The Deterrence of Rapid Metabolic Decline Within 3 Months After Teplizumab Treatment in Individuals at High Risk for Type 1 Diabetes

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End points that provide an early identification of treatment effects are needed to implement type 1 diabetes prevention trials more efficiently. To this end, we assessed whether metabolic end points can be used to detect a teplizumab effect on rapid β-cell decline within 3 months after treatment in high-risk individuals in the TrialNet teplizumab trial. Glucose and C-peptide response curves (GCRCs) were constructed by plotting mean glucose and C-peptide values from 2-h oral glucose tolerance tests on a two-dimensional grid. Groups were compared visually for changes in GCRC shape and movement. GCRC changes reflected marked metabolic deterioration in the placebo group within 3 months of randomization. By 6 months, GCRCs resembled typical GCRCs at diagnosis. In contrast, GCRC changes in the teplizumab group suggested metabolic improvement. Quantitative comparisons, including two novel metabolic end points that indicate GCRC changes, the within-quadrant end point and the ordinal directional end point, were consistent with visual impressions of an appreciable treatment effect at the 3- and 6-month time points. In conclusion, an analytic approach combining visual evidence with novel end points demonstrated that teplizumab delays rapid metabolic decline and improves the metabolic state within 3 months after treatment; this effect extends for at least 6 months.

Type 1 diabetes is an autoimmune disease resulting in insulin deficiency because of the destruction of pancreatic β-cells (1). In the Type 1 Diabetes TrialNet TN10 Anti-CD3 prevention study, a single 14-day course of the Fc receptor–nonbinding anti-CD3ε monoclonal antibody teplizumab was able to delay the onset of diabetes by 32.5 months in a group of autoantibody-positive, high-risk individuals (2,3). In a follow-up longitudinal analysis of that study (3), after a decline in C-peptide responses before entry into the trial, teplizumab treatment improved average C-peptide area under the curve (AUC) over a period of 6 months. In addition, treatment reversed an observed decline in insulin secretion before enrollment.

These findings suggested that metabolic end points could be of value for gaining more precise information about the effect of teplizumab. The utility of metabolic end points to examine the teplizumab effect could then potentially be generalized to assess preventive treatments in other clinical trials. C-peptide responses from oral glucose tolerance tests (OGTTs) or mixed-meal tolerance tests have mainly been used to evaluate β-cell function both before and after the diagnosis of diabetes (4). However, several studies have now shown that combined stimulated glucose and C-peptide markers improve the prediction of type 1 diabetes, the identification of heterogeneity within autoantibody-positive populations, and the detection of subtle changes in β-cell function (5–11).

Thus, we have reasoned that these combined measures could be used to examine two important questions: *1)* Among individuals at high risk for type 1 diabetes, is there a substantive teplizumab effect on β-cell function within 3 months after the 14-day course of treatment, and if so, does the effect persist for at least 6 months? *2)* Is there a prospect of rapid β-cell decline among high-risk individuals within 3 months if teplizumab is not administered? We used two composite glucose and C-peptide markers to

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address these questions: Index60 (8,9) and the C-peptide AUC/glucose AUC ratio (AUC ratio). In addition, we used OGTT-derived glucose and C-peptide response curves (GCRCs) on two-dimensional (2d) grids. This recently introduced methodology has extended the concept of combining glucose and C-peptide measures by using a joint qualitative and quantitative approach. It has already facilitated studies of the metabolic natural history of type 1 diabetes (12), the metabolic heterogeneity of the disorder at diagnosis (13), and the metabolic effect of a potential preventive treatment (14). In another study, GCRCs (from mixed-meal tolerance tests) were a basis for studying associations of metabolic changes with miRNAs after the diagnosis of type 1 diabetes (15).

The findings presented here show that teplizumab effectively preserves and possibly improves β-cell function soon after treatment in individuals at high risk for type 1 diabetes and that this action persists. They also show that although none in the placebo group had been diagnosed within 3 months after randomization, there was already marked metabolic deterioration in that period. Moreover, the findings demonstrate that using composite glucose and C-peptide end points in tandem with GCRCs provides qualitative and quantitative insights into the timing and magnitude of preventive treatment effects that are missed by using diagnostic end points alone in type 1 diabetes prevention trials.

