention of many researchers and clinicians as a result of encouraging preclinical animal data in T1DM. The most important advantages are: (1) Wide range of sources; (2) Self-renewal capacity; (3) Multidifferentiation capacity; and (4) Strong immunomodulatory potential. MSCs are also immunoprivileged, well-tolerated and safe[98]. The clinical studies vary in MSC origin, dose, route of transplantation, administration frequency and in eligible patients' characteristics (Table 1).

Hu *et al*[99] studied the long-term effects of Wharton's jelly-derived MSC in newly diagnosed T1DM patients. Group 1 was treated with parenteral solution of Wharton's jelly-derived MSCs by intravenous delivery, while the control group received normal saline. In the treatment group HbA$_{1C}$ reached its lowest value after half a year and then began to fluctuate. Fasting C-peptide showed a progressive increase, reaching its maximum after 1 year; 3/15 patients were insulin-free, and 8 had their insulin dose halved after 2 years. As the study follow-up period lasted 2 years, exceeding the average 1.5 year honeymoon period, the therapeutic effect was due to MSCs[99]. This was one of the first studies to prove the safety and effectiveness of MSCs.

Thakkar *et al*[100,101] used the combination of adipose-derived insulin-secreting mesenchymal stem cells and bone marrow-derived (BM-) hematopoietic stem cells, comparing autologous (group 1) and allogeneic (group 2) stem cells. The study procedure was as follows: Resection of adipose tissue from the abdominal wall, collected in proliferation medium, bone marrow aspiration, conditioning treatment with bortezomib, methylprednisolone, ATG, and finally injection of the mixed inoculum. Autologous stem cell therapy offered better long-term control of hyperglycemia, but the two groups fairly differed in baseline mean C-peptide levels[100]. Although the two treatment methods showed significant differences in carbohydrate metabolism, the results before and after stem cell therapy were not statistically analyzed within the groups, thus lacking conclusive information about the efficacy of MSCs. As an early-result, the group reported preliminary data of 10 patients[101]. After an approximately 3-year

