TOPPLE T1D

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

Protocol Version 3.0 January 7, 2022

NCT04279613

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Trial Phase: 1

(Protocol TN-27)

VERSION 3.0

January 7, 2022

Pre-assigned Submission Tracking Number (STN): IND 24252

Sponsored by the National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK)

PREFACE

The Type 1 Diabetes TrialNet Protocol TN-27, NNC0361-0041 in New Onset Type 1 Diabetes, describes the background, design, and organization of the study.

The protocol will be maintained by the TrialNet Coordinating Center over the course of the study through new releases of the protocol, or issuance of updates either in the form of revisions of complete chapters or pages thereof, or in the form of supplemental protocol memoranda.

Abbreviations

ADA	American Diabetes Association
AE	Adverse event
AUC	Area Under Curve
APC	Antigen-presenting cell
BMI	Body Mass Index
CBC	Complete blood count
CD	cluster of differentiation (CD25, CD69, CD74, CD210)
CFR	Code of Federal Regulations
cGMP	Current Good Manufacturing Practice
CHO	Carbohydrates
CMV	Cytomegalovirus
COVID-19	Coronoavirus Disease 2019
CRF	Case report form
CRS	Cytokine Release Syndrome
DIA	Drug-induced antibody
DPT-1	Diabetes Prevention Trial of Type I Diabetes
DSMB	Data and Safety Monitoring Board
EBV	Epstein-Barr virus
FDA	US Food and Drug Administration
FWA	Federal-wide Assurance
GAD	Glutamate decarboxylase
GCP	Good Clinical Practice
HbA1c	Hemoglobin A1c
HBsAg	Hepatitis B surface antigen
HIV	Human immunodeficiency virus
ICA	Islet cytoplasmic antibodies
IEC	Independent Ethics Committee
IGRA	Interferon-γ release assays
IND	Investigational New Drug

IRB	Institutional Review Board
JC	John Cunningham Virus
JDRF	Juvenile Diabetes Research Foundation
MAP	Mechanistic Analysis Plan
NIDDK	National Institute for Diabetes and Digestive and Kidney Disease
NIH	National Institute of Health
NCI-CTCAE	National Cancer Institute *Common Terminology Criteria for Adverse Events*
NOD	Nonobese diabetic
OGTT	Oral Glucose Tolerance Test
OHRP	Office for Human Research Protections
PBMC	Peripheral Blood Mononuclear Cell
PCR	Polymerase chain reaction
PD	Pharmacodynamics
PI	Principal Investigator
PK	Pharmacokinetics
PML	Progressive Multifocal Leukoencephalopathy
QA	Quality Assurance
SAE	Serious adverse event
SAP	Statistical Analysis Plan
SC	Subcutaneous Formulation
SOE	Schedule of events
SOP	Standard operating procedure
T1D	Type 1 diabetes mellitus
ZnT8	Zinc Transporter 8

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1 INTRODUCTION

1.1 Study Overview

Title	NNC0361-0041 in New Onset Type 1 Diabetes
IND Sponsor	National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK)
Conducted By	Type 1 Diabetes Trial Network (TrialNet)
Protocol Chair	Robin S. Goland, MD
Accrual Objective	To assess safety and tolerability as well as target engagement and pharmacokinetics (PK) in patients within 4 years of diagnosis of T1D
Study Design	The trial 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).
Treatment Description	Recombinant supercoiled plasmid encoding four human proteins: (pre-proinsulin (PPI), transforming growth factor β1 (TGF-β1), interleukin-10 (IL-10), and interleukin-2 (IL-2) is administered subcutaneously via syringe and needle, once every week for 12 weeks.
Study Duration	Individual subjects will be followed for 12 months from initial dosing.
Objective	The primary objective of this study is to investigate the safety and tolerability of ascending subcutaneous weekly doses of NNC0361-0041 plasmid in patients with T1D.
Primary Outcome	The primary endpoint is the number of adverse events recorded during the on-treatment period.
Secondary Goals	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
Major Inclusion Criteria	Diagnosed with T1D less than 48 months prior, male or female, aged 18-45 years, positive results for ≥1 islet autoantibody, peak C-peptide of ≥0.2 pmol/mL after mixed-meal tolerance test (MMTT)

2 BACKGROUND AND SIGNIFICANCE

2.1 Introduction

Type 1 diabetes mellitus (T1D) is an immune-mediated disease in which insulin-producing beta cells are completely or almost completely destroyed, resulting in life-long dependence on exogenous insulin. It is a chronic and potentially disabling disease that represents a major public health and clinical concern. The number of patients being diagnosed with T1D is increasing each year (1).

For individuals living with T1D, continuous exogenous insulin therapy is needed to prevent ketoacidosis and other catabolic effects of insulin deficiency and to promote anabolism for normal growth and development. Dynamic insulin regimens, accounting for carbohydrate intake and exercise, must be combined with frequent blood glucose monitoring in order to optimize glycemic control, and to prevent hypo- and hyperglycemia. In addition to the unrelenting financial and psychological burdens on patients and their families from day-to-day management of this disease, individuals with T1D are at risk for both short-term and long-term complications.

Short-term complications include severe hyperglycemia resulting in diabetic ketoacidosis (DKA), a life-threatening condition. As reported in the SEARCH study, almost one-third of individuals up to 19 years of age present with DKA at the time of clinical diagnosis; the risk was highest for those ages 0 to 4 (~39%) and lowest in the 15-19-year-old group (~23%) (2). In addition to the cost of hospitalization, a rare but significant concern associated with DKA is cerebral edema, which is associated with a high risk for morbidity and mortality (3). Recurrent DKA after the new onset period remains a significant clinical problem, with 3% of T1D Exchange participants experiencing DKA in the prior 3 months. Another significant acute risk is hypoglycemia, which can result in impaired cognition and if severe, coma and death (4). Long-term complications for those living with T1D include visual impairment and blindness, renal failure, vascular disease and limb amputation, peripheral neuropathy, and stroke. The resultant financial burden of T1D to the affected individual, and to society, is of tremendous impact but difficult to calculate (5); the overall DM burden was recently estimated at $327 billion in the US, accounting for 1 in 4 health care dollars, with a 26% increase from 2012 to 2017. Of these, $14 billion are estimated in T1D-associated annual health care costs in the US, consuming more than 1 in 7 health care dollars (6).

