

TN-18 Statistical Analysis Plan

TRIAL FULL TITLE	<b>CTLA-4 Ig (Abatacept) FOR PREVENTION OF ABNORMAL GLUCOSE TOLERANCE IN RELATIVES AT-RISK FOR TYPE 1 DIABETES MELLITUS (Protocol TN-18)</b>
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## 1. Abbreviations and Definitions

AE	Adverse Event
ADA	American Diabetes Association
AGT	Abnormal Glucose Tolerance
AUC	Area Under the Curve
BMI	Body Mass Index
DSMB	Data Safety Monitoring Committee
ITT	Intent to Treat
OGTT	Oral Glucose Tolerance Test
PH	Proportional hazards
SAE	Serious Adverse Event
SAP	Statistical Analysis Plan
T1DM	Type 1 Diabetes Mellitus

## 2. Introduction

### Preface

The rationale for this study is that antigen-specific T-cells are thought to play a central role in the pathogenesis of autoimmune diseases. Abatacept binds to CD80/CD86 receptors on antigen presenting cells, thereby inhibiting their binding to the co-stimulatory molecule CD28 on T-cells. By inhibiting full T-cell activation, Abatacept also affects the downstream inflammatory cascade.

### Purpose of the analyses

Analyses of study data will be conducted to address all objectives and other interrelationships among elements of study data of interest to the investigators and of relevance to the objectives of the study. Analyses by sex, age, and race/ethnicity are also planned.

All primary analyses will be conducted under the intention-to-treat principle whereby all outcome data in all randomized subjects will be included, regardless of treatment compliance.

### Primary Outcome

The primary outcome is the elapsed time from random treatment assignment to the development of confirmed abnormal glucose tolerance or diabetes among those enrolled in the primary analysis cohort consisting of subjects with insulin autoimmunity and absence of metabolic abnormalities (normal OGTT).

The study endpoint is realized with either OGTT criteria for abnormal glucose tolerance or diabetes or clinical criteria for diabetes.

### OGTT criteria for abnormal glucose tolerance or diabetes:

The presence of an OGTT consistent with abnormal glucose tolerance or diabetes on two sequential dates. A subject with abnormal glucose tolerance or diabetes on an OGTT should undergo a repeat OGTT as soon as possible, but no less than one day apart. Aim to repeat within one month. The time of abnormal glucose tolerance or diabetes will then be taken as

the date of the confirmatory abnormal OGTT. The definition of abnormal glucose tolerance or diabetes is:

- a. Fasting plasma glucose  $\geq 110$  mg/dL (6.1 mmol/L) and  $< 126$  mg/dL (7 mmol/L) or
- b. 2 hour plasma glucose  $\geq 140$  mg/dL (7.8 mmol/L) and  $< 200$  (11.1 mmol/L), or
- c. 30, 60, 90 minute plasma glucose during OGTT  $\geq 200$  mg/dL (11.1 mmol/L)

### Onset of Diabetes:

Criteria for diabetes\_onset (T1DM) are based on glucose testing, or the presence of unequivocal hyperglycemia with acute metabolic decompensation (diabetic ketoacidosis). One of the following criteria must be met on two occasions as soon as possible but no less than one day apart for diabetes to be defined: 1. Symptoms of diabetes plus casual plasma glucose concentration  $\geq 200$  mg/dL (11.1 mmol/L). Casual is defined as any time of day without regard to time since last meal. The classic symptoms of diabetes include polyuria, polydipsia, and unexplained weight loss. 2. Fasting plasma glucose  $\geq 126$  mg/dL (7 mmol/L). Fasting is defined as no caloric intake for at least 8 hours. 3. 2 hour plasma glucose  $\geq 200$  mg/dL (11.1 mmol/L). The test should be performed using a glucose load containing the equivalent of 1.75g/kg body weight to a maximum of 75g anhydrous glucose dissolved in water. It is preferred that at least one of the two testing occasions involve an OGTT. Cases identified will be confirmed as having diabetes if the glucose values to make these determinations were obtained in a TrialNet laboratory as part of an OGTT. Cases diagnosed with diabetes by symptoms and casual glucose  $\geq 200$ mg/dL or by other criteria than the above will be adjudicated by the TrialNet Endpoint Adjudication Committee.

The date of arriving at the study endpoint is the date of the second occasion that meets either of the above criteria or the date in which the participant presented in a state of DKA.

## **3. Primary Analysis**

The study design is a randomized double-blind placebo-controlled trial. The primary objective of the study is to assess the effect of CTLA4-IG therapy versus placebo on the risk of developing abnormal glucose tolerance (AGT) as measured from an OGTT.