**Table 1 Summary of clinical trials using mesenchymal stem cells in type 1 diabetes mellitus**

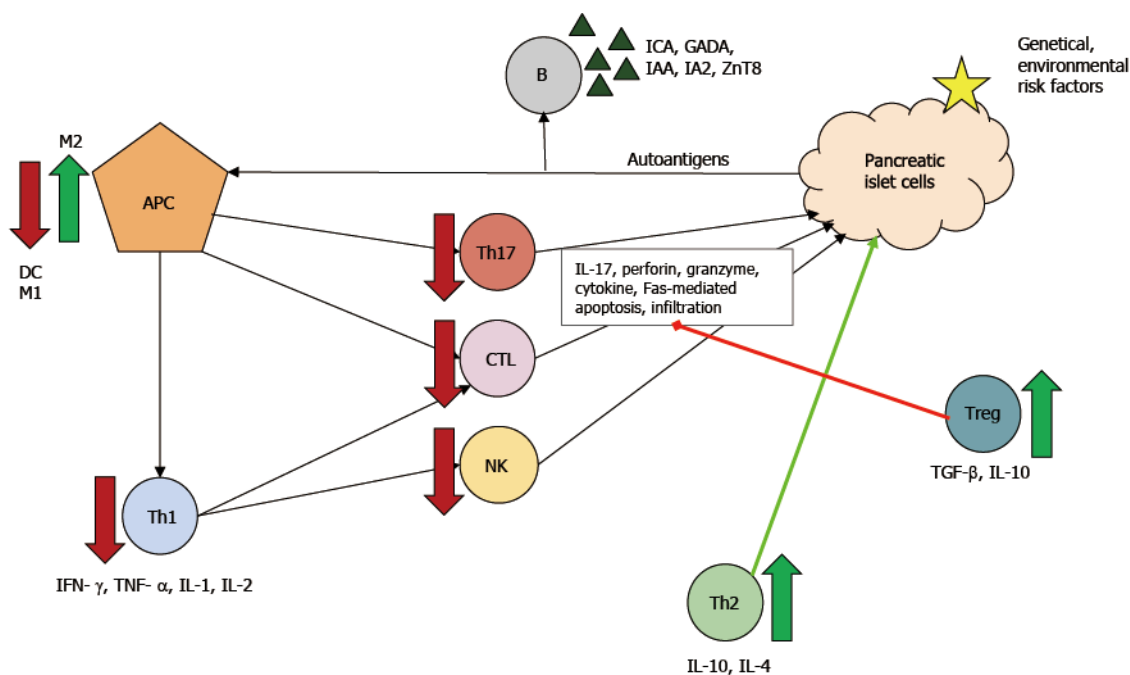| Ref. | Patient characteristics | Treatment | Therapeutic outcomes |
| --- | --- | --- | --- |
| Wu *et al*[105], 2022[1] | *n* = 14; aged 27-47 yr | Intrapancreatic: Allogeneic UC-MSC + autologous BM-MNC | Insulin independence: No |
| China, 8 yr | Duration of T1DM: 10-24 yr | | Insulin requirement: Improvement at 1 yr but no difference at 8 yr |
| | | | FCP and HbA$_{1C}$: Significant improvement |
| | | | Significantly lower occurrence of diabetic complications |
| Izadi *et al*[108], 2022 | *n* = 20; aged 8-40 yr | BM-MSC | Insulin independence: No |
| Iran, 12 mo | Duration of T1DM: < 1 yr (*n* = 11) and > 1 yr (*n* = 9) | | Insulin requirement, FCP, HbA$_{1C}$: Significant improvement |
| | FCP (*n* = 11): 0.92 ± 0.57 ng/mL | | Number of hypoglycemic events decreased |
| | | | Patients with early onset of T1DM benefit more |
| | | | Adverse effects: Possible mild injection site reactions |
| Lu *et al*[106], 2021 | *n* = 27; aged 8-55 yr | IV (2x): Allogeneic UC-MSC | Insulin independence: 3 subjects |
| China, 12 mo | Median duration of T1DM: 2.3 mo | | Insulin requirement, HbA$_{1C}$: No improvement |
| | FCP: 100 pmol/L ( 0.3 ng/mL) | | SCP: improved in adult-onset T1DM subgroup |
| | | | Adverse effects: Mild fever |
| Dantas *et al*[103], 2021[2] | *N* = 7; Aged 16-35 yr | Allogenic AD-MSC + 2000 UI/d cholecalciferol | Insulin independence: 1 subject |
| Brazil, 6 mo | Duration of T1DM: ≤ 4 mo | | Insulin requirement: Stable at 6 mo |
| | FCP: 0.80 ± 0.38 ng/dL | | FCP and HbA$_{1C}$: Significant improvement |
| | | | Adverse effects: Transient headache, mild local reactions, immediate tachycardia, thrombophlebitis + other mild effects |
| Araujo *et al*[102], 2020 | *n* = 8; aged 16-28 yr | Allogenic AD-MSC + 2000 UI/d cholecalciferol | Insulin independence: 2 subjects |
| Brazil, 3 mo | Duration of T1DM: ≤ 4 mo | | Insulin requirement, HbA$_{1C}$: Decreased significantly at 3 mo |
| | | | FCP: Only initial improvements, with the same results at the 3-mo visit |
| | | | Adverse effects: Transient headache, mild local reactions, immediate tachycardia, thrombophlebitis + other mild effects |
| Cai *et al*[104], 2016 | *n* = 21; aged 18-10 yr | Intra-pancreatic: Allogeneic UC-MSC + autologous BM-MNC | Insulin independence: No |
| China, 12 mo | Duration of T1DM: 2-16 yr | | Insulin requirement, HbA$_{1C}$: Decreased significantly |
| | FCP: < 0.1 pmol/mL (< 0.3 ng/mL) | | FCP: Markedly increased |
| | | | Adverse effects: Transient abdominal pain, bleeding |
| Carlsson *et al*[107], 2015 | *n* = 9; aged 18-40 yr | IV: Autologous BM-MSC | Insulin independence: No |
| Sweden, 12 mo | Duration of T1DM: < 3 wk | | Insulin requirement, HbA$_{1C}$, SCP: No significant improvement |
| | SCP: > 0.1 nmol/L (> 0.3 ng/mL) | | Adverse effects: No |
| Thakkar *et al*[100], 2015 | *n* = 20; aged 8-45 yr | Into portal + thymic circulation and subcutaneous tissue: | Insulin independence: No |
| India, 24 mo | Duration of T1DM: > 12 mo | | Insulin requirement: Decreased |

