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# Teplizumab treatment may improve C-peptide responses in participants with type 1 diabetes after the new-onset period: a randomised controlled trial

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## Abstract

**Aims/hypothesis—**Type 1 diabetes results from a chronic autoimmune process continuing for years after presentation. We tested whether treatment with teplizumab (a Fc receptor non-binding anti-CD3 monoclonal antibody), after the new-onset period, affects the decline in C-peptide production in individuals with type 1 diabetes.

**Methods—**In a randomised placebo-controlled trial we treated 58 participants with type 1 diabetes for 4–12 months with teplizumab or placebo at four academic centres in the USA. A central randomisation centre used computer generated tables to allocate treatments. Investigators, patients, and caregivers were blinded to group assignment. The primary outcome was a comparison of C-peptide responses to a mixed meal after 1 year. We explored modification of treatment effects in subgroups of patients.

**Results—**Thirty-four and 29 subjects were randomized to the drug and placebo treated groups, respectively. Thirty-one and 27, respectively, were analysed. Although the primary outcome analysis showed a 21.7% higher C-peptide response in the teplizumab-treated group (0.45 vs 0.371; difference, 0.059 [95% CI 0.006, 0.115] nmol/l) ($p$=0.03), when corrected for baseline

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**Duality of interest**
K. C. Herold and J. A. Bluestone have a patent pending concerning the use of anti-CD3 mAb in combination therapies. J. A. Bluestone has a patent for teplizumab.

**Contribution statement**
KCH, SEG, JD and JAB designed the trial. KCH, SEG, SMW, PAG, FW-L, LD, JS, SMR, SA, MYJ, AWM and JD acquired and analysed the data. FW-L and LD performed additional mechanistic studies. All of the authors contributed to writing the manuscript. KCH was the trial sponsor. KCH is the guarantor of this work, had full access to all the data and takes full responsibility for the integrity of data and the accuracy of data analysis. All of the authors approved the final manuscript.

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imbalances in $HbA_{1c}$ levels, the C-peptide levels in the teplizumab-treated group were 17.7% higher (0.44 vs 0.378; difference, 0.049 [95% CI 0, 0.108] nmol/l, $p$=0.09). A greater proportion of placebo-treated participants lost detectable C-peptide responses at 12 months ($p$=0.03). The teplizumab group required less exogenous insulin ($p$<0.001) but treatment differences in $HbA_{1c}$ levels were not observed. Teplizumab was well tolerated. A subgroup analysis showed that treatment benefits were larger in younger individuals and those with $HgbA_{1c}$ <6.5% at entry. Clinical responders to teplizumab had an increase in circulating CD8 central memory cells 2 months after enrolment compared with non-responders.

**Conclusions/interpretations—**This study suggests that deterioration in insulin secretion may be affected by immune therapy with teplizumab after the new-onset period but the magnitude of the effect is less than during the new-onset period. Our studies identify characteristics of patients most likely to respond to this immune therapy.

**Trial registration—**ClinicalTrials.gov NCT00378508

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### Keywords

Autoimmunity; Immune therapy; Type 1 diabetes

## Introduction

Studies from the past 25 years have described type 1 diabetes as a chronic immune-mediated disease involving a progressive destruction of insulin-producing beta cells often beginning years before clinical presentation and continuing for years after diagnosis [1]. Clinical experience is consistent with a chronic rather than acute failure of beta cell function after the onset of disease. Many newly diagnosed patients show marked improvement in glucose tolerance and reduced insulin requirements [2, 3]. Indeed, experience from the DCCT suggests that for at least 2–3 years after clinical onset, many patients still retain significant levels of insulin production reflected by a stimulated C-peptide level of at least 0.2 nmol/l [4]. Retention of insulin production has been associated with improved prognosis including reduced rates of retinopathy, nephropathy, neuropathy and even hypoglycaemia [5, 6]. This suggests that treatments that are able to arrest the decline in insulin production, even after the new-onset period, may have clinical benefit [7, 8]. However, with time the majority of patients lose the ability to make insulin: from the DCCT, only 3% of participants diagnosed before the age of 18 years and 8% of participants diagnosed as adults retained clinically significant insulin production after 5 years.

Several questions remain about the optimal patients for immune intervention. Most studies have been performed in participants with new-onset type 1 diabetes, generally defined as within 100 days of diagnosis [9–14]. There are at least two reasons for this. First, since C-peptide levels decline with time the proportion of participants with clinically significant levels, who could potentially benefit from a therapy that arrests beta cell destruction, becomes smaller in participants with longer duration of type 1 diabetes [4]. Second, experimental evidence suggests that immune interventions are most effective when there are greater levels of residual insulin production or in the period closer to onset. Anti-CD3 monoclonal antibody (mAb) and ciclosporin treatment were shown to be most effective in those with higher levels of insulin production, and GAD65 immunisation was also most effective in participants treated within 6 months of diagnosis [10, 15, 16]. These observations imply that there may be a window of treatment opportunity in the new onset



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period related to the immunological events in the peridiagnosis period or to beta cell intrinsic factors. In addition, a previous study suggested that younger participants may show greater responses to immune therapy–a surprising finding since the rate of decline in younger participants is more rapid than in adults [14]. Data from the DCCT showed that glucose levels may affect beta cell function, yet $HbA_{1c}$ levels have largely been considered as an outcome rather than a modulator of immune response [17]. The relationship between glucose control and responses to immune therapy has not been evaluated.

We and others have previously shown that humanised FcR non-binding anti-CD3 mAb can arrest the decline in beta cell function when it is given to patients with type 1 diabetes in the new onset period [9, 10, 18]. We postulated that it may be possible to stop the decline in beta cell function in those with significant levels of C-peptide production, even after the first 100 days following diagnosis. We therefore conducted a randomised double-blind placebo-controlled study to determine whether teplizumab attenuates the decline in C-peptide response in patients with type 1 diabetes of 4–12 months duration.

