

Timing of Immunotherapy in Type 1 Diabetes: The Earlier, the Better?

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ABSTRACT

In 1986, *The New England Journal of Medicine* published George Eisenbarth's (Eisenbarth. 1986. *N. Engl. J. Med.* 314: 1360–1368) model of type 1 diabetes (T1D) as a chronic autoimmune disease. In 2019, the same journal published the results of the teplizumab trial, which showed the anti-CD3 mAb delayed T1D progression in high-risk individuals. Although teplizumab is the first immunomodulatory agent to demonstrate significant delay in disease progression, it is also one of the few tested prior to clinical disease onset. Is it possible, then, that this trial's success is as much about the agent as it is about its timing? This commentary will review the landscape of immune intervention in T1D since 1986, discuss the teplizumab trial results, and finally, speculate on whether current paradigms for T1D immune intervention should focus less on disease development as a continuum and more on the stages of T1D progression as distinct disease processes. *ImmunoHorizons*, 2021, 5: 535–542.

INTRODUCTION

The loss of insulin-producing β cells in the pancreas has been recognized as the cause of type 1 diabetes (T1D) since the early 1900s. Without insulin, chronic hyperglycemia leads to irreversible dehydration, metabolic acidosis, and ultimately death if not treated with s.c. insulin injections. It was not until 1986, however, when George Eisenbarth (1) published his seminal work in *The New England Journal of Medicine*, that T1D was recognized as an autoimmune disease. The Eisenbarth model of T1D suggested that, in genetically susceptible individuals, evidence of autoimmune β cell destruction could be detected by the presence of autoantibodies against islet proteins in the circulation years before the onset of clinical disease (1). At that time, he acknowledged that, although “remarkably little” was known about the immunology of T1D, the “data that link autoimmune phenomena to type 1 diabetes” highlighted some “obvious” directions for future research, including characterization

of putative Ags, development of diabetogenic autoreactive T cell lines, and improved understanding of genetic factors (1). Simultaneously, the assertion that β cell destruction was immune-mediated thrust immunomodulation as a therapeutic tool into the T1D research forefront. Indeed, attempts to suppress or alter the immune system to prevent β cell destruction and change the course of disease progression have characterized the 35 years since the publication of Eisenbarth's work.

Eisenbarth's model described six stages of T1D development, beginning with genetic susceptibility. Genes within the MHC HLA class II region were recognized as high risk, with over 90% of T1D patients carrying one of the HLA DR3 or DR4 alleles (2, 3). Candidate gene association studies and genome-wide linkage analysis have since identified important non-HLA genes associated with T1D, including insulin (*INS*) and the protein tyrosine phosphatase, nonreceptor type 22 (*PTPN22*), and the IL-2 receptor (*IL2RA*) (4). Eisenbarth (1) proposed that, in individuals with genetic risk, an

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Abbreviations used in this article: ATG, antithymocyte globulin; DKA, diabetic ketoacidosis; ITN, Immune Tolerance Network; T1D, type 1 diabetes.

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environmental triggering event led to active autoimmunity that could be characterized by immunological abnormalities, including the presence of β cell-targeted (islet) autoantibodies in the serum. These immunological abnormalities preceded β cell destruction, and importantly, at the time of autoantibody seroconversion, insulin secretion remained normal. Over time, glucose-stimulated insulin secretion declined, leading to impaired glucose tolerance and, finally, overt hyperglycemia and a clinical diagnosis of T1D. In addition to recognizing T1D as a chronic, progressive, autoimmune disease, the Eisenbarth model also implied that at-risk individuals had the potential to be identified prior to overt hyperglycemia and clinical diabetes diagnosis, based on the presence islet autoantibodies early in disease progression.

