# Targeted immune interventions for type 1 diabetes: not as easy as it looks!

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**Purpose of review**
Although insulin is lifesaving and sustaining for those with type 1 diabetes (T1D), curing the disease will be much more complex than simple replacement of this hormone. T1D is an autoimmune disease orchestrated by T cells, and includes many arms of the immune response. Tremendous effort has gone into understanding its underlying immune, genetic, and environmental causes, and this progress has led to immunologically based clinical trials in T1D. This review will focus primarily on the clinical trials of the past decade that have attempted to translate these fundamental findings.

**Recent findings**
It is known that powerful, nonspecific immune suppressants can temporarily slow the course of newly diagnosed T1D, yet are too toxic for long-term use, especially in children. Recent clinical trials to reverse T1D have used newly developed therapies that target specific components of the immune process believed to be involved with T1D. Although well justified and designed, no recent approach has resulted in clinical remission and few have had any effect on disease course.

**Summary**
Advances in our fundamental understanding of how the human diabetes immune response is activated and regulated coupled with lessons that have been learnt from the most recent era of completed trials are guiding us toward the development of more effective, multipronged therapies to ablate diabetes autoimmunity, restore immune tolerance, preserve β cells, and, ultimately, improve the lives of patients with T1D.

**Keywords**
autoantibodies, autoantigens, immune therapy, regulatory T cells, T cells, type 1 diabetes

## INTRODUCTION

Type 1 diabetes (T1D) affects up to 3 million North Americans, is primarily a disease of childhood, and is increasing in incidence, especially in young children [1–3]. It is an autoimmune disease specific for the insulin-producing β cells in the pancreas [4]. Generally, all, or nearly all, β cells are destroyed and individuals are left with the inability to produce insulin, with life-threatening consequences. Insulin, discovered almost 100 years ago, is lifesaving but is required daily and even with the best-managed regimens, T1D patients have increased risks for morbidity and mortality, reaffirming that insulin is not a cure for this disease [4,5].

Steady progress since the 1970s has led to the recognition that T1D is an autoimmune disease with an underlying genetic component and one or more unidentified environmental triggers [6–9]. The current paradigm for initiation of T1D is that genetically susceptible individuals encounter an environmental trigger that activates the β-cell autoimmune response, which expands over months or years and results in near-total β cell loss [10–12]. Recent studies have also suggested that individuals prone to T1D have heightened markers of β cell stress, although it is uncertain whether these reflect inherent defects with repair of cellular damage or are because of excess metabolic demands [13,14▪]. It remains a possibility that, depending on the underlying genetics, different individuals may be susceptible to different triggers [15]. The event or antigen that incites T1D has remained elusive, and it is currently not possible to

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## KEY POINTS

- T1D is the result of a multifaceted immune attack on pancreatic β cells.
- Agents that directly affect the immune system (immune suppressants and modulators) have had the most and most reliable success in modifying the course of T1D.
- Although well founded and successful in rodent models of T1D, recent trials using agents targeting T cells, B cells, or cytokines have had less than the expected effect on the course of T1D.
- Future trials incorporating data from more detailed studies in human T1D and combining targeted therapies, specifically those which combine targeting effector cells and enhancing regulation, may hold the most promise for inducing durable remission in T1D.

identify individuals before the onset of β cell autoimmunity.

One of the significant successes in T1D over the past decades has been the identification of autoantibodies to β cell antigens [16–18]. These autoantibodies are required for the diagnosis of T1D, but it is unclear what role they play in T1D pathogenesis [17]. Nevertheless, the presence of autoantibodies significantly predates the clinical onset of disease, suggesting they may play a role in disease progression [10,16,19]. The odds of developing and the time to clinical disease can be predicted in asymptomatic individuals depending on the number of positive autoantibodies, which is now part of the entry criteria in T1D preventive trials.

The area that has experienced the greatest advances and has provided the foundation for the most promising clinical trials to prevent or reverse T1D is the study of the contribution of T cells to T1D [20–22]. It is apparent that β-cell antigen-specific T cells orchestrate other components of the immune response to β cells and are directly involved in β cell killing [23,24]. In humans, both CD4 and CD8 T cells are found infiltrating islets in newly diagnosed T1D [25]. In rodent models, either CD4 or CD8 T cells can adoptively transfer disease. CD8 T cells likely are directly involved with β cell killing, as MHC I is expressed on β cells, and CD4 T cells likely impact pathogenesis via an indirect route [22,26]. Both cell types secrete a number of proinflammatory cytokines, such as TNFα, IFNγ, interleukin (IL)-6, and IL-1, which not only recruit and activate accessory cells, thereby magnifying the inflammatory process, but also are directly toxic to β cells [26–28].

