JCI The Journal of Clinical Investigation

Alefacept provides sustained clinical and immunological effects in new-onset type 1 diabetes patients

Mark R. Rigby, … , Mario R. Ehlers, the Immune Tolerance Network (ITN) T1DAL Study Group

J Clin Invest. 2015;125(8):3285-3296. https://doi.org/10.1172/JCI81722.

Clinical Medicine | Clinical trials

BACKGROUND. Type 1 diabetes (T1D) results from destruction of pancreatic β cells by autoreactive effector T cells. We hypothesized that the immunomodulatory drug alefacept would result in targeted quantitative and qualitative changes in effector T cells and prolonged preservation of endogenous insulin secretion by the remaining β cells in patients with newly diagnosed T1D.

METHODS. In a multicenter, randomized, double-blind, placebo-controlled trial, we compared alefacept (two 12-week courses of 15 mg/wk i.m., separated by a 12-week pause) with placebo in patients with recent onset of T1D. Endpoints were assessed at 24 months and included meal-stimulated C-peptide AUC, insulin use, hypoglycemic events, and immunologic responses.

RESULTS. A total of 49 patients were enrolled. At 24 months, or 15 months after the last dose of alefacept, both the 4-hour and the 2-hour C-peptide AUCs were significantly greater in the treatment group than in the control group ($P = 0.002$ and 0.015, respectively). Exogenous insulin requirements were lower ($P = 0.002$) and rates of major hypoglycemic events were about 50% reduced ($P < 0.001$) in the alefacept group compared with placebo at 24 months. There was no apparent between-group difference in glycemic control […]

Alefacept provides sustained clinical and immunological effects in new-onset type 1 diabetes patients

Mark R. Rigby,[1] Kristina M. Harris,[2] Ashley Pinckney,[3] Linda A. DiMeglio,[1] Marc S. Rendell,[4] Eric I. Felner,[5] Jean M. Dostou,[6] Stephen E. Gitelman,[7] Kurt J. Griffin,[8] Eva Tsalikian,[9] Peter A. Gottlieb,[10] Carla J. Greenbaum,[11] Nicole A. Sherry,[12] Wayne V. Moore,[13] Roshanak Monzavi,[14] Steven M. Willi,[15] Philip Raskin,[16] Lynette Keyes-Elstein,[3] S. Alice Long,[11] Sai Kanaparthi,[2] Noha Lim,[2] Deborah Phippard,[2] Carol L. Soppe,[17] Margret L. Fitzgibbon,[18] James McNamara,[18] Gerald T. Nepom,[11] Mario R. Ehlers,[17] and the Immune Tolerance Network (ITN) T1DAL Study Group[19]

[1]Section of Pediatric Endocrinology and Diabetology, Department of Pediatrics, Riley Hospital for Children, Indiana University School of Medicine, Indianapolis, Indiana, USA. [2]Biomarker Discovery Research, ITN, Bethesda, Maryland, USA. [3]Biostatistics, Rho Inc., Federal Systems Division, Chapel Hill, North Carolina, USA. [4]Creighton Diabetes Center, Omaha, Nebraska, USA. [5]Department of Pediatrics, Division of Pediatric Endocrinology, Emory University School of Medicine, Atlanta, Georgia, USA. [6]Division of Endocrinology, Department of Medicine, University of North Carolina School of Medicine at Chapel Hill, Durham, North Carolina, USA. [7]Department of Pediatrics, Division of Endocrinology, UCSF, San Francisco, California, USA. [8]Department of Pediatric Endocrinology, University of Arizona, Tucson, Arizona, USA. [9]Department of Pediatrics, Division of Pediatric Endocrinology and Diabetes, University of Iowa, Iowa City, Iowa, USA. [10]Barbara Davis Center for Childhood Diabetes, University of Colorado School of Medicine, Aurora, Colorado, USA. [11]Benaroya Research Institute at Virginia Mason, Seattle, Washington, USA. [12]Department of Pediatrics, Massachusetts General Hospital, Boston, Massachusetts, USA. [13]Pediatric Endocrinology, Children's Mercy Hospital, University of Missouri, Kansas City, Missouri, USA. [14]Center for Endocrinology, Diabetes, and Metabolism, Children's Hospital Los Angeles, Department of Pediatrics, USC Keck School of Medicine, Los Angeles, California, USA. [15]Department of Endocrinology, Children's Hospital of Philadelphia, Philadelphia, Pennsylvania, USA. [16]Department of Internal Medicine, Division of Endocrinology, The University of Texas Southwestern Medical Center, Dallas, Texas, USA. [17]Clinical Trials Group, ITN, San Francisco, California, USA. [18]Division of Allergy, Immunology, and Transplantation, National Institutes of Allergy and Infectious Diseases, Rockville, Maryland, USA. [19]The ITN T1DAL Study Group Members and their affiliations are detailed in the supplemental material.

BACKGROUND. Type 1 diabetes (T1D) results from destruction of pancreatic β cells by autoreactive effector T cells. We hypothesized that the immunomodulatory drug alefacept would result in targeted quantitative and qualitative changes in effector T cells and prolonged preservation of endogenous insulin secretion by the remaining β cells in patients with newly diagnosed T1D.

METHODS. In a multicenter, randomized, double-blind, placebo-controlled trial, we compared alefacept (two 12-week courses of 15 mg/wk i.m., separated by a 12-week pause) with placebo in patients with recent onset of T1D. Endpoints were assessed at 24 months and included meal-stimulated C-peptide AUC, insulin use, hypoglycemic events, and immunologic responses.

RESULTS. A total of 49 patients were enrolled. At 24 months, or 15 months after the last dose of alefacept, both the 4-hour and the 2-hour C-peptide AUCs were significantly greater in the treatment group than in the control group ($P = 0.002$ and 0.015, respectively). Exogenous insulin requirements were lower ($P = 0.002$) and rates of major hypoglycemic events were about 50% reduced ($P < 0.001$) in the alefacept group compared with placebo at 24 months. There was no apparent between-group difference in glycemic control or adverse events. Alefacept treatment depleted $CD4^+$ and $CD8^+$ central memory T cells (Tcm) and effector memory T cells (Tem) ($P < 0.01$), preserved Tregs, increased the ratios of Treg to Tem and Tcm ($P < 0.01$), and increased the percentage of PD-1^+CD4^+ Tem and Tcm ($P < 0.01$).

CONCLUSIONS. In patients with newly diagnosed T1D, two 12-week courses of alefacept preserved C-peptide secretion, reduced insulin use and hypoglycemic events, and induced favorable immunologic profiles at 24 months, well over 1 year after cessation of therapy.

TRIAL REGISTRATION. https://clinicaltrials.gov/ NCT00965458.

FUNDING. NIH and Astellas.

Note regarding evaluation of this manuscript: Manuscripts authored by scientists associated with Duke University, The University of North Carolina at Chapel Hill, Duke-NUS, and the Sanford-Burnham Medical Research Institute are handled not by members of the editorial board but rather by the science editors, who consult with selected external editors and reviewers.

Role of funding source: NIAID had a role in the design and conduct of the study; collection, management, analysis, and interpretation of the data; preparation, review, or approval of the manuscript; and decision to submit the manuscript for publication.

Conflict of interest: Linda A. DiMeglio has received personal fees from Sanofi and grants from Novo Nordisk. Peter A. Gottlieb has received grants from Pfizer, Novartis, and Omni BioPharmaceuticals Inc. and has a patent for the treatment of type 1 diabetes with α1-antitrypsin. Carla J. Greenbaum has received grants from Novo Nordisk and Novartis. Nicole A. Sherry has received grants and personal fees from MacroGenics Inc. and Novo Nordisk. Philip Raskin has received personal fees from Janssen Pharmaceuticals Inc., Boston Therapeutics, and GlaxoSmithKline, as well as research support from Amylin Pharmaceuticals, Andromeda Biotech Ltd., AstraZeneca Pharmaceuticals LP, Boehringer Ingelheim, Intarcia Therapeutics Inc., Lilly, Merck, Novo Nordisk, and Pfizer Inc. Gerald T. Nepom has received honoraria from Genentech, Pfizer Inc., and GlaxoSmithKline.

Submitted: March 3, 2015; **Accepted:** June 9, 2015.
Reference information: *J Clin Invest.* 2015;125(8):3285–3296. doi:10.1172/JCI81722.

Table 1. AEs in alefacept- and placebo-treated subjects

	Total participants (*n* = 49)	Events	Alefacept participants (*n* = 33)	Events	Placebo participants (*n* = 16)	Events
Serious AEs	1 (2%)	1	1 (3%)	1	0	0
Serious AEs related to study drug	0	0	0	0	0	0
AEs	49	1783	33	1,076	16	707
AE related to study drug	44 (90%)	563 (32%)	29 (88%)	365 (34%)	15 (94%)	198 (28%)
AEs by severity						
Grade 1	48 (98%)	515 (29%)	32 (97%)	360 (34%)	16 (100%)	155 (22%)
Grade 2	47 (96%)	1,165 (65%)	31 (94%)	659 (61%)	16 (100%)	506 (72%)
Grade 3	29 (59%)	97 (5%)	20 (61%)	53 (5%)	9 (56%)	44 (6%)
Grade 4	5 (10%)	5 (<1%)	3 (9%)	3 (<1%)	2 (13%)	2 (<1%)
Grade 5	0	0	0	0	0	0
Injection reactions	10 (20%)	26 (2%)	6 (18%)	18 (2%)	4 (25%)	8 (1%)
Hypersensitivity reactions	1 (2%)	1 (<1%)	1 (3%)	1 (<1%)	0	0
Lymphopenia	3 (6%)	8 (<1%)	3 (9%)	8 (<1%)	0	0
Infection with EBV, cytomegalovirus, or tuberculosis	2 (4%)	3 (<1%)	1 (3%)	1 (<1%)	1 (6%)	2 (<1%)
Infection	42 (86%)	179 (10%)	28 (85%)	127 (12%)	14 (88%)	52 (7%)
Asymptomatic hepatic injury	19 (39%)	37 (2%)	12 (36%)	28 (3%)	7 (44%)	9 (1%)
Major hypoglycemic event	45 (92%)	1,134 (64%)	30 (91%)	609 (57%)	15 (94%)	525 (74%)
Pregnancy	1 (2%)	1 (<1%)	1 (3%)	1 (<1%)	0	0

Introduction

Type 1 diabetes (T1D), one of the most prevalent chronic diseases of childhood that also presents in adults (1, 2), results from destruction of insulin-producing β cells by self-reactive T cells that have escaped central and peripheral tolerance (3). Insulin therapy is lifesaving but is required daily, heightens risks for major hypoglycemia, and lessens but does not avert other serious complications, including death (4). There is a need for safe interventions to preserve β cell function, reduce hypoglycemia, and improve short- and long-term outcomes (5).