Longitudinal, prospective, and natural history studies have since solidified the predictive power of autoantibodies in T1D. In individuals with high genetic risk, the presence of two or more islet autoantibodies is associated with a nearly 80% risk of developing T1D within 15 years (5–7). These findings led to the development of a new system to diagnose T1D based on predictable stages: stage 1 T1D is the presence of two or more islet autoantibodies with normal glucose tolerance; stage 2 T1D is the presence of two or more islet autoantibodies with impaired glucose tolerance but without clinical symptoms of hyperglycemia; and stage 3 T1D is the onset of symptomatic disease (8) (Fig. 1). The adoption of this new staging paradigm was associated with new hope that the recognition of an early T1D diagnosis, prior to clinical symptoms, could enhance clinical trial design and encourage the adoption of early interventions during presymptomatic periods. To date, however, clinical

trials have been offered primarily to individuals immediately after stage 3, upon clinical T1D diagnosis, and until recently have largely failed to meet meaningful clinical endpoints. Despite demonstration of successful mechanism in animal models of T1D, human clinical trials of immunomodulatory agents have resulted in repeated disappointment, causing some to question the pertinence of immune intervention in human T1D altogether.

The evolution of immune intervention in T1D

It was not long after the publication of Eisenbarth's model of T1D as an autoimmune disease that results of the first trial testing an immune intervention in T1D were published. In the double-blind trial, 122 individuals with newly diagnosed T1D received either the calcineurin inhibitor cyclosporin or placebo (9). Although insulin independence was achieved in the cyclosporin group, diabetes remission did not last after the drug was stopped, and the toxicities associated with long-term cyclosporin treatment (nephrotoxicity, malignancy risk) made it difficult to justify its use as a curative therapy. However, this trial and others confirmed the immune etiology of T1D and revealed the potential to modulate immune responses to change the course of disease. Given the adverse side effects and risks associated with immunosuppression, especially in children, the goals of immune therapy in T1D shifted to from immunosuppression to tolerance induction. In the early 2000s, research consortia, including the Immune Tolerance Network (ITN) and the T1D TrialNet conducted a multitude of clinical trials aimed at inducing tolerance to pancreatic islets with immunomodulatory agents. These trials were almost universally conducted in new-onset T1D patients and used a primary endpoint of endogenous insulin secretion as measured by the change in baseline C-peptide area under the curve during a mixed meal tolerance test (10). In 2002, Herold et al. (11) demonstrated that a short course of an anti-CD3 Ab given after diagnosis could preserve some islet cell function, results that were reproduced by another group using a different anti-CD3 Ab (12); however, the benefit was limited to 1–2 y posttreatment, and dose-related adverse events, including cytokine release syndrome and EBV activation, further limited its use. These trials provided proof of concept that autoimmune destruction could be alleviated through immunomodulation rather than generalized immune suppression and that single treatments could have lasting effects. In the years that followed, phase 2 clinical intervention trials in recent-onset T1D patients included the B cell-depleting therapy rituximab (13), the CTLA4-Ig fusion protein abatacept (14), alefacept, a fusion protein that binds CD2 on effector memory T cells (15), and a follow-up trial that tested two courses of teplizumab given one year apart (16). Although each of these trials demonstrated short-term preservation of β cell function as estimated by measurements of C-peptide, by two years posttreatment, the slopes of C-peptide decline in the treatment and control groups had paralleled, and none demonstrated long-term benefit (17, 18).

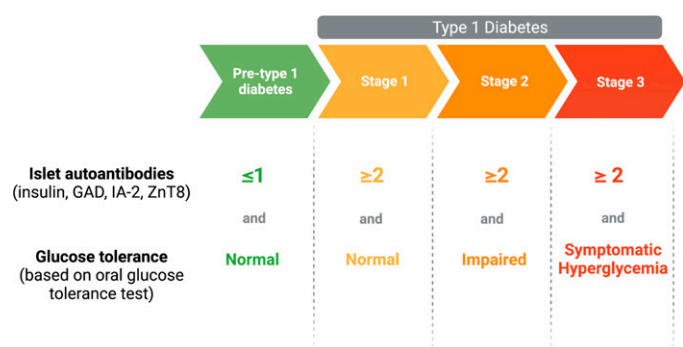


FIGURE 1. Stages of T1D.