| | 2 groups with a mean C-peptide: | Group 1: Autologous IS-AD-MSC+ HSC | HbA$_{1C}$, C-peptide: Sustained improvement |
| --- | --- | --- | --- |
| | Group 1: 0.22 ng/mL | Group 2: Allogeneic IS-AD-MSC+ HSC | Adverse effects: No |
| | Group 2: 0.028 ng/mL | | |
| Dave *et al*[101], 2015[3] | *n* = 10; aged 9-29 yr | Into portal + thymic circulation and subcutaneous tissue: autologous IS-AD-MSC+ HSC | Insulin independence: No |
| India, 27 mo | Duration of T1DM: 2-15 yr | | Insulin requirement: Decreased |
| | Pre-IV C-peptide: 0.22 ng/mL | | HbA$_{1C}$, C-peptide: Sustained improvement + significantly lower GADA levels |
| | | | Adverse effects: No |
| Hu *et al*[99], 2013 | *n* = 15; aged < 25 yr | IV (2x): Allogeneic WJ-MSC | Insulin independence: 3 subjects |
| China, 24 mo | Duration of T1DM: < 6 mo | Control group: normal saline | Insulin requirement: 8 patients more than 50% reduction |
| | C-peptide: ≥ 0.3 ng/mL | | HbA1C: Significantly decreased; FCP: Significantly increased |
| | | | Adverse effects: No |

[1]Follow-up trial to Cai *et al*[104], 2016.
[2]Extension trial to Araujo *et al*[102], 2020.
[3]Preliminary data of Thakkar *et al*[100], 2015.
AD: Adipose-derived; BM: Bone marrow-derived; FCP: Fasting C-peptide; GADA: Glutamic-acid-decarboxylase antibody; HbA$_{1C}$: Glycated hemoglobin; HSC: Hematopoietic stem cell; IS-AD: Adipose-derived insulin-secreting; IV: Intravenous; MNC: Mononuclear cell; MSC: Mesenchymal stem cell; SCP: Stimulated C-peptide; T1DM: Type 1 diabetes mellitus; UC: Umbilical cord-derived; WJ: Wharton's jelly-derived.

**Figure 3 Immunoregulatory function of mesenchymal stem cells in type 1 diabetes mellitus.** The effects of mesenchymal stem cells ultimately result in downregulation in the proliferation and function of dendritic cells, cytotoxic T lymphocytes (CTLs), helper T (Th) type 1 and type 17 cells, natural killer (NK) cells and type 1 macrophages (M1). Meanwhile, mesenchymal stem cells increase the number of type 2 macrophages (M2) and regulatory T cells (Tregs), which can inhibit effector T cells and stimulate Th type 2 protective cells. Red arrow/line: Downregulation/inhibition; Green arrow/line: Stimulation. APC: Antigen-presenting cell; B: B cell; DC: Dendritic cell; GADA: Glutamic-acid-decarboxylase antibody; IA2: Islet tyrosine phosphatase 2 antibody; IAA: Insulin autoantibody; ICA: Islet cell antibody; IL: Interleukin; IFN-γ: Interferon γ; TGF-β: Transforming growth factor β; TNF-α: Tumor necrosis factor α; ZnT8: Zinc transporter 8 antibody.

follow-up, increased C-peptide secretion, decreased exogenous insulin requirement, improved HbA$_{1C}$ and significantly lower GADA levels have been found.

Similar results were obtained in the studies of Araujo *et al*[102] and Dantas *et al*[103], where a single dose of adipose-derived MSC infusion were combined with daily 2000 IU vitamin $D_3$ supplementation. Compared to control subjects on traditional treatment, improved $HbA_{1C}$ levels and reduced insulin doses have been found 3-mo after MSC infusion[102], while basal C-peptide levels remained the same at first but significantly improved for the 6-mo follow-up measurement[102,103]. It has to be mentioned though that most patients reported transient headache and local reactions, and further mild but resolving adverse events were also reported by a significant amount of the study population. Although the results of this study further strengthened the efficacy and safety of adipose-derived MSCs, all positive effects were somewhat overshadowed by the significant number of adverse events.

