Prevention and Reversal of Type 1 Diabetes—Past Challenges and Future Opportunities

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Over the past three decades there have been a number of clinical trials directed at interdicting the type 1 diabetes (T1D) disease process in an attempt to prevent the development of the disease in those at increased risk or to stabilize—potentially even reverse—the disease in people with T1D, usually of recent onset. Unfortunately, to date there has been no prevention trial that has resulted in delay or prevention of T1D. And, trials in people with T1D have had mixed results with some showing promise with at least transient improvement in β-cell function compared with randomized control groups, while others have failed to slow the decline in β-cell function when compared with placebo. This Perspective will assess the past and present challenges in this effort and provide an outline for potential future opportunities.

THE BEGINNINGS
The first randomized, double-masked, controlled trials with sufficient statistical power to give confidence for the outcome were conducted in the mid-1980s with cyclosporine (1,2). Two large studies were conducted—the French cyclosporine study that included 122 patients with type 1 diabetes (T1D) aged 15–40 years who had been symptomatic for 6 months or less and were on insulin therapy for 2 months or less (1) and the Canadian-European cyclosporine study that included 188 subjects aged 9–35 years who had been symptomatic for 14 weeks or less and were on insulin therapy for 6 weeks or less (2). Both trials used as their primary outcome the achievement of remission defined two ways. First, “complete remission” was defined as good metabolic control (fasting glucose <140 mg/dL [7.8 mmol/L], postprandial glucose <200 mg/dL [11.1 mmol/L], HbA1c ≤7.5%) in the absence of insulin treatment. Second, “partial remission” was defined as good metabolic control with insulin dose <0.26 units/kg per day. Both studies found a greater proportion of cyclosporine patients than placebo patients achieving and maintaining remissions. In both studies, cyclosporine could be given for 1 year, with stopping rules in place that led to blinded substitution of placebo for cyclosporine if remission was lost. Although both studies found that cyclosporine had superior efficacy than placebo, the magnitude and duration of benefit did not appear sufficient to justify cyclosporine treatment in clinical practice, given the potential of cyclosporine-induced nephrotoxicity. The importance of the studies, however, was that they demonstrated the impact of immune intervention on the evolution of T1D, in a sense fulfilling Koch postulates, which were developed for infectious diseases, but the response to immune therapy can be considered as indicative that T1D is immune mediated.

CHALLENGES COMPLICATING PREVIOUS STUDIES
Since the completion of the early trials, particularly during the past decade, a number of additional randomized, double-masked, adequately powered, controlled
clinical trials have been conducted using many different immunological strategies. For the most part, these have been disappointing, with none showing unambiguous benefit in preserving β-cell function. Some studies have shown transient benefit with anti-CD3 monoclonal antibodies targeting T cells (Fig. 1A and B) (3–6), an anti-CD20 monoclonal antibody (i.e., rituximab) targeting B cells (Fig. 1C) (7,8), and with a costimulation blocking agent (i.e., abatacept) that prevents immune activation (Fig. 1D) (9,10). One pilot study combining antithymocyte globulin (ATG) and granulocyte colony-stimulating factor (GCSF) suggested benefit (Fig. 1E) but awaits confirmatory results in a larger trial (11).

A number of other strategies have been without benefit in preserving β-cell function (12–17). In addition, some studies had ambiguous effects, not meeting the primary outcome measure (i.e., preservation of β-cell function) but showing potential benefit either on secondary outcome measures or mechanistic measures or in only a subgroup of subjects (18–20).

Thus, most immune intervention trials in T1D have either failed to achieve success in preserving β-cell function or have met that hurdle but have nonetheless shown only a transient effect. This has resulted in a flurry of editorial and commentary articles in peer-reviewed journals that take a bleak look at the field. Rather than looking negatively on the results to date, an opportunity exists to examine the details of previous studies to identify what can be learned and what can be applied to future studies. To that end, several problems are notable, and these are detailed below.