The power of islet autoantibodies to predict T1D led to the reclassification of T1D diagnosis in 2015. Following the publication of the scientific statement by the JDRF, the Endocrine Society, and the American Diabetes Association (8), T1D was diagnosed in stages. Stage 1 is diagnosed in individuals with two or more islet autoantibodies and normal glucose tolerance. Stage 2 is diagnosed in individuals with two or more islet autoantibodies and impaired glucose tolerance based on an oral glucose tolerance test. Stage 3 is diagnosed in individuals with clinical symptoms of hyperglycemia (stage 3 replaces what had previously been described as “new-onset” T1D). The figure was created with BioRender.com.

More is better?

Out of this dearth of durable β cell preservation using single, immune-modulating interventions rose the proposition that combination therapy, using synergistic agents targeting different aspects of the immune response, was a potential alternative to safely improve efficacy (19). Recommendations published in 2010 by the ITN in collaboration with the JDRF, cited the “abundantly clear” need to target multiple biological pathways to induce lasting remission, given the complex immunological defects that drive T1D (19). Included in these recommendations was a consensus priority ranking of combination immune therapies that was based, in large part, on the safety and efficacy profiles of immunomodulatory agents as monotherapies. It is not surprising, then, that combinations of anti-CD3 with both Ag-specific therapies (oral insulin, glutamic acid decarboxylase-alum) or anti-inflammatory agents (IL-1R antagonist, IL-1 inhibitors) topped this list (19). In 2015, Haller and colleagues (20) published the results of a small clinical trial that demonstrated preservation of C-peptide in individuals with established T1D (disease duration greater than 4 mo and less than 2 y) using a combination of antithymocyte globulin (ATG) and pegylated G-CSF, two agents that prevented T1D in NOD mice when used together but failed to show efficacy when used alone. Interestingly, when ATG and G-CSF were tested in individuals at disease onset (<100-d disease duration), the addition of G-CSF failed to enhance preservation of C-peptide compared with ATG alone (21). In both studies, as in previous intervention trials, the effects were not long standing. They did, however, challenge the notion that only successful monotherapies should be tested in combination.

In addition to combination therapy with immune agents, there is growing support for combinations of agents that target the immune response and the β cell itself. In a recently published phase 2 clinical trial, an anti-IL-21 mAb was used to target CD8⁺ T cell trafficking to the islet, and a GLP-1 agonist (liraglutide) was added to prevent β cell apoptosis (22). Although efficacy was similar to that of the ATG/G-CSF and anti-CD3 trials, it remains to be seen whether this combination provides a safer alternative to combinations of immune agents.

The prevention–intervention continuum

Teplizumab’s modest success in preserving β cell function in new-onset T1D patients (11) prompted the inevitable question: could β cell function be preserved for a longer period of time if treatment was initiated earlier? Post hoc analysis of the Protégé study, a phase 3 trial that assessed the safety and efficacy of teplizumab given in two doses (at onset and again 12 mo later) found that individuals who responded to anti-CD3 therapy were generally younger and had treatment started earlier in diagnosis, supporting the concept that initiating anti-CD3 therapy earlier in disease course may improve response (23). In 2019, over 30 y after the recognition of T1D as an immune-mediated disease, evidence that immune modulation could significantly alter T1D progression was published (24). In a phase 2, randomized, placebo-controlled, double-blind trial, Herold

et al. (24) demonstrated that the anti-CD3 Ab teplizumab could delay T1D onset in high-risk individuals. All participants had at least two islet autoantibodies and dysglycemia at study entry, and received either a 14-d infusion of teplizumab or placebo. The median time to the diagnosis was 48.4 mo in the teplizumab group and 24.4 mo in the placebo group, which resulted in a median 2-y delay in diabetes diagnosis in the teplizumab group. Individuals who received teplizumab had higher frequencies KLRG1⁺TIGIT⁺CD8⁺ T cells, which were shown to be associated with an unresponsive or “exhausted,” phenotype (24, 25).

Extended follow-up of this trial examined the metabolic and immune implications of teplizumab treatment and found that, in addition to modulating pathogenic T cell signatures, treatment with teplizumab improved β cell function as measured by C-peptide responses and insulin secretion rates (26). Improvements in β cell function were accompanied by increased frequencies of TIGIT⁺KLRG1⁺ memory CD8⁺ T cells and decreased secretion of inflammatory cytokines, supporting CD8⁺ T cell functional exhaustion as a mechanism of action. Further, the benefit of a single infusion persisted as follow-up continued. Median time to T1D diagnosis in the teplizumab-treated group was 5 y, compared with 2 y in the placebo group. Of the 44 participants randomized to the teplizumab group, eight remained disease free (18%) over 5 y after treatment compared with only two of the initial 32 participants randomized to the placebo group (6%) (26).

