TOPPLE T1D

A multiple ascending dose trial investigating safety, tolerability and pharmacokinetics of NNC0361-0041 administered subcutaneously to patients with type 1 diabetes mellitus

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Protocol TN27

Protocol title:

A multiple ascending dose trial investigating safety, tolerability and pharmacokinetics of NNC0361-0041 administered subcutaneously to patients with type 1 diabetes mellitus

Trial Phase: 1

Statistical Analysis Plan (SAP)

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Author: Jeffrey Krischer
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TrialNet Approver
Brian Bundy, PhD
University of South Florida

Novo Nordisk Approver
Lars Holm Damgaard
Novo Nordisk Senior Statistical Director

Signature

Signature

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1 AMENDMENTS FROM PREVIOUS VERSION(S)

This version (dated June 23, 2023) is an update to the initial SAP for the TN27 protocol.
Previous SAP version and date: V1.1 February 28, 2021

2 INTRODUCTION

This document describes the planned statistical analyses for protocol TN27, current version dated January 7, 2022. This analysis plan is meant to supplement the study protocol. Any deviations from this analysis plan will be described in the Clinical Study Report.

2.1 STUDY DESIGN

This is a placebo-controlled, double-blinded within cohorts, randomized, multiple ascending dose trial with a staggered trial design. A total of 48 patients with T1D are planned to be studied in 4 cohorts of 12 patients (9 on active and 3 on placebo treatment). Patients will be randomized in a 3:1 fashion within each cohort to receive recombinant supercoiled plasmid NNC0361-0041 or placebo. The randomization is not stratified. A centralized random permuted block design will be used to balance treatment assignments within cohorts.

Each treatment is defined as 12 weekly doses. Should it not be possible to administer all 12 doses, patients will be followed, nonetheless, for 12 months according to the protocol Schedule of Assessments.

2.2 STUDY OBJECTIVES

Primary Objective:
To investigate the safety and tolerability of ascending subcutaneous weekly doses of NNC0361-0041 plasmid in patients with T1D.

Secondary Objectives:
To investigate effect on target engagement, immune response, and beta cell function following ascending subcutaneous weekly doses of NNC0361-0041 plasmid in patients with T1D.

3. Population Definitions

Up to 12 patients will be randomized to treatment vs. placebo within each of 4 cohorts. The goal is to have a minimum of 9 patients within each cohort to have received at least 10 of the planned 12 treatment doses.

Randomized:	Met eligibility criteria and randomly assigned to a treatment arm without consideration of actually receiving treatment.
Randomized and partially treated:	Met eligibility criteria, randomly assigned to a treatment arm and received at least 1 – 9 doses.
Randomized and treated:	Met eligibility criteria, randomly assigned to a treatment arm and received at least 10 doses.
Pretreatment period	From signing of informed consent until 1st treatment

On-treatment period	From 1st treatment through visit 15 (or 5 weeks after last treatment for subjects not receiving all injections)
Follow-up period	From visit 15 (or 5 weeks after last treatment) through visit 17

Up to 2 additional individuals within each cohort may be enrolled to complete the target 9 patients randomized and treated to account for those who are randomized and partially treated.

4. SAFETY and INSULIN SECRETION ANALYSES

4.1 SAFETY

Clinical data (vital signs, AEs) from the first 48 hours for the first two patients enrolled within a cohort (sentinel cases) will be evaluated by the TrialNet Medical Monitor and the Novo Nordisk Safety group to assess whether accrual within the cohort can continue. A safety analysis to support the decision to proceed to the next dose level will be conducted when at least 9 patients have received the first four injections and have had 4-week assessments (visit 6.) This analysis is conducted by TrialNet DMC and the NovoNordisk Safety group and will include cumulative data from previous cohorts when available.

4.2 INSULIN SECRETION ANALYSIS

The insulin secretion analysis is conducted when all subjects have completed their final visits through visit 17 (1 year after first treatment).

