

Teplizumab for treatment of type 1 diabetes (Protégé study): 1-year results from a randomised, placebo-controlled trial

Nicole Sherry, William Hagopian, Johnny Ludvigsson, Sunil M Jain, Jack Wahlen, Robert J Ferry Jr, Bruce Bode, Stephen Aronoff, Christopher Holland, David Carlin, Karen L King, Ronald L Wilder, Stanley Pillemer, Ezio Bonvini, Syd Johnson, Kathryn E Stein, Scott Koenig, Kevan C Herold, Anastasia G Daifotis, for the Protégé Trial Investigators*

Summary

Background: Findings of small studies have suggested that short treatments with anti-CD3 monoclonal antibodies that are mutated to reduce Fc receptor binding preserve β -cell function and decrease insulin needs in patients with recent-onset type 1 diabetes. In this phase 3 trial, we assessed the safety and efficacy of one such antibody, teplizumab.

Methods: In this 2-year trial, patients aged 8–35 years who had been diagnosed with type 1 diabetes for 12 weeks or fewer were enrolled and treated at 83 clinical centres in North America, Europe, Israel, and India. Participants were allocated (2:1:1:1 ratio) by an interactive telephone system, according to computer-generated block randomisation, to receive one of three regimens of teplizumab infusions (14-day full dose, 14-day low dose, or 6-day full dose) or placebo at baseline and at 26 weeks. The Protégé study is still underway, and patients and study staff remain masked through to study closure. The primary composite outcome was the percentage of patients with insulin use of less than 0.5 U/kg per day and glycated haemoglobin A_{1c} (HbA_{1c}) of less than 6.5% at 1 year. Analyses included all patients who received at least one dose of study drug. This trial is registered with ClinicalTrials.gov, number NCT00385697.

Findings: 763 patients were screened, of whom 516 were randomised to receive 14-day full-dose teplizumab (n=209), 14-day low-dose teplizumab (n=102), 6-day full-dose teplizumab (n=106), or placebo (n=99). Two patients in the 14-day full-dose group and one patient in the placebo group did not start treatment, so 513 patients were eligible for efficacy analyses. The primary outcome did not differ between groups at 1 year: 19.8% (41/207) in the 14-day full-dose group; 13.7% (14/102) in the 14-day low-dose group; 20.8% (22/106) in the 6-day full-dose group; and 20.4% (20/98) in the placebo group. 5% (19/415) of patients in the teplizumab groups were not taking insulin at 1 year, compared with no patients in the placebo group at 1 year (p=0.03). Across the four study groups, similar proportions of patients had adverse events (414/417 [99%] in the teplizumab groups vs 98/99 [99%] in the placebo group) and serious adverse events (42/417 [10%] vs 9/99 [9%]). The most common clinical adverse event in the teplizumab groups was rash (220/417 [53%] vs 20/99 [20%] in the placebo group).

Interpretation: Findings of exploratory analyses suggest that future studies of immunotherapeutic intervention with teplizumab might have increased success in prevention of a decline in β -cell function (measured by C-peptide) and provision of glycaemic control at reduced doses of insulin if they target patients early after diagnosis of diabetes and children.

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Massachusetts General Hospital, Boston, MA, USA (N Sherry MD); Pacific Northwest Diabetes Research Institute, Seattle, WA, USA (W Hagopian MD); Division of Pediatrics, Department of Clinical and Experimental Medicine, Faculty of Health Sciences, Linköping University, Linköping, Sweden (Prof J Ludvigsson MD); TOLL Diabetes Hormone Research Institute, Indore, Madhya Pradesh, India (S M Jain MD); Endocrine Research Specialists, Ogden, UT, USA (J Wahlen MD); Division of Pediatric Endocrinology and Metabolism, Le Bonheur Children's Hospital and University of Tennessee Health Science Center, Memphis, TN, USA (Prof R J Ferry Jr MD); Atlanta Diabetes Associates, Atlanta, GA, USA (B Bode MD); Research Institute of Dallas, Dallas, TX, USA (S Aronoff MD); MacroGenics, Rockville, MD, USA (C Holland MS, D Carlin PhD, K L King MS, E Bonvini MD, S Johnson PhD, K E Stein PhD, S Koenig MD, Anastasia G Daifotis MD); PAREXEL International, Durham, NC, USA (R L Wilder MD); American Biopharma Corporation, GaitHERSburg, MD, USA (S Pillemer MD); and Yale University, New Haven, CT, USA (Prof K C Herold MD) Correspondence to: Dr Nicole Sherry, Massachusetts General Hospital for Children, 175 Cambridge Street Room 537, Boston, MA 02114, USA nsherry@partners.org

*Members listed in Appendix pp 1–2

Introduction

In type 1 diabetes mellitus, pancreatic insulin-secreting β cells are progressively destroyed by autoreactive CD4+ and CD8+ lymphocytes.¹ When clinical hyperglycaemia occurs, about 30% of β -cell function remains intact, but these cells are not fully functional because of inflammation and glucotoxicity.^{2,3} Residual endogenous insulin secretion synergises with exogenous insulin therapy to create an interim period with fewer hypoglycaemic events and markedly lower overall glycaemia.⁴ Immunotherapy aims to preserve endogenous insulin secretion, by attenuation of the activated, autoreactive T cells that probably mediate β -cell killing, to prolong this interim period and lessen complications.⁴ However, in view of the long experience with exogenous insulin therapy and the slow appearance of serious complications, new interventions should have reasonably low systemic toxic effects.

Regimens of chronic immunosuppression — eg, ciclosporin — have shown promise for attenuation of the loss of insulin secretion in new-onset disease, but have unacceptable toxic effects (potential risk of infections and tumours from continuous immunosuppression and nephrotoxicity). Antigen-specific therapies to restore β -cell tolerance have shown low toxic effects but little efficacy.^{5,6} Non-antigen-specific short-course therapies, such as anti-CD3 and anti-CD20, have had more success.^{7,8} Of these, anti-CD3 had a durable effect, with efficacy up to 4 years after one 1-week treatment in a pilot study, and longlasting efficacy in non-obese diabetic mice.^{9,10}

Teplizumab is a humanised, anti-CD3 monoclonal antibody that has been mutated to greatly reduce Fc receptor and complement binding.¹¹ In an early trial of anti-CD3 antibody,¹² 24 patients with recent-onset diabetes were randomised equally to receive open-label teplizumab (34 mg cumulative dose for one 14-day course in a 70 kg individual) or no antibody for 14 days, with daily dose based on previous transplantation trials. At 12 months, C-peptide response to a mixed meal was maintained in 60% of treated patients versus 8% of controls ($p < 0.03$).

In a trial of otelexizumab,¹³ another monoclonal anti-CD3 antibody with reduced binding to the Fc receptor, β -cell function was preserved in patients receiving otelexizumab and their insulin needs were decreased up to 48 months after treatment. Adverse events, including Epstein-Barr virus reactivation, were more frequent than in the teplizumab trial,¹² which is consistent with the higher cumulative dose.¹⁴ A much lower dose of 3.1 mg otelexizumab was subsequently used in a phase 3 trial, but the primary efficacy outcome of change in C-peptide at month 12 was not met.¹⁵

We undertook a phase 3, multicentre, randomized study (Protégé) to assess the safety and efficacy of teplizumab, and we report results at 1 year. By contrast with previous studies of one dose cycle, our study included a second dose cycle at 6 months.

Methods

For the Protégé study protocol see http://www.macrogenics.com/content/file/protocol_v8.pdf and http://www.macrogenics.com/content/file/protocol_v9.pdf

See <http://www.macrogenics.com/content/file/appendix> for Appendices

Patients. The Protege study was undertaken in 83 academic centres, hospitals, and clinics in North America (USA, Canada, and Mexico), India, Israel, and Europe (Czech Republic, Estonia, Germany, Latvia, Poland, Romania, Spain, Sweden, and Ukraine; appendix pp 1–3). The study procedures in effect during the Protege study are described

most completely in protocol version 8; the statistical analysis was revised shortly before unblinding for analysis of the primary outcome and is described in protocol version 9. Participants were eligible if they met the following criteria: aged 8–35 years; body mass of at least 36 kg; type 1 diabetes mellitus diagnosed for 12 weeks or fewer, according to American Diabetes Association criteria,¹⁶ with need for injected insulin therapy; detectable fasting or stimulated C-peptide; and positive autoantibody titre against an islet-cell antigen (ICA-512/IA-2), glutamic acid decarboxylase (GAD 65), or insulin, within 2 weeks of initiating insulin treatment. Exclusion criteria focused on medical disorders that would potentially confound results or interfere with safe completion of the trial, including serious cardiovascular disorders, active infections, recent participation in a clinical trial, vaccination, or pregnancy (appendix p 3).

The research protocol was approved by institutional review boards, and all participants or guardians gave written informed consent.

Randomisation and masking. An independent third party, United BioSource Corporation (San Francisco, CA, USA), generated the allocation schedule by computer with block randomisation (block size of five), and managed the distribution and assignment of study drugs at all sites via a controlled access interactive telephone system. Randomisation was stratified by country and age group (8–11, 12–17, and 18–35 years). Patients were randomly assigned (2:1:1:1 ratio) to receive treatment in one of four parallel groups: a 14-day course of escalating doses of intravenous teplizumab, with a total cumulative dose of about 9034 $\mu\text{g}/\text{m}^2$ (14-day full-dose group); a 14-day course of escalating doses of intravenous teplizumab, with a total cumulative dose of about 2985 $\mu\text{g}/\text{m}^2$ (14-day low-dose group); a 6-day course of escalating doses of intravenous teplizumab plus 8 days of intravenous placebo, with a total cumulative dose of about 2426 $\mu\text{g}/\text{m}^2$ (6-day full-dose group); or a 14-day course of intravenous placebo (placebo group). All treatments were repeated at week 26. Further details about randomisation and dosing are provided in appendix pp 3–4.

Dosing was double blind and double dummy, with use of two vials for each dose (full dose and low dose, or matching placebos) and numbered codes to conceal allocation. All patients and study personnel were masked to the treatment codes, block sizes, and laboratory measurements that might reveal allocation (such as serum teplizumab concentrations and T-cell proteins), with a few exceptions for safety issues, laboratory validation, and drug supply.

Procedures. From February, 2007, groups of about ten patients in each age stratum received open-label teplizumab according to the 14-day full-dose regimen to assess safety and tolerability before randomisation of patients in the double blind study. Enrolment was staggered: patients aged 8–11 and 12–17 years were enrolled after the independent data monitoring committee reviewed and approved results from the initial dosing regimen of the age 18–35 group. Treatment was repeated at 26 weeks and patients were followed up for a total of 24 months, with the last patient's visit in March, 2010. Data for the open-label phase are not presented here.

We did not prespecify regimens to adjust insulin use, but investigators were instructed to aggressively treat diabetes, attempt to keep HbA_{1c} at 6.5% or lower, and maintain an insulin dose of at least 0.25 U/kg per day. Patients recorded insulin use in diary cards at screening and for 3 days before each visit at days 91, 140, 364, 546, and 728; daily doses in U/kg were calculated from these data and weight measurements from the corresponding office visits. Use of inhaled insulin, exenatide, or other agents that stimulate pancreatic β -cell regeneration or insulin secretion were not permitted during the study.

Blood samples were used to measure HbA_{1c} and area under the curve (AUC) of C-peptide during 4 h after a mixed meal² (appendix p 5). Epstein-Barr virus (EBV) and cytomegalovirus (CMV) seropositivity were documented by

measurement of anti-CMV IgG, anti-EBV IgG, or anti-EBV IgM at screening and days 28, 91, 140, 210, 273, 364, and 728. For seropositive patients, viral load was measured by use of semi-quantitative PCR (appendix p 5).

Investigators reported abnormal laboratory values and other adverse events that were coded according to the Medical Dictionary for Regulatory Activities and graded according to the Common Terminology Criteria for Adverse Events (version 3.0). Clinically significant hypoglycaemic events, requiring assistance by another individual for parenteral dextrose, glucagon, or equivalent intervention, were reported as adverse events. Patients permanently discontinued treatment if they had protocol-defined dose-stopping events, including pregnancy, anaphylaxis, abnormal liver function tests, abnormal blood cell counts, or certain adverse events rated grade 3 or higher (appendix pp 3–4). Many of the patients who stopped dosing continued to participate in the study. Patients received a non-steroidal anti-inflammatory drug for at least the first 5 days of the treatment cycle, or longer if the investigator judged it to be appropriate to prevent adverse events, and concomitant need for an antihistamine was decided by the investigator.

The trial is planned to last for 2 years, but we report results for the first year because analyses of primary and main secondary outcomes were prespecified to occur at 1 year. After the 1-year review by the data monitoring committee, the study was changed to continue safety assessments, but minimise blood draws for biomarker measurement. The primary composite outcome was the percentage of patients from each treatment group with insulin use of less than 0.5 U/kg per day and HbA_{1c} of less than 6.5% at 1 year. A second primary outcome was mean change from baseline in HbA_{1c}. Secondary outcomes at 1 year were mean change from baseline in AUC of C-peptide, and a composite of insulin use of less than 0.5 U/kg per day and HbA_{1c} of less than 7%.