The notion that an intervention with modest success and positive safety data, when used at disease onset, could achieve clinically meaningful endpoints when used at an earlier stage informs the current paradigm of immune intervention trials in T1D. As discussed in their review of the ITN’s experience, Ehlers and Nepom (10) posit that therapies aimed at “preserving beta cell function in recent-onset disease are excellent candidates for broader trials that include high-risk nondiabetic individuals with early signs of islet autoimmunity.” Haller et al. (21) conclude their discussion of low-dose ATG’s modest success and acceptable safety profile in new-onset patients by suggesting that it should be tested for prevention. At first glance, it appears that the results of the teplizumab trial would affirm this. In light of teplizumab’s success in relatives of individuals with T1D, Jacobsen et al. (27) reviewed and compared β cell preservation in recent-onset clinical trials to prioritize therapies that should be tested in future prevention trials, under the premise that success of an agent used in recent-onset T1D “should increase the probability of efficacy in prevention.” Although the notion that immune modulators should show efficacy at later stages before being tested at earlier stages is reasonable, the evolution of the autoimmune response throughout disease progression is not uniform and remains incompletely understood. Therefore, clinging too tightly to this framework may ultimately hinder the progress of immunotherapy in T1D.

Reassessing current approaches to immune intervention trials

The results of the teplizumab trial call to question the prioritizing of immunomodulatory agents that show efficacy at disease onset for prevention trials (10, 27). In follow-up analysis of responders compared with nonresponders, teplizumab was most effective in individuals whose responses to the oral glucose tolerance test at baseline were below the median, suggesting that teplizumab works best in individuals with more advanced disease and that efficacy is not improved with earlier intervention in individuals with stage 2 T1D (26). Teplizumab selectively targets CD8⁺ effector T cells; therefore, it may not be efficacious at an earlier stage when CD8⁺ T cells are playing a lesser role in disease pathogenesis (Fig. 2A, 2B). Asserting that immune agents must demonstrate efficacy in later stages of disease before being tested at earlier stages implies that the factors driving the auto-immune response are uniform throughout a continuum of disease progression when, in fact, they are not. Transcriptional profiling in an elegant set of NOD mouse models and controls demonstrated that the earliest detectable difference between mice who will develop diabetes and controls who will not is a type I IFN-dependent gene expression signature that appears prior to the appearance of autoantibodies and prior to T and B cell infiltration of the islet (28). A type I IFN-inducible transcriptional signature is also detectable in the blood of children genetically at risk for T1D, before the development of islet autoantibodies, but is not detected in children with established disease, indicating that type I IFN signaling pathways are more responsible for driving presymptomatic stages of disease (29, 30). Type I IFN signals through its receptor to activate JAKs and phosphorylate STAT proteins, and JAK-STAT and IFN signaling pathways have been implicated in numerous autoimmune diseases (31, 32). Small-molecule inhibitors of JAK proteins have been used in clinical trials to treat rheumatoid arthritis and alopecia areata, and recent studies in NOD mice revealed that JAK1/JAK2 inhibitor AZD1480 was able to prevent and reverse T1D by inhibiting MHC class I upregulation on β cells, thereby reducing interaction between β cells and immune cells and reducing lymphocytic infiltration of the islets (31). Currently, repurposing of JAK inhibitors for use in new-onset human T1D intervention is being tested in clinical trials worldwide (31, 33). It is quite possible that the efficacy of these interventions will not be superior and may even be inferior to other immunomodulatory agents tested at diabetes onset for the same reasons teplizumab was found to be more efficacious in individuals with more active disease: the intervention tested is most likely to be efficacious when its target is most active. Therefore, it is possible that the contribution of type I IFN signaling is greatest in stage 1 T1D and that altering type I IFN signaling will have the most benefit when therapy is initiated at stage 1 (Fig. 2C). If this is the case, requiring efficacy at stage 3 before moving forward with testing at earlier stages could ultimately hinder our progress toward definitive prevention.