5 ANALYSIS SETS

5.1 FULL ANALYSIS POPULATION

The full analysis (FA) population will include all patients who are randomized with study drug assignment designated according to the initial randomization. In exceptional cases subjects from the FA may be excluded. In such cases the exclusion will be justified and documented. The subjects in the FA will contribute to the analyses 'as treated'.

5.2 SAFETY ANALYSIS POPULATION

The safety analysis (SA) population will include all randomized patients who receive at least one dose of study medication, with treatment assignments designated according to actual study medication received. The SA population will be the primary population for safety and treatment evaluations.

5.3 EVALUABLE POPULATION FOR PHARMACOKINETICS

The PK concentration population is defined as all patients in the SA population who have at least one dose.

5.4 ANALYSIS SUBGROUPS

Of particular interest for safety outcomes and potential worsening of insulin secretion is the subset of the safety analysis population who receive at least 10 doses of their assigned treatment (FA10). Those not included in FA10 will also be analyzed as a separate subgroup. AEs reported through 4 weeks (visit 6) will also be a defined subgroup for safety assessments.

5.5 TREATMENT MISALLOCATIONS

Patients who were randomized but not treated with study medication will be reported under their randomized treatment group (FA). However, they are by definition excluded from the safety analyses.

Patients who were randomized but received incorrect treatment, will be reported under their randomized treatment group (FA) but will be reported under the treatment they actually received (SA) for all safety analyses and treatment evaluations.

5.6 PROTOCOL DEVIATIONS

All deviations will be described when they appear and relate to the statistical analyses or analyses sets. No patients will be excluded from primary analyses based on eligibility evaluations.

6 ENDPOINTS AND COVARIATES

6.1 SAFETY ENDPOINTS

The primary endpoint is the number of adverse events recorded during the on-treatment period through visit 15. No formal statistical analysis will be made for the primary outcome. The primary endpoint will be presented by treatment groups in a summary table including number of AEs, number of subjects with at least 1 AE and percentage of exposed subjects with at least 1 AE. Placebo will be pooled across cohorts for comparison. AEs will also be summarized by system organ class, preferred term, seriousness, severity, outcome, and relationship to IMP using descriptive statistics.

Adverse events will be summarized over the entire trial (i.e., on-treatment and follow-up period). (i.e., visit 0 through visit 17 at 12 months). They will be further summarized by whether they occurred up to and including visit 6 (4 weeks) or later. Follow-up visits 16 and 17 will be conducted to obtain 6- and 12-month data on participants' metabolic status and reportable adverse event assessments.

All AEs will be coded using the CTCAE and mapped to latest version of Medical Dictionary for Regulatory Activities (MedDRA). AEs will be summarized by system organ class, preferred term, seriousness, severity, outcome, and relationship to IMP using descriptive statistics.

6.2 SECONDARY ENDPOINTS

$\Delta AUC_{0\text{-}2h,C\text{-}peptide,MMTT}$; Relative change in the area under the plasma C-peptide concentration-time curve from time 0 to 2 hours during MMTT from baseline to 3 months (visit 14) between groups. The comparison will be blinded until the last patient has been followed for 12 months.

Additional Outcomes and Analyses

It is the aim to perform additional analysis to get information of the appearance and disappearance of Plasmid DNA and RNA at various time points during the trials conduct period:

- Plasmid DNA and RNA concentrations at selected time points before and from 1 hour to up to 48 hours after the first dose (Visits 0-2, days 0-2)
- Plasmid DNA and RNA concentrations before second dose (Visit 3, day 7 +/- 1 day)
- Plasmid DNA and RNA concentrations before dose 5 (Visit 6, day 28 +/- 1 day)
- Plasmid DNA and RNA concentrations before dose 8 (Visit 9, day 49 +/- 1 day)

- Plasmid DNA and RNA concentrations at selected time points after last dose (Visit 14, day 84 +/- 3 days; Visit 15, day 120 +/- 14 days; Visit 16, day 180 +/- 14 days; and Visit 17, day 365 +/- 14 days)

Additional analysis of the impact of study drug on metabolic outcomes (Section 5.4) will be determined at 1-, 3-, 6-, and 12-months following treatment including analysis of changes of these parameters from baseline.