MISLEADING PILOT STUDIES

The Diabetes Prevention Trial–Type 1 Parenteral Insulin Trial was a fully powered, randomized, controlled clinical trial that enrolled 339 relatives of patients with T1D who were estimated to have at least a 50% risk of developing T1D in the next 5 years (21). To enroll these

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**Figure 1**—There has been a progressive decline in β-cell function, as measured by C-peptide, even in most studies that have been “successful,” thus showing only a transient benefit, as depicted for the anti-CD3 monoclonal antibody teplizumab (A) (3,4), the anti-CD3 monoclonal antibody otelixizumab (B) (5,6), the anti-CD20 monoclonal antibody rituximab (C) (7,8), and the costimulation blocker abatacept (D) (9,10). E: Also shown is the preservation of β-cell function in a pilot study (only 25 subjects randomized) with the combination of low-dose ATG plus GCSF (11), in which there is preservation of β-cell function at 1 year, something needing confirmation in a larger study.
subjects, more than 93,000 relatives were screened. The trial showed no difference in the rate of development of T1D (Fig. 2A) (21). This was a surprising outcome, as two pilot studies had suggested that the intervention—low-dose insulin—could delay the disease. One of those apparently promising pilot studies was a nonrandomized study of 12 individuals offered the intervention, 7 of whom declined and were analyzed as a comparison group, along with a group of historical control subjects of undefined number (Fig. 2B) (22). Life-table analysis suggested a statistically significant ($P = 0.002$) and dramatic difference in rate of development of T1D. Interestingly, the treated group was younger (mean age 10 years) than the untreated group (mean age 20.7 years), and one would have expected younger individuals to progress faster, which did not happen. In the other pilot trial, 14 subjects were randomized to insulin treatment or to the control group, with the insulin group having longer diabetes-free survival ($P < 0.03$) (23).

The first study in T1D using GAD in a vaccine formulation with alum (GAD-alum) randomized 70 subjects, aged 10–18 years, within 18 months of diagnosis, to receive either two doses of GAD-alum or two doses of alum alone (24). The primary outcome measure, fasting C-peptide at 15 months, showed no difference between groups. However, mixed-meal tolerance test (MMTT)-stimulated C-peptide was higher in GAD subjects who were treated within 6 months of diagnosis, but this included only 11 subjects treated with GAD and 14 treated with placebo (Fig. 2C). On the basis of this secondary subgroup outcome, three additional studies—all enrolling subjects within 3 months of diagnosis—were undertaken, including two phase 3 trials. A TrialNet study enrolled 145 subjects aged 3–45 years (Fig. 2D), a European phase 3 trial enrolled 334 subjects aged 10–20 years (Fig. 2E), and a U.S. phase 3 trial enrolled...
In all three of these trials, there was no evidence of a treatment effect with GAD-alum. Yet, it should be appreciated that the success of GAD in mice was approached differently. For example, the GAD-alum studies perhaps used the wrong formulation (with adjuvant). The studies could have used GAD-alum by the wrong route (subcutaneous) and at the wrong time (after clinical diagnosis of T1D). Although GAD vaccine still may be able to prevent or delay the development of T1D if used as a vaccine prior to disease onset, the results of these trials have greatly diminished the enthusiasm for GAD that once existed in our field.

Another approach considered was DiaPep277. DiaPep277 is a peptide derived from positions 437–460 of the human heat shock protein 60 (Hsp60), named peptide 277, that is alleged to induce anti-inflammatory T cells. The first report of its use in T1D was a pilot study in which 35 subjects, aged 16–55 years, with recent onset T1D, received either three doses of DiaPep277 or three doses of placebo (26). Primary outcome was glucagon-stimulated C-peptide at 10 months, which was higher in the DiaPep277 group than in the placebo group, an effect that was sustained with follow-up to 18 months (27). Four additional phase 2 studies showed results that were ambiguous at best, with no clear benefit (28–30). Nonetheless, the sponsor mounted two full-scale phase 3 trials. The first enrolled 457 subjects, aged 16–55 years, with recent-onset T1D, who received injections of DiaPep277 or placebo quarterly for 2 years (14,15). The initial report alleged that glucagon-stimulated test (GST) of C-peptide at 24 months was improved in the DiaPep277 group versus the placebo group, although there was no difference in MMTT-stimulated C-peptide.