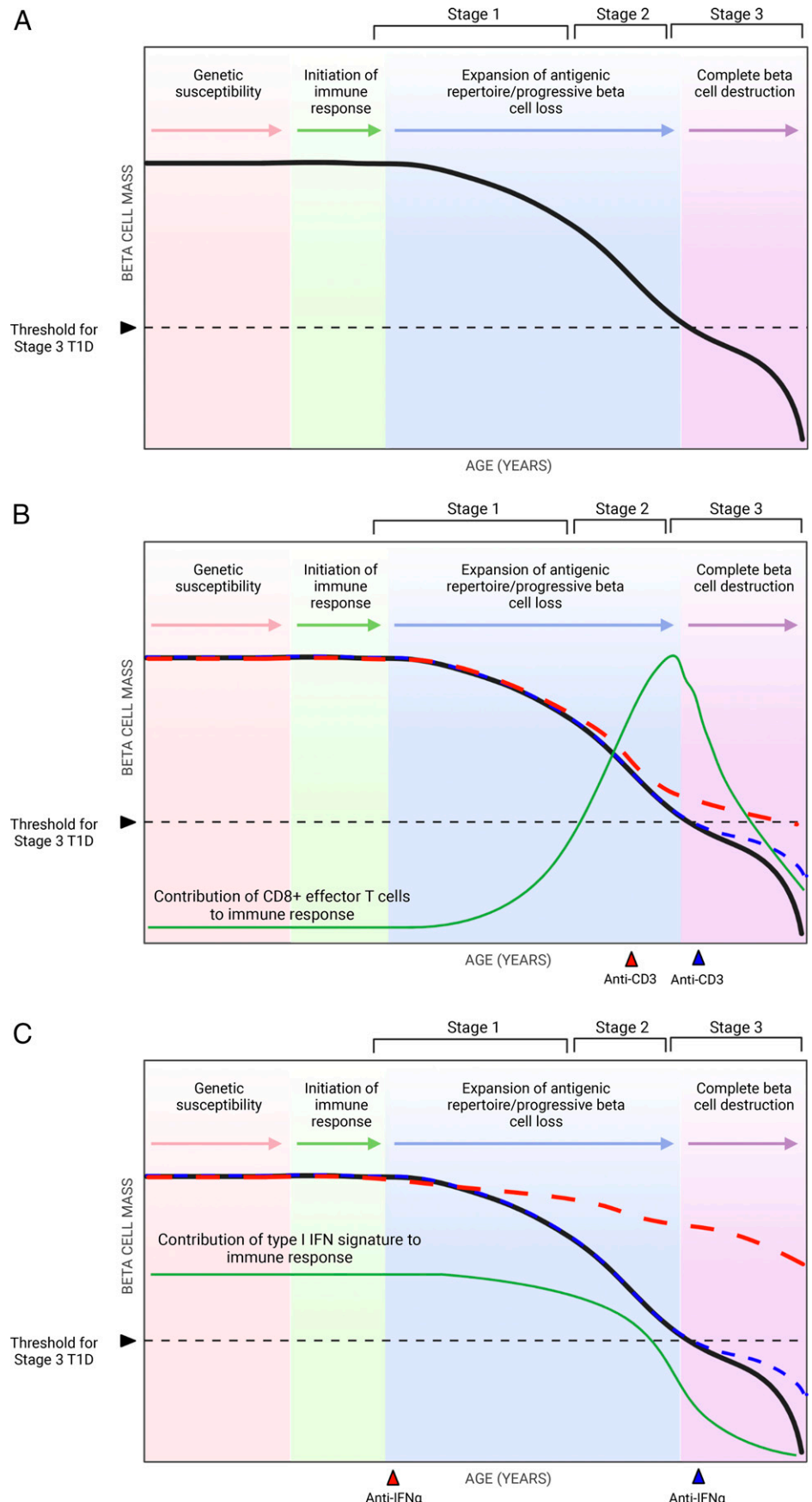
Studies of the B and T cell compartments in peripheral blood of individuals at risk for T1D further support the