RESEARCH DESIGN AND METHODS

Trial Procedures and Design

The design of the phase 2, randomized, placebo-controlled, double-blinded TrialNet TN10 Anti-CD3 prevention study (ClinicalTrials.gov identifier: NCT01030861) has previously been reported in detail (2). Institutional review board approval was obtained at each participating site, with written informed consent and assent obtained before trial entry. Inclusion criteria were age ≥8 years at randomization, a history of a relative with type 1 diabetes, stage 2 diabetes (positive titers for two or more islet autoantibodies [anti–glutamic acid decarboxylase 65, micro-insulin autoantibody, anti–islet antigen 2, anti–zinc transporter 8, and/or islet cell antibodies] and dysglycemia). HbA$_{1c}$ levels were in the normal range (median [interquartile range]: teplizumab 5.2% [4.9–5.4%]; placebo 5.3% [5.1–5.4%]). Participants were randomly assigned to teplizumab or saline and treated with a 14-day outpatient course administered as an intravenous infusion. OGTT C-peptide and glucose values were tested by Northwest Lipids Research Laboratories using the Tosoh C-peptide and Roche glucose assays. OGTTs with glucose values in the diabetic range were excluded from this analysis.

AUC values for C-peptide and glucose were calculated using the trapezoidal rule (16). AUC ratio values were obtained by calculating the ratio × 100. Index60 was calculated as 0.3695 × (log fasting C-peptide [ng/mL]) + 0.0165 × 60-min glucose (mg/dL) − 0.3644 × 60-min C-peptide (ng/mL) (13). GCRCs were generated by plotting

mean glucose (y-axis) and C-peptide values (x-axis) from OGTTs (30, 60, 90, and 120 min) on a 2d grid.

Analytic Components

Supplementary Fig. 1 includes six key components used in the analyses of metabolic change: 2d grids with glucose as the y-axis and C-peptide as the x-axis, GCRCs, centroids (central point of GCRCs), vectors, directional quadrants for vectors, and angles. Shown in Supplementary Fig. 1A are two hypothetical GCRCs from the same individuals plotted on 2d grids from OGTT mean glucose and C-peptide values at 30, 60, 90, and 120 min. One of the GCRCs is typical for 6 months before diagnosis, while the other is typical for the time of diagnosis. A centroid for each GCRC is calculated (see Supplementary Material for equation). Marked changes in GCRC position and shape from 6 months before diagnosis to diagnosis are indicative of metabolic decline. The arrow is the vector for the positional change of the GCRC centroid from 6 months before diagnosis to the GCRC centroid at diagnosis. Note that the direction and magnitude of the vector are functions of the decrease in the C-peptide and the increase in the glucose from baseline to 6 months.

In Supplementary Fig. 1B, a right triangle has been overlaid onto Fig. 1A. The right triangle is formed from the vector (the hypotenuse) and the changes in glucose (vertical side) and C-peptide (horizontal side). The formation of the right triangle provides a means for calculating an angle of the vector. As shown in Supplementary Fig. 1B, the calculated angle for this analysis is defined as the angle between the vector (hypotenuse) and the horizontal side of the right triangle.

Supplementary Fig. 1C shows a hypothetical example of vectors for each of the four directional quadrants: right lower quadrant, right upper quadrant, left upper quadrant, and left lower quadrant. They all emanate from a baseline centroid, with changes in glucose and C-peptide fixed at 0. The directional quadrant for the vector thus depends on whether the change of glucose and C-peptide is positive or negative. The calculated angles between the horizontal and the vector are also shown.

To provide quantitative comparisons that complement GCRC visual comparisons, we developed two novel end points on the basis of vectors and angles that indicate changes in GCRC movement over a 6-month period: the within-quadrant end point (WQE) and the ordinal directional end point (ODE). Both end points use Cox regression modeling that is based on GCRC movement into four potential quadrants reflecting changes in glucose and C-peptide. Refer to the Supplementary Material, including Supplementary Fig. 2, for information about the development of these two end points.

