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

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Summary

Background Type 1 diabetes results from autoimmune targeting of the pancreatic β cells, likely mediated by effector memory T (Tem) cells. CD2, a T cell surface protein highly expressed on Tem cells, is targeted by the fusion protein alefacept, depleting Tem cells and central memory T (Tcm) cells. We postulated that alefacept would arrest autoimmunity and preserve residual β cells in patients newly diagnosed with type 1 diabetes.

Methods The T1DAL study is a phase 2, double-blind, placebo-controlled trial in patients with type 1 diabetes, aged 12–35 years who, within 100 days of diagnosis, were enrolled at 14 US sites. Patients were randomly assigned (2:1) to receive alefacept (two 12-week courses of 15 mg intramuscularly per week, separated by a 12-week pause) or a placebo. Randomisation was stratified by site, and was computer-generated with permuted blocks of three patients per block. All participants and site personnel were masked to treatment assignment. The primary endpoint was the change from baseline in mean 2 h C-peptide area under the curve (AUC) at 12 months. Secondary endpoints at 12 months were the change from baseline in the 4 h C-peptide AUC, insulin use, major hypoglycaemic events, and HbA_{1c} concentrations. This trial is registered with ClinicalTrials.gov, number NCT00965458.

Findings Of 73 patients assessed for eligibility, 33 were randomly assigned to receive alefacept and 16 to receive placebo. The mean 2 h C-peptide AUC at 12 months increased by 0·015 nmol/L (95% CI –0·080 to 0·110) in the alefacept group and decreased by 0·115 nmol/L (–0·278 to 0·047) in the placebo group, and the difference between groups was not significant (p=0·065). However, key secondary endpoints were met: the mean 4 h C-peptide AUC was significantly higher (mean increase of 0·015 nmol/L [95% CI –0·076 to 0·106] *vs* decrease of –0·156 nmol/L [–0·305 to –0·006]; p=0·019), and daily insulin use (0·48 units per kg per day for placebo *vs* 0·36 units per kg per day for alefacept; p=0·02) and the rate of hypoglycaemic events (mean of 10·9 events per person per year for alefacept *vs* 17·3 events for placebo; p<0·0001) was significantly lower at 12 months in the alefacept group than in the placebo group. Mean HbA_{1c} concentrations at week 52 were not different between treatment groups (p=0·75). So far, no serious adverse events were reported and all patients had at least one adverse event. In the alefacept group, 29 (88%) participants had an adverse event related to study drug versus 15 (94%) participants in the placebo group. In the alefacept group, 14 (42%) participants had grade 3 or 4 adverse events compared with nine (56%) participants in the placebo group; no deaths occurred.

Interpretation Although the primary outcome was not met, at 12 months, alefacept preserved the 4 h C-peptide AUC, lowered insulin use, and reduced hypoglycaemic events, suggesting efficacy. Safety and tolerability were similar in the alefacept and placebo groups. Alefacept could be useful to preserve β-cell function in patients with new-onset type 1 diabetes.

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Introduction

Type 1 diabetes is a T-cell-mediated autoimmune disorder that targets the insulin-secreting β cells in the islets of Langerhans.[1] Disease onset usually occurs in childhood or adolescence. Patients with type 1 diabetes require lifelong therapy with exogenous insulin and are at substantial risk for increased morbidity and mortality. At diagnosis, substantial islet function remains and, in the absence of active destruction, residual β cells might be salvageable.[1] Even modest endogenous insulin production might substantially improve long-term outcomes.[2]

Although trials in the 1980s and 1990s suggested that non-specific immune suppressants (eg, cyclosporine) might slow progression or even reverse type 1 diabetes while on therapy, the risks of life-long immune suppressant therapy outweighed the benefits.[3–5] Over the past two decades, more selective immunomodulatory drugs with lower risk profiles have been developed; but, although effective in some autoimmune diseases, trials of these agents in type 1 diabetes have shown so far either no efficacy, efficacy of small duration, or efficacy in a subgroup of patients only.[6–12]

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In type 1 diabetes, effector T cells are directly implicated in β-cell destruction.[1] CD2 is a surface protein expressed on most human T cells, but expression is highest on effector memory T (Tem) cells and central memory T (Tcm) cells, and most prominently on highly pathogenic armed effector T cells.[13,14] The endogenous ligand for CD2 in man is CD58 (LFA3), which is found mainly on antigen-presenting cells.

Alefacept (LFA3-Ig) is a dimeric fusion protein that was the first biological drug approved by the US Food and Drug Administration for moderate-to-severe plaque psoriasis.[15] Clinical response in psoriasis is improved with repeated courses of alefacept, resulting in a proportion of patients achieving sustained remissions even after drug discontinuation.[16–19] Alefacept disrupts the CD2–CD58 interaction, thereby blocking T-cell costimulation. Alefacept also induces granzyme-mediated apoptosis of T cells by crosslinking CD2 with CD16 expressed on natural killer cells and monocytes.[20] Results from psoriasis clinical trials[14,21,22] have shown that alefacept mainly depletes Tem cells and to a lesser extent Tcm cells, consistent with expression of CD2 on these cells. The effects of alefacept on regulatory T cells (Tregs) have not been studied.

In the "inducing remission in new-onset type 1 diabetes with alefacept" (T1DAL) trial, we postulated that treating patients with newly diagnosed type 1 diabetes with alefacept would target pathogenic effector T cells, arrest further destruction of β cells, and stabilise endogenous insulin production.

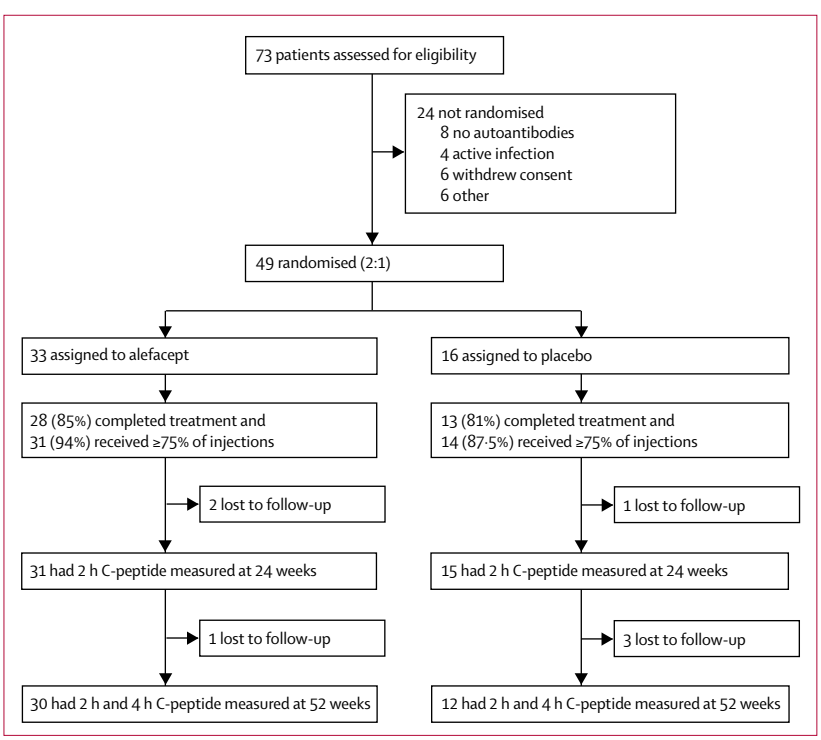

Figure 1: **Trial profile**

Methods

Study design and participants

This is a phase 2, multicentre, randomised, placebo-controlled, double-blind clinical trial in which participants with newly diagnosed type 1 diabetes received either two 12-week courses of alefacept separated by a 12-week pause, or matching placebo.