Although it is believed that β-cell antigen-specific T cells are a necessary component of autoimmune diabetes, their very presence is not sufficient for disease because such cells are also found in healthy individuals; and, not all genetically predisposed mice develop diabetes (e.g., in NOD colonies only ~50% of male and ~80% of female develop disease despite harboring autoreactive T cells) [20,29,30]. This strongly suggests that there are critical peripheral tolerance mechanisms that play a role in restraining self-reactive T cells that have escaped central (thymic) tolerance. Although there are a number of mechanisms of peripheral tolerance (including anergy and exhaustion), evidence now points to active regulation to be the primary mechanism of peripheral tolerance in T1D.

In the past decade, the concept of a T cell population that can suppress the activity of effector T (Teff) cells has re-emerged. Most focus has been on a subset of CD4 T cells that express the transcription factor FoxP3 [31▪,32]. The role of FoxP3 and regulatory T cells (Tregs) is most obvious in conditions wherein there is genetic disruption of FoxP3, leading to multisystem autoimmunity in humans (the IPEX syndrome) and in mice (the Scurfy mouse), which lack Tregs [33–37]. NOD mice seem to have a loss of (functional) Tregs early in life concordant with the development of diabetes, and adoptive transfer of Tregs (either isolated directly from congenic mice or ex-vivo expanded) can prevent and even reverse disease [33,35]. Although the data from human studies have been more difficult to interpret, it now seems that although healthy individuals and those with T1D have similar circulating levels of Tregs, Tregs in T1D have functional deficits, that is, reduced suppressive ability [35,38–41]. Tregs exert suppression through secretion of certain immunomodulatory cytokines (e.g., IL-4, IL-10, and TGFβ) and via direct interaction with T cells or antigen presenting cells (APCs) [42–45].

These observations have led to the concept that the development of autoimmunity (including T1D) is dependent on the 'balance' of self-reactive Teff cells and Tregs (Fig. 1). Although frequently thought of in terms of stoichiometric ratios, there are likely functional considerations of the T cells that must also be accounted for. For example, simply having sufficient numbers of Tregs may not be enough to prevent disease, and the functional state – either baseline (genetically determined) suppressive activity or impaired activity due to effects of the immunologic microenvironment – should be taken into consideration. Alternatively, it is well known that there are differences in activation requirements of naive (Tn) and memory T (Tmem) cells, and that Tmems expand much more robustly than Tns (Fig. 1b). Thus, depending on the maturation stage and time after antigen encounter, Tregs may have