Statistical analysis. Statistical analyses were done in accordance with a prespecified analysis plan (appendix p 6). The second primary analysis, secondary analyses, and post-hoc analyses were regarded as exploratory and hypothesis generating because they were originally planned only to be assessed if the primary outcome was significant. Patients with missing composite outcome data were counted as non-responders. Composite outcome analyses were done with Cochran-Mantel-Haenszel tests, stratified by age-group, to compare teplizumab groups with placebo. The target sample sizes were 200 patients for the 14-day full-dose group and 100 patients for each of the other three groups. With the planned sample sizes and an overall type 1 error rate of 0.05, the power for the primary composite outcome with a binomial test, which is more conservative than Cochran-Mantel-Haenszel test, was more than 99% to detect group differences of 50% versus 5% and about 90% for differences of 30% versus 5%; these differences were based on a previous study.¹²

ANCOVA models, adjusted for age-group and baseline values, were used to compare C-peptide and HbA_{1c} values in teplizumab groups with the placebo group; missing values were imputed by use of last observation carried forward at the request of regulators. Safety analyses of adverse events and exploratory efficacy analyses of dichotomous outcomes were done with Fisher's exact test. Analyses of treatment or region differences with respect to baseline characteristics were done with Cochran-Mantel-Haenszel tests for categorical outcomes or ANOVA tests for continuous outcomes. If normality assumptions relating to the ANOVA or ANCOVA models were not met, then post-hoc exploratory analyses used non-parametric Wilcoxon rank-sum tests to detect differences between treatment groups.

Two-sided testing was done at an α level of 0.05. All efficacy analyses controlled for multiple treatment group comparisons with placebo.¹⁷ However, because the primary outcome did not differ, subsequent analyses compared the 14-day full-dose regimen with placebo. These analyses were done for hypothesis generation, therefore no adjustment for multiple comparisons was made. Primary and secondary efficacy analyses included all patients who received at least one dose of study drug, and safety analyses included all randomly assigned patients.

The statistical analysis plan predefined subgroup analyses of the primary and main secondary outcomes based on age group (8–11, 12–17, and >17 years), geographical region (USA, India, Europe and Israel, and Mexico and Canada), and time from diagnosis (defined as first physician visit related to diabetes symptoms) to randomisation (≤ 6 weeks and > 6 weeks). SAS (version 9.2) was used for all analyses.

This trial is registered with ClinicalTrials.gov, number NCT00385697.

Role of the funding source. The study was designed by MacroGenics, with substantial input from advisers and site investigators. MacroGenics had direct oversight or participation in every stage of the study, including pharmacovigilance, data collection, data interpretation, and writing of the report. Data analyses were done by MacroGenics and an independent academic centre (Data Analysis Center, Department of Biostatistics and Medical Informatics, University of Wisconsin, Madison, WI, USA); the centre provided data to the independent data monitoring committee, which was supplemented by information from MacroGenics as requested. After initiation of the trial, Eli Lilly partnered with MacroGenics for development of teplizumab. The Juvenile Diabetes Research Foundation and Eli Lilly did not participate in study design, data collection, data analysis, data interpretation, or writing of the report. All authors had full access to the data, and the corresponding author had final responsibility for the decision to submit for publication.

Results

Between July, 2007, and June, 2009, 763 patients were screened for the double-blind study, of whom 516 were randomised (figure 1). All randomised patients were included in safety analyses, and 513 patients who received treatment were eligible for primary and secondary efficacy analyses. In the first treatment cycle, 85 patients stopped treatment because of protocol-defined dose stopping events, adverse events, or other reasons (withdrawal from treatment or study, low C-peptide at day 140, insufficient autoantibodies at study entry, pregnancy, or loss to follow-up). In the second treatment cycle at week 26, 18 of 428 patients stopped treatment because of dose-stopping events, adverse events, or other reasons (withdrawal of dosing, insufficient autoantibodies at study entry, or drug supply issue). 1-year follow-up was completed by August, 2010. Many patients who stopped or withdrew from treatment continued to participate; 12 patients withdrew completely from study participation and four patients were lost to follow-up, leaving 497 patients who continued long-term follow-up after 1 year.

Baseline characteristics were well balanced across treatment groups (table 1), but varied by region (appendix p 7). Most notably, patients in India had higher HbA_{1c} and insulin use, lower AUC of C-peptide (characteristic of later disease stage), and lower frequency of anti-ICA512 antibodies than did those in other regions.

HbA_{1c} and insulin use decreased after patients entered the study, and then increased after day 91 (appendix pp 8–9). Mean changes in HbA_{1c} from baseline were essentially identical in the 14-day full-dose and placebo groups at all times. The 14-day full-dose group had numerically larger decreases in mean insulin use relative to the placebo group, and the difference was maintained at all times (appendix pp 8–9). Differences between the 14-day full-dose and placebo groups were not significant for the primary and secondary outcomes (table 2). The amount of missing data for these outcomes was generally small. Results were largely unchanged in sensitivity analyses in which alternative methods were used for imputation of missing data.

AUC of C-peptide was analysed with non-parametric methods because data were not normally distributed. Median change from baseline to 1 year was lower in the 14-day full-dose group than in the placebo group (figure 2A, table 2). The shift between treatment groups is evident from the empirical cumulative distribution curves in the overall

population, in which 40% of the 14-day full-dose group had a preservation or increase in AUC of C-peptide relative to baseline compared with 28% of the placebo group (figure 3A).

Change in insulin use from baseline did not differ between the 14-day full-dose group and placebo (table 2). However, at any given HbA_{1c} threshold, a greater percentage of patients in the 14-day full-dose group achieved the threshold at lower insulin dose cutoffs than in the placebo group (figure 4A, appendix p 10). This effect was most notable at insulin doses of lower than 0.5 U/kg per day. 5% (19/415) of patients receiving teplizumab were not taking insulin at 1 year compared with no patients in the placebo group (p=0.03), and 15 of these 19 patients had HbA_{1c} of less than 7%. In the Diabetes Prevention Trial,⁵ insulin was given to the relatives of patients with type 1 diabetes at a dose of 0.25 U/kg per day because this dose did not increase the occurrence of hypoglycaemia.⁵ We therefore used the dose of 0.25 U/kg per day as a cutoff in subsequent analyses. The proportion of patients who achieved HbA_{1c} of less than 7% and insulin use of less than 0.25 U/kg per day was greater in the 14-day full-dose group than in the placebo group for the study overall (table 2, figure 4A, appendix p 10) and at each study visit (figure 5). A similar effect was seen at HbA_{1c} of less than 6.5% and insulin use of less than 0.25 U/kg per day (p=0.02).

The lower reduction in median AUC of C-peptide in the 14-day full-dose group than in the placebo group was evident in three predefined subgroups (table 3): children aged 8–11 years (figure 2B, figure 3B), the US region (figure 2C, figure 3C), and patients randomised at 6 weeks or fewer after diagnosis (appendix p 11). Because of violations of normality assumptions, inferential comparisons were made with non-parametric methods.

In the subgroup of the US region, the proportion of patients who achieved an HbA_{1c} of less than 7% at each low dose insulin cutoff was higher in the 14-day full-dose group than in the placebo group (figure 4B). A similar pattern was seen in children aged 8–11 years and patients randomised at no more than 6 weeks after diagnosis, but data are not shown because sample sizes were small. Furthermore, in the subgroups of children aged 8–11 years, the US region, and patients randomised at 6 weeks or fewer after diagnosis, the proportion of patients who achieved HbA_{1c} of less than 7% and insulin use of less than 0.25 U/kg per day was greater in the 14-day full-dose group than in the placebo group at all timepoints (appendix pp 12–14).

In the safety analyses, the proportion of patients who had adverse events (512/516 [99%]) and serious adverse events (51/516 [10%]) was similar across the four study groups: 99% (207/209) and 9% (19/209) for the 14-day full-dose group; 99% (101/102) and 11% (11/102) for the 14-day low-dose group; 100% (106/106) and 11% (12/106) for the 6-day full-dose group; and 99% (98/99) and 9% (9/99) for the placebo group. The proportion of patients with infection did not differ between the four treatment groups (table 4). Although the study population had a high occurrence of anti-EBV IgG at baseline (441/513 [86%]), 22 of 415 (5%) patients receiving teplizumab became positive for anti-EBV IgG or IgM during the study compared with seven of 98 (7%) receiving placebo, and only one patient (teplizumab group) had a small transient increase in EBV viral load. 77% (318/415) of patients treated with teplizumab (150/207 [72%] in the 14-day full-dose group, 78/102 [76%] in the 14-day low dose group, and 90/106 [85%] in the 6-day full-dose group) and 13% (13/98) of those receiving placebo developed anti-drug antibodies, defined as a titre of more than 1:100 when samples were obtained at 28 or 56 days.

Rash, the most common clinical adverse event in the teplizumab groups, occurred in a higher proportion of patients than in the placebo group (table 4). With median onset at day 6 (IQR 5–11), rash was usually mild to moderate (218/220 [99%]), self-limited in all but one patient, most often maculopapular (132/220 [60%]), and sometimes pruritic 56/220 (25%). Mild cytokine release syndrome was infrequent in the teplizumab groups (22/417 [5%]) and was not recorded in the placebo group (table 4). The safety profile was characterised by transient, small increases in aminotransferases, and mild, transient decreases in the neutrophil and leucocyte

concentrations (appendix pp 15–17). A more profound, but transient drop occurred in the lymphocyte concentration (nadir at day 6), as reported in previous studies^{12,13} (appendix pp 15–17). The proportion of patients who were not able to complete all drug doses because of lymphopenia and protocol-defined stopping rules for alanine or aspartate aminotransferase increases, neutropenia, and reduced platelet counts was higher in the teplizumab groups (39/415 [9%]) than in the placebo group (2/98 [2%]).

Discussion

Our study of immune therapy in recent-onset type 1 diabetes did not show any significant between-group differences in the primary and secondary outcomes (**see Panel**). The primary composite outcome has not been used or validated previously, but was used to ensure that low insulin use would be accompanied by adequate glycaemic control. The exact parameters chosen ($\text{HbA}_{1\text{c}} < 6.5\%$ and daily insulin use $< 0.5 \text{ U/kg}$) were derived from a previous unblinded, uncontrolled study of teplizumab,¹² and might not translate to our study population, which was older (mean age of 19 vs 14 years), recruited from different regions of the world (vs USA only), masked to treatment, and treated later after diagnosis (< 12 weeks vs < 6 weeks). Moreover, the patients recruited into this study might differ from previous cohorts. For example, 20% of the placebo group met the primary outcome criteria in our study compared with 5% in the previous trial.¹² The differences might be due to improved overall treatment practices for type 1 diabetes in the 9 years between the studies, and to demographic differences.

Exploratory analyses in all patients who received treatment and in subgroups showed effects similar to those seen in previous, smaller studies of anti-CD3 antibody: C-peptide secretion was preserved, allowing glycaemic control to be achieved at a lower insulin dose in the teplizumab groups than in the placebo group. A greater proportion of patients in the teplizumab groups were able to discontinue or use very low doses of insulin than in the placebo group. Investigators were instructed to maintain an insulin dose of more than 0.25 U/kg per day, which might have diminished the effect of teplizumab on insulin use. Although discontinuation of insulin was never a study objective and was driven entirely by glycaemic status, reduced insulin requirement with maintenance of glycaemic control supports a biological effect of teplizumab. Studies of other immune modulators have not consistently achieved this outcome.⁸ During the course of Protégé, accumulating evidence suggested that C-peptide concentration might be a more reliable outcome because C-peptide is a more direct indicator of endogenous insulin secretion than is $\text{HbA}_{1\text{c}}$ or insulin use, or both, and it is independent of manipulation by patients or physicians. Our results support this view, showing a treatment effect on C-peptide changes in exploratory analyses.

The effect of teplizumab was only seen with the highest dose (two courses of 17 mg, 6 months apart, in a 70 kg individual on the basis of a cumulative dose of $9034 \mu\text{g/m}^2$ and a body surface area of 1.92 m^2), yielding a cumulative dose per cycle that was half that in the previous study of teplizumab¹² (34 mg cumulative dose for one 14-day course in a 70 kg individual). Otelixizumab has a similar specificity to the ϵ -subunit of CD3, but is produced by different methods, and has different affinity and structural features from teplizumab.²⁰ The teplizumab dose seems to be lower than the dose used in the phase 2 trial of otelixizumab (48 mg cumulative dose),¹³ but higher than the dose in the phase 3 trial (3.1 mg cumulative dose).²¹ The dose regimen we used was not associated with severe reactions or biological signs of EBV reactivation, which was a problem in the otelixizumab trial, and, thus, had an acceptable safety profile in view of the seriousness of type 1 diabetes.¹⁴ More research is needed to establish whether higher teplizumab doses (eg, closer to that in the previous study¹²) might improve efficacy without impairing safety, and whether repeated dosing improves efficacy or is countered by the development of teplizumab antibodies and late timing after diagnosis.

Limitations of this study include the failure to achieve the primary outcome, necessitating the use of post-hoc (hypothesis-generating) analyses, and a need for additional follow-up to examine long-term safety and efficacy; 2-

year results are forthcoming soon. Another limitation was the lack of extensive data for hypoglycaemia, which was not captured on diary cards. Future studies could benefit from documenting hypoglycaemic events more thoroughly, particularly because hypoglycaemia is an important clinical problem.

The study also identified subgroups that might be more likely to respond to treatment. Larger treatment effects were associated with younger age, recruitment from the USA, and earlier treatment. The reasons for these differences might be as much a reflection of the behaviour of the study subpopulations as the effect of the drug, but this possibility does not detract from their potential importance. This finding underscores the heterogeneity in type 1 diabetes and could have implications for showing efficacy of immune interventions in future trials. For example, the rate of β -cell decline is faster in children than in adults. Immunotherapy might, therefore, be particularly effective for improvement of clinical management of the disease in children—an important finding because the management of diabetes in children is particularly challenging, with higher rates of both hyperglycaemia and hypoglycaemia.^{4,7} Also, US patients generally had higher C-peptide concentrations, lower insulin use, and lower HbA_{1c} at baseline than did patients in other regions, suggesting a more advanced stage of disease progression in these other regions, especially India. The increased response in patients from the USA and those treated within 6 weeks of diagnosis suggests that there is an advantage to treating early in the course of disease. Future studies might benefit from use of metabolic entry criteria that indicate a fairly early stage of disease.

