Teplizumab Preserves C-Peptide in Recent-Onset Type 1 Diabetes

Two-Year Results From the Randomized, Placebo-Controlled Protégé Trial

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Protégé was a phase 3, randomized, double-blind, parallel, placebo-controlled 2-year study of three intravenous teplizumab dosing regimens, administered daily for 14 days at baseline and again after 26 weeks, in new-onset type 1 diabetes. We sought to determine efficacy and safety of teplizumab immunotherapy at 2 years and to identify characteristics associated with therapeutic response. Of 516 randomized patients, 513 were treated, and 462 completed 2 years of follow-up. Teplizumab (14-day full-dose) reduced the loss of C-peptide mean area under the curve (AUC), a prespecified secondary end point, at 2 years versus placebo. In analyses of prespecified and post hoc subsets at entry, U.S. residents, patients with C-peptide mean AUC >0.2 nmol/L, those randomized ≤6 weeks after diagnosis, HbA_{1c} <7.5% (58 mmol/mol), insulin use <0.4 units/kg/day, and 8–17 years of age each had greater teplizumab-associated C-peptide preservation than their counterparts. Exogenous insulin needs tended to be reduced versus placebo. Antidrug antibodies developed in some patients, without apparent change in drug efficacy. No new safety or tolerability issues were observed during year 2. In summary, anti-CD3 therapy reduced C-peptide loss 2 years after diagnosis using a tolerable dose. ***Diabetes*** **62:3901–3908, 2013**

Immunotherapy that directly inhibits β-cell destruction is an unfulfilled need for treatment of autoimmune type 1 diabetes. Although it may eventually be useful in prediabetes, treatment at clinical onset is an excellent opportunity when patients are easily identified and functional β-cell mass remains (1). Preservation of residual β-cell function, represented by higher levels of C-peptide, facilitates better glycemic control to lessen retinopathy, nephropathy, hypoglycemia, and ketoacidosis (2–4). Immunotherapy given at diagnosis aims to prolong and augment this effect by preventing further β-cell death and possibly also by enabling living β-cells to recover function after resolution of inflammation (5). Clinical trials of different agents have had modest success in this regard, but treatment responses have often waned within 2 years (6–8).

Teplizumab is a nonactivating, Fc-modified, anti-CD3 monoclonal antibody thought to attenuate activated autoreactive T cells mediating β-cell death. These T cells disappear from the peripheral circulation during immunotherapy but return within weeks after stopping treatment (9). Preclinical and clinical studies suggest that the drug may induce regulatory T-cell activity, suggesting augmented immune tolerance (10).

Protégé was a large, randomized, placebo-controlled, double-blinded trial of immunotherapy in type 1 diabetes (11). Recently diagnosed patients (8–35 years of age) were randomized to receive daily infusions of placebo or one of three teplizumab regimens at baseline and at 6 months. The primary outcome, a composite of insulin <0.5 units/kg/day and HbA_{1c} <6.5% (48 mmol/mol) at year 1, had not been validated previously and did not achieve statistical significance. In exploratory analyses, a significant improvement in area under the curve (AUC) mean C-peptide during a 4-h mixed-meal tolerance test (MMTT) was observed in the group treated with a full-dose 14-day course. In certain prespecified subgroups, the AUC mean C-peptide differences versus placebo appeared to be most pronounced in recently diagnosed patients, patients in the U.S., and in younger patients. The drug was generally well tolerated.

A recent study reported that teplizumab treatment reduced β-cell death at 1 year, but the differences versus placebo were not significant earlier, at 6 months (12). The acute (i.e., within 1 year) effects of immunotherapy on β-cell function may not occur through the same mechanisms as longer-term effects that have greater clinical importance. Improvement in C-peptide responses may be seen in type 1 diabetes trials, even with therapies that do not affect immune responses, through mechanisms that may involve recovery of dysfunctional β-cells when inflammation is acutely resolved (5,13). To be of value, a lasting effect on β-cell function and survival is needed.

The objective of this report is to characterize the efficacy and safety of teplizumab over 2 years and identify characteristics associated with response to therapy. Regarding efficacy, we focus on the 14-day full-dose regimen

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See accompanying commentary, p. 3669.

that was administered versus placebo, because at 1 year, efficacy was seen in the 14-day full-dose arm but not in the reduced-dose or curtailed-dose arms (11). Emphasis is given to AUC mean C-peptide because this has become the preferred measure of efficacy in type 1 diabetes immunotherapy (14). To explore the potential implications for dosing in future studies, we also describe the pharmacokinetics and pharmacodynamics of teplizumab, the effect of antidrug antibodies, and the safety profiles of all three dosing regimens.

RESEARCH DESIGN AND METHODS

Details of the trial methodology were published previously (11) and are summarized briefly here and in the Supplementary Data online. Participation was restricted to patients with type 1 diabetes diagnosed according to American Diabetes Association (ADA) criteria (15) within the prior 12 weeks and who required injected insulin therapy. Inclusion also required detectable levels of fasting or stimulated C-peptide and autoantibodies to one or more standard islet autoantigens. Exclusion criteria focused on medical disorders, such as active infections, that might confound results or interfere with safe trial completion. The research protocol was approved by institutional review boards, and all participants or guardians gave written informed consent.