The cumulative incidence of AGT over time since randomization within each treatment group will be estimated from a Kaplan-Meier estimate of the "AGT-free" survival function. The difference between groups in the cumulative incidence functions, and the associated hazard functions, will be tested at the 0.05 level, two-sided, using the Cox regression including age as a covariate (1, 5). The critical value for the test statistic, and confidence intervals in this primary analysis will be determined by the group-sequential procedure.

Data from the TrialNet Pathway to Prevention Study may be used to determine participant eligibility. Outcomes from other studies may be used in meta-analyses, but not in the evaluation of this study's efficacy.

## **4. Secondary Outcomes and Analyses**

### **4.1 Subgroup analyses.**

Subgroup analyses will be conducted comparing the effects of CTLA4-Ig versus placebo on the risk of AGT with a test of the group by subgroup factor interaction in a Cox proportional hazard (PH) Model. Subgroups of the population classified by age (children  $\leq 12$  years of

age, adolescents 13-17 years and adults  $\geq$  18 years), gender, race/ethnicity (white, nonwhite and Hispanic yes/no), and specific antibody status at baseline. Differences in the treatment effect between subgroups will be tested using Likelihood Ratio Test (LRT) in a Cox PH model (1).

#### **4.2 Covariate analyses.**

Similar analyses will be conducted using the values of quantitative baseline factors. They include age, weight, BMI, HbA1c, HLA (DR3/4 vs. others), autoantibody titers, basal C-peptide, stimulated C-peptide ( peak and AUC), glucose (fasting, 2-hour, AUC and measures of insulin secretory rate (0-30 min) and sensitivity (ISI) (6,7) modeled from the OGTT) at study entry. The dependence of the treatment effect on the quantitative levels of a covariate will also be assessed by a covariate by treatment group interaction in a PH model. The ISI can also inform on the treatment effect independent of C-peptide.

The association of demographic, genetic, immunologic, metabolic, and lifestyle factors, the presence of illness and concomitant meds, both at baseline and over time, with the risk of AGT onset will be assessed in Cox PH Models over time. The effects of changes in longitudinal factors on AGT risk will be assessed using time-dependent covariates for these factors.

Using a step-up procedure additional covariates will be tested and included in the model only if they improve the log-likelihood at 0.10 level (2-sided). This will be accomplished with the treatment assignment variable included but the inclusion/exclusion of the candidate covariates will be completely independent of the treatment variable's impact on the model. The LRT associated with treatment variable in the full, adjusted model will be used for the test of treatment effect described in the previous paragraph. Thus, the adjustment of the significance level, as with multiple testing, is unnecessary.

#### **4.3 Longitudinal analyses.**

Longitudinal analyses will assess the effects of CTLA4-Ig versus placebo treatment on immunologic and metabolic markers over time up to the onset of AGT. Differences between groups in the mean levels of quantitative factors over time will be assessed using a normal errors linear model for repeated measures. Differences between groups in the prevalence of qualitative factors over time will be assessed using generalized estimating equations for categorical measures. Generalized estimating equations may also be employed for the analysis of quantitative factors if the assumption of multivariate normal random errors is violated.

#### **4.4 Diabetes.**

Additionally, as noted in section 4.9.2 of the protocol, subjects will be able to be followed for the occurrence of diabetes. The treatment arms will be compared on the corresponding incidence rates of Type 1 diabetes using the logrank statistic. Subgroup analyses analogous to those described for the AGT endpoint will be conducted on the endpoint of Type 1 diabetes.

Additional, pre-specified secondary analyses are identified as follows:

1. Although there is limited power, the AGT-free proportion at 1,2 and 3 years after randomization will be calculated and compared using the Kaplan-Meier estimates of the proportions and the Greenwood estimates of the variance.
2. Evidence that the HR is not constant over the period of follow up will be assessed and the risk of diabetes will be assessed in a PH model. The primary test for treatment effect is based on the Cox model, which assumes a proportional hazard between treatment groups. If there is a true treatment effect but it is not proportional over the follow-up period the test will have substantially less statistical power than stated in the statistical section of the protocol. Therefore, to establish a reasonable guide to pursue the possibility of a non-proportional treatment effect we will use the guideline requiring a significance level of 0.10 or less of the LRT from the standard Cox model. Graphical diagnostics using Schoenfeld residuals will be employed to explore evidence for a monotonically decreasing effect of treatment over the follow-up period. Also, plotting the Kaplan-Meier time-to-AGT rates by treatment group on the log-log scale will provide visual assessment of any diminishing effect of treatment (equal distance separation of curves indicates proportional hazard). We will characterize any such decreasing effect of treatment by model parameterization with monotonically decreasing benefit over the follow-up time. Initially, a treatment interaction with log-transformed time-on-study will be fit to the data and the log likelihood improvement in the fit noted. Other time transforms will be explored only to characterize mathematically the rate of the diminishing effect. Of particular interest will be characterizing the point in follow-up where the hazard ratio is 1. Any variations in proportional hazards other than a monotonically decreasing effect will not be of interest because of the possibility that it is simply random error.