Another pilot study involved the use of Bacillus Calmette-Guérin (BCG) vaccine in six subjects with long-standing T1D who were randomized to receive either two doses of BCG or placebo (32). The authors claimed that the BCG subjects, and one of the three control subjects who developed acute Epstein-Barr virus (EBV) infection, showed increases in dead insulin-autoreactive T cells and induction of regulatory T cells. They also claimed that there was transient increase in C-peptide levels not seen in a nonrandomized cohort of subjects with T1D in their institution. They have garnered a lot of publicity for this very tiny study. Although the authors reported an increase in C-peptide levels using an ultrasensitive assay, there are no data that the trivial increases in C-peptide have any biological importance. Moreover, two larger previous randomized clinical trials of BCG, involving 26 and 47 subjects, showed no effect of BCG on preservation of β-cell function, but in both trials there was a trend to greater decline of β-cell function in the BCG group than in the control group (33,34).

What can one then conclude about the value of pilot studies in this area? Essentially, the bottom line is that pilot studies must be viewed with great caution and definitive answers can only be obtained by adequately powered randomized controlled trials.

### Failure to Distinguish Transient Symptoms from Serious Adverse Events

A number of interventions used in recent-onset T1D have shown transient symptoms related to cytokine release...
### Table 1—Immune intervention trials in T1D

<table>
<thead>
<tr>
<th>Study name</th>
<th>Intervention</th>
<th>Outcome</th>
<th>Result</th>
<th>Year reported</th>
<th>Reference</th>
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<td>Finnish TRIGR pilot</td>
<td>Casein hydrolysate formula</td>
<td>Autoantibodies</td>
<td>Apparent benefit</td>
<td>2010</td>
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<td>Casein hydrolysate formula</td>
<td>Autoantibodies</td>
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<td>2014</td>
<td>67</td>
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<td>FINDIA</td>
<td>Insulin-free whey-based formula</td>
<td>Autoantibodies</td>
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<td>BABYDIET</td>
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<td>TRIGR</td>
<td>Casein hydrolysate formula</td>
<td>Diagnosis of T1D</td>
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<td>Ongoing</td>
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<td><strong>Secondary prevention studies</strong></td>
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<td>70</td>
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<td>ENDIT</td>
<td>Nicotinamide</td>
<td>Diagnosis of T1D</td>
<td>No difference</td>
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<td>No difference</td>
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<td>21</td>
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<td>Diagnosis of T1D</td>
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<td>2005</td>
<td>20</td>
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<td>Injected insulin</td>
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<td>Nasal insulin</td>
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<td>No difference</td>
<td>2008</td>
<td>52</td>
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<td>DIPP sibling cohort</td>
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<td>INIT II</td>
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<td>Remission</td>
<td>Benefit</td>
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<td>Canadian-European cyclosporine</td>
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<td>C-pep ≥0.6 mmol/L or noninsulin treated</td>
<td>Benefit</td>
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<td>Azathioprine + glucocorticoids</td>
<td>Azathioprine</td>
<td>and prednisone</td>
<td>Peak C-pep/glucose ratio</td>
<td>Benefit</td>
<td>1988</td>
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<td>Azathioprine, children</td>
<td>Azathioprine</td>
<td>Partial remission</td>
<td>No difference</td>
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<td>76</td>
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<td>Linomide French trial</td>
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<td>Glucagon-stimulated C-pep</td>
<td>Benefit</td>
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<td>BCG</td>
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<td>1998</td>
<td>33</td>
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<td>BCG</td>
<td>BCG vaccine</td>
<td>Primary, remission; secondary, MMTT C-pep</td>
<td>No difference</td>
<td>1999</td>
<td>34</td>
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<td>Oral insulin</td>
<td>Glucagon-stimulated C-pep</td>
<td>No difference</td>
<td>2000</td>
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<td>Italian oral insulin</td>
<td>Oral insulin</td>
<td>Fasting C-pep</td>
<td>No difference</td>
<td>2000</td>
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<td>U.S. oral insulin</td>
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<td>Loss of C-pep</td>
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<td>Herold anti-CD3</td>
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<td>MMTT C-pep</td>
<td>Benefit</td>
<td>2002, 2005</td>
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<td>Keymeulen anti-CD3</td>
<td>Otelixizumab</td>
<td>C-pep after clamp</td>
<td>Benefit</td>
<td>2005, 2010</td>
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<td>Protégé</td>
<td>Teplizumab</td>
<td>Insulin &lt;0.5 unit/kg + HbA1c &lt;6.5%</td>
<td>No difference</td>
<td>2011, 2013</td>
<td>35, 36</td>
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<td>Protégé Encore</td>
<td>Teplizumab</td>
<td>Insulin &lt;0.5 unit/kg + HbA1c &lt;6.5%</td>
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<td>2011</td>
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<td>DEFEND-1</td>
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<td>No difference</td>
<td>2014</td>
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<td>DEFEND-2</td>
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<td>MMTT C-pep</td>
<td>No difference</td>
<td>2014</td>
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<td>AbATE (ITN study)</td>
<td>Teplizumab</td>
<td>MMTT C-pep</td>
<td>Benefit</td>
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<td>Benefit</td>
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<td>GAD pilot</td>
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<td>Fasting C-pep</td>
<td>Apparent benefit in secondary outcome in subgroup</td>
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<td>No difference</td>
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<td>MMTT C-pep</td>
<td>No difference</td>
<td>2012</td>
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<td>GAD U.S. (DiaPrevent)</td>
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<td>DiaPep277 peptide</td>
<td>Glucagon-stimulated C-pep</td>
<td>Benefit</td>
<td>2001, 2007</td>
<td>26, 27</td>
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<td>DiaPep277 peptide</td>
<td>MMTT C-pep</td>
<td>No difference</td>
<td>2007</td>
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*Continued on p. 1002*
syndrome, including headache, fever, and hypotension, at the time of infusion of the treatment. These symptoms are not intolerable and are both transient and fully reversible. Thus, they do not constitute major adverse effects that would justify withdrawal of the subject from the study. Cytokine release syndrome has been seen with infusions of anti-CD3 monoclonal antibodies (3,5), rituximab (7), and thymoglobulin (11,18). Yet, although the infusions have been completed within the first days or within the first month after randomization, beneficial effects from these interventions have been seen 12 months to 4 years after randomization (3–7,11). Thus, subjects may have some discomfort, but there needs to be a reality check as to the potential benefit versus the discomfort. A few days of symptoms early on need to be balanced with a sustained beneficial outcome over a protracted time frame.