Patients were randomly assigned (2:1:1:1) to one of four parallel treatment groups, with an escalating dose, 14-day course of daily intravenous treatment starting at baseline, and another 14-day course at week 26. For each treatment course, the 14-day full-dose group (n = 209) received a total cumulative teplizumab dose of ~9,034 μg/m^2, the 14-day low-dose group (n = 102) received a total of ~2,985 μg/m^2, the 6-day full-dose group received a total of ~2,426 μg/m^2 over 6 days, followed by 8 days of placebo, and the placebo group (n = 99) received 14 days of placebo infusions. Randomization was stratified by age-group (8–11, 12–17, and 18–35 years) and by country. Dosing was double-blind (patients and study personnel) to conceal allocation. Patients received a nonsteroidal, anti-inflammatory drug (e.g., ibuprofen) and/or antihistamine (e.g., diphenhydramine) to minimize adverse events during each treatment cycle.

Intensive diabetes care was provided for all patients. Investigators were instructed to aggressively treat diabetes to a target HbA_{1c} of ≤6.5% and to maintain an insulin dose of ≥0.25 units/kg/day, but insulin adjustment algorithms were not prespecified. Patients used diary cards to record insulin use at screening and for 3 days before each visit at days 91, 140, 273, 364, 448, 546, 616, and 728. Use of agents that might affect islet growth, endogenous insulin secretion, insulin sensitivity, or immune function was not permitted during the study.

HbA_{1c} was measured, and a 4-h MMTT was performed at a screening visit and on days 140, 364, 546, and 728 (HbA_{1c} was also measured on days 273, 448, and 616), and the total AUC mean C-peptide during the MMTT was then calculated (1). After interim analyses determined that the primary end point at 1 year was not met, patients not yet at day 728 continued follow-up, but AUC mean C-peptide, flow cytometry, and anti-drug antibodies were no longer measured to reduce the burden on participants and cost. Anti-cytomegalovirus (CMV) IgG, anti–Epstein-Barr virus (EBV) IgG, and IgM titers were measured at screening and days 28, 91, 140, 210, 273, 364, and 728 to evaluate seropositivity for EBV and CMV; semiquantitative PCR was used to measure viral load for seropositive patients.

Adverse events, including clinically significant hypoglycemia, and abnormal laboratory values were reported by investigators, coded using the Medical Dictionary for Regulatory Activities, and graded using the Common Terminology Criteria for Adverse Events (version 3.0).

Statistical analysis. Changes from baseline for AUC mean C-peptide, a prespecified secondary end point, HbA_{1c}, and other measures in teplizumab groups were compared with the placebo group using mixed-model repeated-measures analysis (MMRM) models, adjusted for age-group and baseline values. A Mantel-Haenszel test stratified by age-group (8–11, 12–17, and 18–35 years) or Fisher exact test was used for exploratory efficacy analyses of dichotomous outcomes. Two-sided testing was done at an α level of 0.05. Subset analyses compared the 14-day full-dose regimen with placebo; age-groups, regions, and time from diagnosis to randomization were prespecified subsets (11). These analyses were done for hypothesis generation because the primary outcome was not significant at 1 year; therefore, we did not adjust for multiple comparisons. Similar analyses were conducted for the other treatment groups; however, no meaningful findings were observed, so the results are not presented.

The 1-year analysis used a nonparametric analysis and reported median values for AUC mean C-peptide because the distribution was not normal (11); a last observation carried forward analysis was used at the request of regulators, because too few time points existed for a longitudinal analysis at 1 year. For the current report at 2 years, longitudinal analysis (MMRM) was used instead of last observation carried forward. AUC mean C-peptide change from baseline was calculated using [ln(AUC mean C-peptide$_{\text{Day } x}$ + 1) − ln(AUC mean C-peptide$_{\text{Baseline}}$ + 1)]. The adjusted mean values reported here reflect the logarithm values after adjustment for the covariates (listed above). Consequently, the adjusted means and statistical significance reported here differ from the unadjusted medians and P values reported earlier at 1 year (11). The overall mean of insulin use was calculated for each group using all values after baseline.

Safety and tolerability through year 2 were assessed primarily by summarizing adverse experiences, serious adverse experiences (life-threatening, death, persistent disability, or hospitalization) and adverse experiences of special interest (acute mononucleosis-like illness, infection requiring intravenous antibiotic treatment, demyelinating disease, lymphoma or other malignancy, clinically significant hypoglycemia requiring assistance, grade 3 liver function abnormalities, grade 3 thrombocytopenia, grade 3 neutropenia; and through year 1: rash, grade 4 allergic/hypersensitivity, and grade 4 cytokine-release syndrome).

RESULTS

A high proportion (90% overall) of randomized patients completed 2 years of follow-up (Supplementary Table 1). In the 14-day full-dose group at 2 years, 89% had HbA_{1c} measured and insulin therapy recorded, but only 64% had year 2 AUC mean C-peptide measurements because these were discontinued after final analysis of year 1 data (see RESEARCH DESIGN AND METHODS) (Fig. 1*A*). Baseline characteristics, including diabetes measures (autoantibodies, C-peptide, insulin dose, and HbA_{1c}), were balanced across treatment groups but not geographic regions. In particular, patients in India had less frequent ICA512 autoantibodies, higher HbA_{1c}, higher insulin use, and lower AUC mean C-peptide, the latter suggesting more advanced disease on average than other regions (11).