In recent decades, some clinical trials in new-onset T1D have demonstrated modest or transient preservation of β cell function using generalized or targeted immunomodulation (6–11), but most immunotherapies, as well as dietary intervention, have had no effect (12–17). The greatest clinical efficacy was achieved with a regimen of autologous nonmyeloablative hematopoietic stem cell transplantation. However, it was at the cost of significant short- and long-term morbidity (18, 19).

Alefacept — a fusion protein consisting of two LFA-3 molecules bound to the Fc portion of IgG1 (20) — binds CD2, which is expressed most prominently on $CD4^+$ and $CD8^+$ effector memory T cells (Tem cells) (21), the cells thought to be primarily responsible for β cell destruction in T1D (3). Alefacept interrupts CD2-mediated T cell costimulation and depletes T cells via an NK cell–dependent mechanism (22, 23). Alefacept is effective in plaque psoriasis, which like T1D is considered to be a T cell–mediated autoimmune disease and, in some cases, induces long-term remission off therapy (24, 25). We recently reported the 12-month results of the T1DAL trial (Inducing Remission in New-Onset T1D with Alefacept), which showed improvements in diverse metabolic assessments and effects on T cell subsets (26). However, the durability of these effects off therapy was unknown.

Herein, we report the 24-month clinical, metabolic, and mechanistic findings of the T1DAL trial, testing the hypothesis that specific targeting of memory T cells with alefacept will lead to sustained preservation of β cell function. Our current findings demonstrate continued beneficial effects of alefacept on key metabolic and immunologic outcomes 15 months after cessation of therapy.

Results

Clinical, metabolic, and safety results. As reported previously, of 73 individuals screened, 49 were enrolled in the trial, with 33 patients randomly assigned to receive alefacept and 16 to receive placebo. Demographic and baseline characteristics of the 49 participants enrolled were comparable between the alefacept and placebo groups (26). At 12 months, 3 participants in the alefacept group and 4 participants in the placebo group were lost to follow-up; no additional participants were lost to follow-up between 12 and 24 months (Figure 1).

Alefacept-treated participants had preservation of endogenous insulin production at 24 months, compared with placebo, determined by both the 4- and 2-hour mixed meal tolerance test (MMTT) C-peptide AUC. In the 4-hour evaluation, there was a mean decrease in C-peptide AUC of 0.134 nmol/l (95% CI, 0.002–0.265) in the alefacept group; that amount was less than the placebo group (0.368 nmol/l [95% CI, 0.259–0.476; $P = 0.002$]; for a plot of the actual C-peptide values, see Figure 2A and Supplemental Figure 1; supplemental material available online with this article; doi:10.1172/JCI81722DS1). In the 2-hour C-peptide evaluation, the alefacept group had a mean decrease of 0.185 nmol/l (95% CI,

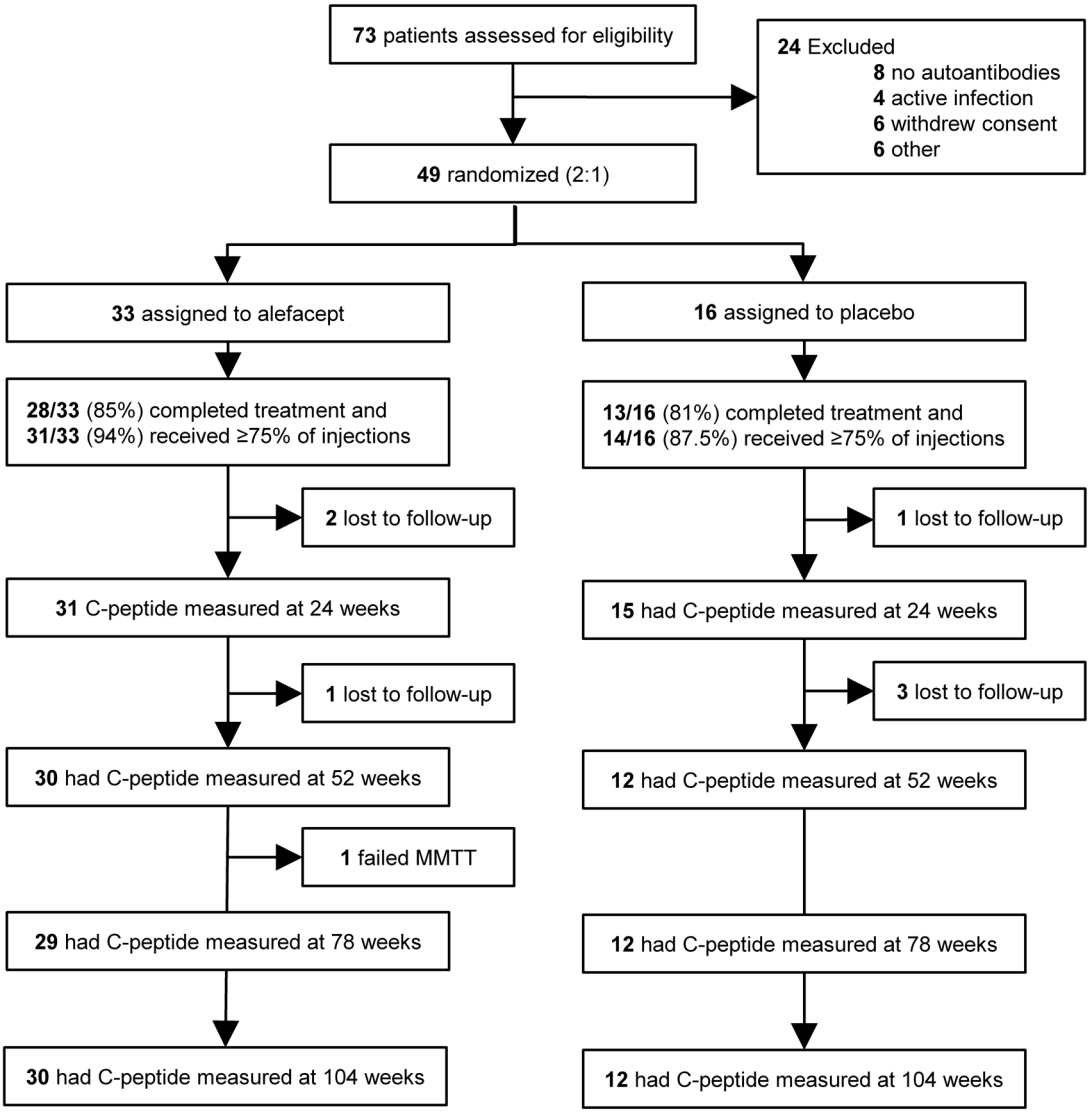

Figure 1. CONSORT diagram showing allocation and disposition of study subjects in the T1DAL trial.

0.065–0.305), whereas the placebo group had a mean decrease of 0.334 nmol/l (95% CI, 0.216–0.453; $P = 0.015$; not shown). Results of sensitivity analyses were consistent with these findings; sensitivity analyses included fitting an ANCOVA model with covariates for sex, age, baseline insulin use, and baseline HbA1c, in addition to baseline AUC, for the 4-hour C-peptide ($P = 0.010$) and the 2-hour C-peptide ($P = 0.077$), and analyzing observed data only for the 4-hour C-peptide ($n = 42$, $P = 0.011$) and for the 2-hour C-peptide ($n = 42$, $P = 0.058$; data not shown).

Both groups achieved good glycemic control, with mean HbA1c levels at 24 months of approximately 7.4%–7.5% in both groups ($P = 0.942$; Figure 2B). At 24 months, participants who received alefacept had lower mean insulin requirements (0.43 U/kg/d) versus controls (0.60 U/kg/d; $P = 0.002$; Figure 2C), equal to an almost 3-fold greater increase in insulin use from baseline in the placebo group (+0.28 U/kg/d) compared with the alefacept group (+0.10 U/kg/d). Alefacept-treated participants had substantially less major hypoglycemia (blood glucose < 55 mg/dl; Figure 2D). Over the entire study, rates of major hypoglycemia were substantially lower in the alefacept group, at 9.6 events/patient/year compared with 19.1 events/patient/year in the placebo group ($P < 0.001$), corresponding to a rate-ratio reduction with alefacept use of 2.0. These lower rates with alefacept occurred both during and off treatment, with rate-ratio reductions of 1.6 during weeks 1–52, and 2.5 during weeks 53–104 (Figure 2D).