## Methods

### Participants and study design

The study was designed as a randomised double-blind placebo-controlled trial with 1:1 randomisation to teplizumab or placebo infusions (NCT00378508). Patients recruited from four clinical sites (Yale University, University of California San Francisco, University of Colorado and Children's Hospital of Philadelphia) were eligible if they were between the ages of 8 and 30 years and diagnosed with type 1 diabetes at least 4 but not more than 12 months before enrolment. The participants in the study all gave written informed consent and the investigations were approved by each institution's institutional review board.

The diagnosis of type 1 diabetes was confirmed by clinical history and the presence of at least 1+ autoantibody (islet cell antibody [ICA], anti-GAD65 or anti-ICA512). To qualify for enrolment, individuals needed to have a single stimulated C-peptide level during a 4-h mixed meal tolerance test (MMTT) of at least 0.2 nmol/l, which has been deemed 'clinically significant' [4]. Randomisation was stratified by two categories for time from diagnosis: 4–8 months and 9–12 months. Participants and all study personnel were blinded to the treatment assignment.

All participants followed identical study procedures throughout the trial. They received a 14-day course of either intravenous teplizumab (day 1, 51$\mu g/m^2$; day 2, 103 $\mu g/m^2$; day 3, 206 $\mu g/m^2$; day 4, 413 $\mu g/m^2$; days 5–14, 826 $\mu g/m^2$) or saline (154 mmol/l NaCl) as described previously [14]. Ibuprofen, diphenhydramine (Benadryl) or paracetamol (known as acetaminophen in the USA and Canada) were given for any infusion related reactions. The participants were instructed to continue their standard diabetes management and were seen in follow-up at intervals of 1–3 months during the year. To assess insulin secretion, 4 h MMTTs, with 11 sampling time points were performed at 6 and 12 months of follow-up as previously described [9, 10, 18]. The mean total daily insulin doses taken for 3 days before study visits was determined at each follow-up visit.

### Laboratory tests

C-peptide and $HbA_{1c}$ levels were measured at the Northwest Research Laboratory (Seattle, WA, USA). Chemistries and complete blood counts (CBCs) and differentials were performed in local laboratories. Anti-insulin, anti-GAD65 and anti-IA-2 antibodies were measured at the Barbara Davis Diabetes Center (Aurora, CO, USA), ICA was measured at the University of Florida (Gainesville, FL, USA). Flow cytometry analysis of peripheral

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blood mononuclear cells (PBMCs) was performed in the Immune Monitoring Core at Yale University on samples shipped overnight and stained using methods described below.

### Flow analysis

PBMCs were separated by Ficoll–Hypaque gradient centrifugation. The cells were stained with mAbs to CD4, CD8, CD45RA, CD45RO, CD69, CD127, FoxP3, CD25, CCR3, CCR4, Vα24 and dendritic and myeloid cell markers, and analysed on an LSRII cytometer (Becton Dickinson, San Jose, CA, USA). Subsets of CD4 and CD8 T cells were analysed with the following definitions: CD4 or CD8 central memory (CM) cells; $CD45RO^{+}CD62L^{+}$, CD4 or CD8 effector memory (EM) cells; $CD45RO^{+}CD62L^{-}$, naive cells; $CD45RA^{+}$ [19]. For the analysis, electronic gates were placed on subpopulations of cells and the percentage of the indicated subset was analysed. The absolute number of cells was determined by multiplying the percentage of the subset × %CD4 or CD8 × absolute lymphocyte count that was obtained from the CBC performed in the clinical laboratory.

### Statistical design

The pre-designated primary endpoint was a comparison of the area under the C-peptide secretory response curve (C-peptide AUC) from the MMTT at the 12-month follow-up visit. Secondary endpoints were percentage change in C-peptide response, insulin dose and $HbA_{1c}$. The study was designed to identify a 40% difference in C-peptide AUC. A sample size of 30 participants per group was required for 80% power to detect this difference with a two-sided 0.05 significance level. The primary and secondary endpoints were compared in the modified intent to treat population, which included all participants with at least one post-randomisation MMTT.

A repeated measures linear mixed model was used to test for the effects of teplizumab on outcome variables following randomisation (i.e. at 6 and 12 months). The models included a random effect for participant and fixed effects for treatment, time and time from diagnosis (by stratum) as well as their interactions. Covariate adjustment was also made for the baseline outcome level. Linear contrasts were used to estimate treatment differences and perform hypothesis testing at individual time points. Because of the chance imbalance between the treatment arms at baseline, analyses were further corrected for the baseline $HbA_{1c}$ level. Exploratory subgroup analyses were done post-hoc and included the effects of duration strata, age (i.e. <15 years and ≥15 years, which represents the upper third of age distribution) and $HbA_{1c}$ (i.e. below diagnostic threshold of <6.5% [47.5 mmol/mol] and >6.5% [47.5 mmol/mol]) categories on the C-peptide endpoint. For statistical analyses, the mean C-peptide secretory response over 4 hrs (as nmol/l) and was calculated as: $\log_e$([AUC of the C-peptide from the 240 min MMTT/240] +1) [12, 20]. For presentation, the C-peptide data were converted to the AUC as nmol/l. Comparisons between baseline characteristics were done by ANOVA and the Mantel–Haenszel $\chi^2$ test. Mean±SD, SEM, or 95% CI are reported as indicated. A $p$ value of <0.05 was considered statistically significant.

## Results

### Participants

The baseline characteristics and enrolment of participants are shown in Table 1 and Fig. 1. Of 86 screened patients, 63 met eligibility criteria, 43 in the 4- to 8-month stratum 1 and 20 in the 9- to 12-month stratum 2. Five participants withdrew before the first dose of study drug. Two participants withdrew from the 4- to 8-month stratum after receiving the first dose of study drug: one following transient grade 3 neutropoenia and the other voluntarily withdrew before the MMTT at month 6. These two participants are included in the reporting of adverse events.