Statistical Analyses

Group comparisons were done using t tests and χ^2 tests. Linear regression was used for adjustments of variables,
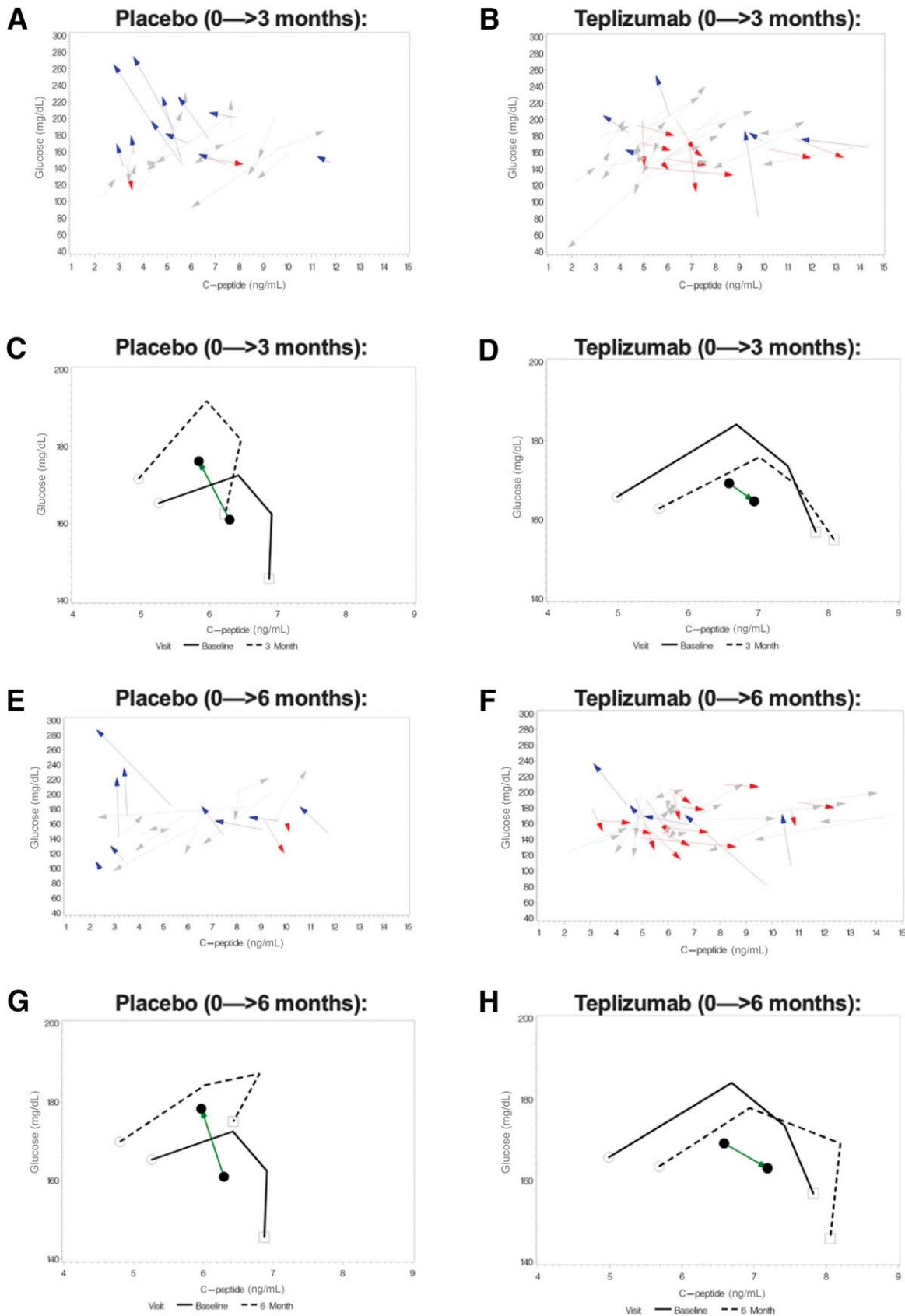
Figure 1—Changes in GCRC vectors show opposite directionality between placebo and teplizumab-treated groups. Individual participant GCRC vectors of change as well as mean treatment group vectors were plotted for the interval from baseline study visit (time of randomization) to 3 months on treatment (A–D) and baseline to 6 months on treatment. A and B: Changes in individual GCRC vectors from baseline to 3 months on treatment are plotted for placebo- and teplizumab-treated groups. High-risk vectors (increasing glucose, decreasing C-peptide) are shown in blue, with low-risk vectors (decreasing glucose, increasing C-peptide) shown in red. Frequency of vector quadrant distribution was significantly different between treatment groups (P = 0.045). C and D: Mean GCRCs are plotted at the time of

while proportional hazards regression was used to develop models for end points. Sex was not included as a covariate in analyses. All analyses were performed using the statistical program SAS 9.4. Two-sided $P < 0.05$ was considered statistically significant.

Data and Resource Availability

Data are available upon reasonable request from the authors.

RESULTS

Relevant baseline characteristics of the participants are shown in Supplementary Table 1. There were no significant differences between the placebo and teplizumab groups for any demographic measure or for glucose or C-peptide levels from baseline OGTTs. Of the 76 participants, there were sufficient OGTT data to analyze 29 placebo-treated and 41 teplizumab-treated individuals at 3 months after randomization and 24 placebo-treated and 44 teplizumab-treated individuals at 6 months after randomization. Five individuals from the placebo group were excluded from the analysis of OGTTs at 6 months because of the diagnosis of diabetes ($n = 3$) or an OGTT with glucose values in the diabetic range but not yet meeting the diagnostic criteria of two consecutive diabetic-range OGTTs ($n = 2$).