HbA_{1c}. Intensive diabetes care with insulin was provided for all patients. There was no significant difference in HbA_{1c} change from baseline comparing the teplizumab and placebo groups over the 2-year study at any time point (see Table 1), suggesting that glycemic control was maintained to a comparable extent across treatment groups.

Efficacy measures during 2 years of follow-up

AUC mean C-peptide. Teplizumab treatment (14-day full-dose) reduced the loss of AUC mean C-peptide at 2 years versus placebo (P = 0.027; Fig. 1*A* and Table 1). The adjusted mean differences in AUC mean C-peptide change from baseline at 2 years favored the 14-day full-dose regimen versus placebo in analyses of all patients, patients in the U.S., and patients randomized ≤6 weeks after diagnosis (Figs. 1*B* and *C* and 2 and Table 2). The results in these prespecified subsets suggested larger treatment effects in patients with characteristics consistent with less advanced disease. Therefore, additional analyses were conducted to explore the treatment effects in other patient subsets at entry defined by *1*) HbA_{1c}<7.5%, the ADA recommendation for type 1 diabetes control in children 13–19 years of age (16); *2*) insulin use <0.4 units/kg/day, the lower limit of typical type 1 diabetes insulin needs (17); *3*) AUC mean C-peptide >0.65 nmol/L, the mean at baseline (11); and *4*) AUC mean C-peptide >0.2 nmol/L, a value including ≥90% of newly diagnosed patients and comparable (18) to an amount of insulin reserve thought to be clinically beneficial (19).

Importantly, patients randomized ≤6 weeks after diagnosis had the largest treatment difference versus placebo among the baseline subsets examined (Fig. 2 and Table 2). Subsets with U.S. residence, HbA_{1c} <7.5%, insulin

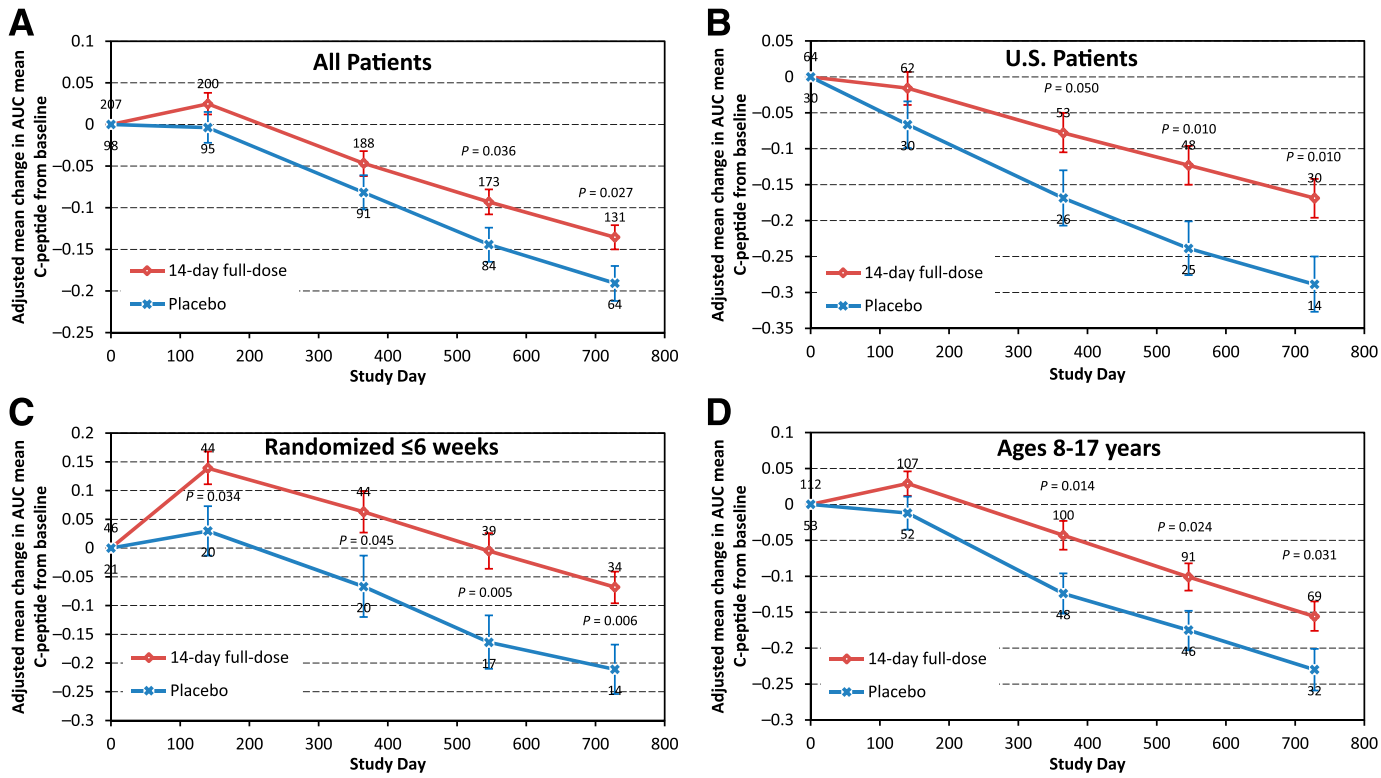

FIG. 1. Adjusted mean changes in AUC mean C-peptide from baseline over time in the 14-day full-dose and placebo groups. Bars indicate standard errors; numbers of patients are above (teplizumab) or below (placebo). *P* values are indicated where significant. Changes in AUC mean C-peptide from baseline were calculated using [ln(AUC mean C-peptide$_{Day\ x}$ + 1) − ln(AUC mean C-peptide$_{Baseline}$ + 1)]. *A*: All patients. *B*: Patients in the U.S. *C*: Patients randomized ≤6 weeks after diagnosis. *D*: Subjects 8–17 years of age.