Target Engagement, Mechanistic Outcomes and Exploratory Analysis
The primary time period for evaluation of target engagement and mechanistic outcomes will be accumulated data through visit #14.

The mechanistic hypothesis underlying this therapy is that NNC0361-0041 is a tolerizing agent (cross reference to protocol section 2.2.2). Due to the low number of transfected cells, it may not be possible to detect any systemic effect. However, evidence supporting this hypothesis would be the detection of plasmid-derived mRNA in the circulation or increase in certain cytokines. Further supporting this hypothesis, will be the demonstration of a change in number (frequency), or change of phenotype in circulating immune cells from baseline. These studies will include, but are not limited to, flow cytometry, measures of gene expression, and cytokine detection. The relationships of such measures will be evaluated with respect to C-peptide, insulin dose, glycemic control, PK and PD markers of therapy, and genotype (including, but not limited to coding and non-coding genes HLA, insulin-VNTR, CD25, PTPN2, and IL10). Additional exploratory analysis for target engagement and mechanistic outcomes to assess the duration of such effects will be done using data through month 12 post-dosing.

6.3 COVARIATES AND STRATIFICATION FACTORS

The potential influences of baseline patient characteristics such as age, gender, C-peptide level following a MMTT at study entry on the primary and secondary endpoints will be evaluated in a multivariate model to see whether they have an effect on the treatment vs. placebo comparison.

7 HANDLING OF MISSING VALUES

7.1 MISSING DATES

In compliance with TrialNet standards, if the day of the month is missing for any date used in a calculation, the 15 of the month will be used to replace the missing date unless the calculation results in a negative time duration (e.g., date of resolution cannot be prior to date of onset; if replacing resolution date with the 15 of the month results in a negative duration, the resolution date will be set to the onset date). In this case, the date resulting in 0 time duration will be used. If the start date is missing for an AE, the AE is considered to be treatment emergent unless the collection date is prior to the treatment start date.

7.2 MISSING VALUES for AUC

For the calculation of AUC following a MMTT, no values will be imputed for missing data; however if a single timed value is unavailable the calculation will proceed with the remaining known timed values. AUC is calcuted using the trapezoidal technique based on observed values and actual measurement times. If more than one timed value is missing the AUC will be considered missing.

7.3 MISSING PK/PD VALUES

Concentrations below the limit of quantification

In all data presentations (except listings), concentrations below the limit of quantification (BLQ) will be set to zero. In listings, BLQ values will be reported as "<LLQ", where LLQ will be replaced with the value for the lower limit of quantification.

Deviations, missing concentrations, and anomalous values

In summary tables, statistics will be calculated having set concentrations to missing if 1 of the following cases is true:

1. A concentration has been noted as ND (i.e., not done) or NS (i.e., no sample);
2. A deviation in sampling time is of sufficient concern or a concentration has been flagged anomalous by the pharmacokineticist.

8 STATISTICAL METHODOLOGY AND STATISTICAL ANALYSES

8.1 STATISTICAL METHODS

8.1.1 Analyses of Continuous Data

Descriptive statistics, including the mean, standard deviation, median, minimum, and maximum values will be provided.

8.1.2 Analyses of Categorical Data

The number and percentage of patients in each category will be provided for categorical variable.

8.2 STATISTICAL ANALYSES

8.2.1 Standard Analyses

Descriptive statistics will be used to summarize study conduct, patient disposition, baseline characteristics, and treatment administration/compliance.

Study Conduct and Patient Disposition - an accounting of the study patients will be tabulated for the FA population. Patients not meeting eligibility criteria will be identified. Patients not completing the study will be listed along with the reason for their premature discontinuation. Reasons for premature discontinuation will be summarized.

Baseline Characteristics - patient characteristics such as age, gender, race, ethnicity, duration of disease will be provided in frequency tables and descriptive statistics for quantitative variables.