Assessments of Metabolic Responses With GCRCs 3 Months and 6 Months After Treatment

To determine whether there was early evidence of a teplizumab effect on β-cell function following treatment, we compared changes of GCRCs between the placebo and teplizumab groups from randomization to 3 months. In Fig. 1A and B, a vector for centroid change from randomization to the 3-month visit is plotted for each individual in the placebo and teplizumab groups. In these vector plots, 11 (37.9%) of 29 individuals in the placebo group vs. 6 (14.6%) of 41 in the teplizumab group had vectors directed toward the left upper directional quadrant (decreasing C-peptide and increasing glucose), suggesting worsening metabolic function. This contrasted with 11 (26.8%) of 41 individuals in the teplizumab group vs. 2 (6.9%) of 29 in the placebo group who had vectors directed toward the right lower quadrant (increasing C-peptide and decreasing glucose), suggesting improving metabolic function. Among all four quadrants, the frequencies of placebo

versus teplizumab-treated participants were significantly different (overall $P = 0.045$) (Table 1).

Depicted in Fig. 1C and D are GCRCs constructed according to glucose and C-peptide mean values for each OGTT time point as well as vectors indicative of GCRC centroid changes from randomization to 3 months. Progressive metabolic dysfunction was evident in the placebo group by 3 months, with the vector for GCRC centroid change over this period directed toward the left upper quadrant (decreasing C-peptide and increasing glucose). In contrast, the vector for GCRC centroid change in the teplizumab group had nearly opposite directionality, which was toward the right lower quadrant (increasing C-peptide and decreasing glucose). The magnitude of the teplizumab effect was evident in the 153° (of a maximum of 180°) directional difference between the placebo vector and the teplizumab vector.

The changes in the AUC ratio and Index60 from baseline to 3 months were consistent with the large difference in directionality of the vectors. There were significant differences in changes between the placebo and teplizumab groups for both ($P < 0.01$ after adjustments for the baseline parameter, age, and BMI) (Table 2). Moreover, there was evidence of improvement in the teplizumab group: The AUC ratio increased ($P < 0.01$), and Index60 decreased ($P < 0.05$). (Adjustments could not be performed in the paired analyses because of multicollinearity.)

Also of note in Fig. 1C and D is the appreciable conformational change in the GCRC of the placebo group from randomization to 3 months. Particularly evident is the increased upward slope between 30 and 60 min, which is typical of changes during the progression to type 1 diabetes (12). In contrast, the slope of the teplizumab group is almost the same.

The pattern for the individual vectors at 6 months was similar to the pattern at 3 months. As shown in Fig. 1E and F, the directionalities of the individual vectors were leftward and upward in the placebo group, suggesting metabolic worsening, whereas they moved rightward and downward in the teplizumab group, suggesting metabolic improvement. Thus, a higher percentage of the placebo group had vectors directed to the left upper quadrant (45.8% of placebo vs. 11.4% of teplizumab), whereas a higher percentage of the teplizumab group had vectors directed toward the right lower quadrant (8.3% of placebo vs. 34.1% of teplizumab). Differences were also evident in the vectors for centroid movement of the GCRCs derived from OGTT mean values

randomization (baseline) and at the time of the OGTT performed after 3 months in the study for the placebo- and teplizumab-treated groups. Mean centroid values for glucose and C-peptide coordinates are depicted as dots within each GCRC, with vectors of change indicated. The change in GCRC centroid values over this period shows directionality toward the upper left portion of the grid for the placebo group, indicating increasing glucose and decreasing C-peptide values. The teplizumab-treated group shows opposite directionality, toward the lower right portion of the grid, suggesting increasing C-peptide and decreasing glucose. E and F: Changes in individual GCRC vectors from baseline to 6 months on treatment are plotted for placebo- and teplizumab-treated groups. Frequency of vector quadrant distribution was significantly different between treatment groups ($P = 0.0044$). G and H: Mean GCRCs are plotted at the time of randomization (baseline) and at the time of the OGTT performed after 3 months in the study for the placebo- and teplizumab-treated groups. At 3 months, $n = 29$ for placebo and $n = 41$ for teplizumab. At 6 months, $n = 24$ for placebo and $n = 44$ for teplizumab. Absolute frequencies for individual vector directional quadrants for each period are displayed in Table 1.

Table 1—Frequencies of individual GCRC vectors of change according to directional quadrants by treatment group from baseline to 3 months and baseline to 6 months

	Baseline to 3-month visit,* n				Baseline to 6-month visit,* n		
	Placebo	Teplizumab	Total		Placebo	Teplizumab	Total
Right upper quadrant	8	15	23	Right upper quadrant	4	13	17
Left upper quadrant	11	6	17	Left upper quadrant	11	5	16
Left lower quadrant	8	9	17	Left lower quadrant	7	11	18
Right lower quadrant	2	11	13	Right lower quadrant	2	15	17
Total	29	41	70	Total	24	44	68

*The χ^2 test showed significantly different distributions between treatment groups at the 3-month ($P = 0.045$) and 6-month ($P = 0.004$) intervals.

(Fig. 1*G* and *H*). The directional difference between the placebo and teplizumab vectors from randomization to 6 months was 138°. The difference in the vector frequencies between the groups among all four quadrants was significant (overall $P = 0.004$) (Table 1).