Some of these trials have used anti-histamine and analgesic prophylaxis to obviate symptoms related to cytokine release. At least one trial also used low doses of glucocorticoids (18) but not in the experimental group, not in
Anti-inflammatory Agents (e.g., Targeting IL-1β or TNF-α)

Immunomodulation (e.g., Anti-CD3 or Anti-CD20 or Costimulation Blockade or ATG)

Drive T-regs (e.g., IL-2 or GCSF or T-reg infusion)

Diabetes-Related Antigen (e.g., Oral Insulin or GAD Vaccine)

Preserve β-Cell Health (e.g., GLP-1 Receptor Agonist)

**Figure 4**—Potential scheme of combination therapy using agents with complementary effects. This scheme includes anti-inflammatory therapy targeting innate immunity, immunomodulatory therapy targeting adaptive immunity, therapy driving regulatory immunity, antigen-based therapy directing regulation to β-cells, and an agent promoting β-cell health. T-reg, regulatory T cell.

The results from these early studies with anti-CD3 monoclonal antibodies—teplizumab and otelixizumab. The first study with teplizumab (given over 14 days) demonstrated a slower decline of β-cell function (by MMTT) at 1 year (3), with sustained improvement on β-cell function at 2 years (4). Meanwhile, the first study with otelixizumab (given over 6 days) showed a slower decline of β-cell function (measured using a hyperglycemic clamp followed by glucagon stimulation) at 18 months (5). After 4 years of follow-up, although β-cell function was not measured, the otelixizumab group had lower insulin requirements despite similar glycemic control as measured by HbA1c (6). Thus, effects of a 6-day treatment course appeared to be evident 4 years later.