Inspection of the plots in Figure 2A suggests that the treatment arms may continue to diverge in year 2 (from week 52–104). There was a mean decrease from month 12–24 in the 4-hour C-peptide AUC of 0.148 nmol/l (95% CI, 0.045–0.252) in the alefacept group, which was less than the change from month 12–24 in the placebo group (0.217 nmol/l [95% CI, 0.107–0.328]), although this did not reach statistical significance ($P = 0.11$). In a post hoc analysis, we divided alefacept-treated subjects into responders and nonresponders using an approach similar to that reported for the AbATE trial (8). As shown in Figure 3, we plotted the percent change from baseline in the 4-hour C-peptide AUC at 2 years for each subject in the alefa-

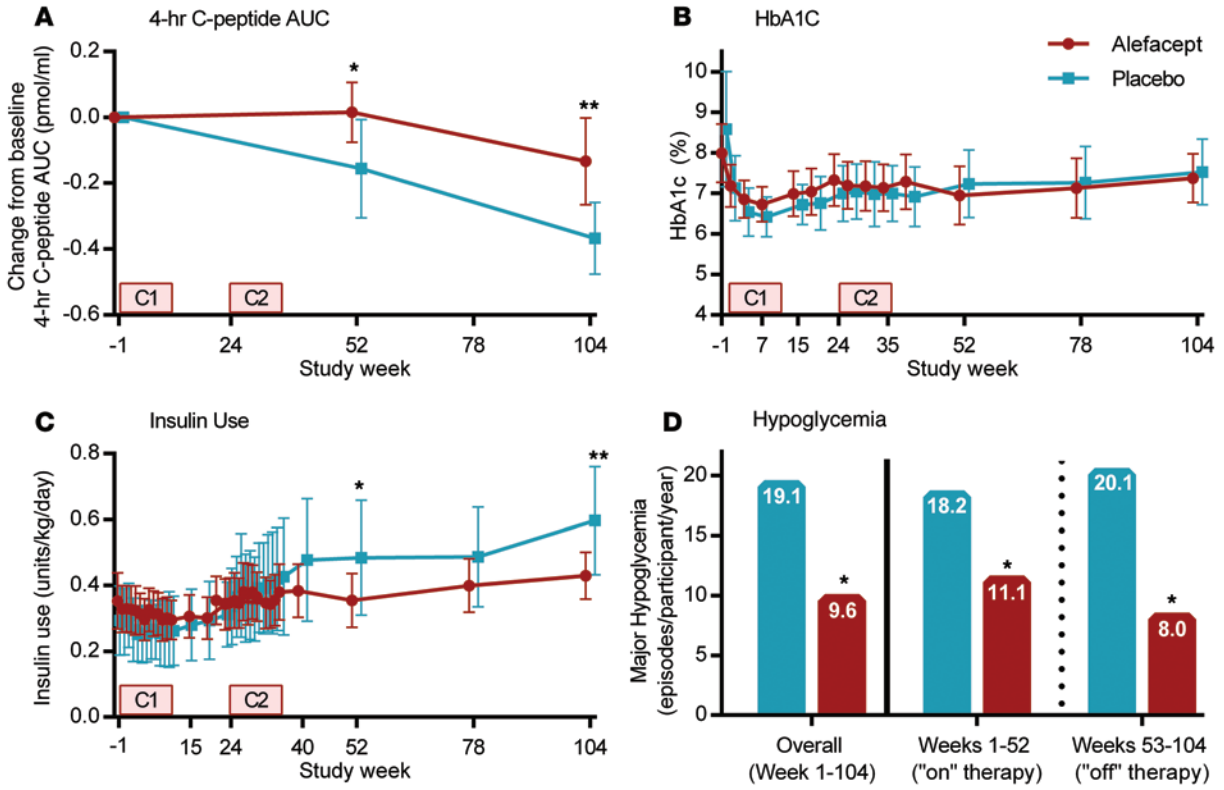

Figure 2. Clinical responses from baseline to 24 months in participants assigned to alefacept and placebo in the ITT sample. (**A**) Change in 4-hour C-peptide AUC. *P = 0.019, **P = 0.002. (**B**) Change in HbA1c. (**C**) Change in exogenous insulin requirements. *P = 0.020, **P = 0.002. Data were analyzed by fitting ANCOVA models with adjustment for baseline levels and plotted as unadjusted means ± 95% CI. P-values are 2-sided. (**D**) Rate of major hypoglycemic events. Event rates between the 2 groups were compared using Poisson regression. *P < 0.001. For all analyses, the number of evaluable subjects (n) at each time point is shown in Figure 1. C1 and C2 denote the two 12-week treatment courses. For additional details, see https://www.itntrialshare.org/T1DAL_fig2.url.

cept and placebo groups. We set 2 thresholds for response: complete preservation of baseline 4-hour C-peptide AUC values (>0%) at 2 years (complete responders) and preservation of 50% or more of baseline 4-hour C-peptide AUC values at 2 years (partial responders). In the alefacept group, 87% (26 of 30 subjects) were partial responders versus 33% (4 of 12) in the placebo group (P = 0.001); 30% (9 of 30) in the alefacept group were complete responders versus 8% (1 of 12) in the placebo group (P = 0.23; data not shown). Of the 9 alefacept-treated subjects who were complete responders at 24 months, 8 were also responders at 12 months (i.e., the 4-hour C-peptide AUC values at 12 months were equal to or greater than the baseline values; not shown), suggesting that response occurred early after alefacept treatment and was not a late phenomenon.

In the responder analysis, we also evaluated the effect of age by dividing subjects into 2 age cohorts: a younger cohort (12–21 years) and an older cohort (22–35 years) (Figure 3). In the alefacept group, the proportion of complete responders did not differ significantly compared with placebo in either age cohort. In contrast, 89% (17 of 19) of alefacept-treated subjects in the younger cohort were partial responders versus 29% (2 of 7) in the placebo group (P = 0.006); in the older cohort, 82% (9 of 11) were partial responders in the alefacept group versus 40% (2 of 5) in the placebo group (P = 0.24; data not shown).

Over the entirety of the study, the proportion of patients who had at least one adverse event (AE) was similar in the 2 treatment groups (Table 1). There was one serious AE in the alefacept group, which was considered unlikely related to the study drug, and 4 participants in the alefacept group had transient, asymptomatic declines in CD4 counts of <250 cells/μl. There were no deaths, opportunistic infections, or cytokine release syndrome (CRS) in either group.

Mechanistic results. Throughout the study, total white blood counts remained unchanged in both groups, but total lymphocytes and $CD4^+$ and $CD8^+$ T cells showed modest declines during the first and second course of treatment in the alefacept group, which rebounded by 78 weeks (Figure 4, A and B). At baseline, CD2 expression was highest on $CD4^+$ Tem, intermediate on $CD8^+$ Tem and central memory T cells (Tcm cells) and $CD4^+$ Tcm, and lowest on naive T cells (Tn cells) and Tregs (Figure 5A). During therapy, the percentage of $CD4^+$ and $CD8^+$ Tn cells increased from baseline in the alefacept group, and $CD4^+$ Tn remained elevated following therapy discontinuation (Figure 5, B and C). Alefacept treatment did not alter the frequency of Tregs during the entire study period (Figure 5D). In contrast, $CD4^+$ and $CD8^+$ Tcm cells decreased by approximately 25%–50% in the alefacept group and, although recovering in year 2, remained lower than in placebo patients at all time points (P < 0.01 for both, Figure 6, A and B). $CD4^+$ and $CD8^+$ Tem cells decreased even more in the alefacept group, approximately 40%–60% at week 35 (P < 0.01 for both), and then recovered in year 2, although $CD4^+$ Tem (but not $CD8^+$ Tem) remained lower compared with the placebo group

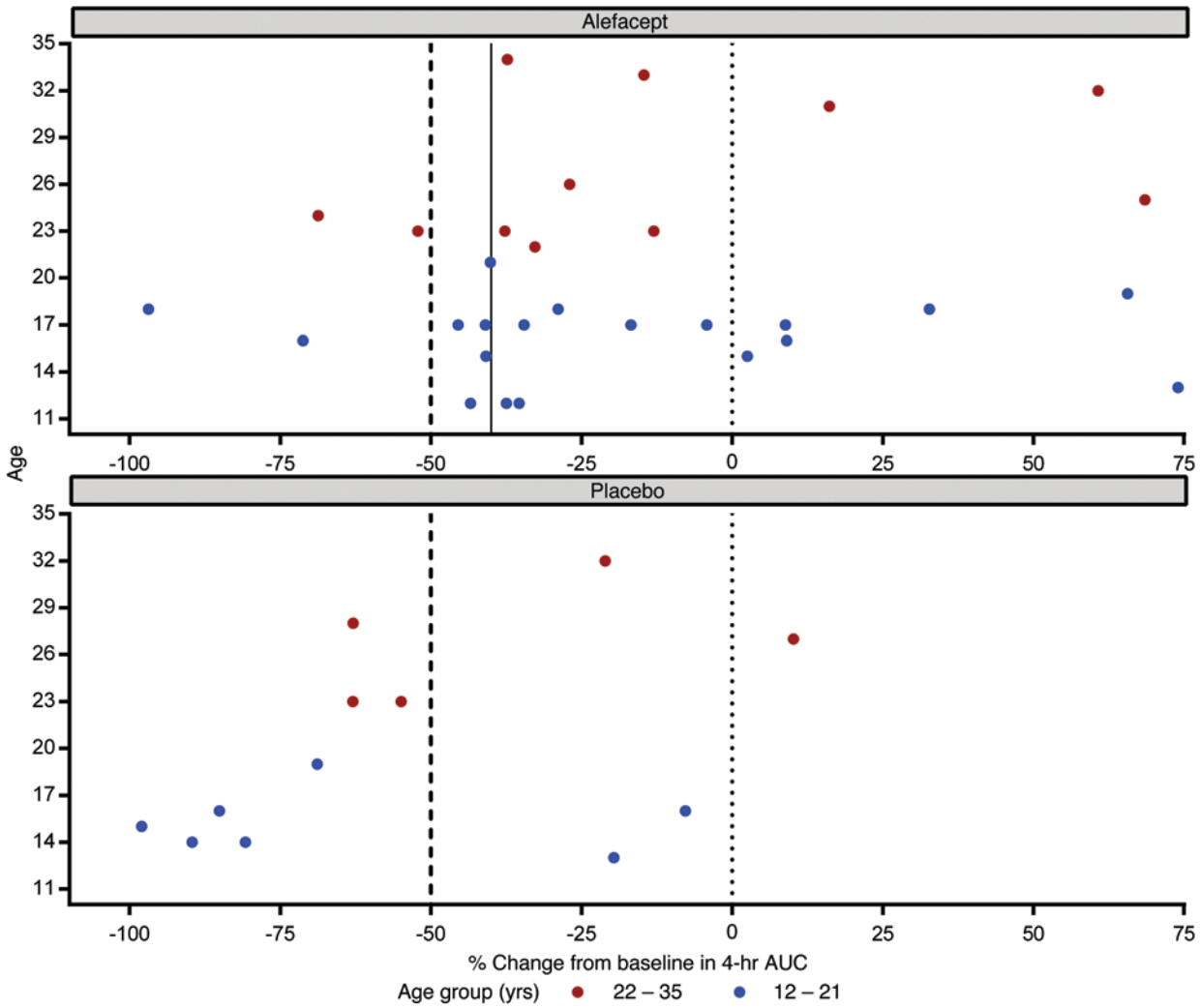

Figure 3. Responder analysis based on thresholds of preservation of baseline C-peptide secretion at 2 years. The % change in 4-hour C-peptide AUC from baseline to 2 years was plotted for each subject as a function of age (blue, younger subjects; red, older subjects); top, alefacept arm; bottom, placebo arm. Subjects to the right of the dotted line (>0% change) are denoted complete responders; subjects to the right of the broken line (<50% decrease from baseline) are partial responders. In the alefacept arm, subjects to the left of the solid line (>40% decrease) are classified as worst responders (n = 9) for comparison with complete responders (n = 9). For additional details, see https://www.itntrialshare.org/T1DAL_fig3.url.

at weeks 78 and 104 (Figure 6, C and D). In the alefacept group, there were no differences in peripheral T cell subsets between complete responders (n = 9) and all others or between complete responders and the 9 subjects with the greatest loss of C-peptide from baseline (see Figure 3) (analyses not shown).