Differences in changes of the AUC ratio and Index60 between the groups were again significant, but less so ($P = 0.005$ and $P = 0.021$, respectively, after adjustments) (Table 2). The increase in the AUC ratio was also again evident within the teplizumab group from baseline to 6 months ($P < 0.01$), but Index60 did not decrease significantly.

In Fig. 1*G* and *H*, it is evident that by 6 months after randomization the shape of the GCRC had become substantially more pathological such that it had assumed a shape resembling the characteristic GCRC shape at diagnosis (12). Specifically, the placebo GCRC from 30 to 90 min had become almost linear, attributable to a slope from 60 to 90 min that had become less downward. The teplizumab GCRC shape changed to a much lesser degree.

Quantitative Assessments of Treatment Effects Using Vector Angles Within Directional Quadrants

The analyses above showed obvious differences in GCRC vectors between the placebo and teplizumab groups in the teplizumab trial within 3 months and at least to 6 months after the 14-day treatment. These differences were corroborated by the differences between the groups in Index60 and AUC ratio. However, those composite glucose and C-peptide measures were not based on the directionality of vectors indicative of GCRC change. Thus, we strived to develop quantitative end points that would be more directly indicative of differences in vectors between placebo and treatment groups. Two such end points were developed: WQE and ODE (see RESEARCH DESIGN AND METHODS and Supplementary Material). Both end points were derived from Diabetes Prevention Trial–Type 1 (DPT-1) changes in GCRC centroid locations at 6-month intervals, which was the shortest interval between OGTTs available for analysis.

The visual difference in movement of the GCRCs from baseline to 3 months between the placebo and treatment groups was confirmed statistically with ODE and WQE (Fig. 2*A* and *B*). ODE values were significantly lower for the teplizumab group ($P < 0.05$ after adjustments). WQE values were also significantly lower in the teplizumab group before adjustments ($P < 0.05$), but they only trended toward a difference after adjustments ($P = 0.072$).

WQE and ODE differences between the placebo and teplizumab groups at 6 months were appreciably greater

Table 2—Comparisons between placebo and teplizumab arms of end points based on metabolic changes from baseline to 3 months and baseline to 6 months

	Arm		P	
End point	Placebo	Teplizumab	Unadjusted	Adjusted[+]
Change from baseline visit to 3-month visit*				
WQE	0.70 (0.62)	0.39 (0.52)	0.026	0.072
ODE	219 (92)	163 (97)	0.018	0.038
ΔAUC ratio	−0.254 (0.80)	0.425 (0.87)	0.001	0.003
ΔIndex60	0.471 (0.92)	−0.271 (0.81)	<0.001	0.002
Change from baseline visit to 6-month visit‡				
WQE	0.66 (0.47)	0.23 (0.46)	<0.001	<0.001
ODE	221 (87)	137 (94)	<0.001	0.002
ΔAUC ratio	−0.379 (1.17)	4.159 (1.42)	0.002	0.005
ΔIndex60	0.481 (1.10)	−0.159 (0.77)	<0.007	0.021

Data are mean (SD). [+]ODE and WQE were adjusted for age, BMI, and baseline AUC ratio. ΔAUC ratio and ΔIndex60 were adjusted for age, BMI, and baseline Index60. *$n = 29$ for placebo arm, $n = 41$ for teplizumab arm. ‡$n = 24$ for placebo arm, $n = 44$ for teplizumab arm.