use <0.4 units/kg/day, AUC mean C-peptide >0.65 nmol/L at entry, or AUC mean C-peptide >0.2 nmol/L also had much larger differences versus placebo compared with subsets of patients from India, higher HbA_{1c}, higher insulin use, and lower C-peptide, respectively. Of note, for patients in the U.S. and India, mean baseline HbA_{1c} was 7.6% (60 mmol/mol) and 9.7% (83 mmol/mol), insulin use was 0.47 and 0.98 units/kg/day, and AUC mean C-peptide was 0.77 and 0.53 nmol/L, respectively (11).

Age-groups were also prespecified for analyses, served as an enrollment stratification criterion, and served as an adjustment covariate in analyses. Although the difference versus placebo was small and not statistically significant in 18- to 35-year-olds, treatment effects were larger in the 8- to 11- and 12- to 17-year-olds, and these groups were combined (Table 2). In the combined 8- to 17-year-old subset, differences in AUC mean C-peptide change from baseline favored the 14-day full-dose versus placebo group ($P < 0.05$ at 1 year and all subsequent time points; Figs. 1*D* and 2 and Table 2). Among age subsets, a large difference versus placebo was seen in 8–11 years of age, but the *P* value was not significant until combined with ages 12–17

TABLE 1
Outcomes at year 2

Outcome	14-day full-dose n = 207	*P* value	14-day low-dose n = 102	*P* value	6-day full-dose n = 106	*P* value	Placebo n = 98
Adjusted mean change in AUC of C-peptide from baseline*[a]	−0.136	0.027	−0.198	0.968	−0.174	0.312	−0.191
Composite of insulin dose <0.5 units/kg/day and HbA_{1c} <6.5%,*[b] n (%)	17 (8.2)	0.775	6 (5.9)	0.402	10 (9.4)	0.859	9 (9.2)
Composite of insulin dose <0.25 units/kg/day and HbA_{1c} <7.0%,[b] n (%)	11 (5.3)	0.070	4 (3.9)	0.183	3 (2.8)	0.339	1 (1.0)
Adjusted mean change in HbA_{1c} from baseline*[a] (%)	0.233	0.706	0.220	0.868	0.149	0.606	0.135
Adjusted mean change in insulin use from baseline[a] (units/kg/day)	0.067	0.963	0.010	0.142	0.105	0.861	0.070

Sample sizes shown are at baseline. Patient numbers at each time point are shown in Fig. 1*A* for AUC mean C-peptide and Supplementary Fig. 1 for insulin use. *Prespecified end points at year 1. [a]Adjusted mean changes from baseline were calculated using MMRM models adjusted for age-group and baseline values; adjusted means for placebo group were calculated using the placebo vs. 14-day full-dose models. AUC mean C-peptide change from baseline was calculated by: [ln(AUC mean C-peptide$_{Day\ x}$ + 1) − ln(AUC mean C-peptide$_{Baseline}$ + 1)]. [b]*P* values were calculated using a Mantel-Haenszel test stratified by age-group (8–11, 12–17, and 18–35 years).

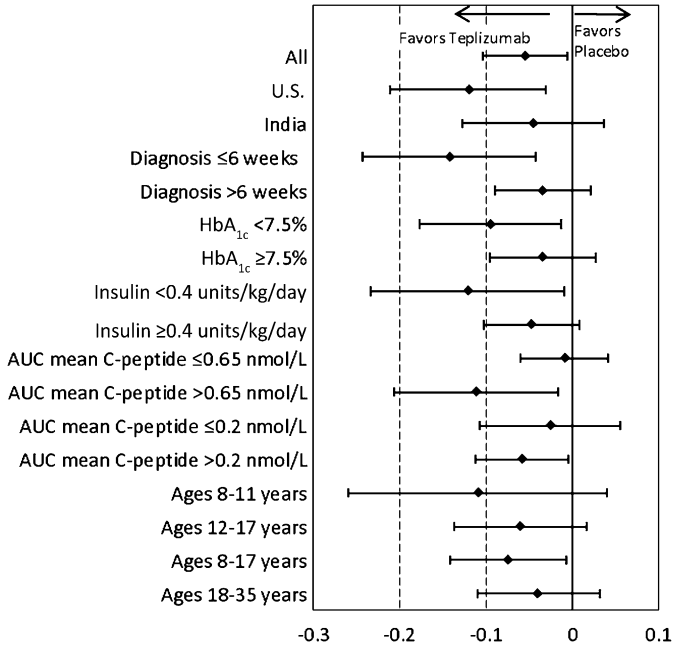

FIG. 2. Adjusted mean difference is shown in AUC mean C-peptide change from baseline at 2 years among subsets at study entry in the 4-day full-dose group vs. placebo. The ◆ indicate least squares means; bars indicate 95% CIs. AUC mean C-peptide change from baseline was calculated using [ln(AUC mean C-peptide$_{\text{Day x}}$ + 1) − ln(AUC mean C-peptide$_{\text{Baseline}}$ + 1)].

years, perhaps due to the smaller number of patients in the youngest group.