The results from these early studies with anti-CD3 led to the initiation of phase 3 clinical trials with both agents. Unfortunately, the phase 3 studies did not meet their primary outcome criteria. For teplizumab, the primary outcome was the combination of HbA1c <6.5% and insulin dose <0.5 units/kg/day (35). This outcome measure was arbitrarily selected without sufficient data to justify its selection. Moreover, by using a composite outcome that requires a subject to meet two criteria, the outcome becomes a dichotomous measure that dilutes the effect of two continuous variables—HbA1c and insulin dose. More important, when the conventional outcome measure of C-peptide was assessed, there was evidence of efficacy both at 1 year (35) and at 2 years (36) following two 14-day courses of teplizumab (at entry and at 26 weeks into the study). This was especially evident in subjects enrolled in the U.S., in younger subjects (age 8–17 years), in subjects enrolled within 6 weeks of diagnosis, and in subjects with higher levels of C-peptide at entry (35). In addition, the phase 3 study also enrolled subjects in South Asia. Although these subjects met the clinical criteria used for enrollment, it is important to note that typical immune-mediated T1D (also called type 1A diabetes) is a disease principally of Europoid Caucasians. Enrollment of Asian subjects may have confounded the results. Another issue was that the control group in the phase 3 study maintained residual C-peptide to a greater extent and longer than expected, which was especially true for adult subjects. This made it more difficult to detect differences between groups.

For otelixizumab, the phase 3 studies used a dose that was one-sixteenth (total of 3.1 mg over 8 days) of that used in the original phase 2 study (total of 48 mg), in an effort to avoid any side effects (37,38). One has to question what the investigators were trying to avoid. The one noninfusion-related side effect seen in the first trial with otelixizumab was transient EBV reactivation (39). Although the authors concluded that such EBV reactivation was of no apparent clinical concern over the long term, others have asserted that this must be avoided at all costs (40). I was Chair of the Data Safety Monitoring Committee for that study, and prior to the study the committee had concluded that transient EBV reactivation was possible and would neither constitute a reason to halt the study nor was a side effect that needed to be avoided. So, in the phase 3 trials with use of the lower dose, side effects were obviated; however, beneficial effects were also completely obviated. This unfortunate dose reduction reminds us that all effective therapies are likely to have some side effects and that if one lowers the dose to eliminate all side effects, the drug may no longer have benefit.

**DOSING ISSUES**

Getting the dose right is important. In addition to the otelixizumab dosing issue, several other examples are worth noting. Interleukin-2 (IL-2) stimulates regulatory T cells at low doses but also stimulates effector T cells at higher doses (41). Thus, it has been noted that the use of IL-2 is a double-edged sword (42). In other conditions (e.g., hepatitis-C virus–induced vasculitis and graft-versus-host disease), low-dose IL-2 has shown beneficial effects (43,44). The first human study of IL-2 in T1D used a relatively high dose and combined its use with rapamycin (45). An adverse effect on β-cell function was observed. A subsequent study using low-dose IL-2 appeared to be relatively safe, without a decrease in β-cell function and with the expected increase in regulatory T cells (46). Therefore, studies are currently under way to study low-dose IL-2 in T1D.

Another example of a dosing issue is thymoglobulin (ATG). In a study using a relatively high dose of ATG, no beneficial effect was seen, and it was observed that there was suppression of both effector and regulatory T cells (18). In another study in which a lower dose of ATG was used, regulatory T cells were not suppressed (11). Interpretation of that study is confounded, however, because low-dose ATG was used in combination with GCSF.
A related issue providing an additional confounder is choosing surrogate agents. A study in NOD mice found that combination therapy with glucagon-like peptide 1 (GLP-1) and gastrin restores normoglycemia (47). This combination resulted in increases in pancreatic insulin content, β-cell mass, β-cell proliferation, and β-cell neogenesis; a reduction in β-cell apoptosis; and a beneficial effect on the immune response (47). It seemed like a natural combination to test in human beings. Yet, rather than testing the combination of GLP-1 and gastrin, a study evaluated the combination of the dipeptidyl peptidase-4 inhibitor sitagliptin and the proton-pump inhibitor lansoprazole, as these agents, respectively, increase circulating levels of GLP-1 and gastrin (48). The primary end point was not achieved, but not all participants had the expected increases in GLP-1 and gastrin levels. Thus, they effectively did not get the doses that may have been needed to achieve the desired effects.