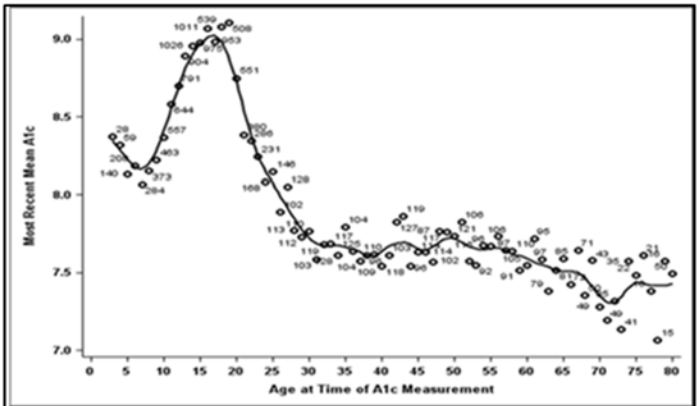


Figure 1: Mean HbA_{1c} by age. Average HbA_{1c} for each year of age was plotted using the most recent HbA_{1c} value available for each of the 16,057 participants in the T1D Exchange Registry. Circles represent the mean HbA_{1c} for each year of age. Numbers next to circles are the *n* for each year of age

The Diabetes Control and Complications study (DCCT) showed that the long-term complications could be reduced with near normal control of glucose levels, but at the cost of an increased frequency of severe hypoglycemia (7). While there have been significant improvements in insulin analogs and insulin delivery systems, such as continuous subcutaneous insulin infusions with insulin pumps, continuous glucose monitoring, and hybrid closed loop systems, normal glucose control, particularly in children, is rarely achieved (4). This is particularly true in the teenage years, where the mean HbA1c as reported in 2015 is as high as 9% and only 1/8 are meeting Hba1c targets. (FIGURE 1 [4]). Glycemic control is also poor among adults, where less than 1/3 meet targets and severe hypoglycemia is a common occurrence in those with long standing disease (4). Strikingly, despite the availability and increasing use of devices to optimize glycemic control, such as sensors, pumps, and hybrid closed-loop systems, glycemic control has worsened over time (4). Moreover, while the frequency of long-term complications including end stage renal disease is decreasing (8), some degree of kidney disease is seen in virtually all those with long-standing T1D (9), and individuals with T1D continue to have reduced life expectancy (10, 11).

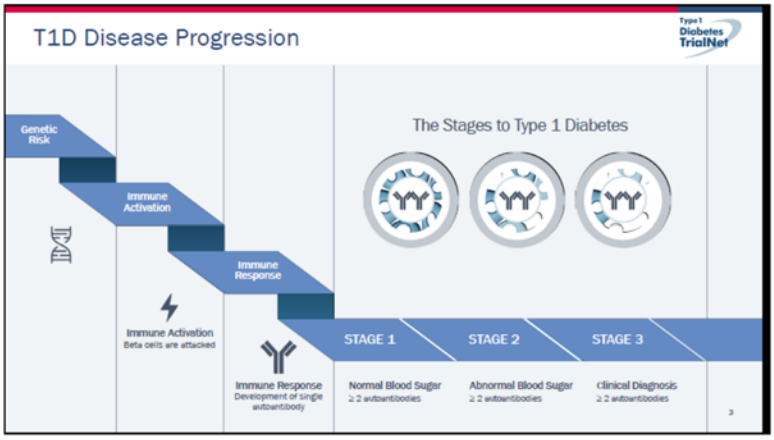
T1D is a particular burden to children and their families, representing one of the most common chronic childhood diseases (12). Recent reports highlight cognitive dysfunction and structural central nervous system (CNS) changes (13). While T1D can occur into adulthood, there is a bimodal peak age of onset, between ages 4-7 and ages 14-16 years (1). The world-wide incidence of T1D is increasing, with the greatest increase in children under the age of 5 years.

2.1.1 Natural History and Stages of T1D

Much is known about the natural history of the type 1 diabetes disease process. Beta cell destruction is initiated in genetically susceptible individuals years before clinical onset of disease (14). The autoimmune process that causes beta cell destruction is clinically silent and can only be identified by the detection of autoantibodies in the blood such as islet cell antibodies (ICA), and autoantibodies to glutamic acid decarboxylase (GAD65), islet antigen 2 (IA-2 Ab/ICA512), insulin (mIAA), zinc transporter 8 (ZnT8 Ab) and others (15, 16, 17). Continued immune-mediated beta cell destruction involving both B and T cells occurs until physiologic insulin demand cannot be met by the remaining beta cells and hyperglycemia ensues (18).

Data gathered from multiple studies during the past 35 years are remarkably concordant with regard to the progression from development of autoantibodies to clinically overt disease. These data, whether from studies of longitudinal cohorts followed from birth (19) or cross-sectionally identified populations at risk (12, 20, 21, 22) indicate that essentially all genetically susceptible

individuals with multiple autoantibodies will eventually progress to clinical disease. Thus, T1D starts with the presence of 2 or more autoantibodies. This observation has led to the concept of progressive stages of T1D (23). Stage 1 is defined as the presence of ≥ 2 autoantibodies with normal glucose tolerance. Stage 2 is the presence of ≥ 2 autoantibodies, with abnormal glucose tolerance. As recently reported, almost 40% of placebo treated individuals at Stage 2 developed clinically overt T1D (Stage 3) within 1 year (24).



The eventual target population for NNC0361-0041 are those at early stages of T1D. Any intervention, which can stop or delay the progression to clinical disease or complete loss of functional residual β-cell mass, is significant as it may reduce the burden of living with clinical disease and/or provide protection against hypoglycemia and provide improved metabolic control resulting in a delay in the micro and macro-vascular complications of diabetes.

2.1.2 Disease Modifying Therapy in T1D

There are at least five immunotherapeutic agents that have been shown to preserve beta cell function in treated as compared to control individuals with recently diagnosed clinical T1D. These include the anti-T cell agents anti-CD3 mAb (teplizumab, otelixizumab)(25, 26), LFA3 Ig (Alefacept) (27), anti-thymocyte globulin (28), anti-CD20 mAb (rituximab) (29) and CTLA4 Ig (Abatacept) (30). Moreover, administration of teplizumab to high risk, asymptomatic individuals has recently been shown to delay progression to clinical disease by a median of 2 years (24).

As an alternative to broad immunosuppression, the promise of antigen therapy is the possibility to restore tolerance or non-responsiveness to specific immune targets. Previous phase 2 trials using antigen therapy both prior to clinical diagnosis to delay disease progression and after diagnosis to preserve beta cell function have largely been disappointing (31). In these trials, protein or peptide antigens have been directly administered with or without adjuvant through various routes (IV, SC, oral, nasal) (31-33).