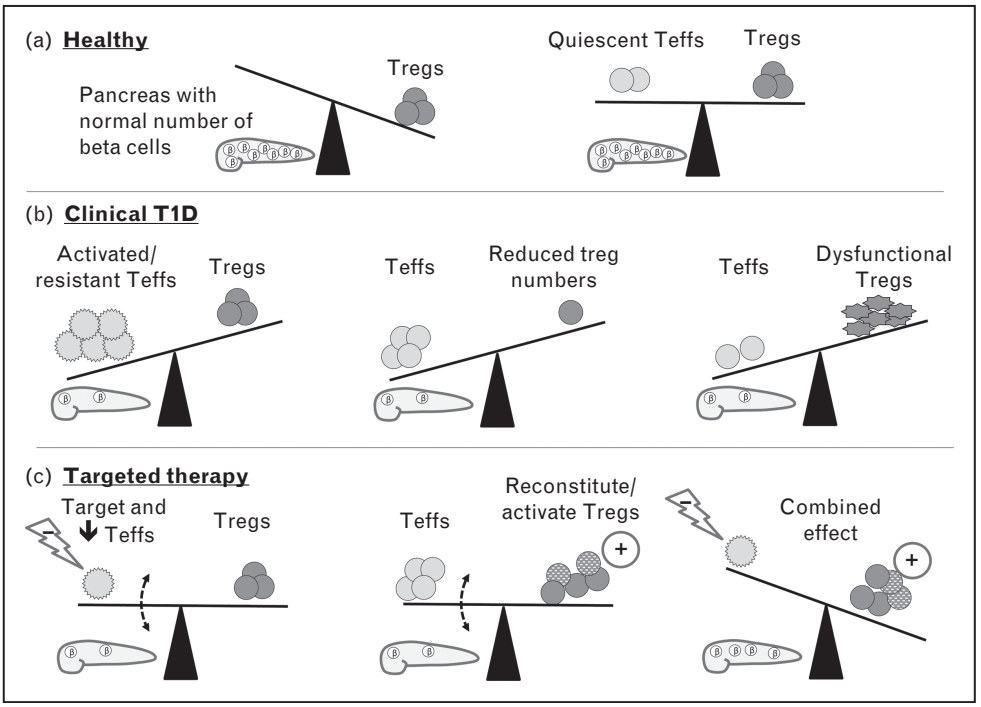


**FIGURE 1.** Balancing effector and regulatory T cells in health, T1D, and with therapies. (a) Individuals free from diabetes may have no circulating β-cell specific effector T cells (Teff; left) or have sufficient, functional peripheral regulatory T cells (Tregs) to counterbalance Teffs (right) and keep β cells free from autoimmune damage. (b) In individuals who develop T1D, Teffs may become resistant to Tregs (left), Treg numbers may diminish (center), or, despite sufficient numbers, Tregs may become dysfunctional (right) resulting in T cell-mediated destruction of β cells. (c) Therapies which temporarily suppress Teffs (left) or bolster Treg number or function (center) may be able to temporarily slow β cell decline, but it may take therapies that both target Teffs and increase β-cell-specific Tregs (right) to have a substantive and long-lasting effect.

vastly different abilities to suppress the Teff cell response [46,47▪]. Indeed, recent studies have suggested that Teff resistance to suppression by Tregs may be a primary defect in T1D [46]. Thus, the paradigm of 'rebalancing' the Teff/Treg ratio to prevent, stabilize, or reverse diabetes autoimmunity may need to take into consideration both quantitative and qualitative factors (Fig. 1c).

Now, it is apparent that there are a number of cells and soluble factors that are involved in the immune dysregulation responsible for β cell loss in T1D. Most of the information on contributions of immune-system components to diabetes pathogenesis has come from preclinical models of T1D, most frequently the NOD mouse and related strains. Based on these findings, approaches to modify the course of diabetes in these models have been developed and have provided the rationale for a number of clinical trials. However, as described in more detail below, although several interventions have been successful in preclinical models, to date, none has translated into similar success in humans. An examination of these trials may provide important insights into human T1D and contribute to the development of future intervention trials.

## IMMUNE THERAPIES IN TYPE 1 DIABETES

In the 1980s and early 1990s, several small-scale clinical intervention trials investigated the use of general immune-suppressive agents on the course of newly diagnosed T1D. For example, in 40 children with new-onset T1D, almost two-thirds were insulin-free about 6 weeks after starting cyclosporine, and this effect persisted for over a year during the time of therapy [48]. Yet once stopped, diabetes returned. Factors associated with response included shorter time from diagnosis, less weight loss, lower HbA1c, and less DKA [48,49]. In another study, children treated with azathioprine and prednisone had evidence of improved glycemic control compared with controls, and in some cases achieved insulin independence, but again, these benefits waned following discontinuation of therapy [50]. These and other examples provided the 'proof-of-principle' that the diabetes autoimmune response, in some cases, could be slowed or even reversed using nonspecific immune-suppressive agents [51,52]. Concern for immune and nonimmune side-effects associated with what would likely need to be indefinite therapy precluded consideration of these as a viable approach. These studies also

suggested that there was a variable response to therapy, suggesting heterogeneity in T1D, even in the pediatric population.