Changes in T cell subsets were reflected in the ratios of Treg to memory cells. Alefacept treatment resulted in increases in Treg/Tem and Treg/Tcm ratios in both $CD4^+$ and $CD8^+$ cells, which peaked at week 35 and remained elevated at most points throughout the study ($P < 0.01$ for overall difference for all 4 ratios; Figure 7, A–D). The most substantial increase was in the Treg/$CD4^+$ Tem ratio, followed by similar increases in the Treg/$CD8^+$ Tem and Treg/$CD4^+$ Tcm ratios.

We also conducted exploratory analyses of the programmed death-1 (PD-1) receptor expression to determine if alefacept influences additional immunoregulatory mechanisms. PD-1 is a negative regulator of T cell activity and is involved in immune therapy–induced β cell sparing in preclinical models of T1D. At baseline, proportions of cells expressing PD-1 were highest (as percent of cells) in the $CD4^+$ and $CD8^+$ Tem subsets, intermediate in Tcms, and lowest in Tns and Tregs (Figure 8, A–D, and Figure 9, A–C). There was a striking increase in the percentage of $CD4^+$ Tem expressing PD-1 in the alefacept group at week 11, and this remained high through most of year 2 (Figure 9A). A more modest increase was also observed in $CD4^+$ Tcm cells in the alefacept group (Figure 8C). There were no changes in PD-1 expression in other T cell subpopulations in either group (Figures 8 and 9). Expression of CD2 was similar between PD-1^+CD4^+ and PD-1^-CD4^+ Tcm and Tem cells at baseline (Figure 9D). In the alefacept group, proportions of PD-1^+CD4^+ Tcms and Tems did not differ between complete responders and all others or between complete responders and the 9 subjects with the greatest loss of C-peptide from baseline (Figure 3) (analyses not shown).

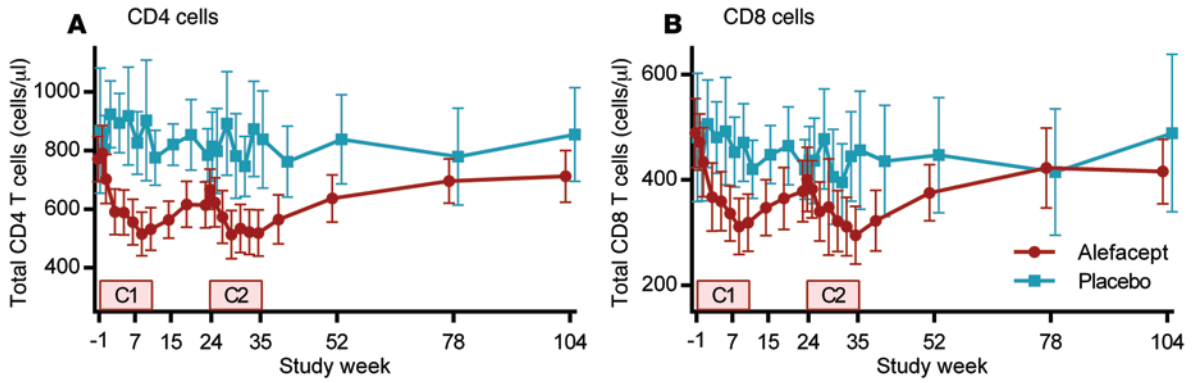

Figure 4. Changes in lymphocyte absolute cell counts from baseline to 24 months. (**A**) Total CD4$^+$ T cells. (**B**) Total CD8$^+$ T cells. Data are mean values ± 95% CI. For all analyses, the number of evaluable subjects (N) at each time point is shown in Figure 1. For additional details, see https://www.itntrialshare.org/T1DAL_fig4.url.

Discussion

Therapeutic options in T1D have changed little since the discovery of insulin in 1921, and even with excellent glycemic control (HbA1c ≤ 6.9%), mortality in those with T1D is twice that of matched controls (4). Hence, there is an urgent need for approaches to stabilize or reverse β cell destruction in T1D. Patients with newly diagnosed T1D have residual β cell function, providing a window of opportunity for a targeted intervention that can preserve islet function for years or decades after diagnosis (3). To this end, immune therapies that may be considered reasonable approaches in the primarily pediatric T1D population (such as anti-CD3 mAbs, abatacept, and rituximab) have been tested but generally have shown only modest success in preserving islet function, and with some, there are concerns of significant side effects, including CRS, EBV reactivation, and progressive multifocal leukoencephalopathy (8–10, 27, 28). A study that reported substantial efficacy was based on a regimen of autologous nonmyeloablative hematopoietic stem cell transplantation, but this was a small (n = 23) open-label trial, and the results were tempered by significant short- and long-term morbidity (18, 19).

The 24-month results of the T1DAL trial provide the most promising proof of concept to date that a brief course of a targeted, well-tolerated immune intervention in the new-onset period can produce lasting clinical and metabolic benefits, long after cessation of therapy. Alefacept significantly preserved endogenous insulin production, reduced exogenous insulin requirements, and, remarkably, reduced the risk of major hypoglycemic events by 50% over the 2-year study period. Severe iatrogenic hypoglycemia is the most concerning risk of intensive management and leads to substantial morbidity and mortality in T1D (4, 29, 30). The ability of a therapy to decrease the rate of major hypoglycemia while achieving good glycemic control with intensive diabetes management may be the most important goal of therapeutic progress in T1D.

A similar magnitude of C-peptide preservation was also observed after longer-term follow-up in patients treated with anti-CD3 mAbs (teplizumab and otelixizumab) (31). However, this efficacy came at the cost of CRS during drug administration and EBV reactivation or EBV-related disease in a significant proportion of treated patients (32). These AEs prompted exploration of a lower dose in larger trials, which resulted in better tolerability but loss of efficacy (33, 34).

The results of the T1DAL trial also provide mechanistic insights with respect to targeting the number and function of pathogenic T cells responsible for T1D. In the absence of broad immune suppression or ablation, depletion of Tem cells has not previously been achieved in T1D trials. In the START trial (antithymocyte globulin in new-onset T1D), in which there was no treatment benefit, Tem cells were unaffected despite robust depletion of all other T cell subsets, including Tregs (13). In sharp contrast to what we observed in T1DAL, antithymocyte globulin led to a significant decrease in Treg/Tem ratios. Interestingly, results from a recently reported pilot study (n = 25) of the combination of antithymocyte globulin and G-CSF in patients with established T1D suggested a treatment benefit and revealed the preservation of Tregs (35). These results suggest that higher Treg/Tem ratios may be an important biomarker of treatment benefit, a hypothesis that needs validation in larger trials and with other agents. It is also unknown whether the alefacept-mediated depletion of Tcm and Tem cells included islet antigen–specific cells, a point that requires further investigation.

An important caveat is that the relationship between changes in peripheral blood T cell subsets and clinical response remains unclear. We performed a post hoc responder analysis based on thresholds of preservation of baseline C-peptide secretion at 2 years (Figure 3). In the alefacept group, complete responders (C-peptide AUC values at 2 years equal to or greater than baseline values) did not differ in terms of frequencies of Tcm, Tem, or Tregs in the periphery when compared with all subjects who did not meet the complete response criterion or when compared only to subjects with the worst response. This finding is consistent with the experience in psoriasis, where treatment with alefacept resulted in a clinical response rate of 40%–60%, but response was poorly correlated with changes in the number of memory CD4$^+$ T cells in the peripheral circulation (36). In contrast, the clinical and histologic response to alefacept in psoriasis was highly correlated with changes in T cells infiltrating the epidermis and dermis (36). Interestingly, psoriasis nonresponders had quantitatively more T cells in skin lesions (36). It is unknown whether the response to alefacept in