## 5. Study Power and Sample Size

The study has been designed to provide 80% power to detect a 40% risk reduction in the rate of AGT using a two-sided test at the 0.05 level after six years of study duration.

Study enrollment will stop on approximately July 31, 2019 and follow-up will continue for up to two additional years. A model of observed events (confirmed abnormal glucose tolerance; AGT) in the placebo group as of June 2019, provides a more realistic estimate of the number that will have developed AGT at the conclusion of the follow up period. The model suggests that the total number of events will be between 64 and 70; resulting in detectable hazard ratios of 0.496 to 0.512. The hazard ratios and difference in cumulative incidence are based on 80% statistical power when testing at the 0.05 level (2-sided).

The final test of significance up through the six-year time point will employ group sequential critical values to protect against inflation in the type I error probability due to interim assessments of the emerging data for review by the DSMB. However, there is only a minimal loss in power with this approach so that the fixed sample size power based on the above information calculation is virtually identical to the group sequential power. Thus, there is no need to adjust for the group sequential critical values in these computations.

Note the original accrual period and the study target sample are only projections since the actual accrual rate and the loss to follow-up rate are unknown. As the study progresses, more accurate projections of the study end date will be computed based on the observed rate of enrollment, the observed number of events, and the observed rate of loss-to-follow-up. This data will be provided

to the DSMB and the TrialNet governing body, and if need be, this document will be amended.

## 6. Interim Monitoring Plan

The Lan-DeMets spending function with an O'Brien-Fleming boundary will be used to protect the type I error probability for the primary outcome analyses, and to assess the significance of the interim results that emerge during the trial.

The spending function that approximates the O'Brien-Fleming boundaries is:

$$\alpha_1(t^*) = 2 - 2\Phi\left[\frac{Z_{\alpha/2}}{\sqrt{t^*}}\right]$$

Where  $t^*$  is the information fraction ( $0 < t^* \leq 1$ ) and  $\alpha$  is the fixed-sample type I error (i.e., 0.025). The DSMB will also be informed if there is a serious lack of evidence of a treatment effect (i.e. futility analysis). The boundaries are based on the paper by Lachin (4). The study should be stopped based on the futility of rejecting the null hypothesis at the completion of the trial if:

$$Z_{HR}(t^*) \geq 0 \text{ when } t^* \leq 0.5 \text{ if } t^* < 0.8 \text{ or } Z_{HR}(t^*) \geq -0.8 \text{ when } t^* \geq 0.8$$

Using Lachin's formulas a onetime use of either boundary for the design parameters above ( $\theta \equiv Z_{1-\alpha} + Z_{1-\beta} = 2.8$ ) raises the type II error to approximately 0.204 and 0.202, respectively. For larger values of  $t^*$  the increase to the error probability is even less. Furthermore, by the laws of probability a single use of each rule will increase the type II error no more than the sum of the increase (i.e.,  $0.15 + 0.004 + 0.002 = 0.156$ ).

The DSMB may terminate the trial prematurely if a statistically significant effect is observed and it is considered that all major trial objectives have been met. The DSMB will also consider early termination due to absence of a treatment effect (i.e. futility) based on computations of conditional power conducted both under the initial study design and under the current trend of the data.

An independent medical monitor will closely monitor the events in the trial as described in section 10.4 of the protocol. Additional analysis will assess potential adverse outcomes of treatment and will assess the incidence of all severe adverse events.

## 7. General Considerations

### 1. Analysis Populations

The Intention to Treat Population (ITT)

*The intention to treat population comprises all randomized (as planned) subjects.*

Full Analysis Population

*The Full Analysis Set (FAS) will comprise all subjects who received any study drug and who participated in at least one post-baseline assessment. These will be analyzed as randomized. FAS will be the primary efficacy population. So, FAS is a subset of ITT.*

## Per Protocol Population

*The Per Protocol Set (PPS) will comprise all subjects who did not substantially deviate (defined as greater than or equal to 75% protocol compliance) from the protocol as to be determined on a per-subject basis before data base lock and unblinding.*

## Safety Population

*All subjects who received any study treatment (including control) but excluding subjects who drop out prior to receiving any treatment.*

## 2. Timing of Analyses

The final analysis will come after the total number of events (confirmed abnormal glucose tolerance) in the placebo group is between 64 and 70. This will result in detectable hazard ratios of 0.496 to 0.512 to achieve the planned 80% statistical power when testing at the 0.05 level (2-sided) for the primary analysis.