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The teplizumab and placebo groups had similar demographics at baseline. The average age was 12.4 (95% CI 11.3, 13.4) years and ranged from 8 to 26 years. Thirty-eight participants were between the ages of 8 and 14 years; the difference in age between the teplizumab and placebo groups was not statistically significant, either overall or in each duration stratum. Despite randomisation, the $HbA_{1c}$ levels were higher in the placebo group ($p$=0.003) (Table 1). Overall, 27 had $HbA_{1c}$ levels <6.5% (47.5mmol/mol) and 30 had $HgbA_{1c}$ levels >6.5% (47.5 mmol/mol).

## Efficacy

In the a priori specified analysis of the pre-designated primary endpoint, the teplizumab group had C-peptide levels that were 21.7% higher than placebo (C-peptide AUC: 0.45 [95% CI 0.40, 0.51] nmol/l vs 0.37 [95% CI 0.32, 0.42] nmol/l, $p$=0.03). The teplizumab group also lost significantly less C-peptide at 12 months as a percentage of the C-peptide at baseline (18% [95% CI 7.43, 28.5] vs 39.0% [95% CI 27.8%, 50.2%], $p$=0.006). At 12 months, 5 of the 58 participants did not have detectable C-peptide (i.e. values during the MMTT below 0.03 nmol/l). All of these participants were in the placebo group (Fisher's Exact test, $p$=0.02).

Since the teplizumab group had lower $HbA_{1c}$ levels at baseline we performed additional analyses adjusting for the baseline $HbA_{1c}$. After adjustment, the teplizumab group had a C-peptide response that was 17.7% higher than that of the placebo group at 12 months ($p$=0.09) (Fig. 2a). Compared with baseline, the teplizumab group lost, on average, 20.6% (95% CI 10.0, 31.1) of the C-peptide responses whereas the placebo group lost 36.8% (95% CI 25.6, 48.0, $p$=0.04) when corrected for the baseline $HbA_{1c}$ and C-peptide levels (Fig. 2b).

These results suggested that the baseline $HbA_{1c}$ may have affected the responses to teplizumab. Indeed, we found that the magnitude of the difference between teplizumab and placebo on C-peptide response was significantly greater in the lower $HbA_{1c}$ category (<6.5% [47.5 mmol/mol]) compared with the higher $HbA_{1c}$ category (>6.5% [47.5 mmol/mol], $p$=0.007) (Fig. 3a,b). In participants with an $HbA_{1c}$ <6.5% [47.5 mmol/mol] $n$=27), the C-peptide levels were 56.3% higher in the teplizumab group compared with the placebo group ($p$=0.001) at 12 months compared with no improvement in C-peptide responses in the teplizumab group with an $HbA_{1c}$ >6.5% (47.5 mmol/mol) when compared with the placebo group ($n$=31) ($p$=0.56). Subjects with lower $HbA_{1c}$ levels at baseline had higher C-peptide levels at baseline (0.725±0.039 [SEM] vs 0.455±0.033 nmol/l, $p$=0.001). Time from diagnosis did not significantly modify the treatment effect on C-peptide response in the mixed model ($p$ for interaction=0.42). When adjusted for baseline $HbA_{1c}$, the teplizumab group in the 4- to 8-month stratum had an 18.5% higher C-peptide level compared with the placebo group whereas the teplizumab-treated participants in the 9- to 12-month stratum showed a 17.5% higher level compared with the placebo-treated participants (both $p$=NS). After adjustment for baseline $HbA_{1c}$, the C-peptide levels declined by 42.5% in the placebo group in the 4- to 8-month stratum (42.5%; 95% CI 28.8, 56.3) compared with a decline of 28.3% in the placebo group in the 9- to 12-month stratum (95% CI 9.94, 45.73, $p$=0.22) (Fig. 2b).

The total insulin requirements were significantly less in the teplizumab group ($p$=0.01) (Fig. 4a). Teplizumab treatment had no significant effect on the $HbA_{1c}$ levels or on change in the $HbA_{1c}$ levels over the 12-month study period (Fig. 4b, $p$=0.67).

## Effect of age on teplizumab response at 12 months

There was a significant difference in the responses of younger (age <15 years) and older participants (≥15 years) ($p$=0.047). When corrected for baseline C-peptide and $HbA_{1c}$ levels,

teplizumab-treated younger participants had C-peptide responses that were 31.7% higher than placebo ($p$=0.02) whereas the teplizumab-treated older participants showed no difference ($p$=0.56) (Fig. 5). Younger participants had a lower C-peptide at baseline than older participants (0.492±0.03 [SEM] vs 0.745±0.042 nmol/l, $p$=0.004). The effect on C-peptide was primarily due to a greater decline in the C-peptide responses in the younger placebo-treated participants who lost 50.2% of the baseline C-peptide response (95% CI 36.5, 63.9, $n$=20) vs 11.2% (95% CI 11.9, 34.2, $n$=7) in the placebo-treated older participants ($p$=0.004); teplizumab-treated participants lost approximately 19% of the response at baseline in both age cohorts.

## Adverse events and safety

In general, teplizumab was well tolerated (Table 2). Five participants experienced serious adverse events during the study, one treated with teplizumab (an allergic reaction—related to injection not drug) and four treated with placebo. One participant discontinued teplizumab because of a transient grade 3 neutropoenia and two placebo-treated participants discontinued treatment (Table 2). The most common adverse events, occurring in at least 40% of participants and more frequent in teplizumab-treated participants, were rash, lymphopoenia and nausea. One participant in the teplizumab group had Epstein Barr virus infection, which resolved without intervention. Two participants treated with teplizumab vs one participant treated with placebo experienced cytokine release syndrome. All adverse events resolved and were expected based on past experiences with teplizumab in type 1 diabetes [18].