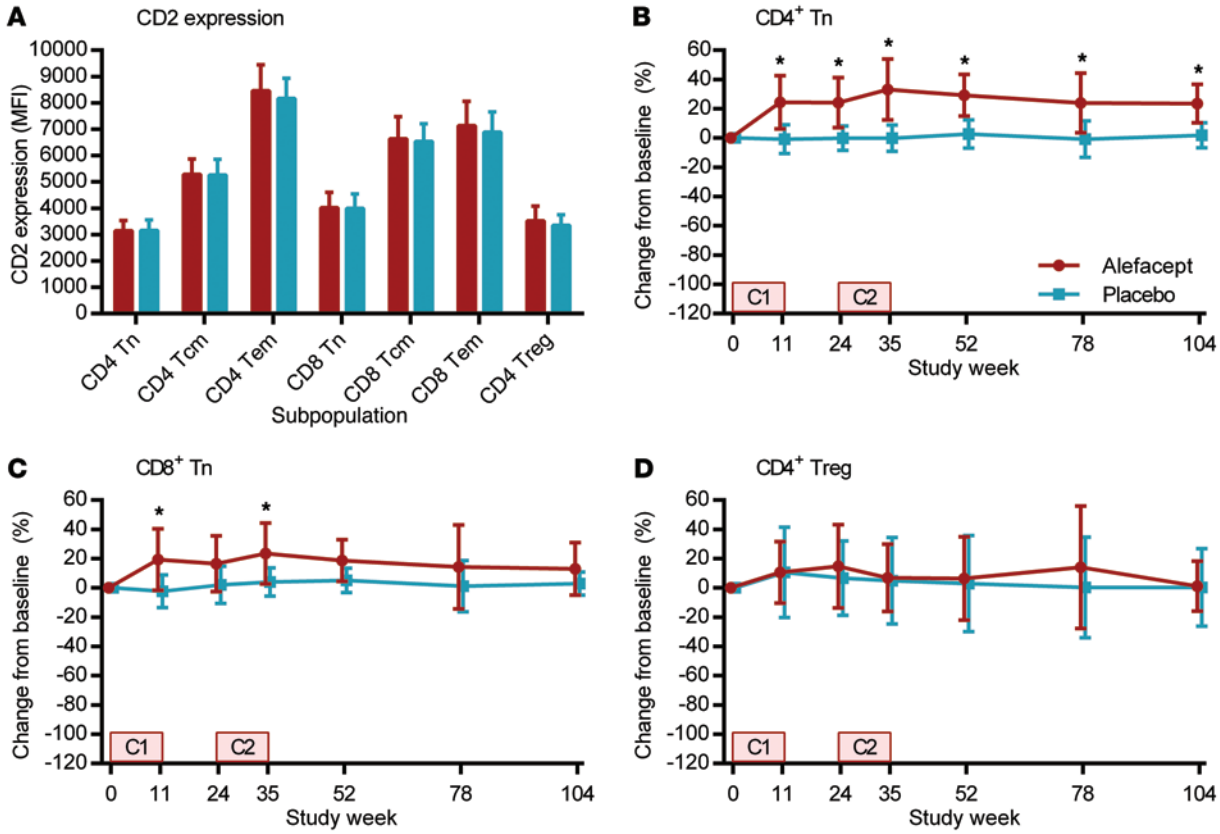

Figure 5. Baseline CD2 expression and changes in lymphocyte populations from baseline to 24 months in participants assigned to alefacept and placebo. (**A**) CD2 expression (mean fluorescence intensity, MFI) at baseline on studied T cell subpopulations. (**B**) $CD4^+$ Tn cells ($CD4^+$ $CD127^+$ $FoxP3^-$ $CD45RA^+$ $CD45RO^-$ $CCR7^+$). (**C**) $CD8^+$ Tn cells ($CD8^+$ $CD45RA^+$ $CD45RO^-$ $CCR7^+$). (**D**) $CD4^+$ Tregs ($CD4^+$ $CD127^{-/lo}$ $FoxP3^+$). Flow cytometry data were log-transformed and analyzed by repeated measures ANOVA, and *P* values were calculated to compare the differences of least square means between treatment groups at every visit. Data are mean values ± SD. Percent change from baseline is presented for T cell subsets as % of $CD4^+$ non-Treg or $CD8^+$ T cells in **B**–**D**. $^*P < 0.01$. For all analyses, the number of evaluable subjects (*n*) at each time point is shown in Figure 1. C1 and C2 denote the two 12-week treatment courses. For additional details, see https://www.itntrialshare.org/T1DAL_fig5.url.

T1D was dictated by quantitative or qualitative differences in lymphocytic infiltrates in the islets, but it may be worth exploring the use of larger doses of alefacept or treatment for longer periods in future trials to overcome a theoretical tissue resistance. In contrast to our results with alefacept, a recent report showed that preservation of C-peptide in new-onset T1D subjects treated with abatacept correlated with a reduction in the proportion of $CD4^+$ Tcm in the peripheral blood collected at the preceding study visit (37).

An interesting finding was that clinical response to alefacept (C-peptide preservation) appeared to be dependent on age. We divided subjects in the alefacept and placebo groups into a younger (age 12–21 years) and older (22–35 years) cohort (Figure 3), based on the results of a recent analysis suggesting that this cut-off demarcates distinct rates of C-peptide decline in the first 2 years after diagnosis (38). We found that in the younger cohort, partial response was significantly ($P < 0.006$) more frequent in the alefacept group than in the placebo group, while in the older cohort, the difference was not significant. This is consistent with the experience with the anti-CD3 mAb otelixizumab, where response was more pronounced in younger subjects (32), and a similar trend was observed with rituximab (9). The biological basis for the effect of age is unclear but warrants further study.

The ability of alefacept to downmodulate T cell activity may also be important. We observed an increase in the percentage of $CD4^+$ Tem and Tcm expressing PD-1 during and after treatment. PD-1 is one of a growing list of immune checkpoint inhibitors that control immune responses and contribute to peripheral tolerance (39). PD-1 is of particular interest because it is upregulated following T cell activation and mediates downregulation of effector functions after binding to cognate ligands (40), thereby suppressing islet infiltration by $CD4^+$ T cells in the nonobese diabetic (NOD) mouse model of T1D (40–43). In a recent study, targeted expression of the PD-1 ligand PD-L1 in neo-islets in diabetic NOD mice led to decreased proliferation and increased apoptosis of infiltrating $CD4^+$ T cells with robust reversal of hyperglycemia, suggesting the PD-1/PD-L1 pathway is strongly tolerogenic in this model (44). Our study is the first report in humans demonstrating an increase in PD-1–expressing $CD4^+$ memory cells with alefacept, or any agent showing benefit in an autoimmune condition. Additional studies are underway to better understand the mechanism of this observation. There was no difference in the intensity of CD2 on $PD\text{-}1^+$ and $PD\text{-}1^-$ T cells at baseline, and thus one hypothesis is that PD-1 was induced in $CD4^+$ memory cells by alefacept, possibly by an

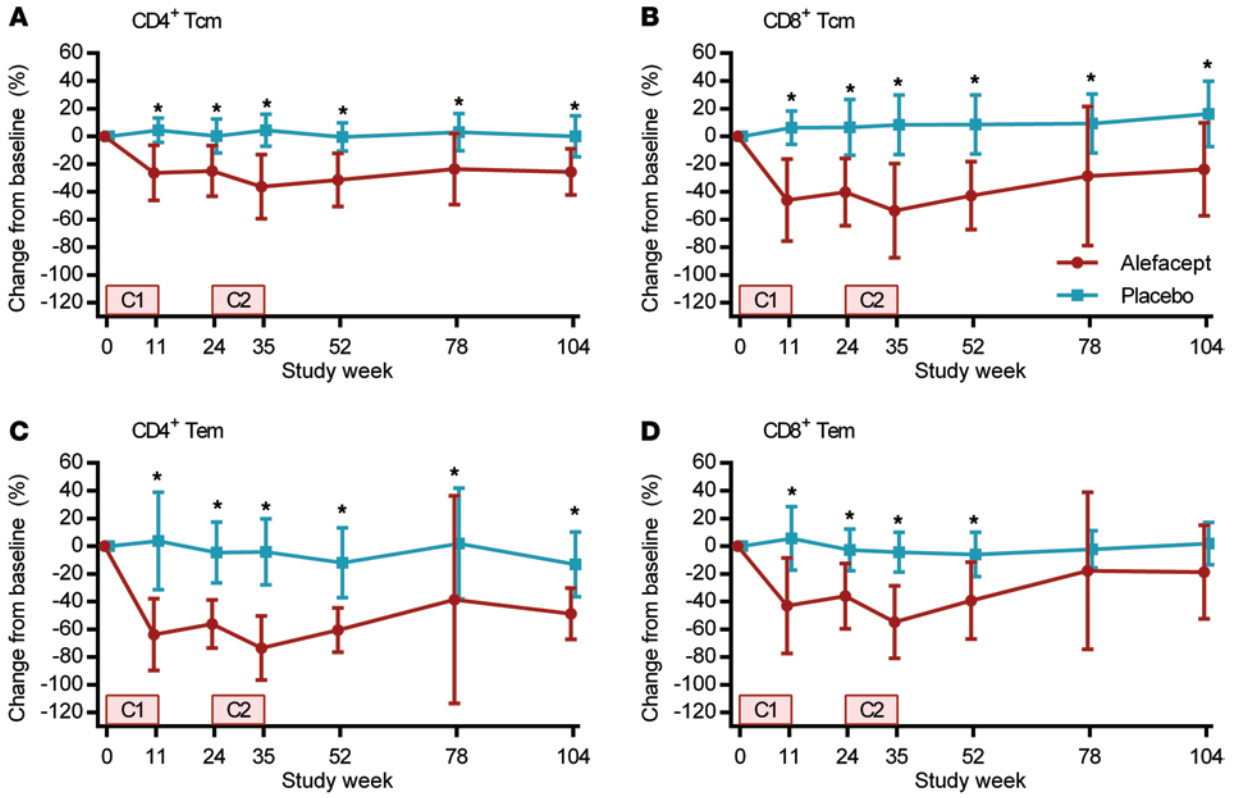

Figure 6. Changes in lymphocyte populations from baseline to 24 months in participants assigned to alefacept and placebo. (**A**) $CD4^+$ Tcm ($CD4^+$ $CD127^+$ $FoxP3^-$ $CD45RA^-$ $CD45RO^+$ $CCR7^+$). (**B**) $CD8^+$ Tcm ($CD8^+$ $CD45RA^-$ $CD45RO^+$ $CCR7^+$). (**C**) $CD4^+$ Tem ($CD4^+$ $CD127^+$ $FoxP3^-$ $CD45RA^-$ $CD45RO^+$ $CCR7^-$). (**D**) $CD8^+$ Tem ($CD8^+$ $CD45RA^-$ $CD45RO^+$ $CCR7^-$). Data were analyzed as described in Figure 5 and are mean values ± SD. Percent change from baseline is presented for T cell subsets as % of $CD4^+$ non-Treg or $CD8^+$ T cells in **B**–**D**. *$P < 0.01$. For all analyses, the number of evaluable subjects (n) at each time point is shown in Figure 1. C1 and C2 denote the two 12-week treatment courses. For additional details, see https://www.itntrialshare.org/T1DAL_fig6.url.

agonist effect (23). Alternatively, $PD\text{-}1^+CD4^+$ Tems may be selectively resistant to alefacept-mediated depletion. As noted for other T cell subsets, the change in $PD\text{-}1^+CD4^+$ Tems did not differ by responder status, which, as speculated above, may relate to differences in the effects of alefacept in peripheral blood versus at the site of pathology.

Although the duration of alefacept therapy was relatively brief (two 12-week courses over 36 weeks), clinical and immunologic benefits continued 15 months following discontinuation of therapy. Thus, it may be possible to restore peripheral tolerance and induce an indefinite off-therapy remission with preserved islet function in T1D. At 12 months, the C-peptide responses in alefacept-treated participants were similar to those at baseline but began to wane in the second year of the study. Coincident with this, the Treg/Teff ratios, reduction of Tems, and increase in PD-1–expressing $CD4^+$ Tcms and Tems also began to decline (compare Figure 2A with Figures 6–9). To maintain longer-lasting immunologic and clinical effects, and to increase the proportion of responders, administration of additional courses of alefacept, higher doses, or combining alefacept with other therapies (e.g., antiinflammatory agents, antigen-specific therapies, or exogenously expanded Tregs) would be worth exploring.