As diabetes-associated autoantigens were identified, the concept of disrupting specific autoimmune processes by administering these antigens – in essence, overwhelming and dampening the autoimmune response by presenting autoantigens in a tolerogenic context – was examined in preclinical models. In some cases, autoantigen therapy prevented or reversed diabetes and thus provided the justification for clinical trials [53–55]. A number of medium-scale to large-scale trials have been conducted evaluating insulin, Hsp60, and GAD on the progression of T1D. In the case of insulin, this has been tested by the oral, intranasal, and parenteral routes with no significant effect [56–59]. In some studies, Hsp60 peptide (also known as DiaPep277) given SQ has slowed β cell loss, but minimally [60–62]. In phase II studies in children and adolescents, GAD65 bound to the adjuvant Alum given SQ seemed to slow β cell loss, but this could not be confirmed in larger phase III trials [63,64]. Some studies evaluated immune responses in participants. Patients receiving the Hsp60 peptide did have increases in IL-10 and dampened T cell responses to antigen, and those receiving GAD-alum had increases in GAD antibodies and increases in proinflammatory cytokines, T cell proliferation, as well as Tregs [65,66▪▪] in response to GAD. Taken together, although autoantigen treatment was successful in preclinical diabetes and may modulate specific aspects of the T1D autoimmune response, after much study, there is little evidence that when given as a monotherapy this approach can modulate the course of disease in humans. Further, in no other autoimmune disease has antigen therapy been shown to slow, prevent, or reverse disease. These and other data presented below would strongly suggest that diabetes autoantigens alone are not able to significantly modify the course of T1D.

Although it seems that diabetes autoantibodies have little role in the pathogenesis or progression of T1D, murine studies demonstrated that agents that deplete B cells can prevent diabetes [67]. Rituximab is a monoclonal antibody to CD20, specifically depletes B cells and is used clinically to treat B cell lineage malignancies, autoimmune disease, and organ transplant rejection. This agent was tested in 8–40-year-old patients diagnosed with T1D within the past 100 days [68]. A four-dose course was associated with what seemed to be a pause in β cell loss, which resumed at 3 months postenrollment. At 12 months, the rituximab group had higher endogenous insulin production than placebo patients, but still lower than baseline. Rituximab caused significant B cell depletion, but the effect on autoantibodies was not reported. It is unclear whether the B cell depletion mediated by rituximab produced clinical efficacy due to their function as APCs, producers of antibodies, or another mechanism [68,69].

Neutralizing proinflammatory and Th1 cytokines has been a successful approach to prevent diabetes in preclinical models and has been one of the most successful approaches to manage other human autoimmune diseases. Both TNFα and interleukin-1β are secreted by immunocytes infiltrating inflamed islets, and both not only assist in propagating inflammation but are also toxic to β cells, and thus may be both directly and indirectly involved in T1D [27,28,70,71]. Some studies have shown elevations in these cytokines in humans with T1D, and treating mice with neutralizing antibodies prevents, and in some cases reverses, disease. In 2009, a small-scale trial of 18 children 7–18 years old studied the effect of etanercept (a recombinant TNFα receptor fusion protein) on disease progress [72]. After 6 months of treatment, those treated with etanercept showed lower HbA1c levels with lower insulin needs and a rise (versus a drop) in C-peptide compared with placebo-treated participants. A larger, confirmatory study has not been conducted. Last year, a publication reported results of two trials using different agents to antagonize IL-1β [73▪▪]. One conducted in Europe used anakinra (an IL-1 receptor antagonist) and enrolled adolescents and adults 18–35-years-old, 35 in the treatment arm and 34 in the placebo arm. Another study was conducted in North America and studied canakinumab (an anti-IL-1β MAb) in 6–45-year-old subjects, 47 in the treatment arm. Neither trial showed any metabolic effect of IL-1β antagonism on T1D course within 1 year. Again, as exemplified for IL-1β blockade, not all agents that can successfully interfere with autoimmunity in murine or other human autoimmune diseases are effective in human T1D; but in the case of TNFα antagonism, there are opportunities for future study.

To date, agents that selectively target T cells have comprised the most numerous T1D intervention trials. In the 1980s and 1990s, monoclonal antibodies against T cells were developed and proved successful to treat organ allograft rejection. In rodent models, antibodies to CD3 can prevent and reverse diabetes [53,74]. The first trials using a biologic agent in T1D used monoclonal antibodies to the CD3. In 2002, a modified form of OKT3 with a mutated (non-Fc receptor binding) Fc region called hOKT3 γ1 (Ala-Ala) (teplizumab) was tested in 12 new-onset patients 7–27 years old [75]. Compared with placebo-treated patients, drug-treated participants

had better maintenance of C-peptide secretion, lower insulin requirements, and lower HbA1c at 1 2months. A follow-up study repeating dosing at 12 months (the AbATE trial) showed lasting metabolic improvement at 24 months, and post-hoc analysis was able to identify responders from nonresponders by lower HbA1c and insulin requirements, lower levels of some types of memory and naive T cells, and lower IFNγ-producing CD8 T cells at baseline [76▪▪]. A large (n = 516), industry-sponsored, multinational phase III randomized controlled trial (Protégé) of teplizumab that tested multiple treatment regimens did not meet its primary end point (which was the percentage of patients with both insulin use of less than 0.5 U/kg/day and HbA1c less than 6.5%) at 12 months [77]. Post-hoc analysis identified factors at baseline associated with C-peptide preservation, including better metabolic control, higher C-peptide response, and time from diagnosis to enrollment.