Insulin use. After an initial decline from baseline, adjusted mean insulin use increased progressively over time (Supplementary Fig. 1 and Table 1). It was prespecified to look at the largest countries in the trial (U.S. and India), and there were important regional differences in insulin use, HbA_{1c}, and C-peptide at study entry, as described above. The overall adjusted mean insulin use (units/kg/day) at all times after baseline in the 14-day full-dose versus placebo groups was 0.59 versus 0.62 for all patients (not significant) and 0.44 vs. 0.50 (P = 0.02) for U.S. patients (data not shown). For individual time points, the difference versus placebo was statistically significant at day 448 in U.S. patients (Supplementary Fig. 1). Compared with placebo, a greater proportion of patients in the 14-day full-dose group met the modified composite end point of HbA_{1c} <7% (53 mmol/mol) and insulin use <0.25 units/kg/day, and the differences were statistically significant at days 91, 273, 364, and 616 (Supplementary Fig. 2). Despite being blind to treatment, at 1 year, 5.3% (11/207) of patients in the 14-day full-dose group were not taking insulin, compared with 0% (0/98) in the placebo group (P = 0.02). At year 2, 3 of these 11 patients remained off insulin, whereas all placebo patients were still taking insulin ($P > 0.05$).

Teplizumab pharmacokinetics, immunogenicity, and effects on T cells. Higher levels of anti-teplizumab (anti-drug) antibodies were seen in cycle 2 than cycle 1 for all three teplizumab regimens (Supplementary Table 2). For typical patients in the 14-day full-dose group who did not make anti-drug antibodies, teplizumab levels peaked on day 14 with concentration minimum and maximums (mean ± SD) of 418 ± 225 and 826 ± 391 ng/mL, respectively. However, teplizumab clearance increased with maximum observed anti-drug antibody concentrations, and some patients demonstrated a strong anti-drug antibody response after ~10 days of cycle 2 dosing, with an abrupt reduction of bioavailability and increase in drug clearance (Supplementary Fig. 3). There did not appear to be a meaningful correlation between anti-drug antibody levels and AUC mean C-peptide changes from baseline (Supplementary Table 3), nor with response using the modified composite HbA_{1c} plus insulin usage end point (data not shown). Additional details are in the Supplementary Data online.

TABLE 2
Adjusted mean change from baseline at year 2 for AUC mean C-peptide in the 14-day full-dose and placebo groups by characteristics at study entry

Baseline subset	Total[a] patients at baseline N	14-day full-dose	Placebo	Adjusted mean difference	P value
U.S.	95	−0.169	−0.289	−0.120	0.01
India	85	−0.102	−0.147	−0.045	0.28
Diagnosed ≤6 weeks	67	−0.068	−0.211	−0.142	0.006
Diagnosed >6 weeks	238	−0.155	−0.189	−0.034	0.23
HbA_{1c} <7.5%	124	−0.153	−0.248	−0.095	0.024
HbA_{1c} ≥7.5%	181	−0.123	−0.157	−0.034	0.28
Insulin (units/kg/day)					
<0.4	74	−0.161	−0.282	−0.121	0.034
≥0.4	231	−0.122	−0.169	−0.047	0.1
AUC mean C-peptide (nmol/L)					
≤0.65	185	−0.104	−0.113	−0.009	0.73
>0.65	120	−0.176	−0.286	−0.111	0.02
≤0.2	31	−0.038	−0.064	−0.025	0.52
>0.2	274	−0.149	−0.207	−0.058	0.036
Ages (years)					
8–11	46	−0.155	−0.264	−0.109	0.15
12–17	119	−0.157	−0.217	−0.060	0.12
8–17	165	−0.156	−0.230	−0.075	0.031
18–35	140	−0.103	−0.142	−0.039	0.28

All analyses were MMRM; P values are for treatment effect from ANCOVA models. Sample sizes are from baseline measurements; see Fig. 1 for sample sizes at each time point. At year 2, ~66% of all patients (~48% of U.S. patients) had AUC mean C-peptide measurements because measurements were discontinued after analysis of year 1 data. C-peptide change of AUC from study entry was calculated using [ln(AUC mean C-peptide$_{\text{Day x}}$ + 1) − ln(AUC mean C-peptide$_{\text{Baseline}}$ + 1)]. [a]Total in the 14-day full-dose and placebo groups combined; overall N = 305.