Cai *et al*[104] investigated the safety and efficacy of umbilical cord-derived MSC (UC-MSC) and autologous BM-mononuclear cell cotransplantation in adult patients. The treatment group received octreotide as a prophylaxis, followed by stem cell infusion into the dorsal pancreatic artery. After 1 year, the C-peptide area under the curve during a 3-h oral glucose tolerance test increased by 105.7% in 20 of 21 responders compared to a 7.7% decrease in the control group showing the robust effect of the treatment against disease progression. Further importance of this trial was that immunological parameters were also assessed. GADA positivity remained unchanged, while IL-10 levels increased, and interferon-γ levels and adenosine triphosphate levels in $CD4^+$ T cells decreased. While the effect of MSCs may be less pronounced in this study due to reduced inflammatory signals in long-standing disease[104], it has to be mentioned that the long-term follow-up analysis of the study population have shown a significantly decreased occurrence of various diabetic complications. Furthermore, the UC-MSC treated patients still had clinically better $HbA_{1C}$ and C-peptide levels, 8 years after the UC-MSC treatment, but the initial difference in insulin requirement leveled off[105]. The combined results of the original and follow-up study[104,105] indicate that UC-MSCs are good candidates for slowing down the progression of T1DM.

Lu *et al*[106] assessed the repeated transplantation of allogeneic UC-MSC in T1DM. The primary efficacy endpoint was clinical remission, defined as a 10% increase from baseline in the levels of fasting and/or postprandial C-peptide. After 1 year, 11 out of 27 in the UC-MSC-treated group maintained clinical remission, whereas only 3 out of 26 in the control group maintained clinical remission. The UC-MSC-treated group showed a decreasing trend in fasting and postprandial C-peptide. Three UC-MSC-treated adults became insulin independent and started using insulin again in 3-12 mo. Among adult-onset T1DM (≥ 18 years of age), UC-MSC treatment showed a protective effect on β-cell function but failed to be protective in juveniles. Three recipients had mild fever after UC-MSC infusion; all of them recovered within 24 h[106]. It seems UC-MSC therapy might be more beneficial for patients with adult-onset T1DM.

Carlsson *et al*[107] tested the efficacy of autologous BM-MSCs in newly diagnosed T1DM patients. Stems cells were harvested from the aspiration of the iliac crest and subsequently administered to the MSC-treated group as an intravenous infusion without premedication. $HbA_{1C}$, fasting C-peptide and insulin requirement were not significantly different compared to the control group[107]. In contrast, Izadi *et al*[108] found improved $HbA_{1C}$ and C-peptide levels, a reduced number of hypoglycemic events and increased anti-inflammatory patterns. Furthermore, early BM-MSC transplantation (< 1 year after disease onset) further improved $HbA_{1C}$ levels and C-peptide levels compared to those who received the transplantation > 1 year after disease onset[108], similar to that of UC-MSC.

Summarizing the available clinical study results of the stem cell therapies, the results about BM-MSC and adipose-derived MSC are more controversial, suggesting that these two therapies may be less effective than UC-MSC therapy in T1DM. It should be noted, however, that based on the results of the studies so far it is recommended to apply these treatments as early as possible. The earlier these treatments are introduced, the greater the preservation of the remaining β cells, thereby the reduction of external insulin requirement and the development of long-term complications can be elongated. Adipose-derived MSCs and UC-MSCs are currently under further investigated in NCT05308836[109] and NCT04061746[110], respectively.

## CONCLUSION

In the management of T1DM the focus remains on the challenges of glycemic control and long-term complications, which could not been fully overcome by new technological advances. Recently, there has been a paradigm change in the treatment of T1DM. The goal now is to cure rather than identify a lifelong 'symptomatic treatment' with insulin supplementation. The crucial future may lie in disease-modifying therapeutic options, which could be used to preserve β cells in the presymptomatic phase of the disease and to cease the destructive autoimmune process as well as to regenerate β-cell function in the clinical phase.

Immunotherapy appears to be a promising disease-modifying therapy in T1DM. Different agents have the potential to target the major autoreactive immune pathways leading to T1DM. Therapies interfering with T cell activation seem to be the most favorable. Regenerative therapy is developing parallel with immunotherapy. MSC therapy stands out from other cell therapies. It is safe, with its

beneficial effects due to immune regulation. However, the clinical trials are limited in their conclusions due to the small patient numbers and short follow-up times. Standardized stem cell processing, transplantation protocols and dosage will be essential for future randomized, double-blinded clinical trials with large patient cohorts. Combining disease-modifying therapies with glucagon like peptide-1 analogues seem to increase efficacy and increase tolerability of interventions.

So far, neither immunotherapies nor stem cell therapies, when used alone, have had ultimate successes in altering T1DM disease course. Their common disadvantage is that their short-term therapy effects are transient. The future for disease-modifying therapies might be the individualized, long-term multimodal approach combining immune, incretin based and regenerative therapeutic options potentially by identifying biomarkers of responders for it to be used in routine clinical treatment.

## ACKNOWLEDGEMENTS

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