A nonglycosylated form of anti-CD3 (ChA-glyCD3; otelixizumab) was tested in the early 2000s in 40 patients 12–39 years old, and it was found to partially preserve β cell function, resulting in less insulin requirements at 6, 12, and 18 months after treatment. Two follow-up phase III industry-sponsored randomized controlled trials (DEFEND-1 and DEFEND-2) enrolled participants 12–45 years old, yet these too failed to meet their primary end points, the change in C-peptide levels at 12 months, perhaps because the studied dose was too low [78,79▪▪].

For T cells to become fully activated, they require both antigen-specific signals (i.e., binding of MHC : peptide from APCs to the T cell receptor) and antigen nonspecific, costimulatory signals (i.e., binding of CD40 and B7 molecules on the APC to CD154 and CD28 on T cells) [80–84]. Blocking T cell costimulation can prevent or dampen T cell responses and is an effective means to modify or prevent diabetes in rodent models. CTLA4-Ig (abatacept) is a fusion protein that binds to B7 molecules and interrupts CD28 signaling in T cells. This agent is a component of therapies in organ transplantation and has been approved for a number of human autoimmune diseases [85]. Abatacept was given for 2 years to 77 patients 6–45 years old and produced a delay in C-peptide decline and lower HbA1c levels with similar insulin use at 2 years compared with placebo individuals [86]. Statistical modeling suggested that a number of factors, including younger [6–12] or older [18–30,31▪, 32–45] age, lower baseline C-peptide, and white race, were associated with a more robust response. However, despite continuous therapy for 2 years, the C-peptide decline resumed in the abatacept group at 6 months.

During the 2000s, technical improvements and advances in immunomodulation resulted in major strides in human islet cell transplantation (ICT) [87]. A variation of the ICT immune protocol from the Edmonton group was assessed in reversing diabetes in new-onset T1D [88,89]. This trial used anti-CD25 (daclizumab) to target CD25-expressing (activated) T cells and mycophenolate mofetil (MMF) as a nonspecific immunosuppressant. In patients 8–45 years old, neither MMF alone nor MMF plus daclizumab had any effect on β cell loss or metabolic parameters over 24 months [89]. This was surprising as this regimen met with some success in ICT (which comprises both alloimmune and autoimmune responses [87]), and anti-CD25 and MMF alone and in combination can delay or prevent autoimmune diabetes in the BioBreeding rat [90]. One possibility is that this regimen inhibited Tregs, which are strongly dependent on signaling through the high-affinity IL-2 receptor that includes the α subunit (CD25) [91].

A number of recent trials have provided an insight on how therapies may modulate Tregs. A phase I trial of IL-2 and rapamycin was tested in nine adults specifically to evaluate whether this could increase Tregs, and its effect on β cell function [92]. IL-2 is known to be involved in Treg survival and function (they express high levels of CD25), while rapamycin inhibits activation and function of Th1 and Th17 Teff cells, and is effective in preventing diabetes in mouse models [93]. This approach transiently increased the numbers of Tregs in the first month after therapy, but concomitantly metabolic parameters were worsened, likely due to unintended Teff activation. A trial of antithymocyte globulin (ATG; the START trial) was based on the concept that significant T cell depletion might eliminate diabetogenic T cells and 'reset' the autoimmune response and the Teff to Treg balance, resulting in long-term remission [94▪▪]. ATG is a rabbit antiserum that depletes human T cells, and is used in organ transplantation and some autoimmune diseases, and analogous therapies can prevent and reverse diabetes in preclinical models. Multiple doses of ATG were given to 38 participants 12–35 years old with new-onset T1D over 1 week. Most recipients acutely developed cytokine release syndrome and serum sickness 7–10 days later. ATG had no effect on C-peptide preservation or metabolic control. In mechanistic evaluations, recipients had acute serum elevations in a number of proinflammatory cytokines during therapy and, interestingly, a preferential depletion of Tregs over effector CD4 and CD8 T cells. In a post hoc analysis, it seems that older participants may have had β cell sparing, while the younger individuals treated with

ATG had an acute loss of β cells in the first 6 months [76▪▪]. A clinical trial of ATG and GCSF is being planned.