Screening, enrolment, and subsequent study visits occurred at 14 participating clinical centres in the USA. For the first ten patients, enrolment was confined to patients aged 16–35 years. The minimum age was subsequently lowered to 12 years after review by the data safety monitoring board (DSMB). Eligible participants had to be 12–35 years of age at time of screening, fewer than 100 days from diagnosis at the time of enrolment, positive for at least one diabetes-associated autoantibody (microassayed insulin antibody [if duration of insulin therapy was fewer than 10 days], glutamic acid decarboxylase-65 [GAD-65] antibody, tyrosine phosphatase-related islet antigen 2 [IA-2] antibody, zinc transporter 8 [ZnT8] antibody, or islet-cell antibody [ICA]), and have a peak stimulated C-peptide of more than 0·2 nmol/L during a mixed meal tolerance test (MMTT). Exclusion criteria were any serological or clinical evidence of infection; a positive purified protein derivative test; past infection with hepatitis B, hepatitis C, or HIV, or clinically active infection with Epstein-Barr virus (EBV), cytomegalovirus, or tuberculosis; substantial past cardiac disease or malignancy; leucopenia, lymphopenia, thrombocytopenia, or anaemia; history of bone marrow transplantation or autoimmune disease associated with lymphopenia; known hypersensitivity to human monoclonal antibodies; liver or renal dysfunction; ongoing use of diabetes drugs other than insulin, or past or current treatment with immune modulators; inoculation with a live vaccine within 6 weeks before enrolment; previous participation in a recent clinical trial (within 6 weeks) or any trial that could have affected type 1 diabetes or immunological status; and women who were lactating, pregnant, or unwilling to defer pregnancy.

The protocol and consent documents were approved by independent institutional review boards. All participants or parents provided written informed consent, and those younger than 18 years provided assent. An independent DSMB undertook regular safety reviews.

Randomisation and masking

Eligible patients were randomly assigned (2:1) to receive either alefacept or placebo. The site-stratified randomisation scheme was computer-generated at the data coordinating centre with permuted blocks of three patients per block. Site personnel randomly assigned participants via an interactive web-based system that sent the treatment assignments directly to the unmasked site pharmacists. All participants and site personnel,

including the independent diabetes educators, remained masked to assignment throughout the study. Site personnel were masked to total lymphocyte, CD4, and CD8 counts on laboratory reports unless patients' CD4 counts decreased to fewer than 250 cells per µL.

Procedures

Participants were brought into the sites' outpatient clinical trial centres to receive the first dose of 15 mg alefacept (Amevive, Astellas Pharma, USA) or equivalent volume of saline (placebo) intramuscularly, and were observed for 30 min. The participants returned to study sites for weekly injections (alefacept 15 mg or placebo) for a further 11 doses. After a 12-week pause, participants returned weekly for an additional 12 doses of alefacept or placebo. The total dosing period was, therefore, 36 weeks.

Patients underwent a 4 h MMTT at screening and 52 weeks, and a 2 h MMTT at 24 weeks; the 4 h MMTT procedure allowed calculation of both 2 h and 4 h C-peptide areas under the curve (AUC). All patients received intensive diabetes management with the goal of achieving age-specific HbA_{1c} and glycaemic targets as recommended by the American Diabetes Association.

Biochemical autoantibody titres were assayed at the Barbara Davis Center (Aurora, CO, USA) with radio-immunobinding assays, and ICA titres were measured at the University of Florida, FL, USA. C-peptide and HbA_{1c} concentrations were measured at the Northwest Lipid Research Laboratory (Seattle, WA, USA). Serum chemistry, haematology, viral load, and serology tests were undertaken at a central laboratory (ICON Central Labs, Farmingdale, NY, USA); clinical laboratory total lymphocyte, CD4, and CD8 counts were done real-time on a fluorescence-activated cell sorting (FACS) Canto II flow cytometer (BD Biosciences, San Jose, CA, USA) at the central laboratory (ICON) that also did the chemical analyses.

Additionally, we froze peripheral blood mononuclear cells obtained at selected timepoints for batched experimental and exploratory flow cytometric analysis after the 12-month endpoint. Analysis was done with the LSR II flow cytometer (BD Biosciences, San Jose, CA, USA) at Benaroya Research Institute (Seattle, WA, USA). Manual sequential gating was done in Flowjo (TreeStar Inc, Ashland, OR). The appendix shows details of antibody panel configurations and definitions of T-cell subpopulations.

Statistical analysis

The primary endpoint was the change in the mean 2 h C-peptide AUC from baseline to 12 months, adjusted for the baseline C-peptide response. Prespecified secondary outcomes were the change in mean 4 h C-peptide AUC from baseline to 12 months, changes of mean 2 h C-peptide AUC over time to month 12, insulin use at month 12, hypoglycaemic events, HbA_{1c} concentration at month 12, and frequency and severity of adverse events in the alefacept group versus placebo group.

Exploratory endpoints included metabolic assessments over the duration of the study; namely, the proportion of patients who were exogenous insulin free (for ≥3 months) with HbA_{1c} lower than 6·5% at 12 months, the proportion of patients with insulin requirements of less than 0·5 units per kg at 12 months, and immunological

	Alefacept (N=33)	Placebo (N=16)
Age (years)		
n	33	16
Mean (SD)	20·30 (6·410)	19·50 (6·154)
Median (min–max)	18·0 (12·0–34·0)	17·5 (13·0–32·0)
Age group		
12–15 years	6 (18%)	6 (38%)
16–35 years	27 (82%)	10 (62%)
Sex		
Female	16 (48%)	4 (25%)
Primary race		
White	32 (97%)	16 (100%)
Other	1 (3%)	0
Ethnic origin		
Not hispanic or latino	30 (91%)	15 (94%)
Hispanic or unknown	3 (9%)	1 (6%)
Height (cm)		
n	30	14
Mean (SD)	170·65 (12·50)	175·13 (11·32)
Median (min–max)	170·6 (144·3–191·5)	174·1 (152·0–190·3)
Weight (kg)		
n	33	16
Mean (SD)	69·16 (20·89)	68·46 (14·99)
Median (min–max)	65·3 (38·3–123·0)	67·0 (37·7–92·1)
BMI (kg/m²)		
n	30	14
Mean (SD)	23·47 (4·97)	22·05 (4·20)
Median (min–max)	22·5 (15·6–37·4)	20·6 (16·3–32·3)
2 h C-peptide AUC (nmol/L)		
n	33	16
Mean (SD)	0·85 (0·42)	0·64 (0·22)
Median (min–max)	0·7 (0·3–1·9)	0·6 (0·2–1·1)
4 h peak C-peptide (nmol/L)		
n	33	16
Mean (SD)	1·13 (0·54)	0·88 (0·30)
Median (min–max)	1·0 (0·3–2·5)	0·9 (0·3–1·7)
HbA_{1c} (%)		
n	33	16
Mean (SD)	7·18 (1·46)	7·13 (1·51)
Median (min–max)	7·2 (4·8–12·2)	6·3 (5·7–11·4)
Insulin use (units per kg per day)		
n	32	14
Mean (SD)	0·33 (0·20)	0·29 (0·17)
Median (min–max)	0·3 (0·0–0·8)	0·3 (0·0–0·7)

Data are number of patients (%) unless otherwise indicated. AUC=area under the curve.

Table 1: **Baseline demographics and laboratory characteristics**

See Online for appendix

assessments (lymphocyte subsets by flow) from baseline to 12 months.