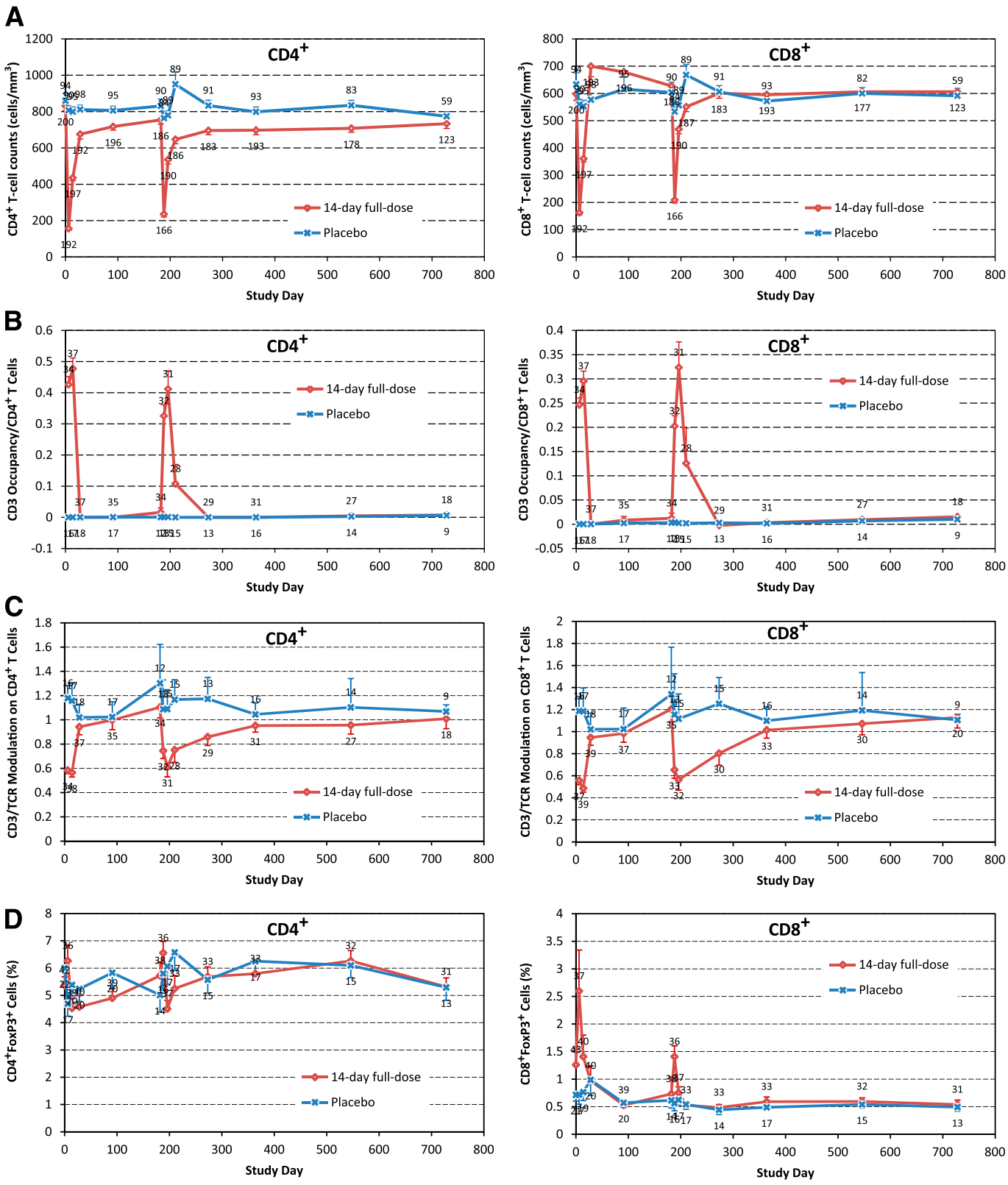

FIG. 3. Flow cytometry for CD4$^+$ (*left*) and CD8$^+$ T cells (*right*). *A*: Cell counts. *B*: CD3 occupancy/cell. *C*: CD3/TCR (T-cell receptor) modulation on cells. *D*: Percentage of cells positive for Foxp3 marker. Symbols indicate means, and bars indicate standard errors. *A* and *C*: Number of patients is above (placebo) or below (teplizumab). *B* and *D*: Number of patients is above (teplizumab) or below (placebo).

Circulating levels of CD4$^+$ and CD8$^+$ T cells were transiently reduced during each cycle of treatment but not in the placebo group (Fig. 3*A*). The effects of teplizumab on CD4$^+$ T cells appeared to diminish at anti-drug antibody levels >5,000 ng/mL (Supplementary Fig. 4). This level was observed in 19% of patients in the 14-day full-dose group after the second course of drug (Supplementary Table 2). During treatment, teplizumab was transiently bound to

TABLE 3
Adverse events in the safety population in the complete 2-year study

Adverse event	14-day full-dose n = 207	14-day low-dose n = 102	6-day full-dose n = 106	Placebo n = 98
Any adverse event	207 (100)	101 (99.0)	105 (99.1)	98 (100)
Adverse event leading to				
Drug withdrawal	35 (16.9)	12 (11.8)	17 (16.0)	5 (5.1)
Study discontinuation	3 (1.4)	1 (1.0)	0	0
Grade 3 or higher adverse event	135 (65.2)	55 (53.9)	71 (67.0)	28 (28.6)
Serious adverse event	23 (11.1)	14 (13.7)	12 (11.3)	12 (12.2)
Deaths	1 (0.5)	1 (1.0)	0	0

Data are n (%).