existence of distinct, stage-dependent functional and immune phenotypes. Dynamic responses in B cells, specifically, were shown to characterize stages of T1D progression among autoantibody-positive individuals followed in TrialNet (34). BCR responsiveness was found to be increased early in autoantibody-positive individuals and decreased as individuals progressed to T1D. Further subgroup analysis of individuals treated with the B cell-depleting therapy rituximab revealed that rituximab responders had significantly increased BCR signaling, suggesting that rituximab may be most effective when BCR hyperresponsiveness is a driver of disease (34). Given the role that B cells play in expanding pathogenic effector T cell subsets as APCs, early targeting of B cells has the potential to minimize T cell-mediated attack as well.

The strongly supported notion that combination therapy (multiple immune agents or an immune agent and a β cell preservation agent) will be required for definitive treatment (19, 35) may also be intimately linked to the timing of immune interventions. Preclinical studies in NOD mice have shown repeatedly that prevention of T1D is more easily achieved when intervention is initiated earlier (36, 37). As the autoimmune response progresses, it inevitably becomes more complicated; clonal expansion of autoreactive T and B cell clones, increased inflammatory responses, epitope spread, and neoepitope formation all contribute to the amplification of the antigenic repertoire (38, 39). In light of this progression, it makes sense that monotherapy at disease onset is unlikely to be sufficient; however, less may be required if therapy is initiated earlier. The lack of clinical trials in earlier stages of T1D reflects a paradox that was acknowledged in a commentary by Schatz et al. (40) in 2003 and persists today: recruiting individuals at highest risk of progression facilitates feasible trial design and power calculations but also increases the likelihood that these trials will fail because the markers used to assess risk denote metabolic dysfunction, which only occurs after the autoimmune response has been fully mobilized and β cell destruction is well underway. In short, by the time glucose tolerance is impaired, both the immune response and β function must be addressed. A single intervention will be too little too late. The holy grail, then, is a single agent that can simultaneously alter the immune response and alleviate β cell stress early in the disease course. Interestingly, targeting the type I IFN signaling pathways may permit this. IFN gene signatures have been detected not only in peripheral blood but also in islets of individuals with T1D. Mechanistic studies suggest that, in addition to MHC class I hyperexpression, dysregulated IFN signaling in islets also leads to β cell apoptosis and generation of β cell autoantigens (41–44). As such, monotherapy targeting type I IFN signaling has the potential to simultaneously alter the autoimmune response and bolster β cell health and function if initiated at the right time. As clinical trials testing JAK inhibitors and other agents targeting type I IFN signaling pathways progress, it will be imperative to remember that failing to meet primary or secondary outcome measures at disease onset may not reflect an inappropriate agent but rather inappropriate timing.

FIGURE 2. Timing and efficacy of immune interventions during T1D disease progression.

T1D disease progression is depicted by the solid black lines in each graph above. (A) Depiction of the original Eisenbarth model of T1D development (1). The *x*-axis represents time in years; the *y*-axis represents β cell mass. Over time, in genetically susceptible individuals, an unknown precipitating event leads to the development of overt immunological abnormalities, resulting in the progressive loss of β cell mass. Presymptomatic stages of T1D development, as defined in 2015 (8), are indicated in the brackets at the top. (B) Graphical representation of how treatment with anti-CD3 Ab alters the decline in β cell mass when initiated at stage 3 (blue dashed lines) or stage 2 (red dashed lines). Contribution of CD8⁺ T cells to immune response is represented by the green overlay to demonstrate that preservation of β cell mass is maximized when anti-CD3 therapy is initiated when contributions of CD8⁺ T cells to immune response are highest. (C) Graphical representation of how treatment with anti-IFN- α may alter the decline in β cell mass when initiated at stage 3 (blue dashed lines) or stage 1 (red dashed lines). Contribution of type I IFN signaling to immune response is represented by the green overlay to demonstrate that preservation of β cell mass is maximized when anti-IFN- α therapy is initiated early in disease course, when its contribution to immune response is highest. The figure was created with BioRender.com.