Another approach is to deliver antigen without adjuvant through plasmid therapy. Such tolerogenic plasmids are designed to reduce an anti-inflammatory response to a given antigen. Plasmid-mediated antigen delivery has been proposed as a suitable platform for antigen-specific tolerance therapy (31-33, 42). This allows for administration of antigen without adjuvant and thus offer the promise of tolerance induction. While no human plasmids have been approved for therapeutic use, dozens of plasmid-based clinical trials for a wide spectrum of prophylactic or therapeutic vaccinations for infectious disease or cancer applications are listed as completed or ongoing. Plasmids have an excellent safety profile with no reported issues such as autoimmunity or host

genome insertional mutagenesis from numerous human clinical trials (34). Plasmid-encoded proinsulin therapy has been studied in T1D. There were no safety issues identified in this dose escalation trial of 80 adults with T1D (35).

2.2 Study Agent: NNC0361-0041

2.2.1 Description

NNC0361-0041 is a novel recombinant supercoiled plasmid (6388) that encodes the following four human proteins, in addition to a modified CD74 (invariant chain): pre-proinsulin (PPI), transforming growth factor β1 (TGF-β1), interleukin-10 (IL-10), and interleukin-2 (IL-2) (Figure 1). The PPI is fused to CD74, lacks a secretion signal, and is degraded for presentation on the cell surface by Human Leukocyte Antigen (HLA) class I and II molecules. The three cytokines are secreted in the local environment. The plasmid has been designed to be non-integrating and non-replicating in human cells. The protein sequences of the four target gene products share a high degree of sequence similarity to the corresponding human endogenous proteins. For PPI, TGF-β1, and IL-2, but not IL-10, codon usage has been altered in order to eliminate nucleotide sequence homology to endogenous genes to minimise risk of homologous recombination. On the protein level, the amino acid sequences of the TGF-β1 and IL-2 gene products are essentially identical to the endogenous counterparts. The plasmid-encoded PPI and IL-10 each carry a C-terminal tag from the viral cleavage element (24 amino acids for the PPI "tag" and 21 amino acids for the IL-10 "tag"). The small translated C-terminal tag has been confirmed not to interfere with the function of IL-10. Specifically, expression of the tagged protein was confirmed using Western blotting and ELISA, while bioactivity was demonstrated in a native receptor dimerization bioluminescence assay.

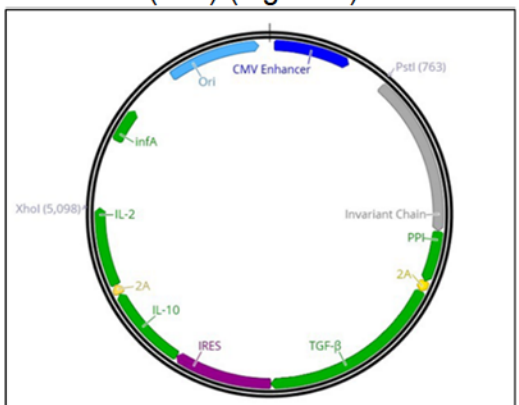


Figure 1 The recombinant DNA plasmid construct (NNC0361-0041), encoding the four human proteins: PPI, TGF-β1, IL-10, and IL-2. Ori: origin of replication; CMV: promoter for cytomegalovirus; PPI: pre-proinsulin; TGF-β: transforming growth factor β1; IRES: internal ribosome entry site; IL 10: interleukin 10; IL-2: interleukin 2. plasmid constructs are expressed and isolated from *Escherichia coli* (*E. coli*).

2.2.2 Rationale for use in T1D

NNC0361-0041 treatment is an immunotherapy intended for chronic administration to halt the autoimmune response against pancreatic beta cells and to preserve endogenous insulin production, thereby preventing progression to clinical disease. Preservation of insulin production after clinical diagnosis is associated with better glycemic control and less short- and long-term complications (36). Halting immune destruction earlier in the disease may delay or prevent progression to clinical disease. Thus, the primary target population consists of subjects at risk of developing T1D. The plasmid induced diabetes prevention is designed to be achieved without suppression of normal protective immune responses.

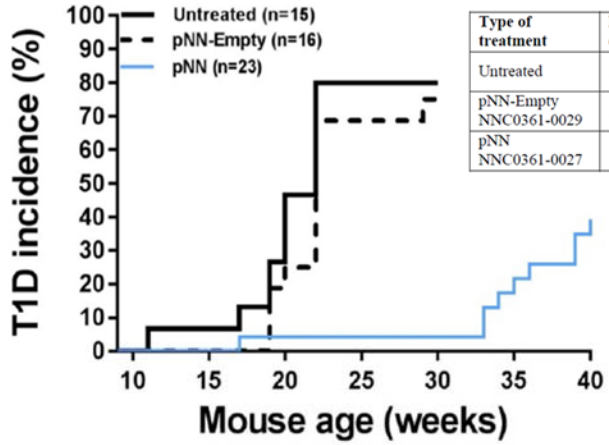
The medical hypothesis for NNC0361-0041 is that antigen presenting cells (APCs) will be transfected with the plasmid via subcutaneous injection, leading to localised expression of PPI and the immuno-modulatory cytokines, TGF-β1, IL-10, and IL-2. PPI will not be secreted but will be presented by HLA class I and II on the surface of the transfected cells to circulating immune cells. The immuno-modulatory cytokines will be locally secreted around the transfected cells in small amounts to promote PPI-specific immune tolerance mechanisms, without causing systemic immunosuppression. PPI-specific T cells, which encounter APCs presenting plasmid-derived PPI, will recognise their antigen within this tolerogenic milieu. As a consequence, the T cells will not attack the antigen (PPI)-positive cells in the pancreas. Thus, antigen presentation will cause a

tolerogenic re-education of the immune cells arresting the ongoing autoimmune attack against the beta cells. This will result in preserved beta cell function, translating into disease prevention.

The plasmid is intended to be injected naked, without adjuvants or enhancers and therefore will have a low transfection capacity. The plasmid is expected to only transfect and express in a limited number of cells. The systemic exposure to plasmid-derived proteins will therefore be minimal and occurrence of acute safety signals is consequently considered unlikely.