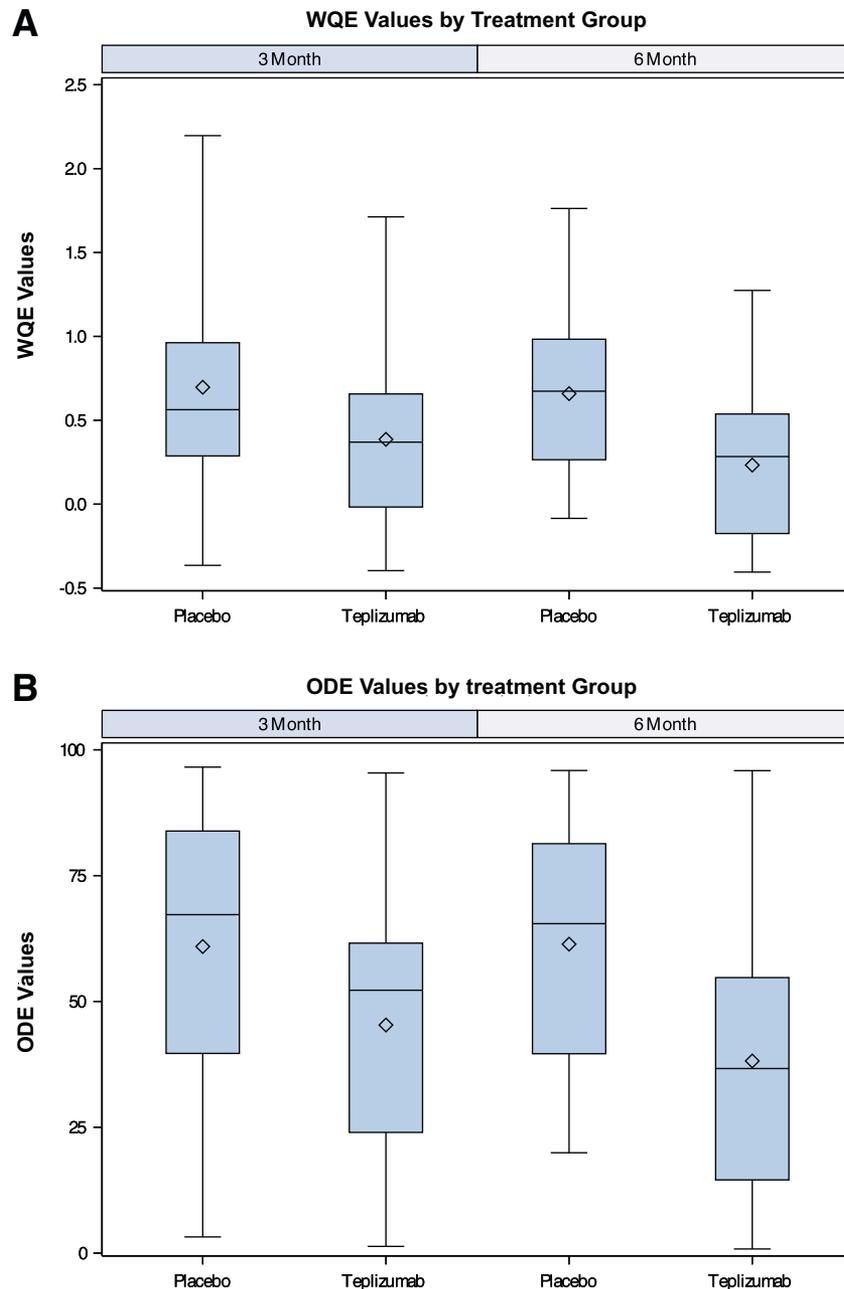**A**

WQE Values by Treatment Group

B

ODE Values by treatment Group

Figure 2—Statistical assessments of a treatment effect using vector angles within directional quadrants. Unadjusted individual values for changes in WQE (*A*) and ODE (*B*) are plotted by treatment group for the periods from baseline to 3 months and baseline to 6 months. For ODE treatment group comparisons at 3 months, $P = 0.018$ before and $P = 0.038$ after adjustments for age and BMI. At 6 months, $P < 0.001$ before and $P = 0.002$ after adjustment. For WQE treatment group comparisons at 3 months, $P = 0.026$ before and $P = 0.072$ after adjustments for age and BMI. At 6 months, $P < 0.001$ before and after adjustment. *$P < 0.05$, ***$P < 0.001$, unadjusted. At 3 months, $n = 29$ in the placebo-treated group and $n = 41$ in the teplizumab-treated group. At 6 months, $n = 24$ in the placebo-treated group and $n = 44$ in the teplizumab-treated group. Line represents the median while open diamond symbol is the mean. Top and bottom of the box represent 25th and 75th percentiles. Top and bottom whiskers represent minimum and maximum.

than at 3 months: WQE was significantly lower for the teplizumab group ($P < 0.01$) as was the ODE ($P < 0.01$). Table 2, which summarizes the differences between the placebo and teplizumab groups according to the end points, shows that whereas Index60 and the AUC ratio differed more between groups at 3 months, WQE and ODE differed more at 6 months.

DISCUSSION

The teplizumab trial showed that a single course of treatment could delay the onset of type 1 diabetes (2). However, that trial and other prevention trials using the standard end point of time to diagnosis have been lengthy. In addition, although teplizumab was very effective in delaying type 1 diabetes, information has been

lacking about the timing of its effect and its impact on the metabolic state. We thus used existing and new metabolic markers to serve as end points in order to gain this information. Such early readouts not only could be helpful for understanding the effects of preventive treatments on β-cell function but also could possibly lead to a shortening of prevention trials.

The measurement of the C-peptide AUC in response to oral glucose before diagnosis or to a mixed meal after diagnosis is often used to assess insulin responsiveness; however, it is not necessarily the most sensitive measure for identifying changes in β-cell function. Multiple studies have suggested that C-peptide loss in type 1 diabetes may be best presented and understood in the context of changes in glucose (5–11). We have used a new approach in this study that melds qualitative and quantitative information to better understand insulin secretory dynamics in response to glucose. Using this approach, the findings provided strong evidence that teplizumab deters severe β-cell decline, and possibly improves function, by 3 months after treatment in a high-risk population. Moreover, an effect persists for at least 6 months after treatment. These findings have important implications since they indicate that even at an advanced stage of metabolic progression before diagnosis, the effect of teplizumab is rapid enough to be highly effective.