We included all randomised patients who received any dose of study treatment in the intention-to-treat analysis for the primary endpoint. We imputed per-protocol, missing month-12 (but not month-6) 2 h C-peptide AUC data as described in the appendix. For the primary inferential analysis on the primary endpoint, we transformed C-peptide AUC values to ln(AUC+1). To compare treatment groups, we fitted 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. We based adjusted means on models fit to untransformed AUC values.

We undertook sensitivity analyses for the 2 h C-peptide AUC and secondary analyses on the 4 h C-peptide AUC using the methods described for the primary endpoint (appendix); we imputed missing 4 h C-peptide AUC values as for the primary endpoint. We based secondary inferential analyses on HbA_{1c} and insulin use on ANCOVA models at every timepoint with adjustment for baseline levels. We used Fisher's exact test to compare the number of patients who were insulin-independent and who had a hypoglycaemic event at month 12. We log-transformed flow cytometry data, and analysed them by repeated measures ANOVA. We calculated p values to compare the differences of least squares means between treatment groups at every visit. For any secondary and exploratory analyses, we did not make corrections for multiple comparisons. We used SAS version 9.2 for all data analyses.

We assumed the 12-month geometric mean 2 h C-peptide AUC in the control group to be 0·384 nmol/L.[23] After transformation, the ln(AUC+1) value in the control group was ln(0·384+1)=0·325 with root mean square error (RMSE)=0·154. We assumed the RMSE would be the same in the control and active groups. With a randomisation of a 2:1 ratio and a two-sided *t* test with a significance level of 5%, a sample size of 66 provided 85% power to detect a 50% improvement of alefacept over control, allowing for a drop-out rate of 10%. Enrolment was halted at 49 individuals after the manufacturer voluntarily withdrew alefacept from the US market.[24] Under the same assumptions, power dropped to 80% to detect a 55% improvement. This study is registered with ClinicalTrials.gov, number NCT00965458.

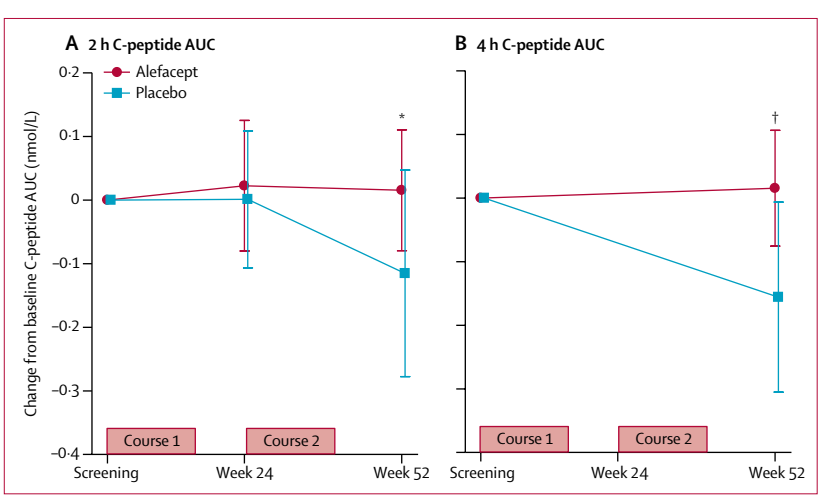

Figure 2: **Population means of change in stimulated C-peptide AUC mean from baseline to 12 months for participants assigned to alefacept and placebo**
(A) 2 h AUCs (primary endpoint). (B) 4 h AUCs (secondary endpoint). Bars represent 95% CIs. We calculated p values using an analysis of covariance with baseline ln(AUC+1) value as a covariate. AUC=area under the curve. *p=0·065. †p=0·019.

Role of the funding source

The Immune Tolerance Network, supported partly by the National Institute of Allergy and Infectious Diseases (NIAID) and the National Institute of Diabetes and Digestive and Kidney Disease (NIDDK) of the US National Institutes of Health (NIH) and Juvenile Diabetes Research Foundation (JDRF), was responsible for study design, data collection, analysis, and decision to submit the report for publication. Astellas Pharma US (Northbrook, IL, USA) provided drug for this study and was not involved in the development, design or implementation of the trial or the interpretation of the results. The writing team had full access to all of the data in the study and had final responsibility for the decision to submit for publication.

Results

Between March 4, 2011, and March 27, 2012, 73 individuals were screened, assessed for eligibility, and enrolled into the trial (figure 1). Final enrolment was curtailed at 49 individuals because of a voluntary withdrawal of alefacept by the manufacturer on Dec 15, 2011.[24] 33 patients were assigned to receive alefacept and 16 to receive placebo. Demographic and baseline characteristics were similar between the alefacept and placebo groups (table 1), with the exception of peak and 2 h AUC C-peptide, which tended to be higher in the alefacept group. The last patient completed the 12-month follow-up in March, 2013.

The alefacept group had a mean increase of 0·015 nmol/L (95% CI −0·080 to 0·110) in the 2 h C-peptide AUC at 12 months, whereas the placebo group had a mean decrease of 0·115 nmol/L (−0·278 to 0·047; figure 2A). After adjustment for baseline C-peptide, the difference between treatment groups was not significant (p=0·065). Secondary analyses included sex and age as covariates (no significant effect) and three sensitivity analyses done on the primary endpoint: no imputation, observed data only (n=42, p=0·183); optimistic imputation (p=0·018); and conservative imputation (p=0·208, appendix). Seven patients in the intention-to-treat population did not have an MMTT at month 12 (three in the alefacept group, four in the placebo group).

With respect to the secondary outcomes, analysis of the change in mean 4 h C-peptide AUC from baseline to

month 12 showed that the alefacept group had a mean increase of 0·015 nmol/L (95% CI –0·076 to 0·106) versus a decrease of –0·156 nmol/L (–0·305 to –0·006) in the placebo group, which was significant after adjusting for baseline C-peptide (figure 2B; p=0·019). Both groups achieved good glycaemic control, with mean HbA_{1c} at 12 months of 6·9% for the alefacept group and 7·2% for the placebo group (p=0·75; figure 3A). Insulin use at 12 months was higher in the placebo group than in the alefacept group (0·48 units per kg per day for placebo *vs* 0·36 units per kg per day for alefacept; p=0·02; figure 3B). Additionally, within the alefacept group, insulin use at 12 months did not increase significantly from baseline (+0·02 units per kg per day, p=0·41), whereas in the placebo group, insulin use increased at 12 months (+0·17 units per kg per day, p=0·02; figure 3B). In the alefacept group, 28 (85%) participants reported 359 major hypoglycaemic events (defined as blood glucose <55 mg/dL), which was significantly fewer than those in the placebo group, for which 15 (94%) participants reported 277 events (mean of 10·9 events per person per year in the alefacept group *vs* 17·3 events per person per year in the placebo group; p<0·0001; table 2).

The study is ongoing and participants remain masked to treatment allocation. No serious adverse events were reported and all patients had at least one adverse event. In the alefacept group, 29 (88%) participants had an adverse event related to study drug compared with 15 (94%) participants in the placebo group (table 2). In the alefacept group, 14 (42%) participants had at least one grade 3 or 4 adverse event (or both), compared with nine (56%) participants in the placebo group; no deaths occurred. Injection site reactions, infections, and asymptomatic hepatic injury (elevated transaminases) were similar between the alefacept and placebo groups. Two patients had suspected EBV infection or reactivation, leading to treatment interruption in one case and discontinuation in the other; treatment assignments remained masked. There were no other opportunistic infections. No patients had cytokine release syndrome or required hospital admission. In the alefacept group, five patients (15%) had transient decreases in CD4 counts to fewer than 250 cells per µL, resulting in temporary dose holding in two patients; this was not noted in the placebo group.