Findings of this study showed that teplizumab had a treatment effect on C-peptide and insulin use while maintaining glycaemic control, particularly in selected, prespecified subgroups, and exemplifies the risks of developing a new outcome without previous validation. The data suggest that future studies intending to examine the effects of CD3 therapy might benefit from recruitment of a population enriched with young patients who are treated early after diagnosis.

Panel: *Research in context*

Systematic review: We searched PubMed Clinical Queries up to June 2, 2011, with the search term “type 1 diabetes immunotherapy”, and identified a systematic review and meta-analysis¹⁸ and a comprehensive, non-systematic review.¹⁹ Findings of several small (n<100) phase 1–2 trials showed some success with antigen-specific and non-specific immune interventions between 6 months and 2 years. Among these trials, anti-CD3 monoclonal antibody showed promise in terms of strength and duration of efficacy balanced by a reasonable safety profile. Interpretation: This phase 3 trial of immunotherapy in recent-onset type 1 diabetes is unique because it has a large sample size (n>500), is multinational, and used two courses of therapy. The primary outcome was not met, but treatment of type 1 diabetes with immunotherapy is an evolving area and a consensus outcome does not exist. Findings of exploratory analyses suggested that teplizumab could help preserve pancreatic β -cell secretion of insulin (as measured by C-peptide), and might decrease the amount of exogenous insulin needed for glycaemic control, particularly in subgroups such as children. These findings could help guide the design of future studies that are needed to assess the short-term and long-term efficacy of this therapeutic strategy.

Contributors

SK, EB, KES, KCH, RLW, SJ, SP, DC, WH, and JL contributed to the study design. All authors contributed to study implementation and supervision of data collection at the sites. AGD, DC, CH, KCH, NS, and SP contributed to planning of protocol-stated analyses and post-hoc analyses. DC and CH designed and did the statistical analysis and verified its accuracy. KLK, CH, DC, and AGD contributed to compiling of the official clinical study report. All authors had full access to the data, helped draft the report or critically revise the draft, contributed to data interpretation, and reviewed and approved the final version of the report.

Conflicts of interest

CH, DC, KLK, EB, SJ, KES, SK, and AGD are employees of MacroGenics and potentially own shares or options in the company. NS, JL, SA, BB, JW, and RJF received research and travel support for this study from MacroGenics. JL has received advisory board support from Johnson & Johnson. RJF has received research support within the past 3 years from Tolerx (to study otelixizumab for recent-onset type 1 diabetes); unrelated research support from the US National Institutes of Health (grants R21 HD059292 and T35 DK007405), Gabrielle's Angel Foundation, Eli Lilly, Diamyd, Pfizer, and Novo Nordisk; and unrestricted research support from Le Bonheur Foundation (Memphis TN, USA). WH chairs the data safety and monitoring board for the BHT-3021-01 insulin plasmid trial (Bayhill Pharmaceuticals). KCH has received research support from MacroGenics, and support from the Juvenile Diabetes Foundation (grant 2008-502) for laboratory studies on patients' samples from Protege. RLW is a former employee of MacroGenics and holds restricted stock in the company, and is now an employee of Parexel International, the lead contract research organisation that coordinated the Protege trial globally. SA has received speaking honoraria from Eli Lilly. SP was formerly employed by MacroGenics and received stock options while employed there. SMJ declares that he has no conflicts of interest.

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Table 1: Demographic and clinical characteristics at baseline.

	14-day full-dose group (n=207)	14-day low-dose group (n=102)	6-day full-dose group (n=106)	Placebo group (n=98)
Age (years)	18.9 (7.6)	17.9 (6.1)	18.1 (6.9)	18.2 (7.3)
8–11	31 (15%)	15 (15%)	16 (15%)	15 (15%)
12–17	81 (39%)	42 (41%)	44 (42%)	38 (39%)
18–35	95 (46%)	45 (44%)	46 (43%)	45 (46%)
Male sex	130 (63%)	62 (61%)	72 (68%)	61 (62%)
White	146 (71%)	74 (73%)	75 (71%)	69 (70%)
Time from diagnosis to randomisation	8.4 (2.6)	8.4 (2.6)	9.0 (4.5)	8.3 (2.6)
History of ketoacidosis	76 (37%)	37 (36%)	45 (42%)	33 (34%)
Number of positive autoantibodies*				
1	18 (9%)	13 (13%)	15 (14%)	11 (11%)
2	91 (44%)	38 (37%)	45 (43%)	45 (46%)
3	98 (47%)	51 (50%)	45 (43%)	42 (43%)
Antibody type				
GAD 65 (≥0.5 U/mL)	194 (94%)	90 (88%)	91 (86%)	89 (91%)
Human insulin (≥2.0 U/mL)	187 (90%)	94 (92%)	89 (84%)	88 (90%)
Islet cell 512 (≥0.8 U/mL)	113 (55%)	58 (57%)	60 (57%)	53 (54%)
HbA _{1c} (%)	8.3% (2.0)	8.4% (2.1)	8.1% (1.8)	8.2% (2.0)
AUC of C-peptide (nmol/L per min)	0.65 (0.54)	0.69 (0.45)	0.68 (0.40)	0.65 (0.44)
Total insulin dose (U/kg per day)	0.63 (0.42)	0.68 (0.41)	0.63 (0.39)	0.65 (0.32)

Data are mean (SD) or number (%). HbA_{1c}=glycated haemoglobin A_{1c}. AUC=area under the curve. *Data are missing for one patient in the 6-day full-dose group.

Table 2: Primary, secondary, and post-hoc exploratory outcomes at 1 year.

	14-day full-dose group (n=207)	14-day low-dose group (n=102)	6-day full-dose group (n=106)	Placebo group (n=98)	p-value*
Primary outcomes					
Composite of insulin dose <0.5 U/kg per day and HbA _{1c} <6.5%	19.8% (n=41)	13.7% (n=14)	20.8% (n=22)	20.4% (n=20)	0.904
Change in HbA _{1c} from baseline (%)	-0.41% (2.3)	-0.33 (2.2)	-0.36 (2.1)	-0.40 (2.7)	0.659
Secondary outcomes					
Change in AUC of C-peptide from baseline (nmol/L per min)	-0.06 (0.36)	-0.14 (0.30)	-0.07 (0.39)	-0.09 (0.42)	0.382
Composite of insulin dose <0.5 U/kg per day and HbA _{1c} <7%	29.0% (n=60)	20.6% (n=21)	26.4% (n=28)	24.5% (n=24)	0.404
Post-hoc exploratory outcomes					
HbA _{1c} (%)	7.9 (2.3)	8.0 (2.2)	7.7 (1.9)	7.8 (2.4)	0.978
Change in insulin use from baseline (U/kg per day)	-0.04 (0.58) -0.07 (0.38) [†]	-0.07 (0.35)	-0.02 (0.35)	0.01 (0.31)	0.601
Median change in AUC of C-peptide from baseline (nmol/L per min; IQR)	-0.06 (-0.25 – 0.12)	-0.13 (-0.33 – 0.01)	-0.08 (-0.31 – 0.11)	-0.14 (-0.30 – 0.02)	0.046
Composite of HbA _{1c} <7.0% and insulin dose <0.25 U/kg per day	13.1% (n=27)	8.8% (n=9)	9.4% (n=10)	3.1% (n=3)	0.006

Data are percentage (n) or mean (SD), unless otherwise indicated. HbA_{1c}=glycated haemoglobin A_{1c}. AUC=area under the curve. *14-day full-dose group versus placebo group. †Excludes one outlier with insulin use of 8.5 U/kg per day.

Table 3: Non-parametric summary statistics and subgroup analysis for change in AUC of C-peptide from baseline to year 1.

	14-day full-dose group (n=207)	14-day low-dose group (n=102)	6-day full-dose group (n=106)	Placebo group (n=98)	p value*
Region					
USA	-0.10 (n=63; -0.30 to 0.16)	-0.17 (n=32; -0.29 to 0.02)	-0.18 (n=30; -0.43 to 0.11)	-0.27 (n=30; -0.50 to -0.14)	0.015
India	-0.00 (n=55; -0.15 to 0.24)	-0.09 (n=27; -0.35 to 0.00)	-0.06 (n=27; -0.15 to 0.42)	-0.04 (n=27; -0.13 to 0.31)	0.824
Europe and Israel	-0.11 (n=70; -0.25 to 0.03)	-0.15 (n=37; -0.39 to 0.01)	-0.05 (n=36; -0.26 to 0.16)	-0.14 (n=34; -0.27 to 0.01)	0.422
Canada and Mexico	0.01 (n=13; -0.02 to 0.21)	-0.02 (n=4; -0.31 to 0.36)	-0.09 (n=9; -0.38 to 0.04)	0.03 (n=5; -0.19 to 0.23)	0.657
Age-group (years)					
8–11	0.02 (n=31; -0.25 to 0.20)	-0.17 (n=15; -0.34 to 0.01)	-0.22 (n=14; -0.38 to -0.03)	-0.30 (n=14; -0.45 to 0.09)	0.047
12–17	-0.09 (n=78; -0.27 to 0.03)	-0.22 (n=40; -0.39 to -0.02)	-0.014 (n=42; -0.40 to 0.04)	-0.20 (n=38; -0.29 to -0.02)	0.114
18–35	-0.06 (n=92; -0.22 to 0.13)	-0.05 (n=45; -0.26 to 0.09)	-0.00 (n=46; -0.12 to 0.30)	-0.07 (n=44; -0.27 to 0.10)	0.959
Time from diagnosis to randomisation					
≤6	0.02 (n=41; -0.13 to 0.39)	-0.04 (n=20; -0.27 to 0.11)	0.17 (n=14; -0.11 to 0.30)	-0.15 (n=16; -0.38 to 0.09)	0.020
>6	-0.09 (n=160; -0.27 to 0.06)	-0.17 (n=80; -0.37 to 0.01)	-0.10 (n=88; -0.35 to 0.05)	-0.14 (n=80; -0.28 to 0.02)	0.332

Data are median (n; IQR) and are in units of nmol/L per min. Missing values were imputed with last observation carried forward. 14 patients (six in the 14-day full-dose group, two in the 14-day low-dose group, four in the 6-day full-dose group, and two in the placebo group) were not included in the last-observation-carried-forward analyses because no C-peptide data were obtained. AUC=area under the curve. *14-day full-dose group versus placebo group; Wilcoxon rank-sum test.

Table 4: All serious adverse events, adverse events occurring in 10% or more of patients in any treatment group, and adverse events of special interest.

	14-day full-dose group (n=209)	14-day low-dose group (n=102)	6-day full-dose group (n=106)	Placebo group (n=99)
Any adverse event				
Total adverse events	207 (99%)	101 (99%)	106 (100%)	98 (99%)
Blood and lymphatic system	181 (87%)*	88 (86%)*	85 (80%)*	51 (52%)
Lymphopenia	153 (73%)*	70 (69%)*	79 (75%)*	19 (19%)
Leukopenia	98 (47%)*	49 (48%)*	50 (47%)*	23 (23%)
Neutropenia	76 (36%)*	39 (38%)*	21 (20%)	20 (20%)
Anaemia	30 (14%)	13 (13%)	10 (9%)	13 (13%)
Thrombocytopenia	21 (10%)	8 (8%)	15 (14%)	10 (10%)
Gastrointestinal disorders	71 (34%)	31 (30%)	44 (42%)*	26 (26%)
Nausea	41 (20%)	16 (16%)	21 (20%)	11 (11%)
Vomiting	30 (14%)*	8 (8%)	14 (13%)*	5 (5%)
General disorders and administration site conditions	89 (43%)	41 (40%)	44 (42%)	36 (36%)
Pyrexia	44 (21%)	18 (18%)	28 (26%)	20 (20%)
Fatigue	22 (11%)	9 (9%)	15 (14%)*	5 (5%)
Chills	20 (10%)*	5 (5%)	13 (12%)*	2 (2%)
Hepatobiliary disorders	24 (12%)	10 (10%)	9 (9%)	9 (9%)
Immune system disorders	18 (9%)	3 (3%)	9 (9%)	3 (3%)
Cytokine release syndrome†	12 (6%)*	2 (2%)	8 (8%)*	0
Infections and infestations	94 (45%)	53 (52%)	55 (52%)	54 (55%)
Upper respiratory tract infection	26 (12%)	19 (19%)	21 (20%)	15 (15%)
Nasopharyngitis	21 (10%)	9 (9%)	13 (12%)	11 (11%)
Acute mononucleosis-like	15 (7%)	4 (4%)	5 (5%)	8 (8%)
Injury, poisoning and procedural complications	22 (11%)	8 (8%)	12 (11%)	8 (8%)
Laboratory investigations				
Blood bicarbonate decreased	83 (40%)	57 (56%)*	38 (36%)	36 (36%)
Haemoglobin decreased	66 (32%)	32 (31%)	38 (36%)	30 (30%)
Aspartate aminotransferase	72 (34%)	25 (25%)	35 (33%)	30 (30%)
White blood cell count decreased	79 (38%)*	27 (27%)	34 (32%)*	18 (18%)
Alanine aminotransferase	72 (34%)*	25 (25%)	31 (29%)*	16 (16%)
Lymphocyte count decreased	46 (22%)*	16 (16%)	25 (24%)*	11 (11%)
Neutrophil count decreased	41 (20%)	17 (17%)	25 (24%)	14 (14%)
Blood alkaline phosphatase	28 (13%)*	18 (18%)	16 (15%)	25 (25%)
Blood sodium decreased	37 (18%)	17 (17%)	14 (13%)	15 (15%)
Platelet count decreased	32 (15%)	12 (12%)	15 (14%)	9 (9%)
Blood calcium decreased	26 (12%)	12 (12%)	11 (10%)	9 (9%)
γ-glutamyltransferase increased	9 (4%)*	7 (7%)	5 (5%)	10 (10%)
Metabolism and nutrition disorders	133 (64%)	64 (63%)	69 (65%)	64 (65%)
Hyponatraemia	66 (32%)	38 (37%)	39 (37%)	33 (33%)
Hypocalcaemia	55 (26%)	21 (21%)	29 (27%)	24 (24%)
Hypoalbuminaemia	20 (10%)	9 (9%)	13 (12%)	8 (8%)
Hypokalaemia	16 (8%)	7 (7%)	9 (9%)	11 (11%)
Hyperkalaemia	20 (10%)	11 (11%)	12 (11%)	11 (11%)
Musculoskeletal and connective tissue disorders	29 (14%)	17 (17%)	14 (13%)	8 (8%)
Nervous system disorders	67 (32%)	30 (29%)	30 (28%)	23 (23%)