CD3 molecules on surfaces of $CD4^+$ and $CD8^+$ T cells (Fig. 3*B*). There was some evidence of down-modulation (Fig. 3*C*) and an increase in the percentage of circulating forkhead box P3 $(Foxp3)^+$ $CD8^+$ (but not $CD4^+$) T cells during teplizumab dosing (Fig. 3*D*).

Safety and tolerability. There were no differences in adverse events or serious adverse events among groups at year 2 (Table 3). Grade 3 adverse events were increased in teplizumab groups, but this difference versus placebo was primarily due to lymphopenia, an expected consequence of the mechanism of action. In particular, no differences were apparent between groups in the incidence of infections overall, or by specific types, with the possible exception of herpes zoster (10 teplizumab patients vs. no placebo patients, all nonserious; Table 4). Information on history was incomplete, but there was no convincing evidence of a history of varicella or varicella vaccination in any of the patients who reported herpes zoster (12–34 years of age). Three events occurred within 28 days of starting cycle 1 of treatment, whereas five occurred after 270 days when drug is no longer detectable in the circulation. Other herpes virus infections, including CMV and EBV, did not appear to increase in frequency during the 2 years of the trial. The most common infection was upper respiratory infection (16.3% of placebo vs. 15.5% of 14-day full-dose patients), which did not differ appreciably between teplizumab groups and placebo (Table 4). As expected, no rashes or cytokine release events occurred during the second year because the drug was not administered during this period.

DISCUSSION

In this report of data from the complete Protégé trial, patients with new-onset type 1 diabetes who received a full course of teplizumab (14-day full-dose) had significant improvement in stimulated C-peptide responses compared with placebo-treated subjects (P = 0.027). This effect was strongest in particular subsets, including children, those randomized ≤6 weeks after diagnosis, and in U.S. participants. As reported earlier, no significant differences were observed between the teplizumab and placebo treatment groups using a previously unvalidated primary composite end point (insulin <0.5 units/kg/day and HbA_{1c} <6.5% at year 1). Although investigators were instructed to treat aggressively to HbA_{1c} <6.5% and to maintain insulin >0.25 units/kg/day, this may have been unrealistic given that, after a nadir of ~6.9% (52 mmol/mol) at 90 days, mean HbA_{1c} increased to ~7.9% (63 mmol/mol) at year 1 (11) and to 8.4% (68 mmol/mol) at year 2. Further, C-peptide may be a more objective and reliable outcome than insulin use and HbA_{1c} because it is a more direct indicator of endogenous insulin secretion and cannot be easily measured or manipulated by patients or their physicians. AUC mean C-peptide is now the most widely used end point for type 1 diabetes interventions and is accepted by the U.S. Food and Drug Administration as a primary end point for these trials (6,8,20–22).

The AUC mean C-peptide treatment difference versus placebo did not appear to change markedly during the second year of follow-up (Fig. 1*A*), although the study was not designed to test hypotheses regarding time-trends. A particularly strong treatment effect was found in patient

TABLE 4
Incidence of infections in the complete 2-year study

	14-day full-dose n = 207	14-day low-dose n = 102	6-day full-dose n = 106	Placebo n = 98
All infections	48.3	48.0	50.9	58.2
Respiratory infection	15.5	20.6	22.6	16.3
Acute mononucleosis-like illness	7.7	4.9	3.8	8.2
Herpes (all)	8.7	8.8	6.6	8.2
Herpes zoster	3.4	1.0	1.9	0.0
Mononucleosis	1.4	0.0	0.9	1.0
Tuberculosis	0.0	1.0	0.0	1.0

Data are %. Herpes zoster cases were presumed but not confirmed; subjects with herpes zoster were asked retrospectively, after study completion, to provide data on their history of chicken pox, herpes zoster, or prior varicella vaccination. The information provided was incomplete (e.g., not all subjects responded to the request, and data for other subjects were provided by family members and not confirmed by a health care professional). The data received supported no prior history of varicella or vaccination in any of the subjects who were reported to have herpes zoster.

subsets that shared characteristics of early type 1 diabetes, including treatment sooner after diagnosis, lower baseline insulin use, greater C-peptide, and lower HbA_{1c} at baseline. The higher baseline HbA_{1c} and lower C-peptide levels suggest the patients in India had more advanced disease. Larger treatment effects on AUC mean C-peptide were also observed in subjects 8–17 years of age, who have a more rapid C-peptide decline, on average, than adults.

The modified composite end point (insulin <0.25 units/kg/day and HbA_{1c} <7.0%) and insulin use also suggested a treatment benefit on insulin use. In the U.S., the overall insulin use was less in the teplizumab-treated subjects compared with those receiving placebo. This trend was not seen when subjects outside the U.S. were included, perhaps reflecting different patterns of insulin use in other countries. Together, the results suggest teplizumab treatment preserves endogenous insulin, thereby reducing needs for exogenous insulin to maintain glycemic control.