2.2.3 Pre-clinical data

NOD mouse data indicate a sustained effect on beta cell preservation in vivo throughout the treatment period with the plasmid. Three times weekly s.c. administration of the mouse plasmid to NOD mice with the sub-clinical autoimmune disease (from 9 weeks of age), decreased the number of mice developing diabetes compared to untreated or empty vector-treated controls. When dosing was discontinued at 30 weeks, diabetes progression resumed, indicating that chronic dosing is required for sustained efficacy (Figure 2).



Type of treatment	Incidence of diabetes	Diabetic mice (%)
Untreated	12 / 15	80%
pNN-Empty NNC0361-0029	12 / 16	75%
pNN NNC0361-0027	1 / 23	4%

Figure 2 Plasmid-induced prevention of diabetes in the NOD mouse model,

Three times weekly s.c. administration of the mouse surrogate plasmid ("pNN"), starting at 9 weeks of age, significantly decreased the number of mice developing diabetes compared to untreated and empty vector-treated ("pNN-Empty") controls. Values in table are the number of diabetic animals out of total group size and corresponding percentage T1D incidence at 30 weeks of age. For the pNN group, plasmid therapy was stopped at week 30 and mice were followed for 10 additional weeks, during which period 8 mice turned diabetic. Blood glucose was followed weekly, and T1D diagnosis was determined with 2 consecutive glycaemia values above 250 mg/dl.

All four plasmid-encoded proteins contribute to the *in vivo* efficacy profile. PPI mimics a key immune target for beta cell destruction during development of T1D (37, 38) and its inclusion into the plasmid confers the antigen-specific nature of the therapy. IL-10 is an important regulatory cytokine involved in self-tolerance and suppresses inflammation and autoimmunity (39). TGF-β1 synergises with IL-10 to induce regulatory T cells (T_{reg}) (40). Finally, IL-2 at low doses can stabilize the T_{reg} lineage to prevent loss of regulatory function in inflammatory environments (41, 42).

As the NNC0361-0041 plasmid is designed to be incapable of replication and integration within a eukaryotic host, the number of transfected cells available to promote insulin-specific immune responses toward tolerance is self-limiting due to normal cellular turnover. This requires chronic treatment but minimises the risks inherent in permanent genetic modifications.

Studies in Primates

Dose toxicity studies in non-human primates to support the FHD trial have been conducted for which full information is available in the investigator brochure.

A 4-week dose range finding study with 4 weeks recovery in cynomolgus monkeys has been completed. In addition to standard toxicity endpoints, C-peptide levels, as well as immune endpoints including cytokine panels, immunophenotyping, and dsDNA and IL-2 DIA analysis was investigated. The study also included an assessment of biodistribution of the plasmid.

There were no adverse treatment related effects on any of the measured toxicity parameters and no meaningful changes in concentrations of any of the cytokines measured during the study compared to pre-treatment values. Plasmid was primarily distributed to skin at injection sites and draining lymph nodes and levels declined in the recovery period.

A 13-week toxicity study with recovery also in cynomolgus monkeys has been completed.

In conclusion, subcutaneous injections of NNC0361-0041 at dose levels up to 20 mg once weekly for 13 weeks was well tolerated in cynomolgus monkeys, with only non-adverse findings noted. These comprised high neutrophil counts and mononuclear cell infiltration at injection sites following dosing with 20 mg once weekly with full recovery at 8- and 13-weeks post-end of treatment. The NOAEL was considered to be 20 mg/once weekly (highest administered dose).

2.2.4 Manufacturing Reference

The drug master file (DMF) submission tracking numbers for the drug substance is MF 21393 and for the drug product/placebo is MF 21394.

3 STUDY DESIGN

3.1 Overview

The proposed first in human dosing (FHD) trial is a placebo-controlled, double-blinded within cohorts, randomised, multiple ascending dose (MD) trial with a staggered trial design. The trial will investigate the safety, tolerability and explore PK as well as target engagement properties following once weekly subcutaneous doses of plasmid NNC0361-0041 for 12 weeks in patients within 48 months of diagnosis of T1D.

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). Within each cohort, sentinel enrollment will occur and safety assessment will occur before remaining participants are enrolled. The treatment period will be 12 weeks with once weekly dosing leading to 12 doses in total. Dose escalation will occur after data safety review (as described in section 4.9.2). An MMTT to assess insulin secretion will be done at screening, 1, 3, 6, and 12 months. The follow-up (FU) period will be 1 week after the last dose, as well as 4, 6 and 12 months after the first dose.

3.2 Entry Criteria

3.2.1 Inclusion Criteria

Potential participants must **meet all** of the following inclusion criteria:

1. Willing to provide Informed Consent
2. Participants must live in a location with rapid access to emergency medical services
3. Age 18-45 years (both inclusive) at the time of signing informed consent
4. Must have a diagnosis of T1D for less than 48 months at randomization
5. Must have at least one diabetes–related autoantibody present (GAD65A; mIAA, if obtained within 10 days of the onset of insulin therapy; IA-2A; ICA; or ZnT8A)
6. Must have stimulated C-peptide levels ≥ 0.2 pmol/ml measured during an MMTT conducted at least 21 days from diagnosis of diabetes and within one month (37 days) of randomization
7. Be willing to comply with intensive diabetes management
8. HbA1c ≤8.5% at screening
9. Subjects who are CMV and/or EBV seronegative at screening must be CMV and/or EBV PCR negative within 37 days of randomization and may not have had signs or symptoms of a CMV and/or EBV compatible illness lasting longer than 7 days within 37 days of randomization
10. Be up to date on recommended immunizations
11. Be at least 6 weeks from last live immunization
12. Be at least 4 weeks from killed vaccine other than flu vaccine
13. Participants are required to receive killed influenza vaccination at least 2 weeks prior to randomization when vaccine for the current or upcoming flu season is available
14. Be willing and medically acceptable to postpone live vaccines during the treatment period and for 3 months following last dose of study drug
15. If participant is female with reproductive potential, she must have a negative pregnancy test at screening and be willing to avoid pregnancy using a highly effective contraceptive method for the 12 months of the study
16. Males of reproductive age must use adequate contraceptive method during the treatment phase and for 3 months following last dose of study drug
17. Participants are required to receive an authorized non-live COVID-19 vaccination and be fully vaccinated, including eligible boosters as indicated, at least two weeks prior to randomization.