Treatment Administration/Compliance - Administration of study medication will be presented for the SA population, by medication administered within each treatment group and will be described in terms of the total number of doses administered, the median (range) of doses administered, dose modifications, dose interruptions, dose delays due to adverse event and other reasons for dose modifications, interruptions or delays.

8.2.2 SAFETY ANALYSES

Safety data will be analyzed in the SA population. Adverse events and select laboratory values (e.g., hematology, blood chemistry and others) may also be summarized by the treatment arms. Only grade 2 or higher AEs are to be reported for visits 16 and 17, as per protocol.

On-Treatment Adverse Events

An overall summary of AEs as described above will be provided. AEs will be summarized for all doses and also by dose. The most commonly reported AEs (5% or more of patients) will also be summarized by preferred term. A summary of AEs by preferred term and maximum CTCAE grade, as well the grouped maximum CTCAE grade group (Grade 1-2 vs. Grade 3-4 vs. Grade 5) will be presented. Only patients who experience AEs during the AE reporting period will be listed.

Treatment Related Adverse Events

Treatment-related AEs are those judged by the Investigator to be at least possibly related to the study drugs [with a cause related to study drug as indicated on the case report form (CRF)] or for which relatedness is recorded as "unknown" by the Investigator. Similar summaries as noted for AEs will be provided for treatment related AEs.

Serious Adverse Events and Death

On-treatment SAEs and treatment-related SAEs will be summarized by MedDRA SOC and preferred term. Patients who experienced a SAE during the SAE reporting period will be listed for all randomized patients. The number and percentage of patients who experienced any serious treatment‐emergent SAE will be summarized for all doses. A summary of SAEs by preferred term, maximum CTCAE grade and grade group will be presented.

Deaths will be summarized by time period (on-treatment vs. during follow-up) and cause of death. Deaths that occurred on or after first dose of study medication and within 28 days after the last dose of study medication are defined as on-treatment deaths. A listing of death data will also be provided and it will include all deaths that occurred during the reporting period for deaths which starts from the signing of the informed consent to the end of the follow up period for death.

Concomitant medications/Follow-up systemic therapy - All medications received during the treatment period will be considered as concomitant medications and will be coded by WHO medical dictionary. Patients who received concomitant medications will be listed. Follow-up systemic therapy for primary diagnosis will be summarized by categories of follow-therapy and will be listed for each patient.

8.2.3 MMTT ANALYSES

Acceleration of beta cell destruction due to immune activation is a potential risk, which will be monitored through $AUC_{0\text{-}2h,C\text{-}peptide,MMTT}$. The plasma C-peptide concentration-time curve from time 0 to 2 hours during MMTT obtained at the end-of dosing day 85 for the treatment and placebo arms will be compared for each cohort (N=3 placebo, and N=9 treated) and at the end of the study by cohort (i.e., pooling the placebo treated patients, N=12 placebo, N=9 treated in each cohort).

The MMTT analysis will be conducted on a transformed scale using the function log $(1+ AUC_{0\text{-}2h,C\text{-}peptide,MMTT})$. This provides better normal distributional behavior by the test statistic. The comparison between the two treatment arms will be based on a test of the difference between the means.

When an adjustment for covariates is desired, the comparison will be made using a Wald test of treatment effect in an ANCOVA model adjusting for gender, baseline age, and baseline.

The study has a 72%-87% power to detect a 50% or less treatment ratio when the coefficient of variation (CoV) ranges from 0.38 to 0.31. This is based upon historical data derived from recent onset patients (REF). Should the observed CoV be larger, there would be a corresponding reduction in power to detect a 50% treatment ratio or a corresponding decrease in the detectable treatment ratio to retain 80% or greater power. The power to detect a 50% or less treatment ratio at the end of the study (using the pooled placebo patients) is uniformly >90%.

Additionally, using a published methodology, the observed transformed AUC C-peptide can be compared visually with the expected AUC C-peptide calculated from TrialNet historical data from untreated recent onset patients.

9.0 REFERENCES

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