## Mechanistic outcomes and immunological characteristics of clinical response

We did not find a statistically significant change in the titres of anti-GAD65 or anti-ICA512 antibodies or differences between the treatment groups (electronic supplementary material [ESM] Fig. 1), although we did find a modest but statistically significant increase in the titre of anti-insulin antibodies in the drug-treated vs placebo-treated group ($p$=0.04). Similar to previous experience, the drug caused transient lymphopoenia (ESM Fig. 2) [14, 18]. We did not find consistent changes in immunological markers between drug-treated younger and older participants or between those with non-diabetic and elevated $HbA_{1c}$ levels but the CD4:CD8 T cell ratio trended lower at month 2 in younger participants ($p$=0.05), a finding we have previously associated with clinical responses to drug treatment [9, 18].

We compared T and B cell subsets among participants who had been treated with teplizumab to identify features that distinguish clinical responders. We designated this subgroup using a previous definition, as those who lost <7.5% of the baseline C-peptide response at 12 months [18]. There was a greater proportion of responders in the teplizumab (13/31, 42%) vs placebo groups (2/27, 7.4%, Fisher's Exact test, $p$=0.003). The clinical responders had a higher C-peptide level at baseline (0.55±0.048 [SEM] vs 0.41±0.037 nmol/l, $p$=0.02) but the insulin use (0.38±0.06 [SEM] vs 0.43±0.03 U $kg^{-1}$ $day^{-1}$, $p$=0.42) and $HbA_{1c}$ levels (6.29±0.24% [45.3±2.57 mmol/mol] [SEM] vs 6.42±0.18% [46.7±1.96 mmol/mol], $p$=0.66) were not significantly different. The numbers of cells in lymphocyte subsets were not significantly different at baseline and there was similar depletion and repletion of lymphocytes in both subgroups (ESM Fig. 2).

We found a significant increase in CD8CM T cells at month 2 in teplizumab clinical responders compared with teplizumab non-responders ($p$=0.018) (Fig. 6a,c). We did not identify significant differences in CD8EM, naive, or in CD4 T cell or $CD4^+$Treg subsets between responders and non-responders (not shown and Fig. 6c).



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## Discussion

In patients treated with a single 14-course regimen of teplizumab, administered up to 1 year following diagnosis of type 1 diabetes, we observed a significant preservation of C-peptide loss after 1 year but this effect was not statistically significant after adjustment for chance imbalances in $HbA_{1c}$ at baseline. However, percentage change in C-peptide from baseline, as well as insulin requirements, were improved in those receiving teplizumab and a greater proportion of placebo-treated participants lost all detectable C-peptide responses. Our exploratory subgroup analyses suggest that younger age and near normal control of blood glucose improves responses to teplizumab.

The effect of the prior glucose control on responses to the drug was unexpected. The participants with lower $HbA_{1c}$ levels had higher baseline C-peptide responses, which may have been a factor in responsiveness to treatment, but younger participants also showed better response to the drug even though their baseline C-peptide levels were lower than in older participants.

Since teplizumab had no direct effect on $HbA_{1c}$, these findings suggest either an effect of prior glucose levels on beta, immune or other cells such as vascular cells during drug treatment [21, 22]. Glucose may stimulate increased levels of IL-1β by beta cells, which could affect responses to anti-CD3 mAb, as we have recently shown, and thus directly or indirectly cause beta cell toxicity [23–25]. We have found a similar effect of baseline $HbA_{1c}$ in a trial of teplizumab in patients with new-onset type 1 diabetes (K. Herold, unpublished data). Interestingly, in a recent trial of abatacept in type 1 diabetes that showed an effect of that drug on the decline of C-peptide, the drug-treated group had a significantly lower level of $HbA_{1c}$ at randomisation (6.31±0.09% vs 6.74±0.16% [45.5 vs 50.2 mmol/mol], $p$=0.01). These observations suggest that good glucose control prior to immune therapy is needed for the responses to immune therapies in patients with established disease but this hypothesis will need further evaluation in larger studies.

Our analysis did not identify differences in the efficacy of teplizumab between the duration strata. The differences that were seen reflected the decline in C-peptide in the two placebo groups and not the response to teplizumab. This may be explained by a slower rate of decline of C-peptide during the second year of disease, a finding recently reported [26]. Therefore those in the longer duration stratum may lose C-peptide more slowly during the period on study. A longer follow-up time may be needed to detect differences in participants whose endpoint occurred after the first year of disease. In addition, participants who were recruited after 8 months may represent a ‘survivor’ group—by requiring the same level of C-peptide for enrolment in the 4-month as in the 9- to 12-month strata, we may have selected for individuals who have less aggressive disease: we do not know whether the same degree of preservation would be seen in those with lower stimulated levels at entry.

Based on data from other trials, it appears that the magnitude of the effects of the drug in this population who enrolled after 4 months following diagnosis, is less than in those studied within the first 100 days after diagnosis. In a previous trial recruiting participants of similar age with new-onset disease (i.e. within 100 days from diagnosis), there was an 84% increase in C-peptide in drug-treated vs control participants [18] at 12 mos. In US participants in the Protégé trial, which also enrolled participants within 100 days from diagnosis ($n$=95), there was a 33% increase in C-peptide at month 12. There are several potential reasons for the difference in the magnitude of response in patients with new-onset vs longer-duration diabetes, such as evolution of the immune response, inflammatory cells that are no longer active following presentation or the effects of an extended duration of damage to beta cells that may render them unrecoverable. The C-peptide responses at entry into new-onset trials

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has been higher than this study (e.g. 0.722±0.04 vs 0.575±0.030 nmol/l) despite our requirement for a stimulated level of at least 0.2 nmol/l. This might affect the responses to drug since we found that the baseline C-peptide responses were significantly higher in clinical responders, consistent with findings reported by Keymeulen et al [10]. However, the response to drug treatment in our study was not restricted to those within the upper half of C-peptide responses at entry—individuals in the upper half of baseline C-peptide responses lost 19.9±8.8% (SEM) of baseline responses at month 12 whereas those in the lower half of baseline responses lost 18.2±5.2% ($p$=0.87).