	14-day full-dose group (n=209)	14-day low-dose group (n=102)	6-day full-dose group (n=106)	Placebo group (n=99)
Headache	53 (25%)*	25 (25%)	26 (25%)	15 (15%)
Renal and urinary disorders	26 (12%)	19 (19%)	18 (17%)	9 (9%)
Proteinuria	24 (12%)	17 (17%)	12 (11%)	9 (9%)
Respiratory, thoracic, and	43 (21%)	17 (17%)	14 (13%)	20 (20%)
Oropharyngeal pain	18 (9%)	7 (7%)	8 (8%)	11 (11%)
Skin and subcutaneous tissue	117 (56%)*	58 (57%)*	61 (58%)*	21 (21%)
Rash (of special interest)†	109 (52%)*	55 (54%)*	56 (53%)*	20 (20%)
Any rash	67 (32%)*	44 (43%)*	37 (35%)*	11 (11%)
Pruritus	32 (15%)*	10 (10%)	11 (10%)	4 (4%)
Any serious adverse event				
Total serious adverse events	19 (9%)	11 (11%)	12 (11%)	9 (9%)
Neutropenia	1 (<1%)	0	1 (1%)	1 (1%)
Lymphopenia	1 (<1%)	0	0	0
Eye disorders (corneal erosion,	1 (<1%)	0	1 (1%)	0
Abdominal pain	1 (<1%)	0	0	0
Coeliac disease	0	0	1 (1%)	0
Gastritis	0	1 (1%)	0	0
Intestinal obstruction	0	0	1 (1%)	0
Vomiting	0	1 (1%)	0	0
Non-cardiac chest pain	0	0	0	1 (1%)
Fever	1 (<1%)	0	0	0
Hepatobiliary disorders	1 (<1%)	0	1 (1%)	0
Immune system disorders	1 (<1%)	0	0	0
Gastroenteritis, viral	1 (<1%)	0	1 (1%)	0
Anal abscess	0	0	1 (1%)	0
Appendicitis	1 (<1%)	0	0	0
Bronchitis	0	1 (1%)	0	0
Cellulitis	0	0	0	1 (1%)
Gastritis viral	0	0	1 (1%)	0

	14-day full-dose group (n=209)	14-day low-dose group (n=102)	6-day full-dose group (n=106)	Placebo group (n=99)
(continued from previous page)				
Gastroenteritis	0	0	1 (1%)	0
Infection	0	0	1 (1%)	0
Paronychia	0	0	0	1 (1%)
Pneumonia	0	1 (1%)	0	0
Pulmonary tuberculosis	0	1 (1%)	0	0
Tuberculosis	0	0	0	1 (1%)
Injury, poisoning, and procedural complications	1 (<1%)	0	0	1 (1%)
Abnormal nuclear MRI of brain	0	0	1 (1%)	0
Diabetic ketoacidosis	5 (2%)	3 (3%)	1 (1%)	0
Hyperglycaemia	1 (<1%)	1 (1%)	1 (1%)	1 (1%)
Diabetes mellitus inadequate	1 (<1%)	2 (2%)	0	0
Hypoglycaemic seizure	1 (<1%)	1 (1%)	1 (1%)	0
Hypoglycaemic unconsciousness	0	0	1 (1%)	1 (1%)
Dehydration	1 (<1%)	0	0	0
Ketosis	0	0	0	1 (1%)
Metastatic malignant melanoma	0	1 (1%)	0	0
Hypoglycaemic coma	1 (<1%)	0	0	0
Complication of pregnancy	0	0	0	1 (1%)
Depression	0	1 (1%)	1 (1%)	0
Epididymitis	0	0	1 (1%)	0
Subclavian vein thrombosis	1 (<1%)	0	0	0

Data are n (%). No significant differences in serious adverse events were noted for any of the intervention groups versus placebo. Other adverse events occurring in less than 10% of patients in any treatment group were: cardiac disorders (palpitations, bradycardia, cardiomyopathy), ear and labyrinth disorders (including ear pain, vertigo, tinnitus), eye disorders, endocrine disorders (including goitre), neoplasms (benign, malignant, and unspecified, including cysts and polyps), psychiatric disorders (including anxiety, insomnia, depression), vascular disorders, pregnancy, puerperium, and perinatal conditions, and reproductive system and breast disorders. * $p < 0.05$ dose versus placebo. †Prespecified adverse event of special interest.

Figure 1: Trial profile.

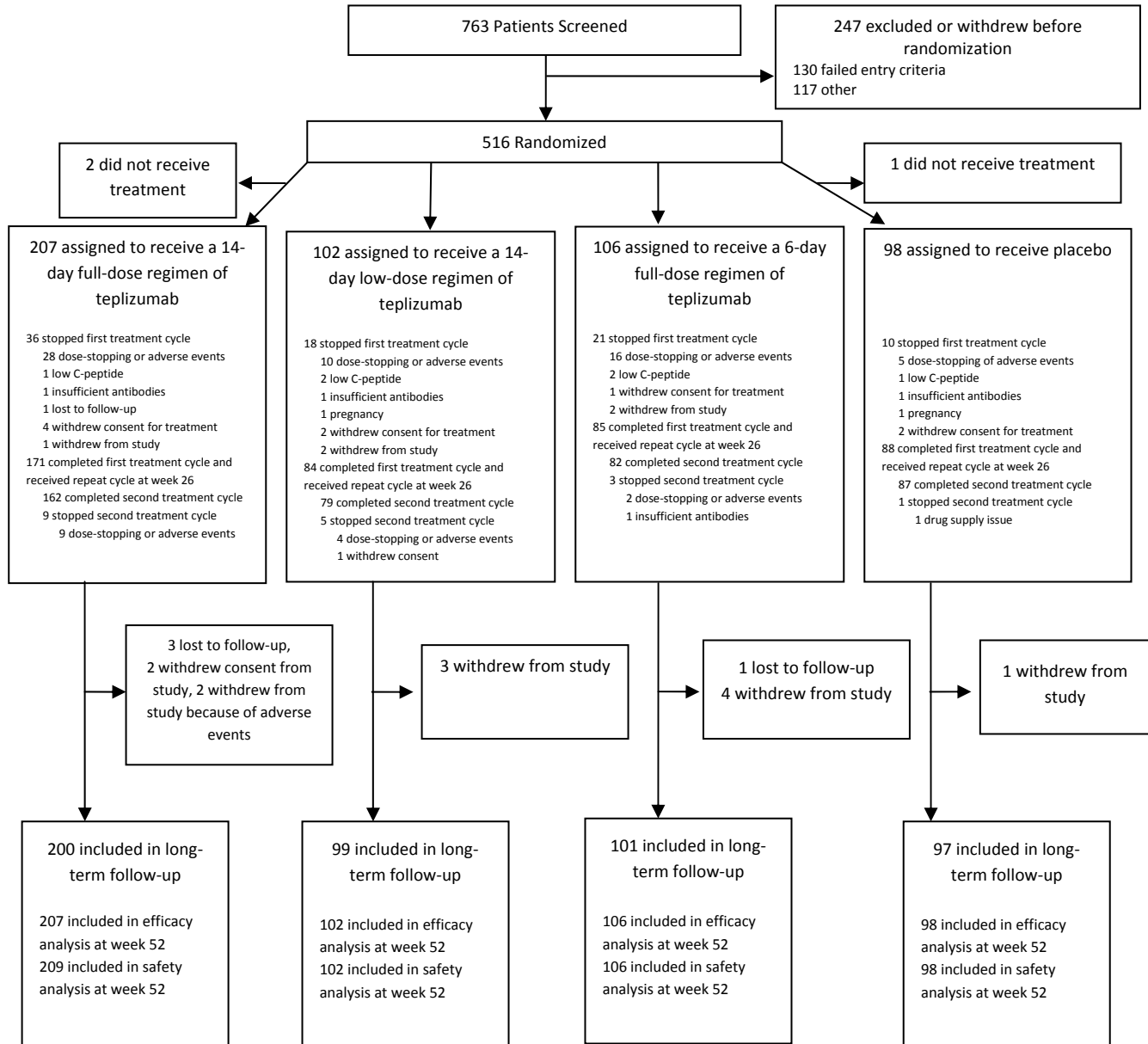
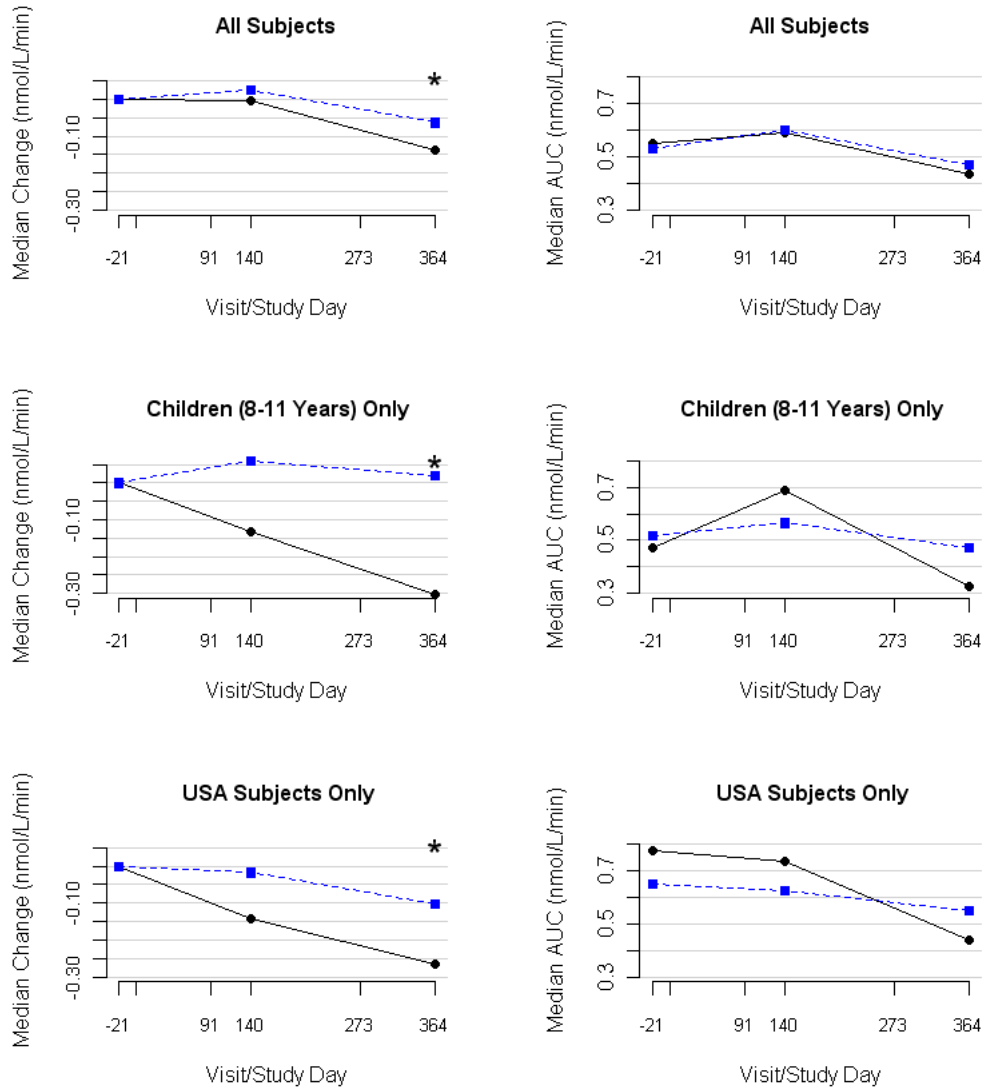


Figure 2: Change in AUC of C-peptide from baseline over time and AUC of C-peptide over time

A. (TOP) All patients. B. (MIDDLE) Ages 8-11 years. C. (BOTTOM) US patients. Statistical testing was not done on absolute AUC values. AUC=area under the curve. * $p < 0.05$ with Wilcoxon rank-sum test.



* Wilcoxon rank-sum test $p < 0.05$ for the change from baseline. Testing on absolute values was not performed.

Figure 3: Cumulative distribution of change in AUC of C-peptide from baseline at 1 year

(A) All patients (n=298). (B) Children aged 8–11 years. (C) US patients. Because of violations of normality assumptions, inferential comparisons were made with non-parametric methods (Wilcoxon rank-sum test).

AUC=area under the curve.