3.2.2 Exclusion Criteria

Potential participants must **not** meet any of the following exclusion criteria:

1. One or more screening laboratory values as stated
    a. Leukocytes < 3,000/μL
    b. Neutrophils <1,500 /μL
    c. Lymphocytes <800 /μL
    d. Platelets <100,000 /μL
    e. Haemoglobin <6.2 mmol/L (10.0 g/dL)
    f. Potassium >5.5 mmol/L or <3.0 mmol/L

g. Sodium >150mmol/L or < 130mmol/L
h. AST or ALT ≥2.5 times the upper limits of normal
i. Bilirubin ≥ 1.5 times upper limit of normal
j. Glomerular Filtration Rate (eGFR) value of eGFR < 60 ml/min/1.73 m^2 as defined by KDIGO 2012 (43)
k. Any other laboratory abnormality that might, in the judgment of the investigator, place the subject at unacceptable risk for participation in this trial

2. Current or ongoing use of non-insulin pharmaceuticals that affect glycemic control within prior 7 days of screening

3. Use of other immunosuppressive agents including chronic use of systemic steroids. Topical products are acceptable (nasal, conjunctival, skin)

4. Have active signs or symptoms of acute infection at the time of randomization

5. Have current, confirmed COVID-19 infection

6. Chronic active infection other than localized skin infections

7. Have evidence of prior or current tuberculosis infection as assessed by PPD, interferon gamma release assay or by history

8. Have evidence of current or past HIV, Hepatitis B infection

9. Have evidence of active Hepatitis C infection

10. Vaccination with a live virus within the last 6 weeks and killed vaccine within 4 weeks (except 2 weeks for flu vaccine)

11. Be currently pregnant or lactating, or anticipate getting pregnant within the one-year study period.

12. Have severe obesity: adults BMI ≥ 40

13. Have a history of malignancies

14. Untreated hypothyroidism or active Graves' disease

15. History of severe reaction to prior vaccination

16. Participation in any clinical trial of an approved or non-approved investigational medicinal product within 30 days after last blood draw (or 5 half-lives of investigational drug, whichever is greater) before screening, or currently enrolled in any other clinical trial

17. Subject is the investigator or any sub-investigator, research assistant, pharmacist, study coordinator, other staff or relative thereof directly involved in the conduct of the trial

18. Supine blood pressure at screening outside the range of 90-139 mmHg for systolic or 50-89 mmHg for diastolic. To exclude white-coat nervousness a single repeat measurement is allowed

19. Have any complicating medical issues or abnormal clinical laboratory results that may interfere with study conduct, or cause increased risk

20. Any condition that in the investigator's opinion may adversely affect study participation or may compromise the study results

3.3 Study Assessments

During the course of the study, participants will frequently undergo assessments of their insulin production, immunologic status, overall health and well-being and diabetes care (see schedule of assessments in Appendix A). The participants' insulin production will be measured by a series of MMTTs conducted regularly during the study. The participants' diabetes control will be evaluated by measuring glycosylated hemoglobin (HbA1c) and clinical records including insulin types, doses, and timing and glucose records. During the course of the study, samples will be drawn for storage in the National Institute for Diabetes and Digestive and Kidney Disease (NIDDK) Repository and at TrialNet Repositories for future analysis.

3.4 Quality Assurance

During the study, duplicate collections of blood samples for assays will be obtained in a small sample of subjects for the purpose of quality surveillance of the performance of the central laboratories.

3.5 Post-treatment Follow-up

At the conclusion of the study or when subjects reach 12 months, subjects may be eligible to participate in further follow-up via participation in the TrialNet Long Term Investigative Follow-Up (LIFT).

4 PARTICIPANT MANAGEMENT

4.1 Screening

After informed consent, subjects will undergo assessments to determine if they meet eligibility criteria. Documentation of the subjects understanding of the risks and benefits of the study will be collected through the Volunteer Understanding Assessment.

4.2 Randomization and Treatment Visit

Eligible study participants will be randomized by the TrialNet Coordinating Center at the baseline visit (visit 0) once eligibility has been confirmed. Subjects will be assigned a study randomization number corresponding to the treatment group assignment. Subjects will be randomized to either the treatment arm or the placebo arm of the study.

Subjects who meet entry criteria will receive a subcutaneous injection of active drug/placebo at the research unit. Subjects will stay in the research unit for an observational period post initial dose to ensure capturing any potential acute safety signals. This period will include an inpatient stay of 48 hours post dosing. For the subsequent visits, subjects will attend the

site for ambulatory dosing visits.

4.3 Intensive Diabetes Management

During the study period, all participants will receive "intensive" management of their diabetes. The goal of the treatment will be to keep the HbA1c levels within the currently recommended American Diabetes Association age-specific target range in the absence of clinically significant or severe hypoglycemia or diabetic ketoacidosis. The primary responsibility for diabetes management will be on the treating or referring diabetes care provider, but the research study team will provide additional support through regular interaction. Records of communication with the participant will provide source documentation of this interaction. Subjects will not be permitted to use non-insulin pharmaceuticals for glycemic control.

The Clinic Monitoring Group (or designated TrialNet Committee) will be evaluating individual HbA1c data outside these ranges, and provide additional guidance to the clinical site as needed to bring glycemic control within goals. Any episode of severe hypoglycemia will be promptly reviewed by the Medical Monitor with recommendations for changes in diabetes management, if any, conveyed to the clinical site in conjunction with the Clinic Monitoring Group.

4.4 Overview of Treatments Administered

The plasmid NNC0361-0041 to be used in this first in human trial is supplied in a 9 mg/ml stock solution and will be prepared as an injection for subcutaneous administration. As described in section 4.4.2 below, the injections will be prepared according to the dose specified for each cohort, beginning with a 1-mg dose in cohort 1 and ending with a final dose of 25 mg in cohort 4 for this trial. The placebo will be administered in the same volume as the treatment. Doses will be administered on a weekly basis for 12 consecutive weeks.

4.4.1 Rationale of Dosing Plasmid NNC0361-0041

The strategy for selecting the starting dose and the clinical dose range is based on the FDA Guidance for Industry, estimating the maximum safe starting dose in initial clinical trials for therapeutics in adult healthy volunteers and the EMA Guideline on strategies to identify and mitigate risks for first-in-human clinical trials with investigational medicinal products. Exposure profiles of the plasmid-derived proteins exerting the pharmacological effect cannot be obtained in animals or in humans (44). Further, no in vitro studies adequately describe the *in vivo* PD effects observed in mice. This prevents the use of PK/PD modelling for establishing the minimal anticipated biological effect level (MABEL)/PAD. Therefore, the strategy for selecting the starting dose is based on the evaluation of effective doses in NOD mice and the no-observed-adverse-effect-level (NOAEL) in the 13-week GLP toxicity study in monkeys (44). Using allometric scaling a starting dose of 1.0 mg/patient (0.0167 mg/kg) results in a margin of 3.9 to the lowest PAD in the mouse disease model.