The effect of age in determining responses was previously identified in the Protégé trial [14] and is confirmed by our findings. Although the baseline C-peptide responses were lower in younger participants, their baseline $HbA_{1c}$ levels were not (6.82±0.17% [51±1.88 mmol/mol] [SEM] vs 6.51±0.24% [47.6±11.7 mmol/mol], $p$=0.29). Since entry into the study required that participants meet a specific stimulated C-peptide level, there may have been a bias in the enrolment of younger participants with less aggressive disease since younger participants in general have lower levels of C-peptide than older participants [27]. Caution is therefore needed in drawing conclusions from these findings.

In previous studies, we had identified an increase in the relative number of $CD8^+$ T cells among clinical responders to anti-CD3 mAb and had shown that the $CD8^+$ T cells from drug-treated participants had suppressor function ex vivo [18, 28–30]. In this trial we found that drug-treated responders could be distinguished from drug-treated non-responders by an increase in the number of CD8CM ($CD45RO^+CD62L^+$) T cells 3 months after treatment. A limitation of our finding is that it was restricted to a single time point and we did not perform corrections for multiple comparisons. We hypothesise that these CD8 T cells may be those associated with the functional responses we have previously identified but further studies will be needed to directly analyse the function of these cells at later time points, and to determine whether CD8CM cells respond differently in drug-treated responders and non-responders when they encounter antigen, analogous to adaptive Tr1 cells [31]. We did not identify consistent changes in T cell subsets in young and older participants or in those with non-diabetic vs elevated $HbA_{1c}$ levels although the differences in the CD4:CD8 T cell ratio and other immunological markers warrant further investigation.

In conclusion, our findings for the primary outcome did not conclusively demonstrate a significant benefit for teplizumab. Nevertheless, our secondary outcomes suggest that treatment with teplizumab may modulate the course and reduce the decline of C-peptide in patients with established disease of up to 1 year duration. The analyses we performed identified clinical (age) and laboratory ($HbA_{1c}$) characteristics that may identify those participants most likely to respond to drug treatment and may serve as a guide to future studies with this or other immunological characteristics tested in this population. These characteristics of ‘responders’ may also provide insights into the mechanisms underlying drug efficacy. Specifically, the $HbA_{1c}$ findings emphasise the need to conduct studies to explore how glucose levels modulate responses to immunological treatments. We also identified immunological determinants of efficacy that may be useful in future trials in tracking responses or selecting those most likely to respond to therapy.

## Supplementary Material

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## Abbreviations

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| **CBC** | Complete blood count |
| **CM** | Central memory |
| **EM** | Effector memory |
| **FcR** | Fc receptor |
| **mAb** | Monoclonal antibody |
| **MMTT** | Mixed meal tolerance test |
| **PBMC** | Peripheral blood mononuclear cell |
| **ICA** | Islet cell antibody |

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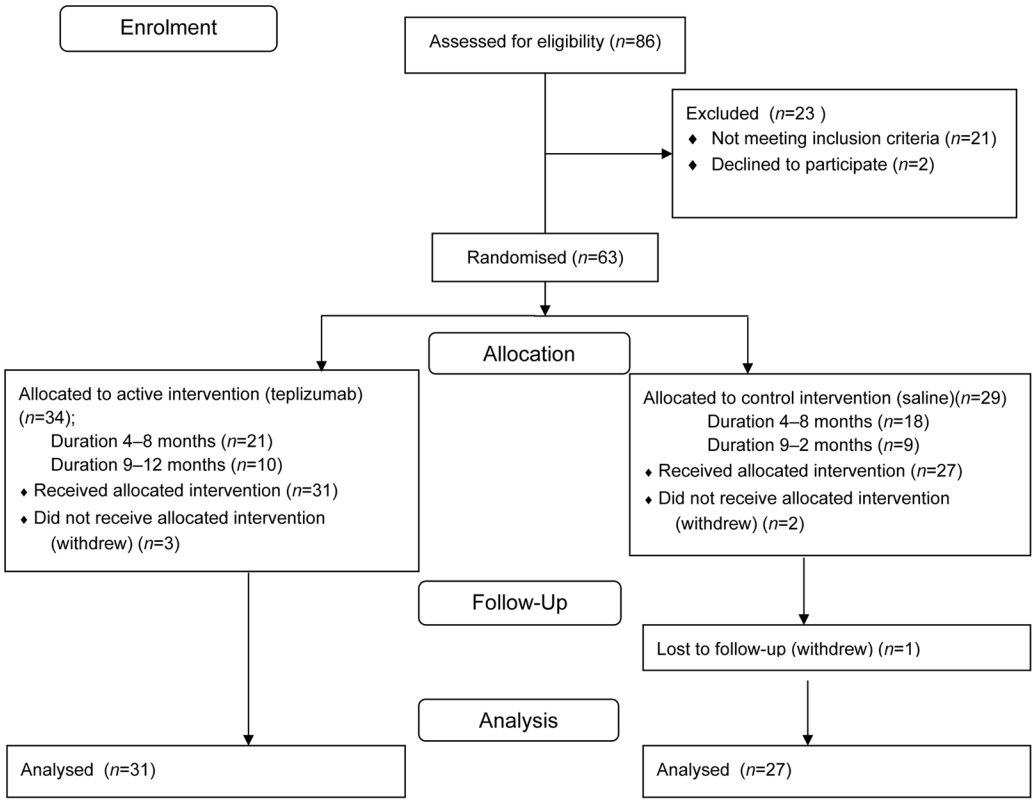


**Fig. 1.**

Enrolment and treatment of study patients. The Figure depicts the flow of patients through the protocol. The reasons for screen failures included insufficient stimulated C-peptide levels, absence of detectable autoantibodies, laboratory abnormalities meeting exclusion criteria, and others. Of the eligible participants, five withdrew before the first dose of study drug. Therefore, 58 participants were included in the endpoint analysis