A limitation of this study was the final sample size (n = 49), which likely contributed to underpowering and inability to meet the prespecified study primary endpoint, the 2-hour C-peptide AUC at 12 months (26). We found that the 4-hour C-peptide AUC generated more robust results at both 12 and 24 months, which may reflect the ability of the 4-hour test to provide more complete data on the insulin response after a mixed meal, allowing for better discrimination between treatment groups (26). In an analysis of data from recent ITN T1D trials, we found that the 2- and 4-hour tests were highly correlated but that the 4-hour test had lower variability (K. Boyle, unpublished observations). The T1DAL C-peptide results from the 4-hour MMTT are consistent with significant treatment effects measured for a range of prespecified metabolic and mechanistic endpoints. Although the trial was too small to detect uncommon AEs, alefacept has been widely used in psoriasis for over a decade with a strong safety record; importantly, alefacept does not blunt immune responses to novel and recall antigens and, based on a 2007 review of available safety data, does not increase susceptibility to infectious disease or malignancy (45). These data suggest that the drug has a profile that would be acceptable for use as an adjunctive therapy in T1D, even in children.

In conclusion, administration of two 3-month courses of alefacept over a 9-month period in patients with new-onset T1D produced extended preservation of endogenous insulin production, reduced insulin requirements, and, importantly, decreased the rate of major hypoglycemia, all coincident with salutary immunologic changes over a period of 2 years, well over a year following

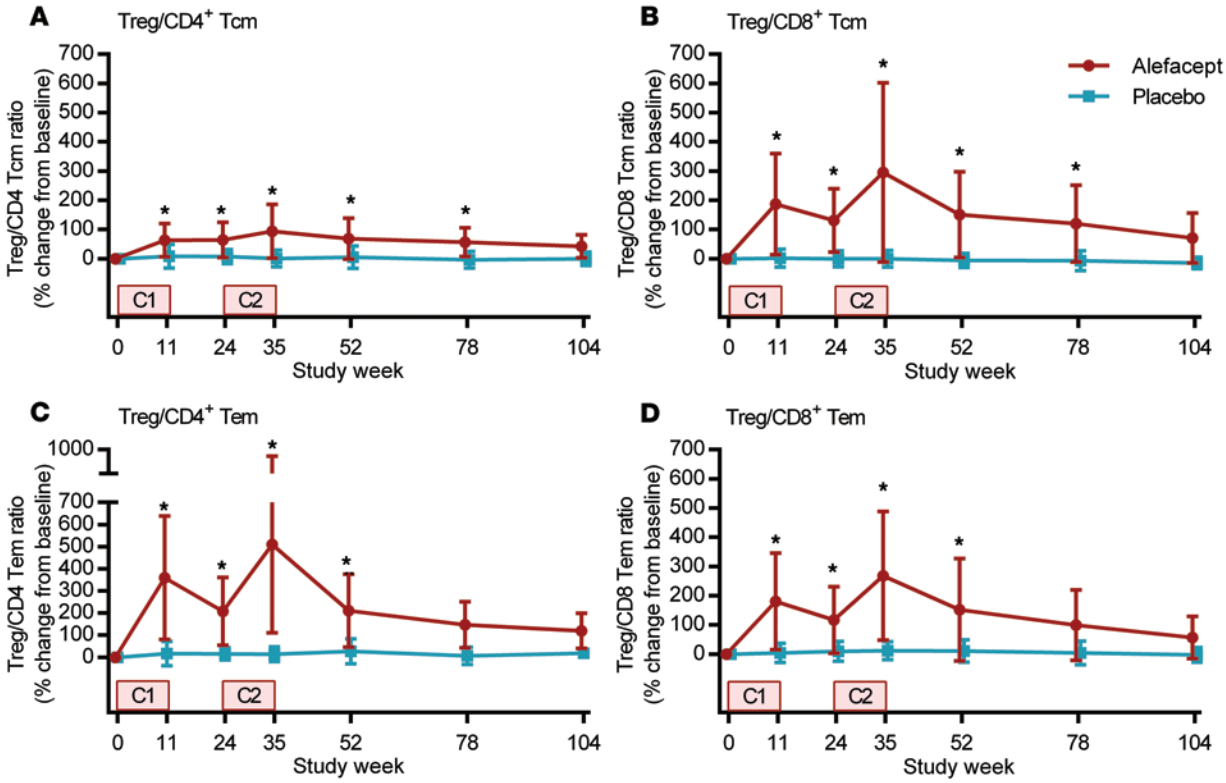

Figure 7. Changes in ratios of Tregs to memory T cells from baseline to 24 months in participants assigned to alefacept and placebo. (**A** and **B**) Ratios of Tregs to CD4⁺ and CD8⁺ Tcm. (**C** and **D**) Ratios of Treg to CD4⁺ and CD8⁺ Tem cells. Flow populations and analyses are as described in Figures 5 and 6. Data are mean values ± SD presented as % change from baseline. *$P < 0.01$. For all analyses, the number of evaluable subjects (*n*) at each time point is shown in Figure 1. C1 and C2 denote the two 12-week treatment courses. For additional details, see https://www.itntrialshare.org/T1DAL_fig7.url.

the last dose. This study provides proof of concept that intermittent dosing with a targeted and well-tolerated immunotherapy can improve islet function in T1D and encourages further investigations to develop an immunologically based therapy for T1D.

Methods

Study design and patients. This was a phase-2, randomized, placebo-controlled, double-blind clinical trial conducted at 14 clinical centers in the United States (see ref. 26 for complete details; the trial protocol is available in the Supplemental Materials; the trial profile is shown in Figure 1) with a 9-month treatment period and 15 months of follow-up (March 2011 to March 2014).

Eligible participants were 12–35 years of age at the time of screening; <100 days from diagnosis at the time of enrollment; positive for at least one diabetes-associated autoantibody (insulin, GAD-65, IA-2, ZnT8, or ICA); and had peak-stimulated C-peptide of >0.2 nmol/l during a MMTT. Exclusion criteria included evidence of tuberculosis, hepatitis B or C, HIV, or active EBV or CMV infection; significant cardiac disease; conditions associated with immune dysfunction or hematologic dyscrasia (including malignancy, lymphopenia, thrombocytopenia, or anemia); liver or renal dysfunction; ongoing use of diabetes medications other than insulin; recent inoculation with a live vaccine; and lactating or pregnant females.

Procedures. Eligible subjects were randomly assigned 2:1 to alefacept or placebo. The site-stratified randomization scheme was computer generated at the data-coordinating center using permuted blocks of size 3. Site personnel randomized subjects via an interactive web–based system, which sent the treatment assignments directly to the unmasked site pharmacists. All subjects and site personnel, including the independent diabetes educators, remained masked throughout the study. Site personnel were masked to total lymphocyte, CD4⁺, and CD8⁺ counts on lab reports unless CD4⁺ counts decreased to <250 cells/μl (26). At outpatient visits, participants received 15 mg alefacept (Amevive, received from Astellas) or equivalent volume of saline (placebo) i.m. weekly for 12 weeks and, after a 12-week pause, 12 additional weekly doses of alefacept or placebo. Participants underwent a 4-hour MMTT at screening, 52 weeks, and 104 weeks; a 2-hour MMTT at 24 and 78 weeks; and intensive diabetes management (30).

Laboratory tests. Autoantibodies were assayed at the Barbara Davis Center (Aurora, Colorado, USA) and the University of Florida; C-peptide and HbA1c at the Northwest Lipid Research Laboratory (Seattle, Washington, USA); and chemistries, hematology, viral loads, and serology at ICON Central Labs.

Immunophenotyping. Cryopreserved peripheral blood mononuclear cells (PBMCs) were batch-analyzed after the month-24 endpoint using an LSR II flow cytometer (BD Biosciences) and gated with Flowjo Mac Version 9.8.1 (Tree Star Inc.) at the Benaroya Research Institute (Seattle, Washington, USA). Antibody panel configurations were changed from the 12-month evaluations to improve discrimination between naive and memory T cell subsets (see Supplemental Tables 1 and 2; ref. 26).