GCRCs and their centroids, together with vectors and their angles, had major roles in the analysis. As was evident from the GCRC changes on the 2d grid, the use of those elements resulted in a visual contrast between the failing metabolic state of the placebo group and the apparent improvement of the teplizumab group. Those elements were also used as a basis for developing the new end points WQE and ODE, which were used in quantitative comparisons of changes between the placebo and teplizumab groups. Together with the AUC ratio and Index60, those end points confirmed the visual impressions that teplizumab deterred rapid metabolic decline in high-risk individuals soon after the 14-day treatment at baseline. Table 3 summarizes how methodology based on GCRC movement and shape enhanced our understanding of the timing, magnitude, and potential clinical importance of the teplizumab effect.

The findings from these analyses highlight advantages of using vectors in addition to scalars as metabolic end points for analyses of treatment effects. Prior analyses of preventive measures have depended on scalars, which only provide information about magnitude, whereas vectors provide information about direction in addition to magnitude. The vectors of the GCRC movements on the 2d grids clearly added information that could not have been ascertained from scalar end points alone.

We had previously used vectors of GCRC change as evidence of an oral insulin effect for preserving β-cell function in a post hoc analysis among individuals at high risk for type 1 diabetes in the DPT-1 and TrialNet oral insulin trials (14). Interestingly, the vectors in the present teplizumab analysis were much farther apart between the placebo and treatment groups than in the oral insulin analysis. Whereas the difference in vector direction between the placebo and teplizumab groups in the teplizumab trial spanned a difference from the left upper directional quadrant (placebo) to the right lower directional quadrant (teplizumab), the difference between the placebo and oral insulin vectors was mostly confined to the right upper directional quadrant. The greater vector separation in the teplizumab trial could be based on differences in therapeutic mechanisms between teplizumab and oral insulin and/or differences in the magnitude of effect. It appears that analyses of preventive treatment effects would be incomplete if vectors are ignored and there is only reliance on scalars.

The detection of treatment effects by WQE and ODE might not generalize to trials at other stages of disease, since participants in the teplizumab trial were selected for high risk. In addition, it is quite possible that other end points relating to GCRC centroids and their vectors for change will be found that are superior to WQE and ODE. Nevertheless, the performance of those end points in the analyses validates visual impressions derived from GCRCs, suggesting that a combined qualitative and quantitative use of vectors to examine metabolic change can be a valuable approach for assessing effects of preventive treatments.

Table 3—Substantive contributions to the analysis from using GCRCs plotted on 2d grids

The rapid pathologic evolution of placebo group GCRCs, evident both in changes of grid location and shape of the placebo group, highlighted the crucial importance of the early treatment effect of teplizumab.

The GCRCs of the teplizumab group had minimal conformational change, with a vector directionality indicative of improvement. This enhanced the quantitative evidence, which suggested that teplizumab administration could reverse pathologic changes.

The striking, almost opposite, difference in directionality (153°) between the placebo vector and the teplizumab vector demonstrated the strength of the teplizumab effect, further reinforcing the value of the medication in high-risk individuals.

At 6 months after randomization, the teplizumab effect was most apparent using quantitative GCRC-derived end points (WQE and ODE); these corroborated visual impressions. Although based on 6-month OGTTs, complementary quantitative information for GCRCs was also provided by those end points at 3 months.

Tracking individual responses with vectors provided a granular way of assessing responsiveness to teplizumab, both qualitatively and quantitatively.

Of note, this report is the first to use changes in Index60 as an end point for demonstrating a metabolic effect in an analysis of a type 1 diabetes prevention study. In aggregate, these and prior results (14) suggest that multiple metabolic end points, including Index60, the AUC ratio, and WQE and ODE, which combine changes in glucose and C-peptide, can provide a readout of treatment effects in type 1 diabetes prevention studies. Subtle variability in treatment group differences detected between the oral insulin and teplizumab trials could reflect differences in study populations or therapeutic interventions. The change from baseline to 3 months in Index60 and AUC ratio differed more between the placebo and teplizumab groups than WQE and ODE, whereas the opposite was evident from baseline to 6 months. Since the development of both WQE and ODE was based on 6-month data, it is not surprising that they performed better from baseline to 6 months. Although the analysis of the teplizumab study suggested that Index60 and AUC ratio could possibly be more sensitive for detecting an early effect, this might not generalize to other trials. The choice of specific metabolic end points for prevention trials will depend on factors that could impact their relevance and performance, such as the target population, objectives, interventions, and trial designs. An advantage of WQE and ODE over other measures is that they directly complement the cogent visual impressions derived from GCRCs (outlined in Table 3).