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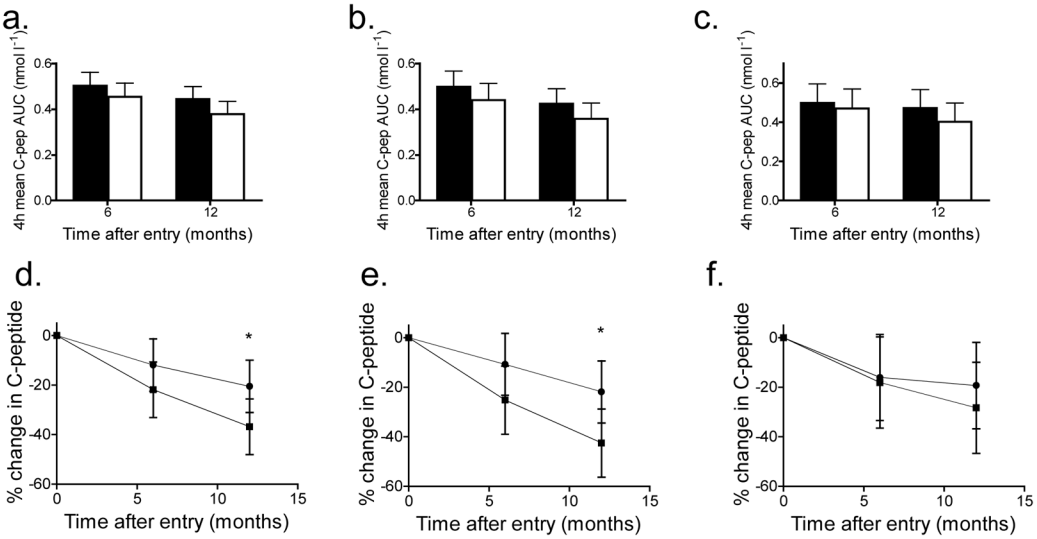


**Fig. 2.**

C-peptide responses to a MMTT in teplizumab- and placebo-treated participants. (**a**–**c**) The mean C-peptide AUC (±95% CI) in participants treated with teplizumab (black bars) and placebo (white bars). The C-peptide AUC at 6 and 12 months adjusting for baseline C-peptide, $HbA_{1c}$ levels and stratum are shown for all participants (**a**, teplizumab-treated, $n$=31; placebo-treated, $n$=27) and for those of diabetes duration 4–8 months (**b**, teplizumab-treated $n$=21, placebo-treated $n$=18) and 9–12 months (**c**, teplizumab-treated $n$=10, placebo-treated $n$=9). (**d**–**f**) The percentage change in C-peptide AUC from baseline (±95% CI) in participants treated with teplizumab (circles) and placebo (squares). The percentage change from baseline at months 6 and 12, adjusted for baseline C-peptide, baseline $HbA_{1c}$ level and stratum is shown for all participants (**d**) and those of diabetes duration 4–8 months (**e**) and 9–12 months (**f**) (*$p$<0.05 teplizumab vs placebo)



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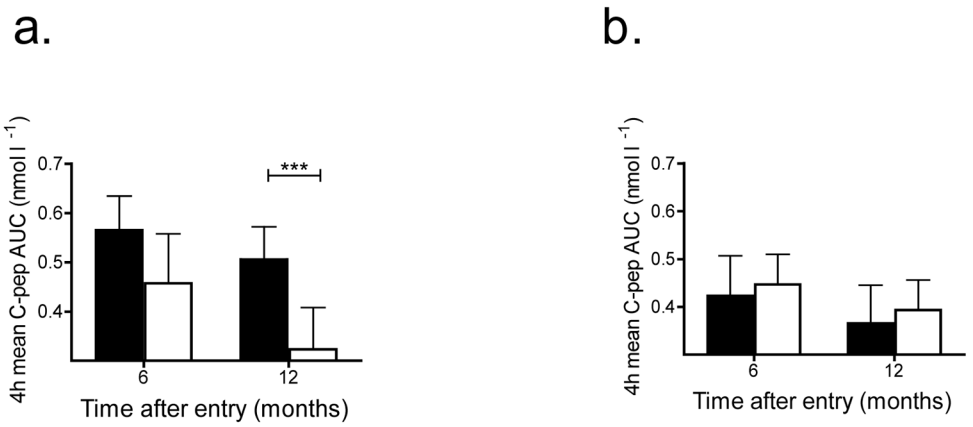


**Fig. 3.**

Effect of baseline $HbA_{1c}$ level on response to teplizumab. The AUC from the MMTT (mean ±95% CI) at 6 and 12 months after enrolment, adjusted for baseline C-peptide, are shown for participants treated with teplizumab (black bar) and placebo (white bar). (**a**) $HgbA_{1c}$ <6.5% (teplizumab-treated $n$=20, placebo-treated $n$=7) and (**b**) >6.5% (teplizumab-treated $n$=10, placebo–treated $n$=19) (***$p$<0.01, teplizumab vs placebo)