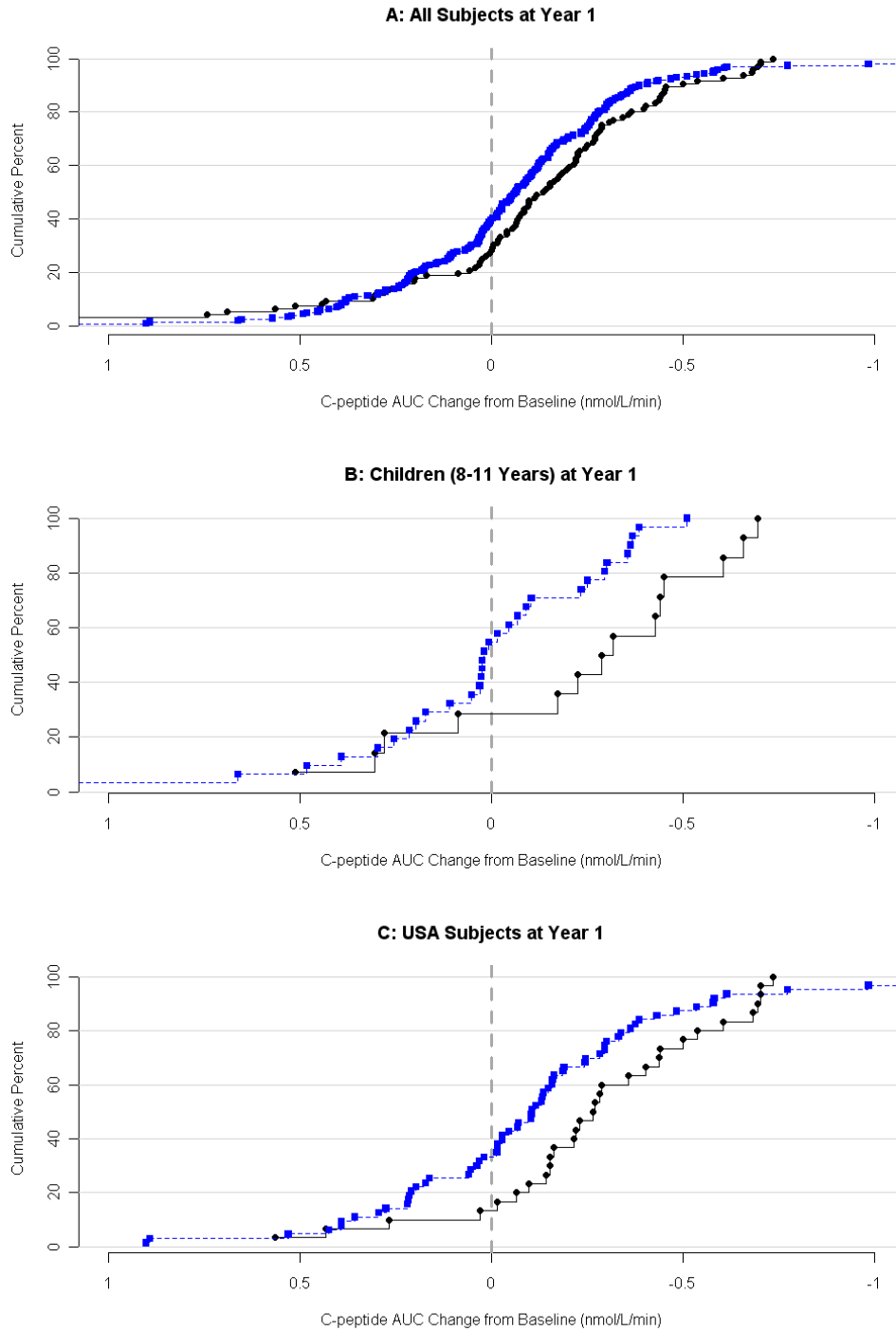
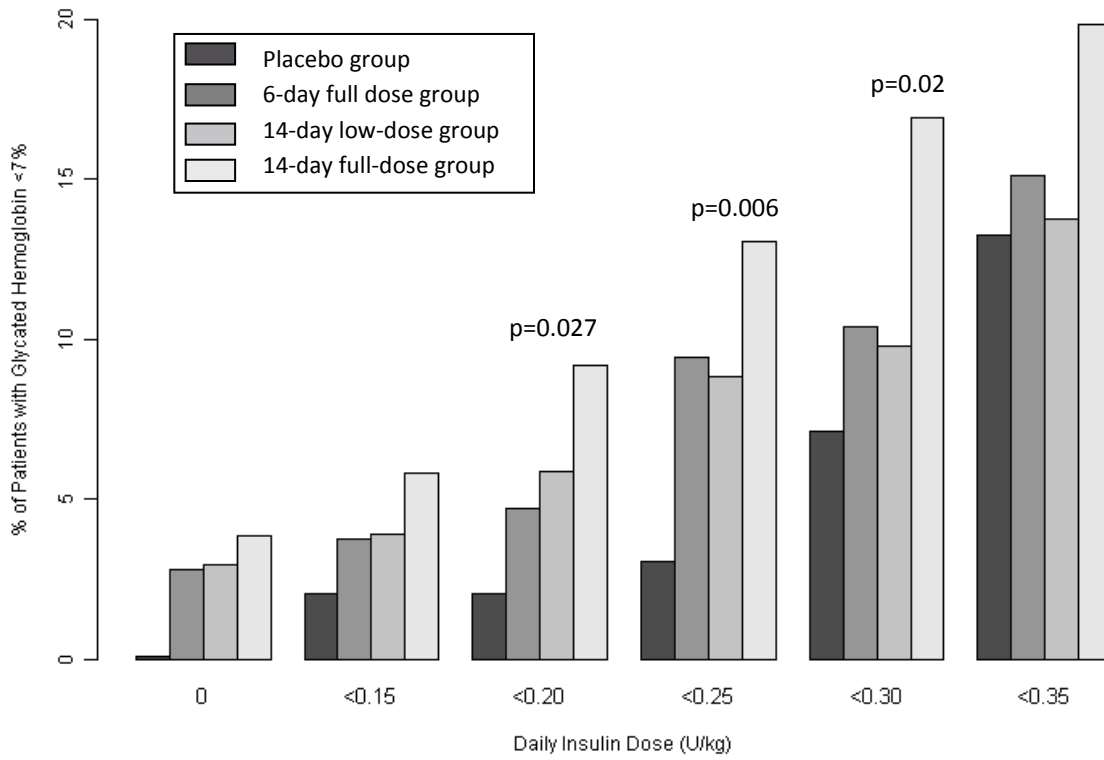


Figure 4: Proportion of patients with HbA_{1c} <7% by daily insulin dose cutoffs

(A) All patients. (B) US Patients. Reported p values are for the 14-day full-dose group versus the placebo group, and were calculated with Fisher's exact test; p values are not shown when p>0.05. Patients with missing values are counted as not meeting criteria. HbA_{1c}=glycated haemoglobin A_{1c}

A.



B.

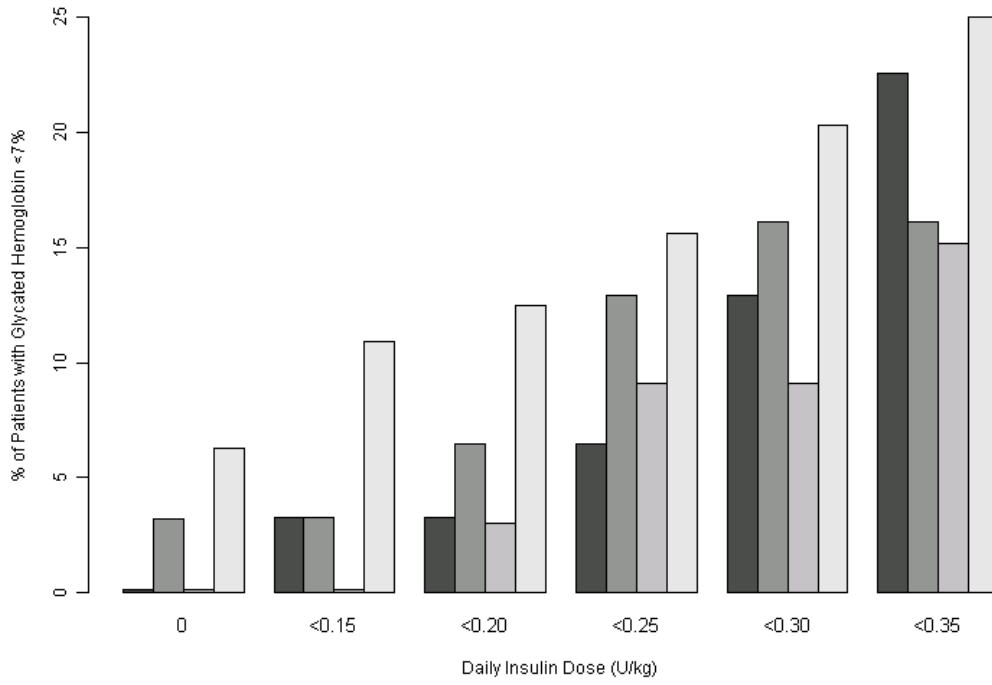
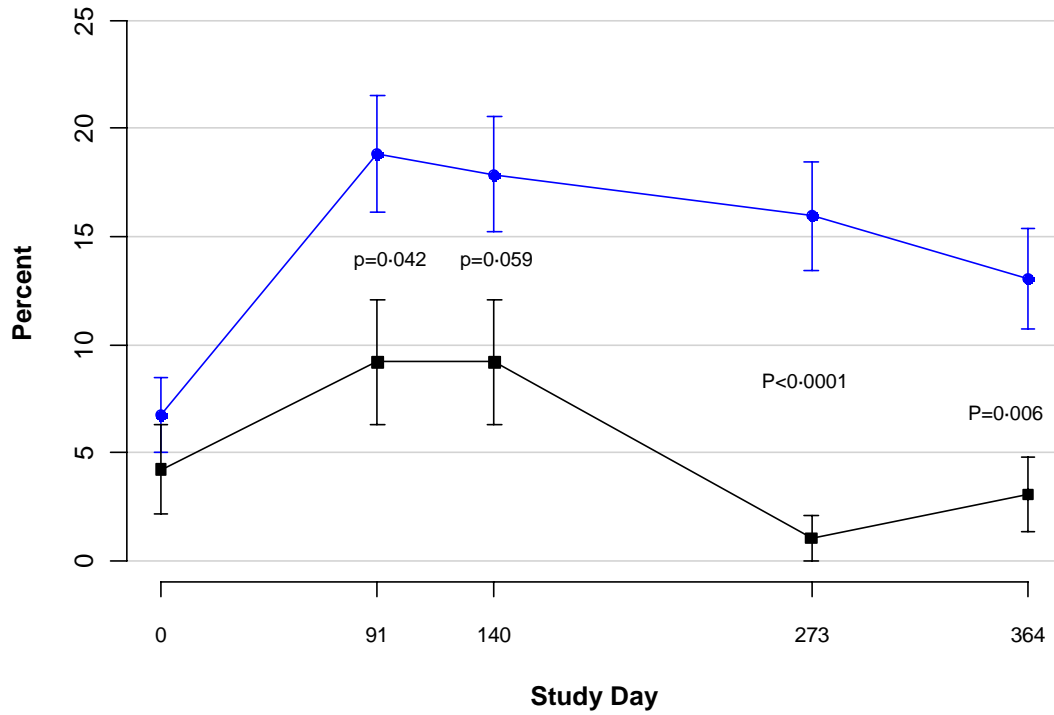


Figure 5: Proportion of patients who met the post-hoc exploratory outcome of HbA_{1c} <7% and insulin use <0.25 U/kg per day at each study visit

HbA_{1c}=glycated haemoglobin A_{1c}.



Number of Subjects Meeting Criteria:

4	9	9	1	3
14	39	37	33	27

Web extra materials

Appendix 1. The investigators and institutions participating in the Protégé trial.