Prespecified exploratory outcomes were the proportion of patients who did not receive exogenous insulin for at least 3 months with HbA_{1c} lower than 6·5% at week 52 (3·4% [one of 29] in alefacept group *vs* 0% [none of 12] in the placebo group, p=1·0) and the proportion of patients who achieved a persistent reduction (at least 3 months) in insulin dose to less than 0·5 units per kg per day at week 52 (48% [14 of 29] in the alefacept group and 27% [three of 11] in the placebo group, p=0·297). A post-hoc analysis was the proportion of patients who achieved glycaemic control as defined by the American Diabetes Association (<7·5% for patients aged 13–19 years and <7·0% for patients aged older than 19 years): at 12 months, 65·5% (19 of 29) in the alefacept group compared with 58·3% (seven of 12) in the placebo group

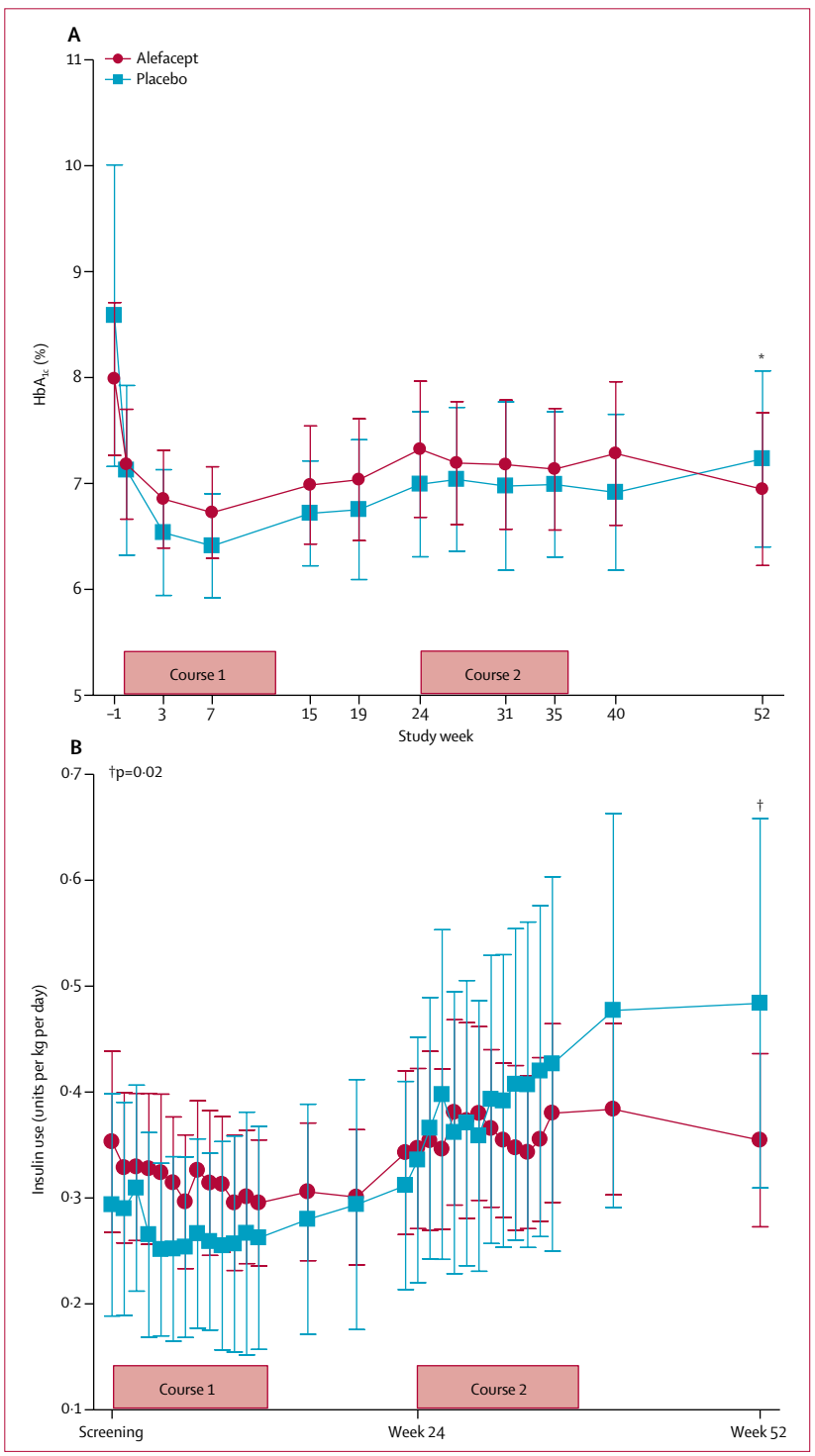

Figure 3: **HbA_{1c} levels and exogenous insulin use in the alefacept and placebo groups**
(A) HbA_{1c} levels (%). (B) Exogenous insulin use (units per kg per day). Bars represent the 95% CIs. Lines connect the mean values across visits for each treatment group. We calculated p values for the change from baseline for both HbA_{1c} and insulin use at week 52 using an analysis of covariance with baseline level as a covariate. *p=0·75. †p=0·02.

	Alefacept		Placebo		Total	
	Participants*† (N=33)	Events‡	Participants*† (N=16)	Events ‡	Participants*† (N=49)	Events‡
Serious adverse events	0	0	0	0	0	0
Serious adverse events related to study drug	0	0	0	0	0	0
Adverse events	33 (100%)	751 (100%)	16 (100%)	433 (100%)	49 (100%)	1184 (100%)
Adverse event related to study drug	29 (88%)	266 (35%)	15 (94%)	139 (32%)	44 (90%)	405 (34%)
Adverse events by severity						
Grade 1	31 (94%)	316 (42%)	15 (94%)	134 (31%)	46 (94%)	450 (38%)
Grade 2	30 (91%)	395 (53%)	16 (100%)	279 (64%)	46 (94%)	674 (57%)
Grade 3	13 (39%)	35 (5%)	9 (56%)	18 (4%)	22 (45%)	53 (4%)
Grade 4	3 (9%)	3 (<1%)	2 (12%)	2 (<1%)	5 (10%)	5 (<1%)
Grade 5	0	0	0	0	0	0
Injection reactions	6 (18%)	18 (2%)	4 (25%)	8 (2%)	10 (20%)	26 (2%)
Hypersensitivity reactions	..	..	..	..	1 (2%)	1 (<1%)
Lymphopenia	..	..	..	..	3 (6%)	8 (<1%)
Infection with EBV, cytomegalovirus, or tuberculosis	1 (3%)	..	1 (6%)	..	2 (4%)	3 (<1%)
Infection	25 (76%)	89 (12%)	11 (69%)	35 (8%)	36 (73%)	124 (10%)
Asymptomatic hepatic injury	6 (18%)	8 (1%)	3 (19%)	5 (1%)	9 (18%)	13 (1%)
Major hypoglycaemic event	28 (85%)	359 (48%)	15 (94%)	277 (64%)	43 (88%)	636 (54%)
Pregnancy	..	..	..	..	1 (2%)	1 (<1%)
Deaths	0		0		0	

Data are number (%). EBV=Epstein-Barr virus. *Percentages for the number of patients with adverse events or serious adverse events are based on the number of individuals randomly assigned to study groups. †Participants who had one or more adverse event(s) are counted only once within each row. ‡Percentages for the number of adverse events are based on the total number of adverse events.