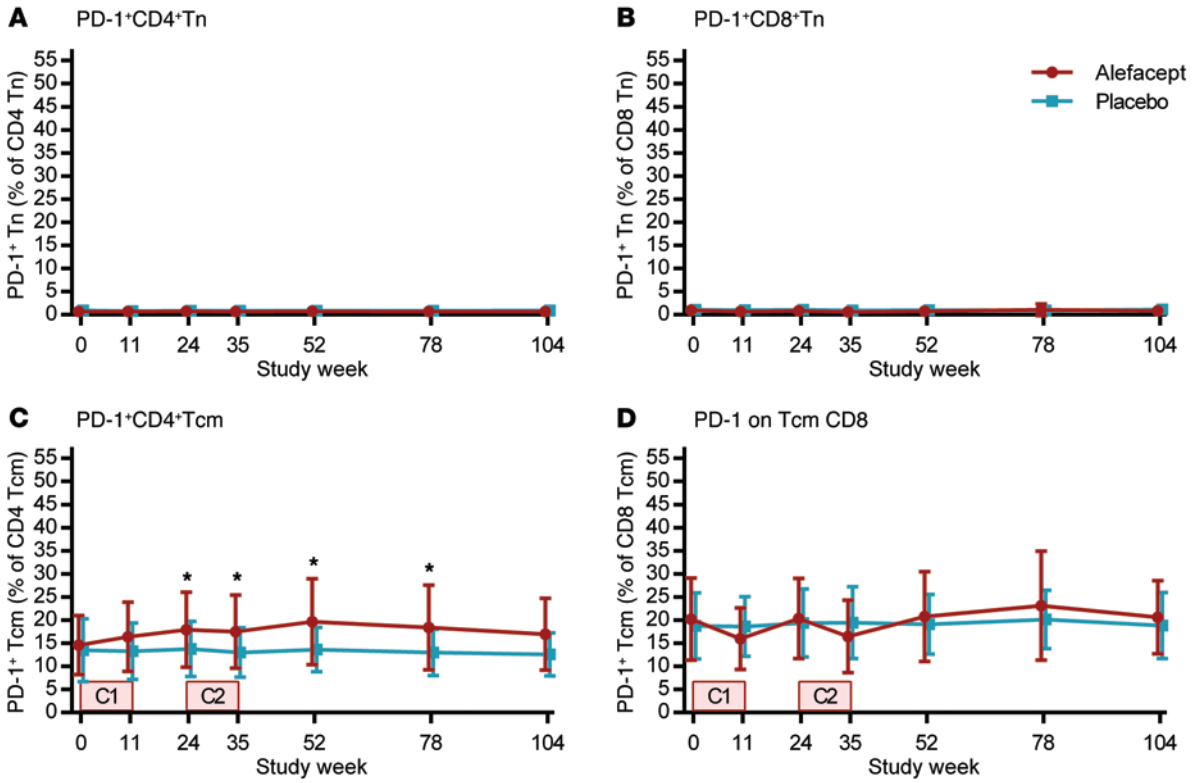

Figure 8. Changes in proportions (% of parent) of PD-1–expressing T cells (naive and central memory) from baseline to 24 months in participants assigned to alefacept and placebo. (**A** and **B**) PD-1–expressing CD4$^+$ and CD8$^+$ Tn cells. (**C** and **D**) PD-1–expressing CD4$^+$ and CD8$^+$ Tcm. Flow populations and analyses are as described in Figures 5 and 6. Data are mean values ± SD. *$P < 0.01$. For all analyses, the number of evaluable subjects (n) at each time point is shown in Figure 1. C1 and C2 denote the two 12-week treatment courses. For additional details, see https://www.itntrialshare.org/T1DAL_fig8.url.

Outcomes. Prespecified outcomes at 24 months included the change in mean 4-hour and 2-hour C-peptide area AUC from baseline, change in mean insulin use, major hypoglycemic events, HbA1c levels, and frequency and severity of AEs in the alefacept versus placebo groups.

Statistics. The original sample size of 66 was calculated to provide 85% power to detect a 50% improvement in the 2-hour C-peptide AUC of alefacept over control at 12 months (details on sample size calculations are shown in the study protocol provided in the Supplemental Materials and in ref. 26). Enrollment was halted at 49 subjects following voluntarily withdrawal of alefacept from the U.S. market; drug discontinuation was driven by business considerations and was not based on safety or regulatory concerns (46). Thus, the power dropped to 80% to detect a 55% improvement.

All randomized subjects who received any dose of study treatment ($n = 49$) were used in the intention to treat (ITT) analysis for the 24-month endpoints. For the primary inferential analysis, C-peptide AUCs were transformed to ln(AUC+1) and treatment groups compared by fitting an ANCOVA model with change from baseline as the outcome and baseline ln(AUC+1) value as a covariate. Means and summary statistics are presented on the untransformed scale. Missing C-peptide AUC data were imputed for 7 subjects in the ITT population who did not have an MMTT at month 24 (3 alefacept, 4 placebo). Secondary inferential analyses on HbA1c and insulin-use were based on ANCOVA models at each time point with adjustment for baseline levels. Hypoglycemic event rates between the 2 groups were compared using Poisson regression. Fisher exact test was used to compare the number of responders (complete or partial) versus nonresponders (subjects who did not meet the criteria for complete or partial response). Flow cytometry data were log-transformed and analyzed by repeated measures ANOVA, and P values were calculated to compare the differences of least square means between treatment groups at every visit. For any secondary and exploratory analyses, corrections were not made for multiple comparisons. SAS version 9.2 was used for all data analyses. P values for these comparisons are 2-sided. Further detail, including methods for handling missing C-peptide data and sensitivity analyses, are described in the Supplemental Methods. Datasets for these analyses are accessible through TrialShare, a public website managed by the ITN (https://www.itntrialshare.org/T1DAL.url).

Study approval. The T1DAL study was conducted according to the Declaration of Helsinki and in accordance with good clinical practice guidelines, performed under an investigational new drug application (IND 105,308), and approved by independent institutional review boards at each participating clinical center. All participants or parents provided written informed consent or assent (<18 years old). An independent data and safety monitoring board (DSMB) conducted regular safety reviews, and the sponsor's medical monitor provided additional study oversight. AEs were recorded and reported according to the standards set forth in the National Cancer Institute Common Terminology Criteria for Adverse Events (NCI-CTCAE), Version 4.0 (May 28, 2009).

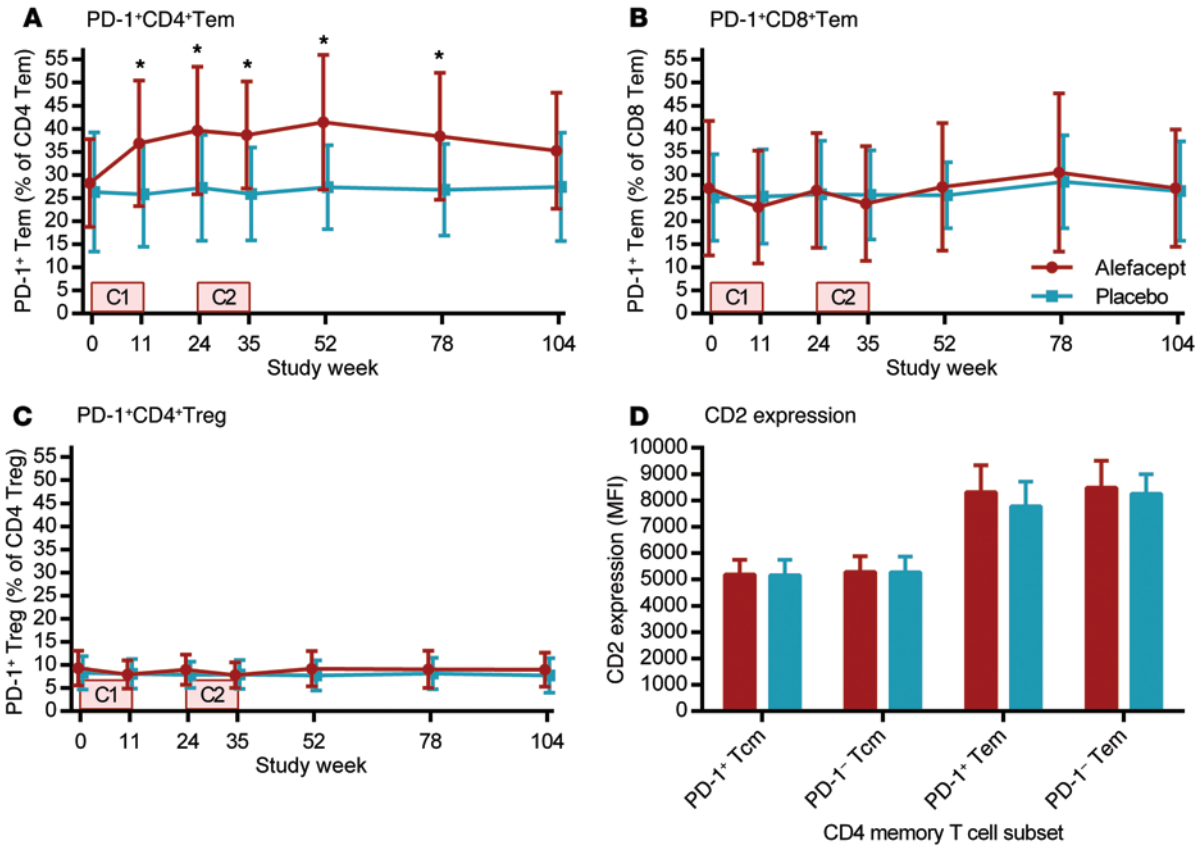

Figure 9. Baseline CD2 expression and changes in proportions (% of parent) of PD-1–expressing T cells (memory and Treg) from baseline to 24 months in participants assigned to alefacept and placebo. (**A** and **B**) PD-1–expressing CD4+ and CD8+ Tem cells. (**C**) PD-1–expressing CD4+ Tregs. (**D**) CD2 expression (mean fluorescence intensity, MFI) at baseline in CD4+ memory T cells. Flow populations and analyses are as described in Figures 5 and 6. Data are mean values ± SD. *$P < 0.01$. For all analyses, the number of evaluable subjects (*n*) at each time point is shown in Figure 1. C1 and C2 denote the two 12-week treatment courses. For additional details, see https://www.itntrialshare.org/T1DAL_fig9.url.

Acknowledgments

Funding/Support: The trial was conducted by the ITN and sponsored by the National Institute of Allergy and Infectious Diseases (NIAID) under Award Numbers NO1-AI-15416 and UM1AI109565. The content is solely the responsibility of the authors and does not necessarily represent the official views of the NIH. Additional funding was provided by the National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK). This project was in part supported, at Indiana University, by the Indiana Clinical and Translational Sciences Institute, funded in part by Grant Number TR000006 from the NIH, National Center for Advancing Translational Sciences (NCATS), Clinical and Translational Sciences Award; at UCSF, by Grant Numbers UL1 RR024131 and UL1 TR000004 from the National Center for Research Resources (NCRR) and the NCATS, NIH; at CHOP, by Grants UL1RR024134 and UL1TR000003 from the NCRR and the NCATS. The contents are solely the responsibility of the authors and do not necessarily represent the official views of the NIH. Astellas provided alefacept (Amevive) and gave input regarding dosage and safety, but had no direct involvement with study design, conduct, or management; data collection, analysis, or interpretation; or manuscript preparation. There are no agreements concerning confidentiality of the data between Astellas, the sponsor, and the authors or the institutions named in the credit lines. The authors provided Astellas a copy of the original manuscript prior to submission. Bayer HealthCare LLC, Diabetes Care provided blood glucose monitoring supplies through an investigator-sponsored research grant. The authors provided Bayer a copy of the original manuscript prior to submission.

Address correspondence to: Mark R. Rigby, Translational Medicine – Immunology Development, Janssen R&D, Pharmaceutical Companies of Johnson & Johnson, 1460 McKean Road, Spring House, Pennsylvania 19477, USA. Phone: 215.540.4724; E-mail: mrigby@its.jnj.com.