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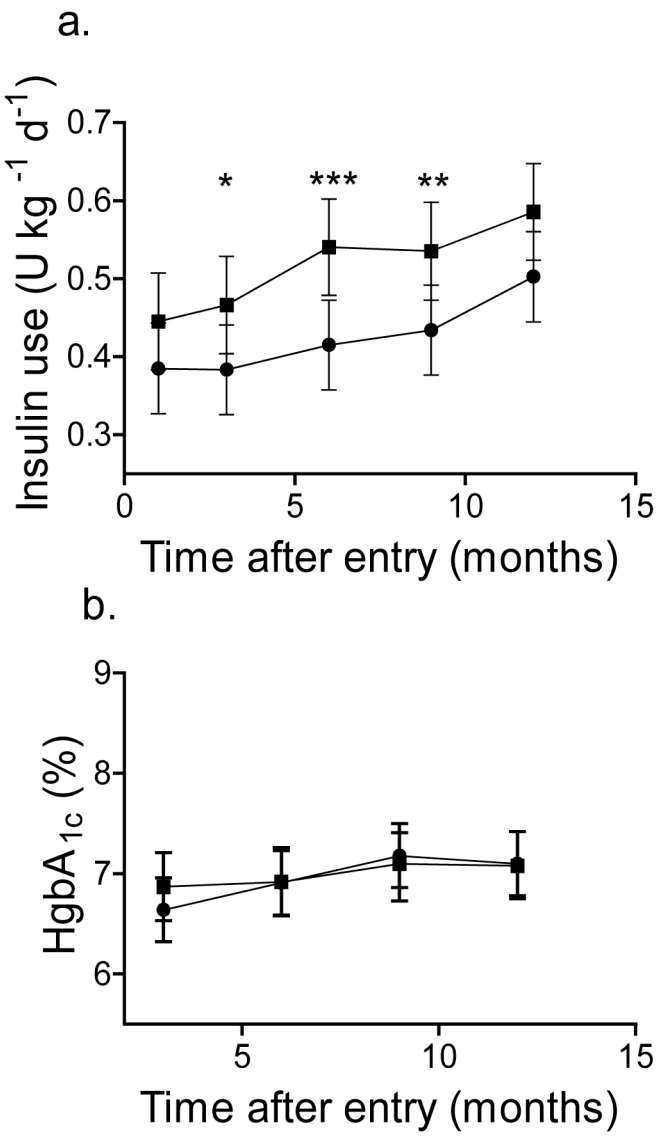


**Fig. 4.**

Insulin use and $HbA_{1c}$ levels in participants treated with teplizumab and placebo. (**a**) The total daily insulin use (mean±95% CI), adjusted for baseline and stratum was compared in all participants treated with teplizumab ($n$=31, circles) and placebo ($n$=27, squares). There was a significant effect of drug treatment on insulin usage ($p$=0.014) (*$p$<0.05, **$p$<0.02, ***$p$<0.01 teplizumab vs placebo). (**b**) The $HbA_{1c}$ levels, adjusted for $HbA_{1c}$ at study entry (mean±95% CI) in all participants are shown. The drug treatment did not have a significant effect on the $HbA_{1c}$ levels ($p$=0.67, teplizumab vs placebo). To convert values for $HgbA_{1c}$ in % into mmol/mol, subtract 2.15 and multiply by 10.929

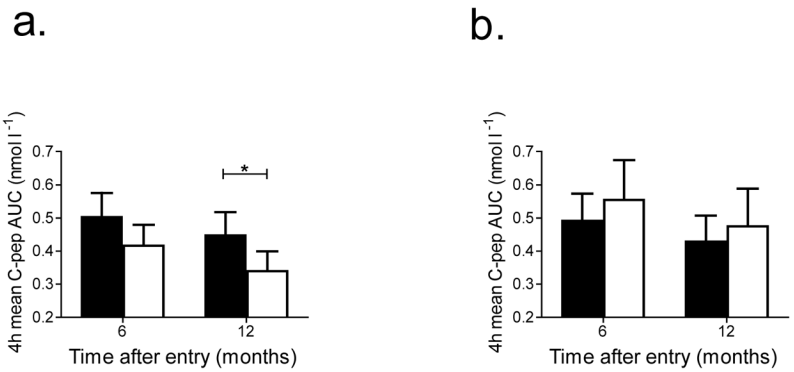


**Fig. 5.**

Effect of teplizumab on C-peptide responses and insulin use in young (age <15 years) and older (>15 years) participants. The C-peptide levels at 6 and 12 months after enrolment, adjusted for baseline C-peptide and $HbA_{1c}$ levels, are shown (mean± 95% CI) for participants treated with teplizumab (black bar) and placebo (white bar). (**a**) Age 8–14 (teplizumab-treated $n$=18, placebo–treated $n$=20) and (**b**) >15 years (teplizumab-treated $n$=13, placebo-treated $n$=7) (*$p$<0.05, teplizumab vs placebo)



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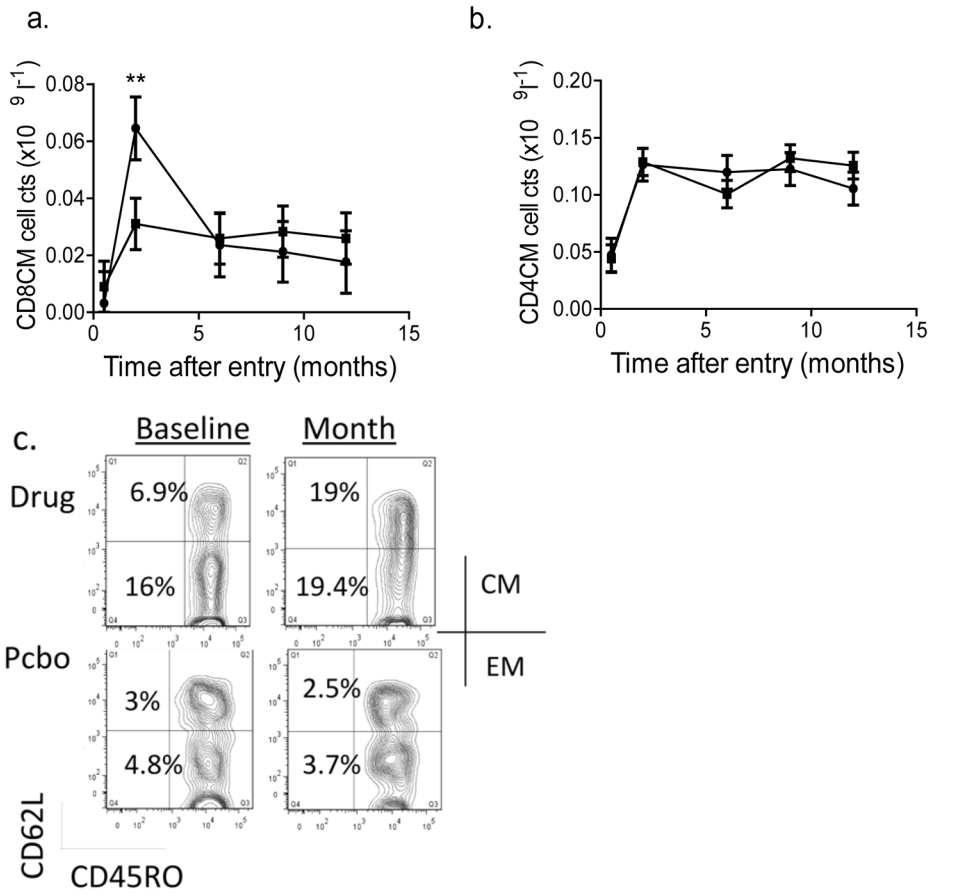