Immunotherapy must meet a high safety standard because clinical type 1 diabetes can be managed using insulin. However, good metabolic control is often difficult to achieve safely with insulin: a recent study of 25,833 type 1 diabetic patients revealed that 7% reported severe hypoglycemic events (seizure or coma) and 8% reported diabetic ketoacidosis in the prior 12 months (23). High doses of anti-CD3 immunotherapy are associated with tolerability/toxicity issues (9,24), whereas low doses appear to be ineffective (11,25). One phase 2 trial of teplizumab (9) used a high dose (37 mg total per course per 1.9-m^2 subject) and observed a high (28%) incidence of grade 2 or greater adverse events associated with infusion (primarily fever, nausea, vomiting, and rigors), whereas the incidence was only 6% in an earlier trial (6). The Protégé trial used a dose of 17 mg total per course (for a 1.9-m^2 patient), comparable with that used in the earlier trial (6), and experienced similar excellent tolerability. Conversely, very low-dose otelixizumab (another nonactivating Fc-modified anti-CD3 monoclonal antibody), dosed at 3.1 mg over 8 days, did not preserve β-cell function in a double-blinded phase 3 study (25), and the Protégé treatment arms with lower cumulative dose were also ineffective (11). Overall, the 14-day full-dose regimen of Protégé appears to provide sufficient drug to influence efficacy measures, with acceptable tolerability and safety.

Treatment-related adverse experiences were mostly limited to the dosing period and generally resolved within 14 days (11). Most (transient cytopenias, transient mild laboratory or clinical manifestations of cytokine release such as rash, headache, nausea, and vomiting) were moderate, manageable, and expected as a manifestation of the intended mechanism of action. Along with transient small increases in aminotransferases, these also represented the main differences versus placebo in year 1 safety analyses (11). Use of effective stopping rules (based on liver function tests to delimit cytokine-release syndrome) served to lessen adverse events compared with earlier studies, allowing 90.6% of treated patients to complete a full course of drug (11).

The observed reduction in circulating $CD4^+$ and $CD8^+$ cells likely reflects transient margination of the T-cell compartment and apoptosis of some activated T-cell subsets. Both may be relevant mechanisms of action of teplizumab, wherein the T-effector cells, which are maintaining an inflammatory environment in the pancreas, are preferentially depleted while regulatory T cells are favored. Flow cytometry analysis of peripheral blood in the treated Protégé patients suggested that Foxp3 expression might be increased in $CD8^+$ but not $CD4^+$ T cells during periods of maximum drug binding to T cells. Previous studies have shown that teplizumab induces activation of $CD8^+$ T cells with regulatory function (26,27). In addition, $CD4^+$ and $CD8^+$ cells are directed to the lamina propria, where they appear to acquire regulatory function, although cell deletion may also be involved in the drug action.

Longer-term changes in patient immune function, such as persistent low CD4 counts (9) and reactivation of EBV infection, were reported from previous studies that used much higher anti-CD3 doses (28). At the lower doses used in Protégé, EBV reactivation was rare, and acute mononucleosis syndrome was not increased versus placebo. A possible dose-related increase in herpes zoster was seen, with 10 cases (that could not be subsequently confirmed) reported among teplizumab patients; no cases occurred in the placebo group. Of note, in a subsequent phase 3, double-blind, randomized study (n = 254) with identical teplizumab dosing (NCT00920582), after 2 years of follow-up, the only patient with herpes zoster was a placebo patient (data on file).

To be meaningful, treatment effects must be maintained for multiple years. Repeated dosing might be advantageous if it increases durability without causing new or cumulative side effects. Protégé did not include an arm with a single drug cycle and cannot answer whether two drug cycles confer greater benefit or duration than a single cycle. Nonetheless, Protégé did not identify any cumulative, persistent, or unexpected safety or tolerability issues. Although high levels of anti-drug antibodies occurred late in the second cycle in about one-sixth of all patients and appeared to accelerate drug clearance, this did not appear to affect efficacy end points.

The large number and diverse characteristics of Protégé patients enables more precise estimates of treatment effects than smaller trials, increases generalizability, and allows for meaningful subset analyses. The 2-year follow-up provides evaluation of efficacy and safety during placebo-controlled double-blind conditions for a longer period than previous trials. The double-blind design reduces the potential for bias. Limitations of the study include the heterogeneous baseline patient disease status, post hoc analyses without adjustment for multiple comparisons, and elimination of AUC mean C-peptide measurements in some patients after the primary analysis at year 1, which may have reduced statistical power. Another limitation is the lack of information on HLA-DQ/DR or other genotypes that might identify patient subsets with greater response.

Rodent studies reported full reversal of diabetes using anti-CD3 immunotherapy, but only when given immediately at disease onset (29,30). In clinical trials, delays due to required screening and enrollment procedures may lead to lower drug efficacy. In actual clinical settings, immunotherapy could be initiated promptly at the time of diagnosis. Further, the peak incidence of diabetes occurs in 8- to 11-year-olds, and subjects 8–17 years of age appeared to have a greater drug response than older patients.

In summary, continued follow-up for a second year demonstrated a benefit of teplizumab treatment on AUC mean C-peptide and a possible benefit on insulin needs. Most importantly, these analyses identified baseline characteristics associated with greater treatment efficacy. No new safety or tolerability issues emerged. These post hoc findings are hypothesis-generating, and confirmation is needed; nevertheless, they suggest that future studies of

CD3 immunotherapy should consider recruiting young patients with better glucose control and greater remaining endogenous insulin secretion and initiating treatment immediately upon diagnosis.

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