Mark R. Rigby's present address is: Translational Medicine – Immunology Development, Janssen R&D, Pharmaceutical Companies of Johnson & Johnson, Spring House, Pennsylvania, USA.

Marc S. Rendell's present address is: Rose Salter Medical Research Foundation, Omaha, Nebraska, USA.

Kurt J. Griffin's present address is: The Sanford Project, Sanford Research, and Sanford School of Medicine, University of South Dakota, Sioux Falls, South Dakota, USA.

Deborah Phippard's present address is: Precision for Medicine, Bethesda, Maryland, USA.

1. Dabelea D, et al. Prevalence of type 1 and type 2 diabetes among children and adolescents from 2001 to 2009. *JAMA*. 2014;311(17):1778–1786.
2. Bruno G, et al. Incidence of type 1 and type 2 diabetes in adults aged 30–49 years: the population-based registry in the province of Turin, Italy. *Diabetes Care*. 2005;28(11):2613–2619.
3. Bluestone JA, Herold K, Eisenbarth G. Genetics, pathogenesis and clinical interventions in type 1 diabetes. *Nature*. 2010;464(7293):1293–1300.
4. Lind M, et al. Glycemic control and excess mortality in type 1 diabetes. *N Engl J Med*. 2014;371(21):1972–1982.
5. Steffes MW, Sibley S, Jackson M, Thomas W. β-Cell function and the development of diabetes-related complications in the diabetes control and complications trial. *Diabetes Care*. 2003;26(3):832–836.
6. Feutren G, et al. Cyclosporin increases the rate and length of remissions in insulin-dependent diabetes of recent onset. Results of a multicentre double-blind trial. *Lancet*. 1986;2(8499):119–124.
7. Cook JJ, et al. Double-blind controlled trial of azathioprine in children with newly diagnosed type I diabetes. *Diabetes*. 1989;38(6):779–783.
8. Herold KC, et al. Teplizumab (anti-CD3 mAb) treatment preserves C-peptide responses in patients with new-onset type 1 diabetes in a randomized controlled trial: metabolic and immunologic features at baseline identify a subgroup of responders. *Diabetes*. 2013;62(11):3766–3774.
9. Pescovitz MD, et al. Rituximab, B-lymphocyte depletion, and preservation of β-cell function. *N Engl J Med*. 2009;361(22):2143–2152.
10. Orban T, et al. Costimulation modulation with abatacept in patients with recent-onset type 1 diabetes: follow-up 1 year after cessation of treatment. *Diabetes Care*. 2014;37(4):1069–1075.
11. Mastrandrea L, et al. Etanercept treatment in children with new-onset type 1 diabetes: pilot randomized, placebo-controlled, double-blind study. *Diabetes Care*. 2009;32(7):1244–1249.
12. Moran A, et al. Interleukin-1 antagonism in type 1 diabetes of recent onset: two multicentre, randomised, double-blind, placebo-controlled trials. *Lancet*. 2013;381(9881):1905–1915.
13. Gitelman SE, et al. Antithymocyte globulin treatment for patients with recent-onset type 1 diabetes: 12-month results of a randomised, placebo-controlled, phase 2 trial. *Lancet Diabetes Endocrinol*. 2013;1(4):306–316.
14. Gottlieb PA, et al. Failure to preserve β-cell function with mycophenolate mofetil and daclizumab combined therapy in patients with new- onset type 1 diabetes. *Diabetes Care*. 2010;33(4):826–832.
15. Long SA, et al. Rapamycin/IL-2 combination therapy in patients with type 1 diabetes augments Tregs yet transiently impairs beta-cell function. *Diabetes*. 2012;61(9):2340–2348.
16. Walter M, Kaupper T, Adler K, Foersch J, Bonifacio E, Ziegler AG. No effect of the 1α,25-dihydroxyvitamin D3 on β-cell residual function and insulin requirement in adults with new-onset type 1 diabetes. *Diabetes Care*. 2010;33(7):1443–1448.
17. Ludvigsson J, et al. GAD65 antigen therapy in recently diagnosed type 1 diabetes mellitus. *N Engl J Med*. 2012;366(5):433–442.
18. Voltarelli JC, et al. Autologous nonmyeloablative hematopoietic stem cell transplantation in newly diagnosed type 1 diabetes mellitus. *JAMA*. 2007;297(14):1568–1576.
19. Couri CE, et al. C-peptide levels and insulin independence following autologous nonmyeloablative hematopoietic stem cell transplantation in newly diagnosed type 1 diabetes mellitus. *JAMA*. 2009;301(15):1573–1579.
20. Krueger GG. Selective targeting of T cell subsets: focus on alefacept — a remittive therapy for psoriasis. *Expert Opin Biol Ther*. 2002;2(4):431–441.
21. Chamian F, et al. Alefacept (anti-CD2) causes a selective reduction in circulating effector memory T cells (Tem) and relative preservation of central memory T cells (Tcm) in psoriasis. *J Transl Med*. 2007;5:27.
22. Miller GT, et al. Specific interaction of lymphocyte function-associated antigen 3 with CD2 can inhibit T cell responses. *J Exp Med*. 1993;178(1):211–222.
23. Haider AS, et al. Novel insight into the agonistic mechanism of alefacept in vivo: differentially expressed genes may serve as biomarkers of response in psoriasis patients. *J Immunol*. 2007;178(11):7442–7449.
24. Ellis CN, Krueger GG, Alefacept Clinical Study Group. Treatment of chronic plaque psoriasis by selective targeting of memory effector T lymphocytes. *N Engl J Med*. 2001;345(4):248–255.
25. Ellis CN, Mordin MM, Adler EY. Effects of alefacept on health-related quality of life in patients with psoriasis: results from a randomized, placebo-controlled phase II trial. *Am J Clin Dermatol*. 2003;4(2):131–139.
26. Rigby MR, et al. Targeting of memory T cells with alefacept in new-onset type 1 diabetes (T1DAL study): 12 month results of a randomised, double-blind, placebo-controlled phase 2 trial. *Lancet Diabetes Endocrinol*. 2013;1(4):284–294.
27. Keymeulen B, et al. Insulin needs after CD3-antibody therapy in new-onset type 1 diabetes. *N Engl J Med*. 2005;352(25):2598–2608.
28. Clifford DB, et al. Rituximab-associated progressive multifocal leukoencephalopathy in rheumatoid arthritis. *Arch Neurol*. 2011;68(9):1156–1164.
29. Seaquist ER, et al. Hypoglycemia and diabetes: a report of a workgroup of the American Diabetes Association and the Endocrine Society. *Diabetes Care*. 2013;36(5):1384–1395.
30. American Diabetes Association. Standards of medical care in diabetes — 2014. *Diabetes Care*. 2014;37(suppl 1):S14–S80.
31. Herold KC, et al. A single course of anti-CD3 monoclonal antibody hOKT3gamma1(Ala-Ala) results in improvement in C-peptide responses and clinical parameters for at least 2 years after onset of type 1 diabetes. *Diabetes*. 2005;54(6):1763–1769.
32. Keymeulen B, et al. Four-year metabolic outcome of a randomised controlled CD3-antibody trial in recent-onset type 1 diabetic patients depends on their age and baseline residual beta cell mass. *Diabetologia*. 2010;53(4):614–623.
33. Ambery P, Donner TW, Biswas N, Donaldson J, Parkin J, Dayan CM. Efficacy and safety of low-dose otelixizumab anti-CD3 monoclonal antibody in preserving C-peptide secretion in adolescent type 1 diabetes: DEFEND-2, a randomized, placebo-controlled, double-blind, multi-centre study. *Diabet Med*. 2014;31(4):399–402.
34. Aronson R, et al. Low-dose otelixizumab anti-CD3 monoclonal antibody DEFEND-1 study: results of the randomized phase III study in recent-onset human type 1 diabetes. *Diabetes Care*. 2014;37(10):2746–2754.
35. Haller MJ, et al. Anti-thymocyte globulin/G-CSF treatment preserves beta cell function in patients with established type 1 diabetes. *J Clin Invest*. 2015;125(1):448–455.
36. Chamian F, et al. Alefacept reduces infiltrating T cells, activated dendritic cells, and inflammatory genes in psoriasis vulgaris. *Proc Natl Acad Sci U S A*. 2005;102(6):2075–2080.
37. Orban T, et al. Reduction in CD4 central memory T-cell subset in costimulation modulator abatacept-treated patients with recent-onset type 1 diabetes is associated with slower C-peptide decline. *Diabetes*. 2014;63(10):3449–3457.
38. Greenbaum CJ, et al. Fall in C-peptide during first 2 years from diagnosis: evidence of at least two distinct phases from composite Type 1 Diabetes TrialNet data. *Diabetes*. 2012;61(8):2066–2073.
39. Murakami N, Riella LV. Co-inhibitory pathways and their importance in immune regulation. *Transplantation*. 2014;98(1):3–14.
40. Francisco LM, Sage PT, Sharpe AH. The PD-1 pathway in tolerance and autoimmunity. *Immunol Rev*. 2010;236:219–242.
41. Pauken KE, Jenkins MK, Azuma M, Fife BT. PD-1, but not PD-L1, expressed by islet-reactive $CD4^+$ T cells suppresses infiltration of the pancreas during type 1 diabetes. *Diabetes*. 2013;62(8):2859–2869.
42. Ansari MJ, et al. The programmed death-1 (PD-1) pathway regulates autoimmune diabetes in nonobese diabetic (NOD) mice. *J Exp Med*. 2003;198(1):63–69.
43. Fife BT, et al. Insulin-induced remission in new-onset NOD mice is maintained by the PD-1-PD-L1 pathway. *J Exp Med*. 2006;203(12):2737–2747.
44. Li R, et al. PD-L1-driven tolerance protects neurogenin3-induced islet neogenesis to reverse established type 1 diabetes in NOD mice. *Diabetes*. 2015;64(2):529–540.
45. Scheinfeld N. Alefacept: its safety profile, off-label uses, and potential as part of combination therapies for psoriasis. *J Dermatolog Treat*. 2007;18(4):197–208.
46. Johnston W. Voluntary US Market Discontinuation of Amevive (alefacept) [Physician Letter]. http://web.archive.org/web/20130401045227/http://www.amevive.com/Physician%20letter.pdf. Milwaukee, Wisconsin, USA: Astellas Pharma US; December 15, 2011. Accessed June 10, 2015.