**Fig. 6.**

Changes in $CD8^+$ T cells in teplizumab-treated participants. (**a**) The number of circulating CD8CM ($CD8^+CD45RO^+CD62L-$) T cells (mean±SEM) is shown in teplizumab-treated responders (circles, $n$=13) and non-responders (squares, $n$=18) after drug treatment, corrected for the baseline counts. The baseline count of CD8CM cells was (mean±SEM) $0.011\pm0.002\times10^9$ cells/l in non-responders and $0.014\pm0.003\times10^9$ cells/l in responders. The number of cells was increased in responders at month 2 compared with the non-responders (**$p$=0.018, drug treated responders vs non-responders). (**b**) The corresponding CD4CM T cell counts (circles, responders; squares, non-responders) are shown. Significant changes in this subpopulation were not detected. (**c**) FACS plots showing CD8CM T cells before and at month 2 in representative drug- and placebo (Pcbo)-treated participants. The baseline count of CD4CM T cells was $0.12\pm0.013\times10^9$cells/l in non-responders and $0.12\pm0.017\times10^9$ cells/l in responders. The staining for CD45RO and CD62L is shown on gated $CD8^+$ lymphocytes. In the data from a representative drug-treated participant shown, the percentage of CD8CM and EM cells (in the corresponding L quadrants) increased from 6.9% and 16% of $CD8^+$ T cells to 19.1% and 19.4% of $CD8^+$ T cells, respectively, whereas in the placebo-treated patient, the CD8CM and EM cells were 3% and 4.8% before and 2.5% and 3.7% after treatment

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## Table 1

Patient characteristics

| Characteristic | All participants | | Stratum 1 (4–8 months) (*n*=39) | | Stratum 2 (9–12 months) (*n*=19) | |
|---|---|---|---|---|---|---|
| | **Drug (*n*=31)** | **Placebo (*n*=27)** | **Drug (*n*=21)** | **Placebo (*n*=18)** | **Drug (*n*=10)** | **Placebo (*n*=9)** |
| Age (years)(±SD) | 12.9±4.18 | 12.0±5.2 | 12.4±3.76 | 10.9±4.24 | 13.4±5.09 | 14.0±5.43 |
| Age category (*n*) | | | | | | |
| < 15 yrs | 18 | 20 | 13 | 15 | 5 | 5 |
| ≥ 15 yrs | 13 | 7 | 8 | 3 | 5 | 4 |
| Sex (% male) | 52 | 63 | 62 | 56 | 40 | 44% |
| Baseline C-peptide AUC (nmol/l) (±SD) | 0.618±0.302 | 0.6±0.43 | 0.611±0.289 | 0.557±0.464 | 0.632±0.342 | 0.686±0.365 |
| Baseline insulin use (U $kg^{-1}$ $day^{-1}$) (±SD) | 0.405±0.141 | 0.386±0.197 | 0.415±0.111 | 0.423±0.178 | 0.384±0.197 | 0.312±0.222 |
| Duration of diabetes (months) | 7.09±2.45 | 7.14±2.44 | 5.61±1.01 | 5.83±1.27 | 10.4±1.13 | 9.76±2.1 |
| Baseline | 6.37±0.787 *** | 7.14±1.21 | 6.28±0.709 | 7.34±1.17 | 6.55±0.94 | 6.62±1.22 |
| $HbA_{1c}$ (%)(mmol/m ol) (±SD) | (46.1±8.6) | (54.1±13.1) | (45.2±7.45) *** | (56.7±12.8) | (48.1±10.32) | (48.9±13.3) |
| Positive for anti-GAD65 antibody (%) | 74 | 55 | 70 | 90 | 80 | 63 |
| Positive for anti-IA-2 antibody (%) | 87 | 73 | 86 | 83 | 80 | 50 |

*** $p<0.01$

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## Table 2

Adverse events

| Reported event | Treatment | |
|---|---|---|
| | **Placebo** | **Teplizumab** |
| Participants with adverse events[a] | 27 (100) | 33 (100) |
| Treatment-related adverse event[a,b] | 25 (92.6) | 30 (90.9) |
| Serious adverse event[a] | 4 (14.8) | 1 (3.0) |
| Event resulting in discontinuation of study medication[a] | 2 (7.4) | 1 (3.0) |
| Event resulting in withdrawal from the study[a] | 0 (0.0) | 0 (0.0) |
| Grade 3 or higher adverse event[a] | 10 (37.0) | 15 (45.5) |
| Fatal adverse event[a] | 0 | 0 |
| Adverse events[c] | 644 (100) | 711 (100) |
| Grade 1 adverse event | 533 (82.8) | 589 (82.8) |
| Grade 2 adverse event | 78 (12.1) | 85 (12.0) |
| Grade 3 adverse event | 25 (3.9) | 21 (3.0) |
| Grade 4 adverse event | 0 (0.0) | 1 (0.1) |
| Grade 5 adverse event | 0 (0.0) | 0 (0.0) |

Data are shown as *n* (%)

[a]Participants reporting at least one event

[b]Includes events with causality ratings of ‘Possible’, ‘Probable’ or ‘Definite’

[c]Graded according to the Common Terminology Criteria for Adverse Events (version 4.0; http://www.acrin.org/Portals/0/Administration/Regulatory/CTCAE_4.02_2009-09-15_QuickReference_5x7.pdf)