**ANTIGEN-BASED THERAPIES HAVE NOT WORKED YET**

The desirability of antigen-based immunotherapy is grounded on the notion that such therapies are specific for T1D and are unlikely to have adverse off-target effects. Thus, they should have a high degree of safety. The two diabetes-specific antigens that have been used are insulin and GAD, both of which had great success in animal models of T1D (49,50). Yet, to date, there has been no unambiguous success with antigen-based therapies in human T1D. GAD-alum vaccine has failed in new-onset T1D (12,13). Injected insulin has failed in prevention trials (21,51). Nasal insulin has failed in prevention trials (52). Oral insulin has failed in new-onset T1D (53–55) and did not meet its primary outcome in a prevention trial (20). An altered peptide ligand of insulin failed in new-onset T1D (56). A plasmid-encoding proinsulin was claimed to have a benefit on β-cell function, but actually the effect was seen in but one of four doses tested and only at one time point (57). Moreover, the number of subjects studied at each dose was small, and there did not appear to be statistical adjustment for multiple comparisons. The field is eagerly awaiting the conclusion of ongoing GAD-alum (58), oral insulin (59), and nasal insulin (60) prevention trials and of the development of an approach that uses a combination of peptides derived from proinsulin (61). Yet, the desirable concept of an antigen-based therapy sustains the efforts to find an effective one, perhaps as a component of a combination therapeutic approach.

**NOT ALL PEOPLE MAY RESPOND TO THERAPY**

In one study using the anti-CD3 monoclonal antibody teplizumab, a group of “responders” to treatment was identified, who at 2 years maintained C-peptide better than the randomized but untreated comparison group (Fig. 3) (62). In that study, responders constituted 48% of subjects treated with teplizumab. Interestingly, the responders not only did better than the comparison group but also, as a group, actually maintained β-cell function essentially at the level seen at randomization, whereas the nonresponders had lost β-cell function at a rate similar to the comparison group. Had the analysis been confined to the total treated group—including both responders and nonresponders—the full retention of β-cell function in nearly half of the subjects would have been missed. Some type of responder analysis should be applied to all intervention studies. Indeed, it has been suggested that a responder analysis be included in the statistical plan for all T1D intervention studies, and for that purpose, the definition of a responder should be the maintenance of 100% of baseline β-cell function (63).

A fundamental question is why some subjects fail to respond. It could be that the immunotherapy used was ineffective (at least at the dose tested), that the immunological process—perhaps a relapsing and remitting one—was in a latent period at the time of drug administration and thus not responsive to immunotherapy, that β-cell mass or β-cell function had already deteriorated to a point of no return, that the immunological processes damaging β-cells are different among individuals, or for some other reason. It is important to assess potential biomarkers that might discriminate responders from nonresponders and thus might be used as enrollment criteria for future use of a given therapy.

**FUTURE DIRECTIONS**

As discussed above, there have been many attempts at immune intervention in T1D, with mixed results. Table 1 provides a listing of all of the major studies to date, including randomized controlled trials and other studies mentioned in this Perspective; not included are small pilot studies not discussed in this article.

It should be appreciated that in the evolution of T1D several immune pathways are involved. This complicates the design of an ideal therapeutic strategy to control the immune system and prevent the loss of β-cell function and β-cell mass. Indeed, if one pathway is controlled, another pathway may become more active. Thus, success may require that a combination approach be used. Such a combination might include (Fig. 4) one or more anti-inflammatory agents targeting innate immunity, such as agents that target IL-1 (IL-1β) or tumor necrosis factor (TNF-α); one or more immunomodulatory agents targeting adaptive immunity, such as anti-CD3, anti-CD20, or costimulation blockade (three agents that already have shown some beneficial effect on preserving β-cell function) or ATG; perhaps some agent that would drive regulatory T cells, such as low-dose IL-2 or GCSF or the infusion of regulatory T cells themselves; any of these perhaps coupled with a diabetes-related antigen that might help target the regulatory T cells to β-cells; and agents that help preserve β-cell health, such as GLP-1 (64). In such a strategy, the combination of agents is selected for having potential complementary effects. It may also be necessary to tailor the selection of agents for different individuals, i.e., we may need to move to a personalized medicine approach with different treatments for different subtypes, if these become better defined (65).

Therefore as outlined, there are many potential interventions that hold promise, particularly if they are used as components of combination therapy. Several new strategies are approaching clinical evaluation. To be successful, we must be patient, yet proceed with diligence. Moreover, it is important that trials be carefully designed, well controlled, and
have adequate sample size to assure valid interpretation.

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