The 0–30-min interval is not included in GCRCs since the magnitude of changes in metabolic measures from 0 to 30 min is much greater than the magnitude at each of the other time intervals. Its incorporation would thus minimize the 30–120-min visualization. Likewise, from a quantitative perspective, the 0–30-min interval would carry an inordinate weight for calculations of centroids and vectors, which would further complicate analyses. Although 0- and 30-min C-peptide and glucose measures are not included in GCRCs, we did adjust for baseline AUC C-peptide and AUC glucose values in the analyses. Now that quantitative measures have been developed to complement the visualization of GCRCs, along with their centroids and vectors, an important future direction will be to study the influence of changes from 0 to 30 min upon changes in form and location of GCRCs.

The applicability of short-term, interim metabolic end points for prevention trials will need further study. Primary metabolic end points have been used successfully in new-onset type 1 diabetes trials, usually with a defined follow-up of 1 year, to assess treatment efficacy for delaying the further loss of insulin secretion. Positive findings of metabolic outcomes in these new-onset trials have been used to justify treatments in prediagnosis prevention trials (17–20). Our findings suggest that information from short-term prevention trials before diagnosis could also be used for deciding whether larger prediagnostic prevention trials are warranted for a particular treatment.

Short-term metabolic end points are likely to have several other utilities for evaluating prediagnosis preventive treatments. They could be used for stopping rules in trials if prespecified effects are not reached. One novel approach for using a metabolic end point would be using it in combination with the diagnosis end point. It is even conceivable that metabolic end points could serve as primary end points for certain trials. These end points could also provide important pharmacologic information from trials, such as the timing of treatment effects, as was evident in the current study.

The prior longitudinal study of teplizumab (3) examined the average effect of teplizumab treatment on C-peptide AUC over a 6-month period. That study neither used GCRC methodology and composite glucose and C-peptide end points to assess the specific timing of a teplizumab effect nor showed the importance of getting ahead of the impending severe loss of β-cell functionality with the use of teplizumab among high-risk individuals. As previously mentioned, the analysis of the oral insulin trials (14) used GCRC vectors, but to a much lesser extent.

This study had some limitations. Three individuals from the placebo group had already developed diabetes at the 6-month time point and so were excluded from the 6-month analysis. This not only decreased the sample size but also likely led to the appearance of less severe metabolic decline in the placebo group. Such bias would attenuate a teplizumab effect at 6 months rather than enhance it. Since all teplizumab trial participants were required to be positive for multiple islet autoantibodies with metabolic abnormalities, future analyses will be needed to determine whether our findings are applicable to at-risk populations with less severe baseline disease, such as individuals who test positive for a single islet autoantibody.

GCRC vectors in right lower and left upper directional quadrants appear to reflect improving or worsening metabolic status, whereas vectors in the right upper and left lower quadrants could reflect more intermediate changes consistent with changes in insulin secretory kinetics or insulin sensitivity. However, gold standard measures of β-cell function, such as glucose-potentiated arginine clamps, would be required to truly define these changes. Quantitative treatment group comparisons (Table 2) were adjusted for BMI and age, but because of the severity of β-cell dysfunction in this population, we chose not to adjust for OGTT-based modeled measures of insulin resistance (21).

WQE and ODE were both significantly associated with diabetes progression in DPT-1 (Supplementary Material); however, the detection of a treatment effect by a metabolic end point is not purely a function of its ability to predict type 1 diabetes. For example, an individual may have an improved C-peptide response to a treatment yet still go on to develop diabetes, while conversely, another individual might not exhibit improved C-peptide secretion to treatment yet not develop type 1 diabetes during follow-up. Thus, a critical future direction is an analysis of

relationships between the ability of end points to detect a treatment effect versus the ability to predict diabetes.

The development of the WQE and ODE involved some complexity, but their applications for studies would mainly be based on standard equations for calculations of angles. Thus, while we have included explanations to describe the development of this novel methodology in this article, practically, implementation of these measurements would simply involve use of an equation similar to other accepted indices, such as Index60 (13).

In conclusion, an analysis of glucose and C-peptide changes that is based on changes of GCRCs and their centroids on 2d grids provided visual evidence of a teplizumab treatment effect that was early enough to deter a rapid decline in β-cell function, and possibly even improve function, in a population at high risk for type 1 diabetes. This was corroborated statistically by composite glucose and C-peptide end points and by novel end points derived from vectors of GCRC change. These findings suggest that changes in GCRC grid location and shape, along with corresponding quantitative measures of directionality, should be integrated into analyses of type 1 diabetes prevention trials. Moreover, they add to the growing evidence that end points that combine both glucose and C-peptide are basic to our understanding of treatment effects in prevention trials. Future directions will involve refining applications of these end points to facilitate assessments of preventive treatments in both smaller and larger trials.

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