Table 2: **Adverse events by grade and type**

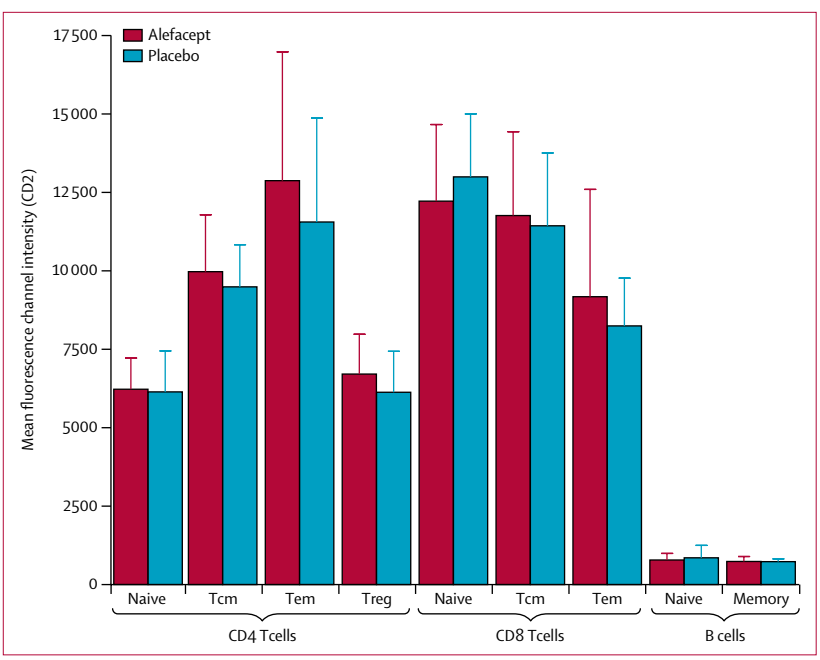

Figure 4: **CD2 expression on lymphocyte subpopulations**
We analysed frozen peripheral blood mononuclear cell obtained at baseline for the mean fluorescence intensity of CD2 by flow cytometry. Values are mean (SD). We defined lymphocyte subpopulations as follows: CD4 naive: $CD3^+CD4^+FoxP3^-CD127^{hi}CCR7^+CD45RA^+$; CD4 central memory T (Tcm): $CD3^+CD4^+FoxP3^-CD127^{hi}CCR7^+CD45RA^-$; CD4 effector memory T (Tem): $CD3^+CD4^+FoxP3^-CD127^{hi}CCR7^-CD45RA^-$; regulatory T cells (Treg): $CD3^+CD4^+FoxP3^+CD127^{lo}$; CD8 naive: $CD3^+CD8^+CCR7^+CD45RA^+$; CD8 Tcm: $CD3^+CD8^+CCR7^+CD45RA^-$; CD8 Tem: $CD3^+CD8^+CCR7^-CD45RA^-$; naive B cells: $CD19^+CD27^-$; memory B cells: $CD19^+CD27^+$.

(p=0·730) had achieved glycaemic control. Analyses of these exploratory endpoints were done with only observed data; we did not impute missing data.

In the T-cell compartment at baseline (before treatment), CD2 expression intensity was highest on the CD4 Tem cells, CD8 naive cells, and CD8 Tcm cells, intermediate on CD4 Tcm cells and CD8 Tem cells, and lowest on CD4 naive and Tregs (figure 4). Total white blood cell counts remained unchanged (figure 5A) but total lymphocyte, CD4, and CD8 cell counts showed slight decreases during the first and second course of treatment in the alefacept group, which largely rebounded to baseline levels by 52 weeks (figure 5B–D). In the CD4 T-cell compartment, the percentage of naive T (Tn) cells increased from baseline by about 25% at week 11 in the alefacept group and remained raised at all later timepoints (p=0·0003 for overall difference; figure 6A). By contrast, CD4 Tcm cells decreased by about 25–30% (p<0·0001; figure 6B) and CD4 Tem cells decreased by 40–60% (p=0·0002; figure 6C) at all timepoints after baseline in the alefacept group. By comparison, CD8 Tn cells decreased by about 25% only at week 11 (p=0·034; figure 6D), Tcm cells decreased by about 35% at all timepoints after baseline (p=0·0003 for overall difference; figure 6E), and Tem cells (defined as $CD45RO^+CCR7^-$ or $CD45RA^+CCR7^-$) did not change (figure 6F and data not shown, respectively) in the alefacept group. Importantly, alefacept treatment did not alter the frequency of Tregs at any timepoint compared with placebo (figure 6G).

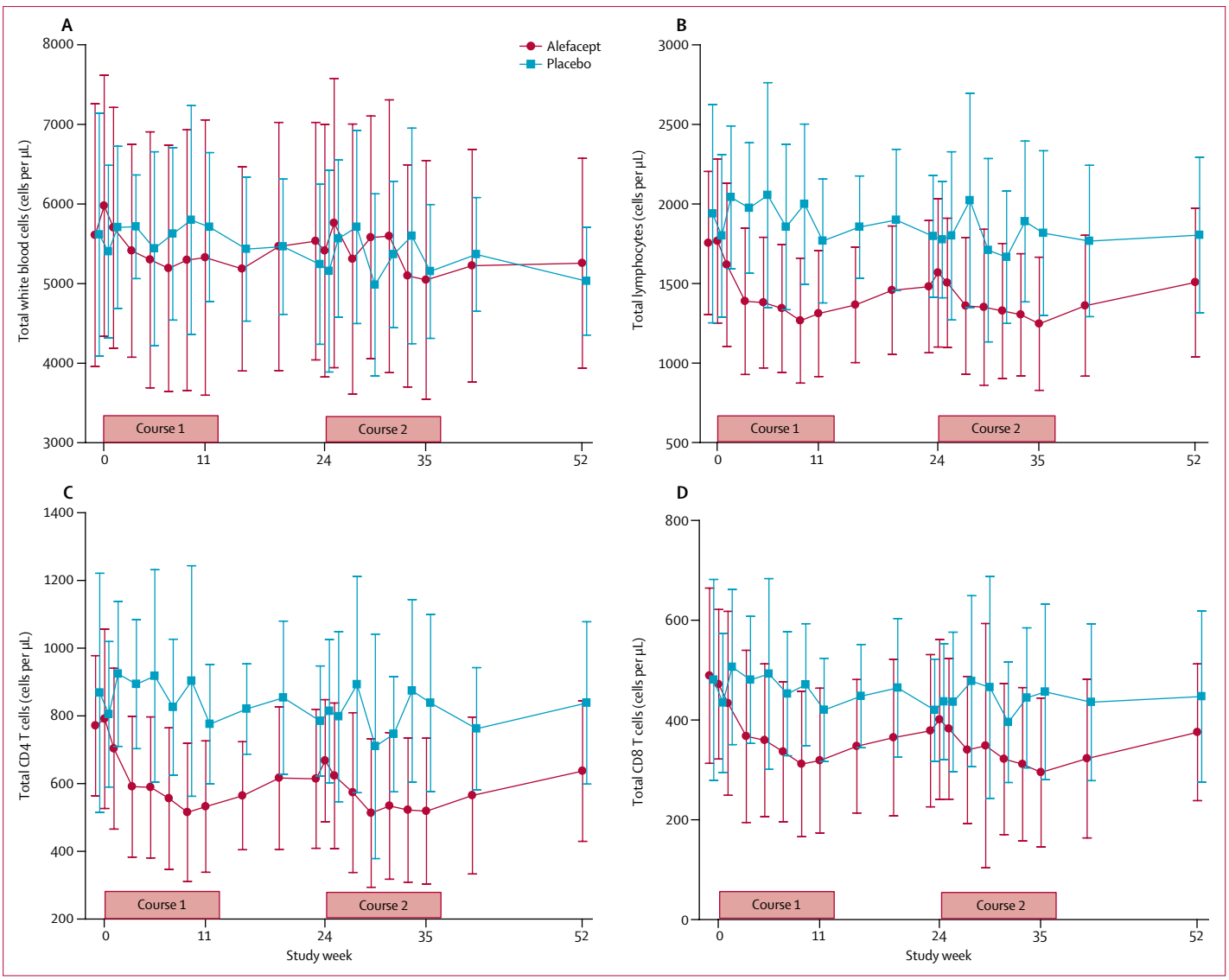

Figure 5: **Absolute cell counts**
Data are mean (SD). (A) White blood cells. (B) Total lymphocytes. (C) CD4 T cells. (D) CD8 T cells. Whole blood was analysed real-time by flow cytometry in a central clinical laboratory.