An ongoing trial evaluating specific depletion of Teff and Tmem cells (the T1DAL trial) recently published its 12-month results [95▪▪]. Alefacept is a fusion protein consisting of an LFA3 head and an IgG tail. The drug preferentially targets memory and effector CD4 and CD8 T cells (which express high levels of CD2, the cognate receptor for LFA3), the cells that seem to be most involved in β cell destruction. The trial randomized 49 participants 12–40 years old (33 to alefacept, 16 to placebo) and found that treated individuals had lower insulin requirements, fewer hypoglycemic episodes, and, in some analyses, preservation of C-peptide at 12 months. In the mechanistic evaluation, it was shown that alefacept significantly depleted CD4 and CD8 effector and memory cells, while sparing Tregs, leading to a favorable Treg : Teff ratio [77]. Additional data from this trial will be forthcoming.

## CONCLUSION

Armed with the knowledge of the immune basis for T1D, the observations that the course of T1D could be modified with nonspecific immune suppressants, and the advent of novel agents to target specific immune processes, the past decade was filled with promise that an approach to reverse and stabilize T1D would be discovered. Unfortunately, despite tremendous effort with nearly a dozen trials enrolling many hundreds of participants, none has been found. In many cases, despite well founded preclinical data and/or experiences from other human autoimmune or alloimmune conditions, there was no apparent impact on the course of T1D. Even in those trials showing some impact on disease course, no approach to induce true clinical remission (i.e., insulin independence) has been found.

Although no approach has been able to achieve frank remission or prolonged β cell preservation, there are a number of lessons from these trials that may help guide the next phase of studies as listed here.

(1) Treatments that are effective in other human autoimmune or alloimmune conditions have marginal or little efficacy in T1D. Possible explanations include: T1D has a unique immunopathogenesis compared with other autoimmune conditions; short-term immune modulation does not restore tolerance and autoimmunity resumes after a variable interval once treatment ends; the residual β cell mass has fallen below a critical threshold and cannot recover even after successful ablation of the autoimmune attack.
(2) As powerful immune modulatory agents have little or no effect in changing the course of T1D, the immune process in T1D seems to be extremely robust, and thus agents with minimal impact on immune responses are unlikely to alter the progression of T1D.
(3) Many interventions that are effective in rodent (primarily NOD) models of T1D are not similarly effective in humans, and therefore the use of rodent models as the prerequisite rationale for human trials may not be appropriate.
(4) In some cases, different subpopulations of patients with T1D seem to respond differently to immune interventions, suggesting significant heterogeneity in human T1D.

Taken together, more in-depth evaluation of existing studies is warranted and further fundamental study of human T1D is needed to guide the next phases of intervention trials in this area.

The human T1D immune response has proven resistant to a number of potent immune interventions that are effective in other human conditions and preclinical models. It thus seems that on a relative scale, T1D autoimmunity is more intractable than a number of other autoimmune diseases or the alloimmune response to organ transplantation, including that to islet allografts. It is also clear that what is shown to be effective in preclinical (rodent) models does not necessarily correlate with efficacy in humans – further putting into question the utility of these models as a litmus test for clinical trials [96]. Certainly, rodent studies have provided critical information for a general understanding of the pathogenesis of T1D, but more translational studies in the clinic are urgently needed.

As the immune response in T1D is more robust and complex than previously considered, trials that interfere with a number of pathways (i.e., through the use of combination therapies) are warranted, and the use of therapies that are likely to have minimal immunological effect (e.g., dietary modification or vitamin supplementation) may be futile. In-depth mechanistic evaluation from some studies has suggested that therapies may differentially impact effector and regulatory cells. There is an emerging consensus that an effective therapy must combine inhibition of Teff cells (by depletion, enhanced suppressibility, or both) with stimulation of Tregs (by increased frequency or function, including ablation of the proinflammatory milieu). Further, if possible, such changes in effector and regulatory cells should be antigen-specific. Such an outcome may require combinations comprising a Teff-depleting agent, a Treg-boosting agent, and an antigen [45,97]. While such combinations will

present substantial practical and regulatory challenges, they will likely be our best shot at inducing a durable remission of autoimmunity in this disease.

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## Conflicts of interest

*There are no conflicts of interest.*

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- ■ of special interest
- ■■ of outstanding interest

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