## 3. Missing Data

In general, missing values will be assumed to be *missing completely at random (MCAR)* unless empirical evidence to the contrary can be established internal to the study. The methodology employed in analyzing time-to-T1D utilizes whatever follow-up has been recorded for each subject (i.e., maximum utilization of follow-up time). Presuming no evidence against MCAR, no methods will be employed to impute additional follow-up of subjects that drop out (i.e., lost to follow-up). All secondary endpoints will use the complete-case analysis approach which limits the analytical cohort to those subjects that have the secondary endpoint of interest measured and recorded. In modeling to adjust for risk factors associated with the endpoint (i.e. covariates), missing values will be assigned the mean from the known covariate cohort. This simple rule will be employed only if the percent missing is less than 10% for the analytical cohort. If the missing is 10% to 20% a separate indicator for missing will be included in the modeling. If the missing exceeds 20% the covariate will be removed from consideration.

## 8. Safety Analyses

Safety will be evaluated with summary of adverse events for the safety population. The following parameters will be assessed during the study:

### Adverse Events

The summary statistics will be produced in accordance with Section 8. Treatment emergent adverse events (AEs) are those events that occur after the baseline assessment. Only Grade 2 or greater adverse events were reported in this study. The incidence of the following AEs will be reported:

A tabular summary of AE will present: Number of subjects with any AE; Number of SAEs with outcome death; Number subjects with SAE; Number subjects with AEs leading to discontinuation of study drug, even if by protocol; Number of subjects with AEs leading to discontinuation of study; Total number of AEs; Total number of SAEs [TABLE].

The Adverse Events summary tables will include number of adverse events, the number of subjects in each treatment group in whom the event occurred, and the incidence of occurrence and should be grouped by system organ class, preferred terms and/or other



interested variables (e.g., relatedness, intensity and seriousness). [TABLE]

When calculating the incidence of adverse events, or any sub-classification thereof by treatment, time period, severity, etc., each subject will only be counted once and any repetitions of adverse events will be ignored; the denominator will be the total population size.

Deaths, Serious Adverse Events and other Significant Adverse Events

All formal testing of adverse effects will be based on the subject as the experimental unit. Thus, for comparing incidence of AE within system organ by treatment group, a one-sided Fisher's exact test will be conducted at 0.05 level (higher incidence in experimentally treated group is the alternative hypothesis). Also, highest AE grade will be determined for each subject and compared by treatment group using a 2 sample Wilcoxon test (one-sided at 0.05). No correction for multiple testing will be employed in order that the statistical power is maintained.

## 9. Reporting Conventions

P-values  $\geq 0.01$  will be reported to 2 decimal places; p-values less than 0.01 and  $>0.001$  will be reported to 3 decimal places; p-values less than 0.001 will be reported as " $<0.001$ ". The mean, standard deviation, and any other statistics other than quantiles, will be reported to one decimal place greater than the original data. Quantiles, such as median, or minimum and maximum will use the same number of decimal places as the original data. Estimated parameters, not on the same scale as raw observations (e.g. regression coefficients) will be reported to 3 significant figures.

## 10. Per Protocol Analysis

Quantifying the evidence of any dose response relationship is part of a complete analysis of any well-run and completed clinical trial. This is especially true when the trial is negative when testing the primary outcome. To explore whether there is evidence that protocol treatment deviations may have played a role in the negative outcome, we plan to assess the treatment hazard ratio by the degree of compliance (percent missed infusions) to the protocol scheduled dose in a quantitative manner.

Using the Cox model we will assess the evidence for an effect of treatment compliance including the entire cohort. The number of infusions of treatment and the treatment dose (in a separate model) will be introduced into the model as a continuous variable (0=received no therapy; 1 = received the full prescribed dose) along with the interaction term with treatment group to determine its effect on risk. The significance level of the interaction term (compliance value if abatacept subject; 0 otherwise) will be considered important if it is approximately 0.05 or less one-sided and the coefficient is negative (indicating lower risk with greater compliance). The procedure for including covariates, such as age, will follow the set up procedure as described above under Primary and Secondary analyses.

## 11. Technical Details

The analysis will be performed in R, S-Plus or SAS. The distributional assumptions as well as other assumptions underpinning the planned analyses will be checked.

## 12. References

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