The changes in T-cell subsets were also reflected in the ratios of Treg to naive and memory T cells (figure 7). Importantly, alefacept treatment resulted in significant increases in the Treg/CD4 Tcm and Treg/CD4 Tem ratios at all timepoints after baseline (p=0·0007 and p=0·0001 for overall difference; figure 7), and an increased Treg/CD8 Tcm ratio (p=0.0003; figure 7; appendix). Thus, with the exception of CD8 Tem cells, the cells that were most affected by alefacept were those that expressed higher levels of CD2 (Tcm and Tem cells) with sparing of Tn and Treg populations.

Discussion

Alefacept targets memory CD4 and CD8 T cells, which are believed to be important in β-cell destruction in type 1 diabetes. Although we did not meet our primary endpoint at 12 months in the T1DAL trial, we did meet three secondary endpoints, suggesting that a memory T cell-targeting agent such as alefacept might be able to assist in preserving residual β cells present at the time of initial diagnosis.

Failure to meet the primary endpoint (change in 2 h C-peptide AUC at 12 months) might have resulted, partly, from reduced power after the planned enrolment target of 66 individuals was curtailed at 49 patients following voluntary withdrawal of alefacept by the manufacturer.[24] By contrast with the 2 h AUC, the 4 h C-peptide AUC was significantly different at 12 months between the treatment groups. This finding might reflect the ability of the 4 h test interval to provide more

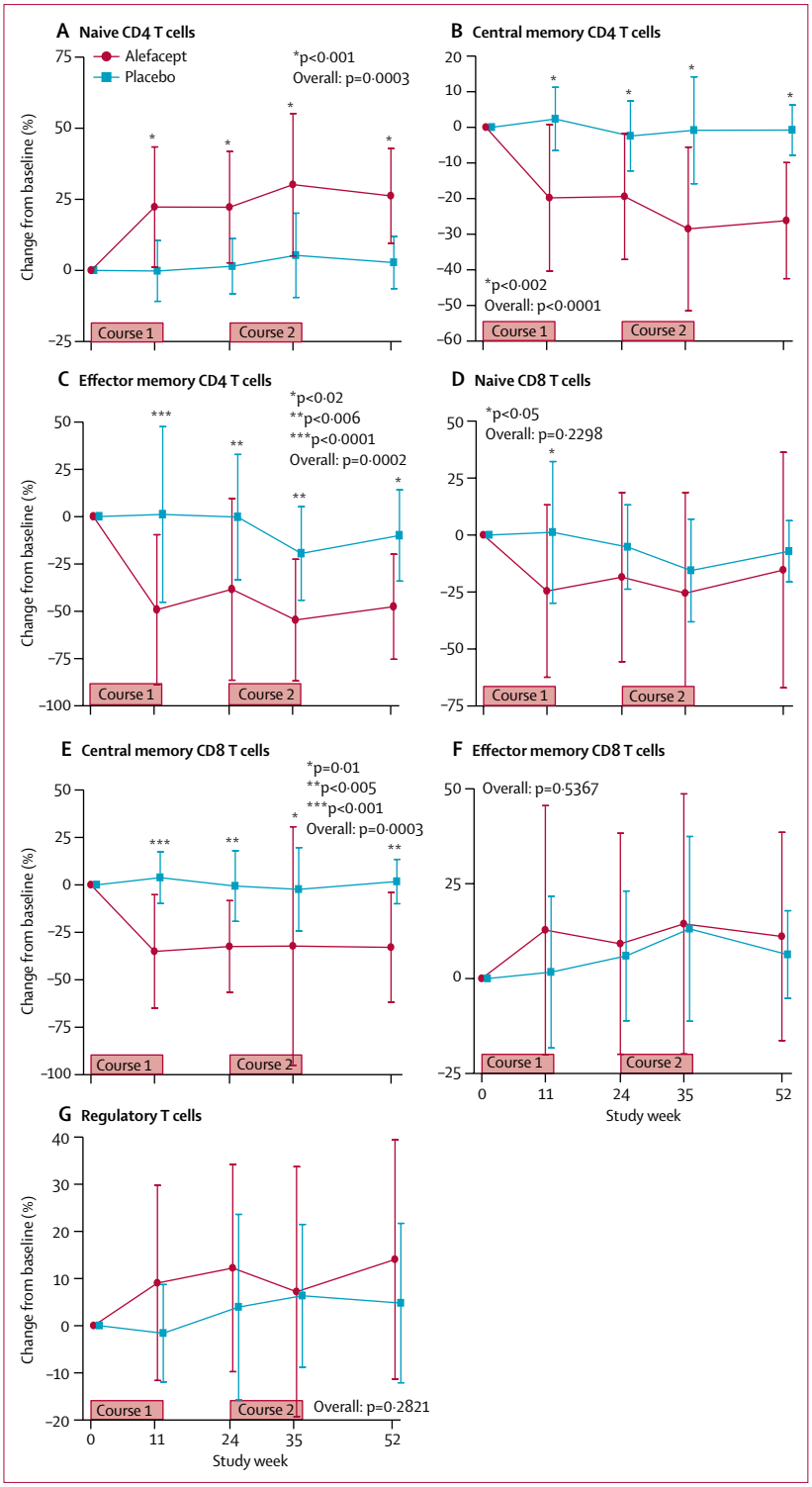

Figure 6: **Change in lymphocyte populations over time**
Frozen peripheral blood mononuclear cells collected at baseline and weeks 11, 24, 35, and 52 were analysed using flow cytometry. Proportions of subpopulations (defined in figure 4) from parent populations were determined and standardised to baseline values. (A, B, C) $CD4^+$ naive T (Tn), central memory T (Tcm), and effector memory T (Tem) cells. (D, E, F) $CD8^+$ Tn, Tcm, and Tem cells. (G) $CD4^+$ Treg. Antibody panels and gating strategies are detailed in the appendix. Values are mean (SD).

complete data on the insulin response after a mixed meal, allowing for better discrimination between treatment groups. It is unclear whether the 2 h or 4 h C-peptide AUC provides more relevant data for type 1 diabetes intervention trials,[25] but the 4 h AUC was chosen as the primary endpoint in the AbATE study.[11] In addition to the 4 h C-peptide AUC data, our findings that insulin use and hypoglycaemic events were also reduced support the notion that alefacept treatment might have resulted in relative preservation of β-cell function at 12 months compared with placebo. However, because of the significant variability in the rate of β-cell decrease during the first year after diagnosis,[26] longer-term follow-up to 24 months will help to better assess these findings.