In the clinical trial it is intended to cover the relevant PAD range in humans. Consequently, the clinical trial should include doses exceeding the equivalent of the highest tested PAD in mice i.e., 100 µg/mouse corresponding to 19.2 mg/subject. Therefore, a maximum dose of 25 mg/patient is proposed for the FHD trial. This allows for pharmacological variation between the efficacy model and the clinical

setting. A 25 mg/patient top dose results in a safety margin of 3.9 in the 13-week GLP toxicity study in monkeys (44). An unmasked physician or nurse is required to administer the study agent in order to keep the treatment arm assignment of each randomized participant masked to the research personnel at each site.

4.4.2 Dosing and Dose Withholding Criteria

Dosing is dependent on the current cohort open to participant enrollment. Table 1 below demonstrates the dose to be administer in each cohort. No routine pre-medication is needed prior to each subcutaneous injection of the study drug.

Table 1: Planned Dosing Levels of Plasmid NNC0361-0041 or Placebo

Cohort	Dose (mg)	Escalation factor	IP volume (mL) (9mg/mL stock solution)	Injection(s) (mL)
1	1	-	0.11	0.11
2	5	5	0.56	0.56
3	12.5	2.5	1.4	0.7 + 0.7
4	25	2	2.8	0.94 + 0.94 + 0.94

Administration of injections will be withheld based on the following criteria:

1. Individuals with fever: Temperature greater than 38.5 °C (101.3 °F) within 48 hours of each dose
2. Individuals with evidence of active infection at time of dosing.

4.5 Infectious Disease Screening

In general, no specific infectious diseases prophylaxis is warranted in this study. In lieu of active serologic or virologic monitoring strategies, all subjects will be counseled on an ongoing basis on the importance of notifying their research centers about the presence of signs or symptoms suggestive of infection. They will also be counseled on the importance of notifying the research center about potential exposures to varicella, influenza, COVID-19 or other infectious illnesses.

Subjects will be assessed for COVID-19 symptoms at each study visit specified in the schedule of assessments. Subjects are required to be free of COVID-19 symptoms for 14 days prior to the screening visit, administration of study drug dose 1 and follow-up visits 15–17. For study drug administration of doses 2–12 and follow-up visit 14, participants are required to be free of COVID-19 symptoms the 7 days (or per current local guidance/CDC guidance) prior to those visits.

Subjects will not be enrolled with active infections, history of current or prior HIV, Hepatitis B, or Tb infection. In addition, all subjects will have CMV and EBV serology determined prior to study enrollment. Subjects who are CMV and/or EBV seronegative at screening must be CMV and/or EBV PCR negative within 30 days of randomization and may not have had signs or symptoms of an CMV and/or EBV compatible illness lasting longer than 7 days

within 30 days of randomization. Additional CMV and EBV seronegative individuals will have samples collected to be stored and tested for viral load if clinically indicated.

4.6 Metabolic Monitoring

Individuals will undergo a 2-hour MMTT for C-peptide measurements to determine eligibility for the study at screening. The investigational drug is not anticipated to have any metabolic effect in subjects with established T1D. However, repeat MMTT procedures will be done at months 1, 3, 6, and 12 to evaluate potential adverse effect of therapy on insulin secretion. HbA1c, blood glucose, and total daily insulin dose will also be monitored according to the schedule of assessments.

4.7 Concomitant Medication

The use of concomitant medications will be assessed at each study visit and recorded on an appropriate source document and CRF. Participants are allowed to use preparations of insulin as advised by the investigator or the referring physician

Participants will be requested not to use any of the following medications during the study:

- Agents that influence insulin sensitivity or secretion (including pramlintide, sulfonylureas, metformin, diphenylhydantoin, thiazide, or other potassium-depleting diuretics, beta-adrenergic blockers, niacin).
- Vaccination with live vaccines from 6 weeks before enrollment to 3 months following last dose of study drug is not permitted. Killed vaccines other than influenza are discouraged during this time period.
- Any medication that may result in immunosuppression or immunomodulation.
- Systemic glucocorticoids

If participants receive, or if the investigator believes that participants must receive, any of the above medications, the case must be discussed with the medical monitor and/or treating physician to determine if alternatives are available. The use of these medications must be documented on the source document and CRF.

4.8 Enrollment Schedule Overview

This study will enroll participants in four cohorts with 12 participants in each cohort. Each subsequent cohort will receive a higher dose of study drug. Within each cohort, 9 individuals will receive active treatment and 3 will receive placebo.

Sentinel Dosing: For the first dose in <u>each cohort</u>, all subjects will be dosed in a sentinel manner. The sentinel dose will be administered in the following pattern:

- Initially two participants (1 with active treatment and 1 with placebo) will be dosed and monitored for an observational period covering twice the predicted t_{max} (48 hours).
- The Novo Nordisk Trial Safety Group and the TrialNet Medical Monitor will review data (as described section 4.9.1) from these two individuals. If approved by both, enrollment of the next three participants will occur (2 with active treatment and 1 with placebo). These three will then be dosed and monitored for 48 hours.
- The Novo Nordisk Trial Safety Group and the TrialNet Medical Monitor will review data (as described section 4.9.1) from these 3 individuals as well as any new data from the first two participants. If approved by both groups, enrollment of the remaining seven participants will occur (6 with active treatment and 1 with placebo).

Cohort Enrollment: Evaluation of accumulated data (as described section 4.9.2) will occur independently by the Novo Nordisk Trial Safety Group and the TrialNet Data Safety Monitoring Board when at least 9 individuals within a cohort have data available from visit 6. If approved by both, randomization of the next cohort will proceed.

Participants who are screened but are not included in the current cohort may rescreen for participation in a later cohort.

4.9 Ongoing Enrollment Decisions

4.9.1 Within Cohort: Evaluation of sentinel dosing

The TrialNet Medical Monitor and Novo Nordisk Trial Safety Group will review data from those receiving sentinel doses (section 4.8) *masked to treatment group* within each cohort. This data includes AE reports regardless of relationship to study drug vital signs and CBC information.