James Lenhard, Christiana Care Research Institute; Stephen Aronoff, Research Institute of Dallas; Bruce Bode, Atlanta Diabetes Associates; Jolene Berg, Diabetes and Glandular Disease Research Associates, PA; Dennis Brenner, Saint Barnabas Medical Center; Holley Allen, Baystate Medical Center; Nicole Sherry, Massachusetts General Hospital; Eda Cengiz, Yale Diabetes Research Program; Marc Rendell, Creighton Diabetes Center; Fernando Ovalle, University of Alabama at Birmingham School of Medicine; Celeste Hart, North Florida Thyroid Center; Richard Guthrie, Mid-America Diabetes Associates, PA; David Liljenquist, Rocky Mountain Diabetes and Osteoporosis Center; Richard Hays; Kevin Ganong, NEA Baptist Clinic Clinical Research Center; Jack Wahlen, Endocrine Research Specialists; Mary Luidens, Division of Endocrinology, Albany Medical College; Roberto Izquierdo, Joslin Diabetes Center; Wayne Moore, The Children's Mercy Hospital; Eva Tsalikian, University of Iowa Children's Hospital; David M. Huffman, University Diabetes & Endocrine Consultants; Barry Reiner.; Peter Gottlieb, University of Colorado Health Sciences Center; William Hagopian, University of Washington; Todd Nebesio, Indiana University School of Medicine; Richard Christensen, Humphreys Diabetes Center; Robert J. Ferry Jr., Le Bonheur Children's Medical Center Hospital and The University of Tennessee Health Science Center at Memphis; Danièle Pacaud, Alberta Children's Hospital; Shayne Taback, University of Manitoba; Miguel Escalante, Hospital Mexico-Americano; Jaime Rodriguez Rivera, Hospital Central; Ignacio Morones Prieto, Endocrinology Department; Hector E. Tamez Perez, Hospital CIMA Santa Engracia; Zdenek Sumnik, Pediatricka klinika UK 2.LF, Fakultni nemocnice v Motole; Jaroslav Skvor, Detska klinika, Masarykova nemocnice v Usti nad Labem; David Neumann, Detska klinika, Fakultni nemocnice Hradec Kralove; Jaroslav Michalek, Pediatricka klinika, Fakultni nemocnice Brno; Jan Vavrínek, Klinika deti a dorostu, Fakultni nemocnice Kralovske Vinohrady; Boris Mankovsky, State Institution "Institute of Endocrinology and Metabolism named after V. Komisarenko of AMS Ukraine"; Yuriy Karachentsev, V. Danilevsky Institute of Endocrine Pathology Problems; Nataliya Zelinskaya, Ukrainian Children Specialised Clinical Hospital Ohmatdet; Gennadiy Lezhenko, Zaporizhzhya Regional Pediatric Hospital; Tatyana Mykhaylychenko, Donetsk Regional Children Clinical Hospital; Oksana Khyzhnyak, Kharkiv Regional Clinical Children Hospital; Maryna Vlasenko, Regional Clinical Endocrinological Dispensary; Gottfried Rudofsky, Innere Medizin I, Endokrinologie und Stoffwechsel, Universitaetsklinikum Heidelberg; Prasanna Kumar, Bangalore Diabetes centre; Richa Chaturvedi, Pushpawati Singhania Research Institute; Uday Phadke, Ruby Hall Clinic; Sailesh Lodha, Fortis Escorts Hospital; Rakesh Sahay, Mediciti Hospitals; K.D. Modi, Medwin Hospitals; Sanjiv Shah, Diabetes Action Center; P.V. Rao, DiabetOmics India Pvt. Ltd; Pramod Gandhi, Gandhi Endocrinology and Diabetes Centre; Parag Shah, Gujarat Endocrine Centre; K.A.V. Subrahmanyam, King George Hospital; Sanjeev Pathak, DHL Research Center; Sanjay Kalra, Bharti Research Institute of Diabetes and Endocrinology; Sunil Jain, TOTALL Diabetes Hormone Research Institute Pvt Ltd; Manojit Mukhopadhyay, B.P. Poddar Hospital and Medical Research Ltd; Sujeet Chandratreya, Endocare Clinic; Aaron Hanukoglu, Pediatric Endocrinology, Wolfson Medical Center; Anat Jaffe, Hillel Yaffe Medical Center; Moshe Phillip, Schneider Children's Medical Center of Israel; Orit Pinchas-Hamiel, Pediatric Endocrine and Diabetic Unit, The Chaim Sheba Medical Center; Naim Shehadeh, Rambam Health Care Campus; Maria Gorska, , Klinika Endokrynologii, Diabetologii i Chorób Wewnętrznych, Uniwersytecki Szpital Kliniczny w Białymstoku; Wojciech Młynarski, Klinika Pediatrii, Onkologii, Hematologii i Diabetologii, Samodzielny Publiczny Zakład Opieki Zdrowotnej Uniwersytecki Szpital Kliniczny Nr 4, im. Marii Konopnickiej Uniwersytetu Medycznego; Anna Noczynska, Klinika Endokrynologii i Diabetologii Wieku Rozwojowego, Samodzielny Publiczny Szpital Kliniczny Nr 1; Maciej Pregiel, Oddział Chorób Wewnętrznych, Powiatowy Zespół Szpitali w Olesnicy; Mieczysław Szalecki, III Oddział Chorób Dziecięcych (Endokrynologiczno-Diabetologiczny), Wojewódzki Specjalistyczny Szpital Dzieciacy im. Władysława Buszkowskiego w Kielcach; Małgorzata Mysliwiec, Oddział Diabetologiczny

Klinika Pediatrii, Hematologii, Onkologii i Endokrynologii; Adriana Dumitrescu, Centrul Medical Sanatatea ta; Nicolae Dragos Hancu, Centrul de Diabet, Nutritie si Boli Metabolice, Spital Clinic Judetean de urgenta Cluj; Valerica Nafornta, S.C. MINIMED S.R.L.; Iosif Szilagy, Sectia de Diabetologie si Boli de Nutritie, Spitalul Judetean Satu Mare; Eugenia Farcasiu, Institutul de Diabet, Nutritie si Boli Metabolice N.C. Paulescu; Ulle Jakovlev, East Tallinn Central Hospital; Tarvo Rajasalu, Tartu University Hospital; Valdis Pirags, P. Stradins Clinical University Hospital; Wilfredo Ricart Engel, Servicio de Endocrinología, Hospital Dr. Josep Trueta de Girona; Marta Botella Serrano, Servicio de Endocrinología, Hospital Príncipe de Asturias; Johnny Ludvigsson, Barnkliniken Universitetssjukhuset. See ClinicalTrials.gov identifier: [NCT00385697](https://ClinicalTrials.gov/ct2/show/study/NCT00385697) for additional information.

Appendix 2: Screening, Randomization, Follow-up Visits

Exclusion criteria

Participation was not permitted if the patient had administration of a monoclonal antibody in the prior year; participation in any type of drug or vaccine clinical trial within the prior 12 weeks; planned therapy with insulin, exenatide or other therapy that may stimulate pancreatic beta cell regeneration or insulin secretion; any medical condition that, in the opinion of the investigator, would interfere with safe completion of the trial; uncompensated heart failure, fluid overload, myocardial infarction, ischemic heart disease or other serious cardiac disease within 12 weeks of enrollment; pregnant or lactating females; prior murine OKT3® treatment; history of epilepsy, cancer, cystic fibrosis, sickle cell anemia, neuropathy, peripheral vascular disease or cerebrovascular disease; recent untreated hypothyroidism or active Graves' disease; eczema, asthma or severe atopic disease requiring treatment within the prior 12 weeks; active infection, such as fever ≥ 38.0 degrees Celsius (100.5 degrees Fahrenheit); known or suspected HIV infection; active hepatitis B or C virus; active or latent tuberculosis; live virus vaccination within the prior 12 weeks or planned during the study; vaccination with an antigen or killed organism was not allowed within 12 weeks before Day 0 or planned within 8 weeks after each dosing cycle; infectious mononucleosis-like illness within the prior 6 months; serologic and clinical evidence of acute infection with Epstein-Barr virus or cytomegalovirus. Use of insulin pumps was permitted.

Screening

The participating centers included clinics, academic centers, and hospitals. An initial 38 patients were enrolled in an open-label portion of the study not presented here. The study was activated in October 2006 and the first patient was randomized into the open-label segment in February 2007; the first patient in the blinded study was randomized in August 2007, and the last patient entered the study in June 2009.

A multi-step enrollment process was used to evaluate the safety and tolerability of open-label treatment with teplizumab prior to randomizing patients in the double blinded study. The DMC continued to monitor safety at scheduled intervals and additionally as appropriate throughout the study.

Randomization and dosing

Dosing in the four arms of the study is described here:

- Arm 1-- 14-Day Regimen: Patients received daily IV doses of 51, 103, 207, and 413 $\mu\text{g}/\text{m}^2$ on Study Days 0–3, respectively, and one dose of 826 $\mu\text{g}/\text{m}^2$ on each of Study Days 4–13. The total dose for a 14-day course is approximately 9034 $\mu\text{g}/\text{m}^2$. For patients weighing 70 kg and having a BSA of 1.92 m^2 , this dosing schedule delivers ~17 mg of teplizumab.
- Arm 2-- 1/3-Dose Regimen: This is the 14-Day Regimen (Arm 1) divided by 3. Patients received daily IV doses of 17, 34, 68, and 136 $\mu\text{g}/\text{m}^2$ on Study Days 0–3, respectively, and one dose of 273 $\mu\text{g}/\text{m}^2$ on each of Study Days 4–13. The total dose for a 14-day course is approximately 2985 $\mu\text{g}/\text{m}^2$. For patients weighing 70 kg and having a BSA of 1.92 m^2 , this dosing schedule delivers ~5.6 mg of teplizumab.
- Arm 3 -- 6-Day Regimen: This regimen is the 14-Day Regimen (Arm 1) curtailed after 6 doses. Patients received daily IV doses of 51, 103, 207, and 413 $\mu\text{g}/\text{m}^2$ on Study Days 0–3, respectively, and one dose of 826 $\mu\text{g}/\text{m}^2$ on each of Study Days 4–5, followed by 8 days of IV placebo (Study Days 6–13). The total dose for a 14-day course is 2426 $\mu\text{g}/\text{m}^2$. For patients weighing 70 kg and having a BSA of 1.92 m^2 , this dosing schedule delivers ~4.6 mg of teplizumab.
- Arm 4-- Placebo: Patients received a 14-day course of IV placebo only.

Study drug dosing was to be permanently discontinued for pregnancy, protocol-defined adverse experiences Grade ≥ 3 during dosing, elevations in total bilirubin (>2 times upper limit of normal), ALT/AST (>3 times upper limit of normal), decreases in platelet count ($<100,000/\mu\text{L}$), neutropenia (<1000 PMN/ μL on 2 consecutive measurements on different days), or hemoglobin (<8.5 g/dL or a reduction of >2 g/dL from pre-treatment level). Dosing Cycle 2 was not administered on Day 140 if any of the following were present: C-peptide $<$ lower limit of detection;

absolute lymphocyte count < 1000/ μ L or <80% of the pretreatment level, platelets < 150,000/ μ L, neutropenia (< 1000 PMN/ μ L on 2 consecutive evaluations on different days), Hgb < 10g/dL, acute EBV or CMV infection, development of HIV I or II, hepatitis B or hepatitis C.

United BioSource Corporation (UBC) generated and maintained the allocation schedule and managed the distribution and assignment of study medications at all sites. Investigators provided patient information to UBC and UBC instructed the investigators which study medications to administer to each patient. All patients and study personnel (except for certain DMC, randomization, and MacroGenics personnel unblinded for safety issues, lab validation and drug supply) were blinded to the treatment codes, to block sizes, and to laboratory measurements that might reveal allocation (such as serum teplizumab levels and T-cell proteins) through 1 year. After the DMC provided notification that the primary outcome was not met, a MacroGenics clinical monitor and statistician were unblinded to evaluate the data and amend Protégé and other teplizumab protocols as needed. All patients and study staff remained blinded through closure of the study, except for authors of this manuscript who were unblinded to the 1-year study results but not individual patient group assignments. The last patient visit is expected in late June, 2011.

Appendix 3. Laboratory measurements

Mixed-meal tolerance tests (MMTT) were performed by measuring C-peptide and glucose levels in blood samples using a validated chemiluminescent assay on the Immulite 2000 at time points 10 minutes (± 5 min) before start of the MMTT, immediately before start of MMTT, administration of Boost®, and at 15 (± 5), 30 (± 5), 60 (± 5), 90 (± 5), 120 (± 5), 150 (± 5), 180 (± 5), 210 (± 5) and 240 (± 5) minutes after the patients finished the meal. The C-peptide lower limit of quantitation was 0.03 nmol/L; the lower limit was 0.17 nmol/L for North America sites before July 2007 and other sites before January 2008. Blood samples were collected at each visit. HbA_{1C} was measured by immunologic method on Roche Modular and Cobas Integra 400. The HbA_{1C} results from the Modular correlated highly with the results from Cobas Integra 400 ($r = 0.9922$). Bio-rad Liquichek Diabetes Control Level 1 and 3 were used to monitor assay quality.

Baseline values for C-peptide 4 hour AUC response to a mixed meal were from a screening visit and for HbA_{1C} from Day 0. All glucose, C-peptide, and autoantibody assays were done in a licensed central laboratory (Esoterix Clinical Trials Services). Autoantibodies to ICA512/IA-2, GAD, and insulin were measured in a licensed central laboratory (Esoterix Clinical Trials Services) at screening only. A Kronus Kit assay for anti-ICA512 was used and met the established acceptability criteria for detection limit, precision, accuracy by inter-assay reproducibility, and standard curve fitting before use; clinical specificity was 99%. Insulin antibodies (IAB) were measured by insulin ¹²⁵I binding capacity, was developed by Esoterix Clinical Trials Services as an in-house RIA (Radioimmunoassay) procedure. The assay was met the established acceptability criteria for detection limit, precision, accuracy, standard curve fitting and linearity. Anti-GAD (glutamic acid decarboxylase) autoantibodies were quantified by IRMA (immunoradiometric assay) using human recombinant ¹²⁵I-labeled GAD. The assay met acceptance criteria for precision, accuracy, specificity and linearity.

Validated quantitative detection of Epstein Barr virus (EBV) DNA in plasma was performed at a licensed central laboratory (ViroMed) using the multicode-RTx PCR assay (qEBV-PCR polymerase chain reaction for viral load). To enable quantitative determinations of input target EBV-DNA, external calibration curves were constructed using dilution panels of DNA extracted from Namalwa cells (EBV-positive lymphoblastoid cell line containing two EBV genomes per host genome) obtained from ATCC (Catalog # CRL-1432). EBV IgM and IgG were measured by Esoterix Clinical Trials Services, using a validated multiplexed, microparticle-based immunoassay (AtheNA Muli-Lyte®, Zeus Scientific, Inc.) for IgM antibodies to EBV, viral capsid antigen, and IgG antibodies to EBV.

Validated quantitative detection of Cytomegalovirus (CMV) DNA in anticoagulated plasma was performed at a licensed central laboratory (ViroMed) using Roche COBAS® AMPLICOR® CMV MONITOR Test. This assay is an in vitro nucleic acid amplification test designed to quantitate CMV DNA by simultaneous amplification of a CMV-specific target region and an internal control amplicon (Quantitation Standard (QS)). Quantitation of the input CMV DNA concentration was determined in the COBAS® AMPLICOR® analyzer by comparison of the CMV signal QS signal for each sample analyzed. The assay has a linear range of 600 to 100,000 CMV DNA copies per mL. CMV IgG were measured by Esoterix Clinical Trials Services, using a validated CMV IgG kit (Catalog# L2KCVG2) on Siemens IMMUNLITE® 2000 chemiluminescent analyzer. Anti-drug antibodies were measured in serum prior to dosing on the 1st day of each dosing cycle, then on study days 28, 56, 91, 210, 244, 273 and 364.