The drug was generally well tolerated. In about 15% of alefacept-treated patients there were transient reductions in CD4 counts to fewer than 250 cells per μL. Compared with placebo, we noted no significant differences in injection site reactions, infections, or other adverse events and, importantly, no cytokine release syndrome or immune complex reactions as have been noted in studies in which other antibody-based drugs were used.[11,27] The observed safety profile for alefacept in patients with type 1 diabetes is similar to the much larger experience for this drug in psoriasis.[19]

CD2 expression at baseline was highest on CD4 Tem cells and CD8 Tn cells and Tcm cells, followed by CD4 Tcm cells and CD8 Tem cells, and then CD4 Tn cells and Tregs. Depletion of T-cell subsets with alefacept was associated with CD2 intensity, with the exception of CD8 Tem cells. Thus, Tregs and CD8 and CD4 Tn cells were largely spared during alefacept therapy, whereas by week 11, CD4 Tem and Tcm populations decreased by 25–50% and remained decreased through 52 weeks. CD8 Tcm cells were also significantly decreased but CD8 Tem cells were unchanged, which was unexpected. By contrast with our results, alefacept treatment decreased CD8 Tem cells in psoriasis;[14,20] differences in the study population (type 1 diabetes *vs* psoriasis, younger *vs* older patients) might play a part in this discrepancy. We noted more variability in CD8 Tem responses in alefacept-treated patients than in CD4 Tem responses (appendix) and it is possible that CD8 Tem depletion was limited to clinical responders; a responder analysis is planned once all patients have reached the 24 month endpoint. Finally, in addition to facilitating depletion, alefacept is thought to impair CD2-mediated costimulation of T cells,[14,15,20] and it is possible that CD8 Tem cells were functionally inhibited via blocking of this process. Additional analyses are required to better understand the effects of alefacept on CD8 Tem cells in patients with type 1 diabetes.

Although a positive effect on preserving β-cell function by alefacept might be explained by depletion of highly pathogenic effector and memory T cells, an important additional finding in this trial was that Tregs were spared with alefacept. Thus, combined with the decline in most memory T-cell subpopulations, the proportions of Treg

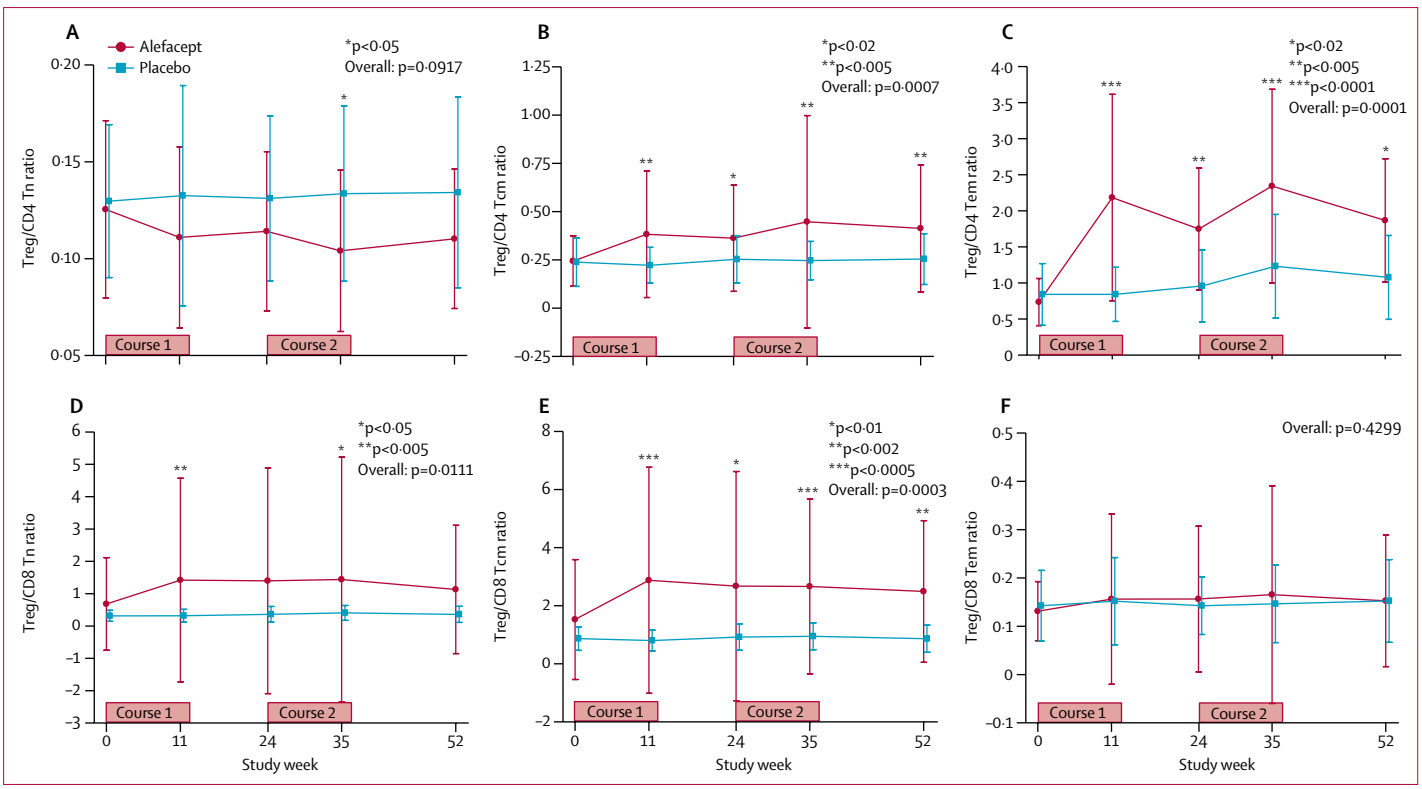

Figure 7: **Ratios of Treg to naive and memory T cells**
Relative numbers of Treg and CD4⁺ and CD8⁺ naive T (Tn), central memory T (Tcm), and effector memory T (Tem) cells (subpopulations as defined in figure 4) were determined by flow cytometry (using the gating strategies described in the appendix), and the ratios of Tregs to the indicated T-cell subpopulations calculated. (A, B, C) Treg/CD4 Tn, Treg/CD4 Tcm, and Treg/CD4 Tem. (D, E, F) Treg/CD8 Tn, Treg/CD8 Tcm, and Treg/CD8 Tem. Values are mean (SD).

per memory T cell were improved. It is possible that the memory populations have been brought under an absolute or functional threshold and are now susceptible to endogenous regulation. By targeting the most pathogenic T cells, while sparing Tregs, alefacept might contribute to reestablishing a state of immune tolerance—which could explain the finding that a proportion of patients with psoriasis given alefacept go into long-term off-therapy remission.[16–18]

The T1DAL trial is the first demonstration, to our knowledge, that it is possible to specifically and effectively deplete memory T cells in new-onset type 1 diabetes, including CD4 Tem cells (panel). This could not be achieved in a study[27] assessing antithymocyte globulin in new-onset type 1 diabetes: despite robust depletion of Tn and Tcm cells, Tem cells were resistant to depletion.[27] Further, Tregs were also strongly depleted by antithymocyte globulin therapy, leading to an unfavourable Treg/Tem ratio.[27] In the T1DAL trial, we have noted the reverse: depletion of Tem and Tcm cells, preservation of Tregs, and an improvement in Treg/memory T cell ratios. Therapies that result in a favourable balance between Tregs and effector T cells are effective in mitigating autoimmunity and result in long-term protection from disease in preclinical models of type 1 diabetes.[28,29] We propose that a targeted depletion of memory T cells, including Tem cells, is an important goal in immune interventions for this disorder, and that an increase in the Treg/memory T cell

Panel: **Research in context**

Systematic review

We searched the PubMed database for articles published up to Aug 1, 2013, with the search terms "immune intervention" AND "type 1 diabetes", and "alefacept". A series of recent, adequately powered randomised trials showed some degree of preservation of β-cell function in patients with type 1 diabetes, as assessed by change in C-peptide secretion in response to a mixed meal tolerance test over time. These trials used anti-CD3, anti-CD20, and abatacept.[7–11] Several other recent trials, notably with anti-IL-1 therapies and with antithymocyte globulin, have failed to show clinical benefit.[12,27] So far, to our knowledge, no randomised, placebo-controlled trials have assessed efficacy of alefacept or other CD2-targeting therapies in patients with new-onset type 1 diabetes.