Continued enrollment after the sentinel dosing within each cohort will require an absence of any of the rules for interruption of the trial/cessation of study (section 4.10) and concurrence by both the TrialNet Medical Monitor and Novo Nordisk Trial Safety Group.

4.9.2 Between Cohorts: Evaluation of accumulated data

The TrialNet Medical Monitor and Novo Nordisk Trial Safety Group will review accumulated data after 9 individuals within a cohort have data available from visit 6 *masked to treatment assignment* and have received the first 5 scheduled doses. This data includes insulin dose, all available MMTT AUC C-peptide values, AE reports regardless of relationship to study drug, vital signs, as well as clinical laboratory data. Clinical laboratory data including normal ranges and C-peptide will be provided by individual participant as well as in summary form.

The DSMB will review the same interim data. Data from placebo participants regardless of cohort will be grouped together.

Dose escalation to the next cohort will require an absence of any of the rules for interruption of the trial/cessation of study (section 4.10) and concurrence by both the DSMB and Novo Nordisk Trial Safety Group.

4.9.3 Participant Replacement

The aim is to have a minimum of 9 of 12 participants in each cohort complete at least 10 doses of study drug/placebo and have visit 14 assessments. If needed, the study can recruit and replace up to two additional participants to each cohort. If a participant is to be replaced in a cohort due to a participant withdrawal, the newly enrolled participant is to be allocated to the same treatment group as the individual who ended their study participation. Participant withdraws will be closely monitored; in the event more than two participants withdraw for any reason from a

cohort both Novo Nordisk and TrialNet will conduct an assessment of the protocol in conjunction with the DSMB and Novo Nordisk Trial Safety Group.

4.10 Interruption of Enrollment/Trial Cessation

This section lists clinical and laboratory adverse events that will necessitate independent review by the Data Safety and Monitoring Board (DSMB) and Novo Nordisk Trial Safety Group to determine whether there should be interruption of enrollment into the study as a whole. As part of their ongoing safety review, the DSMB and Novo Nordisk Trial Safety Group will also make independent judgments regarding other adverse events requiring trial interruption. Either group may interrupt enrollment or study continuation, but both groups must independently agree to continue the study after the evaluation of the event(s).

- Any suspected unexpected serious adverse reaction (SUSAR)
- One or more subjects with severe AEs (CTCAE grade 3 or higher) considered to be related to test drug, regardless of dose cohort (see section 7.1.5 for CTCAE grading definitions)
- Two or more subjects with AEs CTCAE grade 2 or higher, considered to be related to test drug, regardless of dose cohort

5 STUDY ASSESSMENTS

See Appendix 1 for detailed Schedule of Assessments

5.1 General Assessments

Study visits for all groups will occur according to the Schedule of Assessments. General assessments include:

- Medical History (including lifestyle and participant experience assessments*)
- Complete or directed physical exam
- Vital Signs *(some visits will require more frequent collections of these assessments during the treatment phase of the study)*
- Concomitant Medication
- Adverse Events
- COVID-19 Assessment Form

**These visits may occur in-person or remotely. Psychosocial assessments will address participant experiences in the trial and the impact of trial participation and diabetes diagnosis on the participant and family. These assessments may be completed in-person or distributed and completed outside of a scheduled study visit.*

5.2 Laboratory Assessments

The following general clinical laboratory assessments will be performed:

- Chemistry (sodium, potassium, chloride, CO2, glucose, BUN, creatinine)
- Liver function tests (ALT, AST, alkaline phosphatase, total protein, albumin, total and direct bilirubin)
- Hematology (complete blood count with differential and platelets)
- Urine pregnancy test as appropriate

- Antibodies to HIV, hepatitis B (antiHBcAb, HBsAg), hepatitis C (HCV), Cytomegalovirus (CMV IgG, IgM), and Epstein-Barr Virus (EBV IgG, IgM, EBNA)
- CMV and EBV PCR
- Thyroid-stimulating hormone (TSH)
- Coagulation Panel (PT (INR), aPTT, fibrinogen activity)

Additional assessments include:

- Diabetes related autoantibodies (e.g., GAD65A, mIAA, IA-2A, ICA, ZnT8A)
- Immunogenicity (Anti-double-stranded DNA, anti-IL2, anti-IL10, anti-TGFβ1)

Anti-ds DNA, anti-TGF-β1, anti-IL2, and anti-IL-10 antibody samples will be collected according to the Schedule of Assessments. All samples must be drawn prior to trial product administration if trial product administration is planned on the sampling day.

Assessment of antibodies against TGFβ-1, IL-2, and IL-10 in serum will be performed at Novo Nordisk A/S or Novo Nordisk A/S appointed laboratory. The antibody analysis will be performed after last patient last visit (LPLV), therefore the investigators may not be able to review the results of antibody measurements in relation to potential AEs.

5.3 Mechanistic Outcome Assessments

TrialNet and Novo Nordisk (or 3rd party laboratories operating on TrialNet's or Novo Nordisk's behalf) will perform immune and genetic assays to further understand mechanisms that may be underlying the T1D disease process and response to therapy. For this purpose, samples for PBMCs, DNA, RNA, plasma, and serum will be obtained. Mechanistic assays will be used as for exploratory outcomes for this trial. These include assessments related to mechanism of immune changes and plasmid target engagement. HLA screening (HLA Class I and II) will also be performed.

5.4 Metabolic Outcome Assessments

Metabolic assessments will consist of:

- Glucose records and reports of hypoglycemia
- Insulin dose
- HbA1c
- MMTT: 2-hour MMTT at screening and at 1, 3, 6, and 12 months after initial treatment dose
- CGM data will be collected from participants who already use these devices as part of their clinical care

5.5 Visit Windows

Enrollment and initial treatment visit (visit 0) should begin within 1 month (no more than 37 days) from the screening visit (visit -1). Dosing visits (visit #3-#13) should be within +/- 1 day from the

target date. Study visit 14 should be within -1/+1 day of the target date, visit 15 should be within -3/+3 days from the target date. The window for the 6- and 12- month time points (visits 16 and 17) is +/- 14 days.

5.6 Withdrawal from treatment

The aim of the study is to obtain safety and mechanistic data. The study will be conducted as a modified intent to treat protocol in which all subjects who receive at least one dose of study medication will be used for safety analysis. Withdrawal from treatment does not automatically entail withdrawal from the study. Withdrawal from the study will only occur if the participant dies or withdraws consent.