Appendix 4. Definition of Primary Outcome, Sample Size Calculation, Time Log of Statistical Analysis and Related Information

The planned sample sizes for this study were based on the primary composite outcome (insulin dose of <0.5 U/kg/day and $HbA_{1C} <6.5\%$ at 52 weeks after randomization) and previous data from an open-label study that showed $\sim 5\%$ of the control group and nearly half of the group receiving teplizumab achieved this outcome at 52 weeks. The second primary outcome was the mean HbA_{1C} level at 52 weeks after randomization. A hierarchical analysis was planned such that the mean HbA_{1C} outcome would only be assessed if the composite outcome was statistically significant. To test efficacy at 52 weeks, each dose regimen was compared with Placebo. To preserve a two-sided experiment-wise type 1 error rate at 0.05, the Holm stepwise testing procedure was used. The dose with the smallest p-value compared with Placebo was tested at a type 1 error rate of 0.0167. If that comparison was significant, the dose with the next smallest p-value would be tested at a type 1 error rate of 0.025. If that, too, was significant, the third dose regimen would be tested at a type 1 error rate of 0.05.¹³ We used a Mantel-Haenszel test stratified by age group to analyze the composite outcome. All analyses were performed by employees of the sponsor (C.H. & D.C.) using SAS software (SAS Institute, version 9.2) and an independent statistical data analysis center (SDAC; University of Wisconsin – Madison, Department of Biostatistics and Medical Informatics Data Analysis Center). The independent data monitoring committee reviewed interim data analyses produced by the independent SDAC.

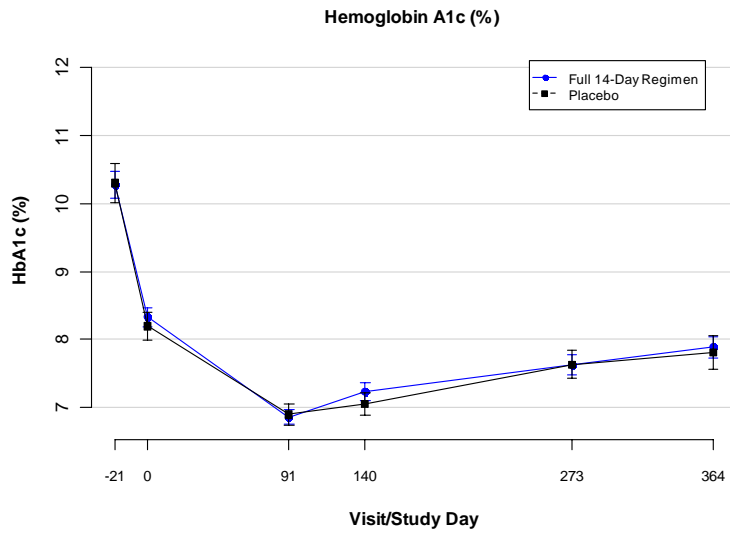
C-peptide response to the mixed meal tolerance test (performed on days 140, 364, 546, and 728) was expressed as the total area under the curve (AUC) over 4 hours using the trapezoidal rule. Missing C-peptide and HbA_{1C} values were imputed by last observation carried forward at the request of regulators.

Appendix 5: Supplementary tables and figures

Table 1. Baseline characteristics by geographic region.

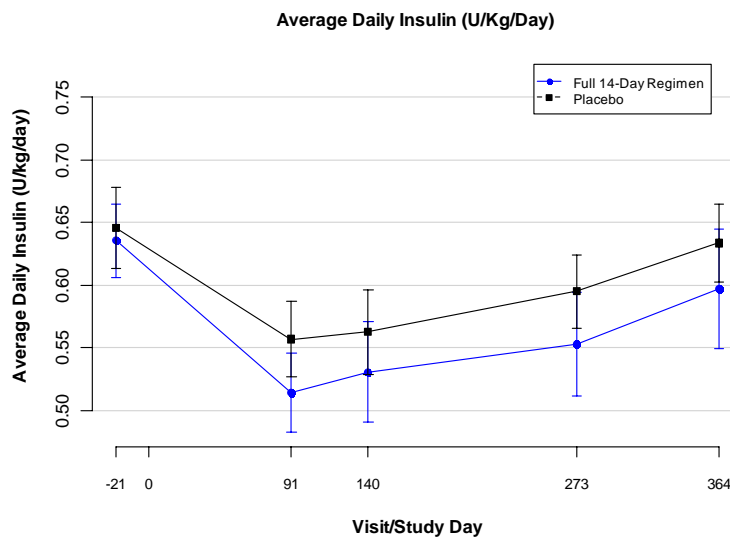
	USA	India	Europe, Israel	Mexico/Canada
Total patients	158	142	179	34
Age – 8–11	27 (17%)	12 (8%)	27 (15%)	11 (32%)
12–17	70 (44%)	49 (35%)	70 (39%)	16 (47%)
≥18	61 (39%)	81 (57%)	82 (46%)	7 (21%)
Mean (SD)	17·3 (6·9)	19·7 (6·5)	18·9 (7·5)	14·7 (5·2)
Gender – Male	98 (62%)	88 (62%)	115 (64%)	24 (71%)
Race – White	15 (94%)	0 (0%)	179 (100%)	34 (100%)
Asian	0 (0%)	142 (100%)	0 (0%)	0 (0%)
Time Diagnosis to Enrollment (wks)	8·8 (2·5)	7·8 (2·6)	8·9 (3·8)	8·0 (2·9)
History of Ketoacidosis	36 (23%)	54 (38%)	82 (46%)	18 (53%)
Antibody type				
GAD 65 (>0·5 U/mL)	141 (90%)	128 (90%)	164 (92%)	28 (85%)
Human Insulin (>2·0 UU/mL)	139 (89%)	127 (90%)	159 (89%)	30 (90%)
Islet Cell 512 (>0·8 U/mL)	103 (66%)	46 (33%)	109 (61%)	25 (76%)
Insulin - (U/kg/day)	0·47 (0·25)	0·98 (0·49)	0·55 (0·26)	0·57 (0·27)
HbA _{1c} – (%)	7·6 (1·3)	9·7 (2·4)	7·6 (1·5)	8·5 (1·6)
C-peptide AUC (nmol/L/min)	0·77 (0·46)	0·53 (0·50)	0·66 (0·39)	0·75 (0·69)

Figure 1. A. (top) Mean HbA_{1C} (%); B. (bottom) Insulin use (U/Kg/d) at each time point, for the 14-Day full dose and Placebo groups.



Number of Subjects Meeting Criteria:

Visit/Study Day	Full 14-Day Regimen	Placebo
-21	98	98
0	204	199
91	96	201
140	97	201
273	97	201
364	98	201



Number of Subjects Meeting Criteria:

Visit/Study Day	Full 14-Day Regimen	Placebo
-21	98	98
0	207	199
91	97	201
140	98	201
273	98	201
364	98	201

Figure 1. C. (top) Mean HbA_{1C} (%); D. (bottom) Insulin use (U/Kg/d) changes from baseline at each time point, for the 14-Day full dose and Placebo groups.

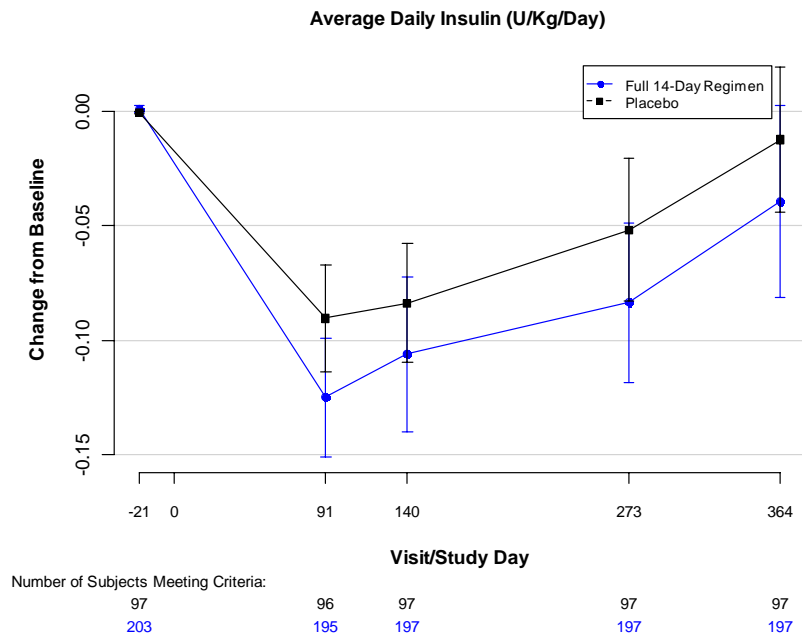
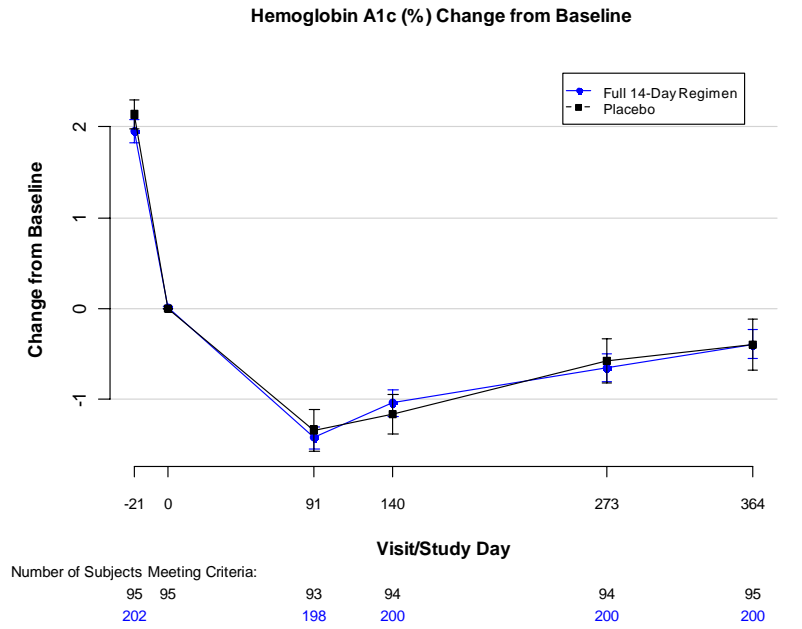
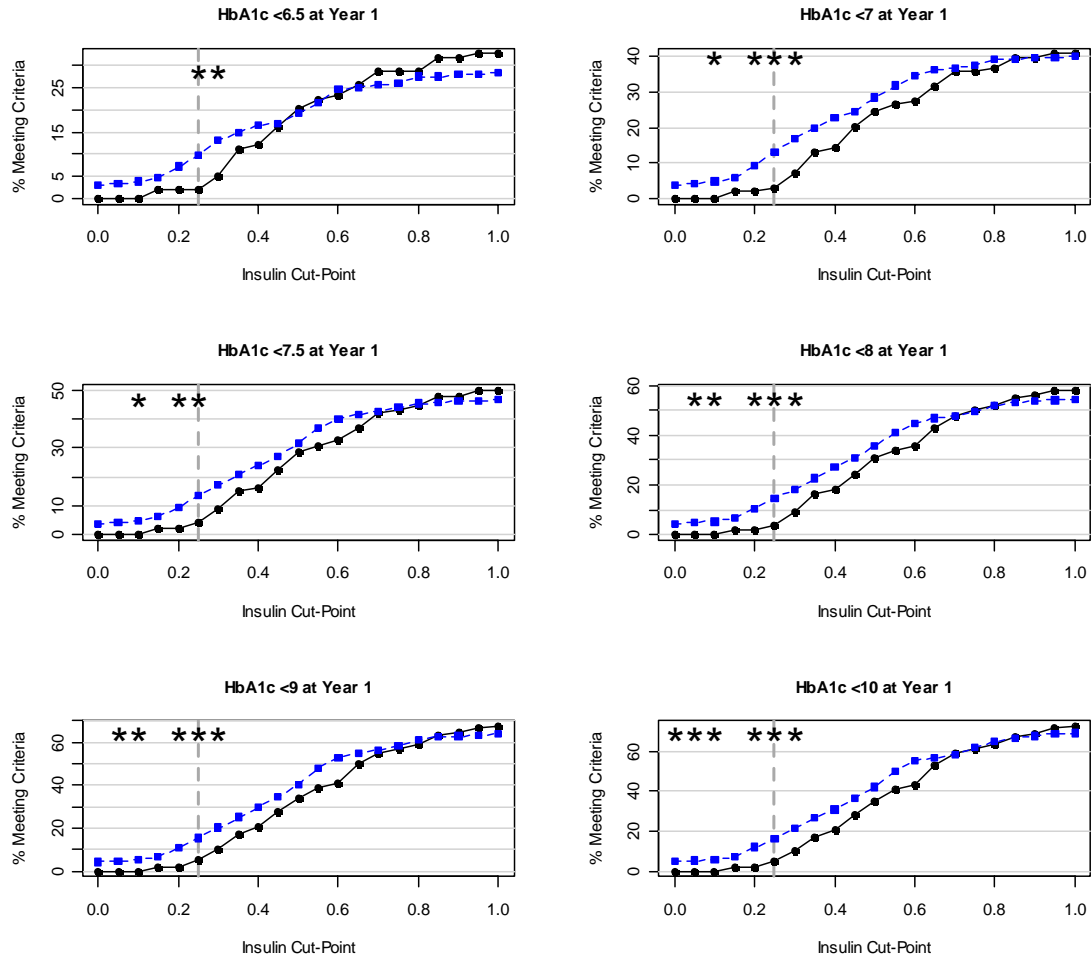


Figure 2. Percentage of patients meeting HbA_{1C} criteria, by insulin use at 1 year. Blue squares: 14-day full-dose group. Black circles: Placebo group.