Challenges to early intervention

Hurdles to testing immune therapies earlier in disease course have been difficult to clear. Safety remains paramount and rightly so, as T1D can be managed with intensive insulin regimens; although, even then, glycemic control is rarely optimal (45). Given the adverse effects of systemic immune suppression, chronic use of agents such as teplizumab is unlikely to be suitable for presymptomatic individuals, particularly in light of the variable rates of progression to stage 3 T1D, which spans months to decades in some cases (46). As such, enthusiasm is rising for more targeted and tissue-specific therapies that modulate rather than suppress the immune system (47, 48). The variable rates of progression to T1D in presymptomatic individuals also highlight the importance of biomarker discovery and identification of intermediate clinical trial endpoints. Metabolic measures, such as stimulated C-peptide, are less likely to be meaningful early in disease course, when the immune response is evolving but β cell destruction has not yet occurred. Therefore, development of immune biomarkers of early breaches in tolerance and alternative, mechanistic endpoints that can be identified in the blood are urgent and pressing needs in the T1D research space. Transcriptional profiling, T and B cell repertoire analysis, microRNAs, metabolomics, and lipidomics, are among current investigations of emerging biomarkers in T1D (49).

At the same time, identification of individuals to enroll in clinical trials is difficult. Currently, screening and natural history studies in the United States require a family history of T1D, excluding over 85% of new T1D diagnoses (50). Although the costs versus benefits of universal screening for T1D continue to be debated (51–54), several groups have implemented universal screening studies for T1D. Recent publication of the Fr1da group study results demonstrated the feasibility of primary care–based screening for islet autoantibodies after screening over 90,000 children aged 2 to 5 y in Bavaria, Germany (55). The primary outcome for this study was presymptomatic T1D as defined by the presence of two or more islet autoantibodies. The prevalence of presymptomatic T1D in this cohort was 0.31%, a total of 280 children. Of these, 62 children developed stage 3 T1D, and importantly, only two (3.2%) presented with diabetic ketoacidosis (DKA), compared to 40% of children in the United States (56) and 20% of children in Germany (57) who present with DKA at diagnosis. DKA has been associated with increased medical costs, poor long-term glycemic control, and adverse neurocognitive outcomes (58–60); therefore, in addition to identification of individuals for prevention trials, universal screening has the potential to decrease the morbidity and mortality associated with DKA at diagnosis, a factor which should also be considered in cost/benefit analysis.

In the United States, the Autoimmunity Screening for Kids study has screened over 10,000 children in the general population for presymptomatic T1D and celiac disease, the two most common autoimmune diseases in children (61). Follow-up analysis of this large-scale screening program is now assessing its feasibility and establishing projections for benchmarks needed

to improve cost-effectiveness (62). Although the cost of screening solely for prevention of DKA is unlikely to provide sufficient benefit, the availability of therapies that delay T1D onset, such as teplizumab, has increased the publicity and push for universal screening. In December 2020, the JDRF launched T1Detect, the first screening program that enables the general population to evaluate T1D risk through using a home Ab testing kit (<https://www.jdrf.org/t1d-resources/t1detect/>). At the same time, advances in our understanding of the genetic contributions to T1D risk have led to the development of several genetic risk scores that incorporate HLA and non-HLA risk loci to predict T1D progression in at-risk individuals (63). It is plausible that, as autoantibody screening in the general population is optimized, the use of genetic risk scores to stratify risk among autoantibody-positive subjects will facilitate identification of candidates for prevention trials.

CONCLUSIONS

In conclusion, the results of the teplizumab trial in relatives at risk for T1D have reinvigorated diabetes researchers, individuals living with T1D, and their families alike. Extended follow-up analysis revealed that teplizumab worked best in individuals with the most advanced stage 2 T1D, making it less likely to be effective at stage 1 and challenging the notion that agents with the most efficacy at later stages of T1D should be prioritized for testing in earlier stages. I would speculate, instead, that the agents used for prevention at each stage in T1D will target unique aspects of the immune response and that identification of those targets must be informed by an understanding of mechanism at each disease stage. Is earlier better? Perhaps. Is earlier different? Absolutely.

DISCLOSURES

The author has no financial conflicts of interest.

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