Interpretation

Alefacept is the first targeted biological drug assessed in patients with new-onset type 1 diabetes that significantly depleted effector and central memory T cells while preserving regulatory T cells. Although the primary endpoint was not met, several key secondary endpoints were significantly different between treatment groups, suggesting that alefacept might preserve β-cell function during the first 12 months after diagnosis. Thus, targeting memory T cells might be a useful strategy in type 1 diabetes, but longer follow-up is required to confirm the preliminary signal of efficacy observed at 12 months in the T1DAL trial.

ratio might be a useful biomarker of treatment response. However, this hypothesis needs to be tested in larger trials with alefacept as well as trials of other agents that specifically deplete memory T cells while preserving or enhancing Tregs. The T1DAL trial is an ongoing study with further assessment of endogenous insulin production planned at 18 and 24 months, as well as other secondary and exploratory endpoints. These ongoing assessments will assist in determining to what extent targeting effector and memory T cells can contribute to arresting diabetes autoimmunity and preserving residual β-cell function in patients newly diagnosed with type 1 diabetes.

Contributors

MRR served as study chair and wrote the first draft of the manuscript. Other members of the writing group included MRE, AP, LK-E, JM, MH, and DP. All authors were involved in the conduct of the trial, and the collection and review of the study data. The writing group had full access to all of the data and made the decision to publish the paper. The authors reviewed and commented on various versions of the paper, and the suggested revisions.

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Conflicts of interest

PR has received research funds, payable to the University of Texas, Southwestern, from Andromeda Biotech. The other authors declare that they have no conflicts of interest.

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Restoring immune balance in type 1 diabetes

Colin McPherson/Corbis

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Insulin replacement therapies—which include multiple daily doses of short-acting and long-acting insulin analogues, insulin pumps, and continuous glucose monitoring—have revolutionised the management of type 1 diabetes. Achieving near-normal glucose control and reduced rates of severe hypoglycaemia are feasible. However, even the most advanced insulin delivery technologies do not replace the capabilities of native β cells. Maintaining even partial β-cell function has consistently been shown to improve glucose control, and reduce the rates of secondary end-organ complications and severe hypoglycaemia. Therefore, to arrest the progression of β-cell destruction remains the ultimate target in managing this condition. But interventions that can achieve these goals need to have sustained effects without the risks of chronic immune suppression.

The immune cells that are thought to cause destruction of insulin-producing β cells reside in the effector and memory subsets of CD4 and CD8 T cells. These cells, particularly the effector memory T cells, express CD2. Therefore, a logical strategy to treat type 1 diabetes was to eliminate CD2 T cells with the LFA3 fusion molecule alefacept, which binds CD2 and results in elimination of T cells expressing this molecule. However, the effect of eliminating these T cells on the disease course was unclear until now. Completed studies with other immune modulators (monoclonal antibodies specific for CD20 [found on B cells, also postulated to be involved in β-cell destruction] or for CD3 [found on T cells]) were successful in preserving β-cell function, but diabetes antigen-specific T cells persisted.[1,2]

In *The Lancet Diabetes & Endocrinology*, Mark R Rigby and colleagues[3] present the 12 month results of a randomised, placebo-controlled, phase 2 trial of alefacept in new onset type 1 diabetes (T1DAL). The primary metabolic endpoint of the study was not met (improvement in 2 h C-peptide area under the curve [AUC] response to a mixed meal in patients treated with drug *vs* placebo). However, the investigators noted a significant improvement in the 4 h C-peptide AUC response to a mixed meal, which was a secondary endpoint. Insulin use decreased in the group receiving alefacept, as did the frequency of major hypoglycaemic events—a secondary endpoint not previously captured in type 1 diabetes clinical trials of immune modulators. Thus, despite the unfortunate selection of the 2 h AUC as the primary endpoint for the trial (the 4 h test shows the complete response since some individuals have a delayed response to the meal[4]), the evidence strongly supports clinical efficacy of this treatment strategy in the first year following diagnosis. Additionally, adverse events were infrequent and not severe. It will be important to determine for how long the differences in responses between the drug and placebo groups persist. And identification of variables that predict clinical responses to treatment, as recently reported in a trial of CD3 monoclonal antibody,[2] would help to identify those with the greatest benefit-to-risk ratio for this treatment.

Using a detailed analysis by flow cytometry, the investigators showed that the frequency of CD4 and most CD8 memory T cells (ie, CD8 effector memory cells, which are sources of interferon-γ, a cytokine thought to be involved in β-cell destruction) were reduced. But regulatory T cells, which can inhibit autoimmune responses, were spared: the ratios of regulatory T cells to effector T cells were generally increased. This finding suggests that alefacept treatment might induce immune tolerance lasting after the drug is withdrawn, which was also suggested in previous studies of this drug in patients with psoriasis who had clinical effects even after they stopped taking the drug.[5] Inducing immunological tolerance in patients with type 1 diabetes is a goal of the study sponsor, the Immune Tolerance Network.

How does this study add to recent reports of other drugs that might induce immunological tolerance that have been trialled in patients with new onset type 1 diabetes? And what might the next studies entail? Like the experience with CD3 monocolonal antibodies, alefacept seems to be successful because it restores a balance in the ratio of regulatory T cells to effector T cells.[6] Elimination of T cells alone, without sparing of regulatory T cells, as occurs in patients treated with antithymocyte globulin, was not effective in preserving β-cell function.[7] Treatment with interleukin 2 and rapamycin, which increased the numbers of CD4 regulatory T cells but also cytotoxic natural killer cells, was not successful either.[8] These natural killer cells might have accounted for the transient worsening of diabetes noted in patients treated with

this drug combination. Therefore, affecting the balance of both regulatory and effector T cells seems paramount, and might be achieved through specific targeting of effector T cells or through enhancement of the number or function of regulatory T cells. Experimentally, this strategy has been accomplished with combinations of drugs such as short-term treatment with alefacept and CTLA4-Ig, anti-interleukin-1 drugs, or diabetes-related antigens together with CD3 monoclonal antibodies.[9] In addition to these immunological strategies, drugs that can enhance the function or the number of β cells are required since it is unclear whether the loss of β cells that results in clinical disease is reparable. One example of such drugs are the GLP-1 receptor agonists, which have been effective in preclinical studies with immune modulators.[10]

These new results, together with the findings from recent trials of a CD3 monoclonal antibody,[2] are leading to mechanism-based strategies to restore the balance between those cells needed for protection against pathogens and those that maintain tolerance to self, rather than broadly eliminating immune cells. It is important to underscore these small successes since, as in other fields such as oncology and infectious diseases, the small achievements acquire greater significance when they are combined. In this regard, the withdrawal of pharmaceutical companies from this and other trials, even before the final outcomes of trials were realised, was disappointing—particularly as we move closer to finally reaching the ultimate goal: to prevent, stop, and even reverse type 1 diabetes.

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I declare that I have no conflicts of interest.

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