*p < 0.05 by Fisher's exact test

Figure 3. Cumulative distribution of C-peptide AUC at 1 year for patients with diagnosis ≤ 6 weeks. The shift between treatment groups is evident in the pre-defined subgroup of patients who were randomized ≤ 6 weeks after diagnosis. Due to violations of normality assumptions, inferential comparisons were made using non-parametric methods.

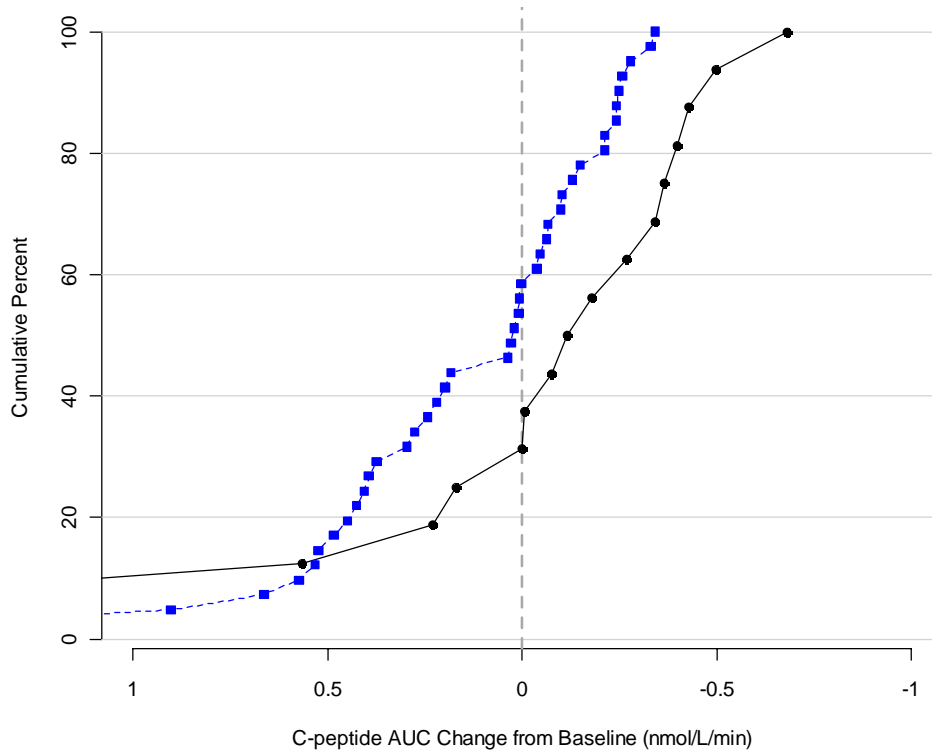
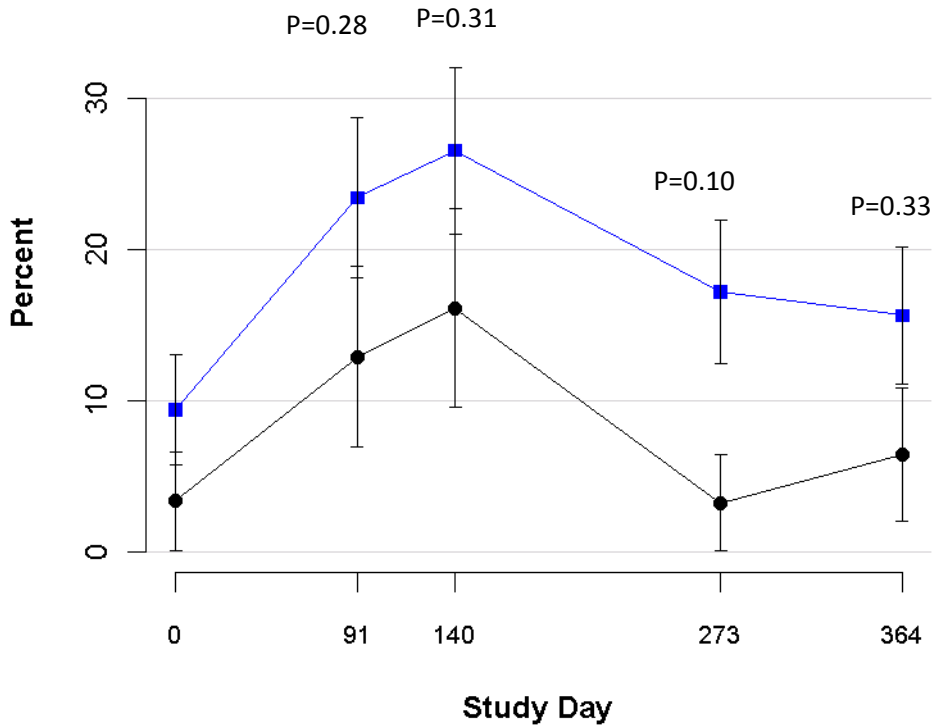


Figure 4: Proportion of patients in the 14-Day full dose (blue squares) and Placebo groups (black dots) who met the post-hoc exploratory outcome of HbA1C <7% and insulin use <0.25 U/kg/d at each study visit. A. US patients. B. Ages 8-11 years. C. Diagnosis ≤6 weeks.

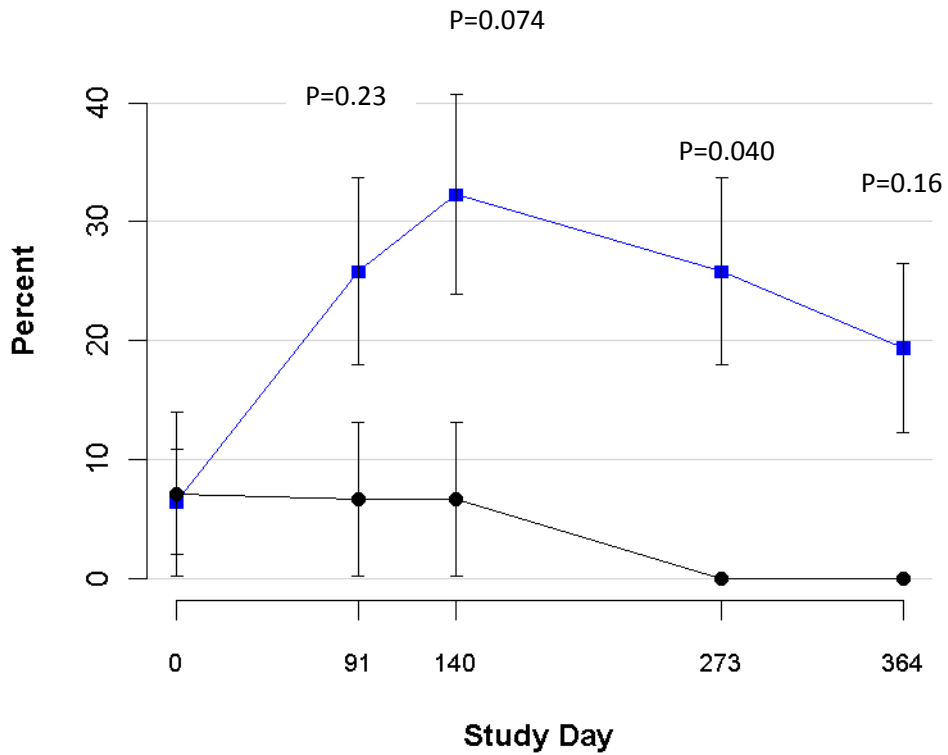
A. US patients.



Number of Subjects Meeting Criteria:

Study Day	14-Day Full Dose	Placebo
0	1	6
91	4	15
140	5	17
273	1	11
364	2	10

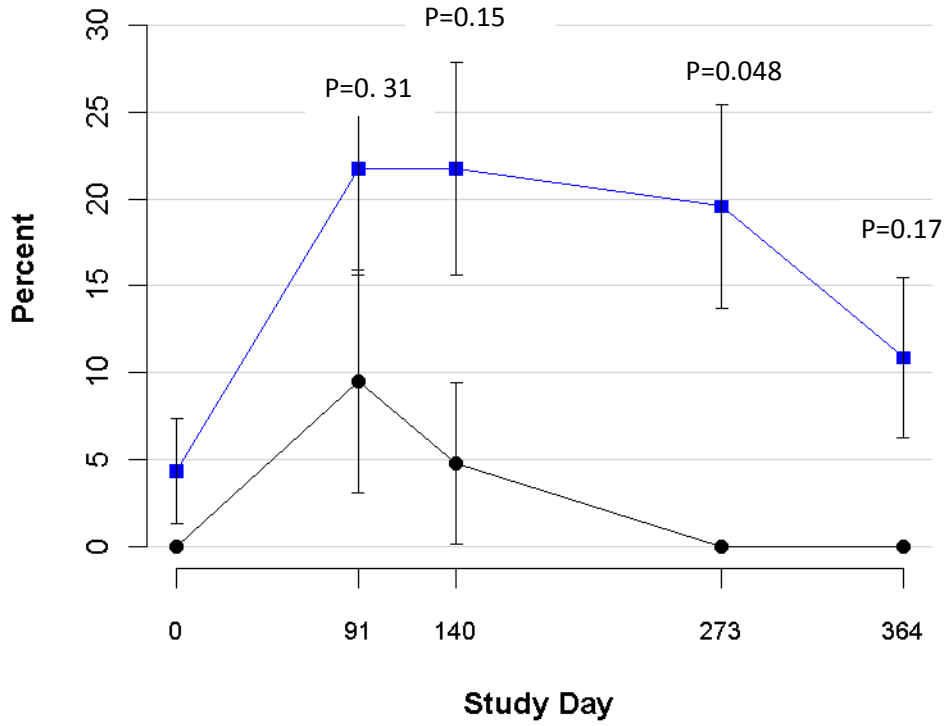
B. Ages 8-11 years.



Number of Subjects Meeting Criteria:

Study Day	0	91	140	273	364
1	1	1	1	0	0
2	2	8	10	8	6

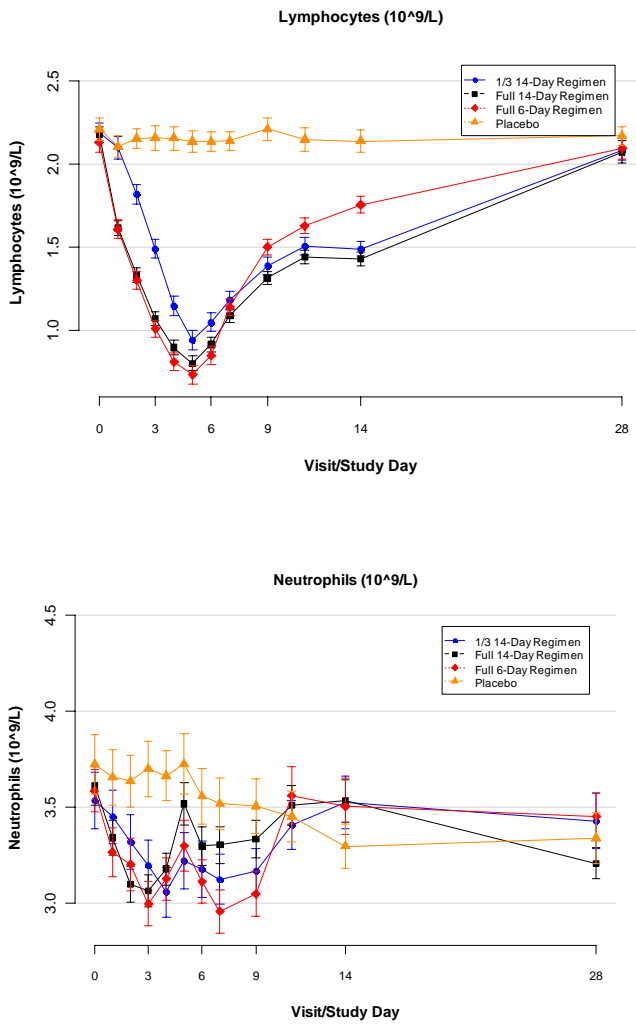
C. Diagnosis ≤ 6 weeks.



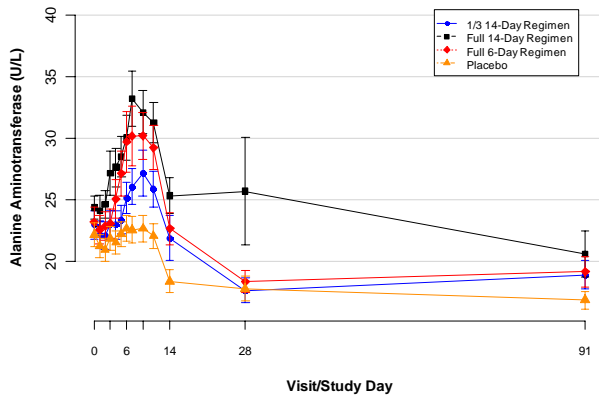
Number of Subjects Meeting Criteria:

0	2	2	1	10	0	9	0	5
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Figure 5. Time course of changes in blood cell counts and indicators of liver function over 1 year.



Alanine Aminotransferase (U/L)



Aspartate Aminotransferase (U/L)