Withdrawal from treatment can occur for a number of reasons, some of which are outlined below:

- Individuals who miss two consecutive doses or more than two doses collectively whether due to missed visit or unable to receive study drug due to withholding rules (section 4.4) will not receive further treatment.
- A participant may elect to discontinue study medications, may be unable to continue them, or may be withdrawn (temporarily or permanently) at the discretion of the Principal Investigator if s/he determines that it is unsafe to continue or there is a significant change in the risk/benefit.
- Individuals who receive a COVID-19 vaccination after start of study drug, will not receive further treatment.
- Should a COVID-19 booster vaccine become recommended by the CDC, individuals who receive a COVID-19 booster while in the treatment phase of the study, will not receive further treatment.

Individuals who are withdrawn from treatment should remain in the study and undergo scheduled assessment visits as appropriate and any additional visits as needed to address ongoing AEs. MMTT tests will not occur while an individual is pregnant.

6 PARTICIPANT SAFETY

The risks of this study are presented in this protocol and in the informed consent form. As a phase 1 study, safety of the participants is the primary aim of the trial and will be carefully assessed during the study. Key elements of this assessment include:

1. Defined parameters for interruption of enrolment in the trial at any point including any SUSAR, a single grade 3 or two subjects with grade 2 adverse events considered to be related to test drug (section 4.10).
2. A requirement that enrolled individuals live in a location with access to emergency response services.
3. A 48 hour on-site observation period after the first dose, a 4 hour on-site observation period for the next 4 doses, and a 2 hour on-site observation period for the remaining doses (section 6.5).
4. Sentinel dosing: after the first two individuals and next three individuals for each dose cohort, a safety review of all accumulated laboratory, AE and injection site reaction data will be performed with approval by the TrialNet Medical Monitor and Novo Nordisk Trial Safety Group required before further enrollment within a cohort (section 4.9).

5. Review of all accumulated safety data and formal approval by Data Safety Monitoring Board and Novo Nordisk Trial Safety Group before proceeding to subsequent dosing cohort (section 4.9).

6.1 General Study Agents considerations

6.1.1 Plasmid

The plasmid DNA vector encodes CD74 anchored to pre-proinsulin (PPI) in a multi-cistronic expression construct. The autoantigen PPI is expressed followed by a 2A peptide element with TGF-β1, an IRES element, IL-10, a subsequent 2A element, and terminal IL-2. The vector will be delivered via subcutaneous injection as outlined in the treatment schedule.

6.1.2 Vector Considerations:

Plasmid therapies have been proposed and tested clinically in a number of indications (PMID: 30445702). While the pre-clinical animal model studies suggest the drug will be safe and result in transient vector responses (see IB for additional information), there remains a potential that a plasmid-based dsDNA vector may induce some host inflammatory response as a function of innate sensing machinery (e.g., as a PAMP/DAMP via TLRs). Moreover, there is a possibility that the inclusion of 2A elements may alter the structure of transgenes. It is currently unknown if this could result in an immune response to the native proteins and/or modified epitopes.

6.1.3 Drug Considerations:

NNC0361-0041 is intended to be injected without adjuvants or enhancers and therefore is expected to only transfect and express in a limited number of cells. The systemic exposure to plasmid-derived proteins will therefore be minimal and occurrence of acute safety signals is consequently considered unlikely.

Autoantigen (Pre-proinsulin)

Rationale: The goal is use host machinery to express ectopic transgenes and facilitate the presentation of PPI peptides in the context of MHC class I and II molecules to host T cells via endogenous antigen processing and presentation machinery. The hypothesis is that both CD4+ and CD8+ T cell responses will be impacted, with the net effect of shifting the balance to favor immune regulation. Potential side-effects may include a delayed hypersensitivity reaction at the injection site or in any site the drug and transgene may ultimately be expressed. While the expectation is that endogenous T_{regs} will be induced in response to the drug, mechanistic and clinical data will also monitor the potential for the transgene to induce the expansion and/or activation of effector cells that might accelerate beta cell loss.

6.1.4 Immunomodulatory Agents:

Transforming Growth Factor – Beta 1 (TGF-β1)

TGF-β1 is a pleiotropic polypeptide that is known for its capacity to function as a negative growth factor to limit T cell proliferation. TGF-β1 is naturally processed by proteolytic activation into its active form that signals through TGF-β1 receptor. On-target implications of localized TGF-β1 are expected to include the induction of both CD25 and FOXP3 in T cells following TCR ligation with reduced effector responses and proliferation. In excess, TGF-β1 can cause tissue remodeling, with reports of renal fibrosis following systemic exposure to high levels of active TGF-β1. Given the receptors for TGF-β1 are widely expressed throughout the innate and adaptive immune system, TGF-β1 can lead to broad immunoregulation, including the downregulation of MHC II expression and costimulatory molecules on host APCs. While no agents are expected to induce mutations or

DNA damage, TGF-β1 signaling has previously been associated with transformation and epithelial-mesenchymal transition (EMT), a process that is favored in tumor cells and facilitates migration and invasion.

Interleukin-10 (IL-10)

Interleukin-10 is most often regarded as an anti-inflammatory class II cytokine capable of inducing a tolerogenic Dendritic Cell (DC) phenotype and subsequent Tr1-like cell response. IL-10 is thought to autoregulate its own expression through the IL-10R and inhibition of p38 signaling. As with TGF-β1, IL-10 exerts a broad array of pleiotropic functions on host and immune cells. Of note, IL-10 is recognized for its capacity to suppress host responses to TLR ligands such as LPS in myeloid lineage cells. Locally, IL-10 has been reported to be produced by mast cells, potentially attenuating the inflammatory byproducts of these cells that may act locally at the injection site. In general, IL-10 suppresses innate inflammation, but can also induce inflammatory subsets at high concentrations. In pegylated forms, IL-10 induces markers associates with T cell activation including PD-1, Lag3, FasL and decreased serum TGβb. Thus, IL-10 is most often regarded as an immunoregulatory cytokine in the context of myeloid exposure to PAMPs but may induce immune activation in a transgene form.

Interleukin-2 (IL-2)

Interleukin-2 is commonly regarded as a T cell growth factor and binds to the high-affinity trimeric IL-2R composed of the common gamma chain (CD132), IL2 beta chain (CD122), and the high affinity alpha chain (CD25). Importantly, the high-affinity trimeric IL2R is constitutively expressed on T_{regs} but can be upregulated on activated conventional T cells. In T_{regs}, IL-2 binding the IL2R induces pSTAT5 signaling and reinforces FOXP3 expression and T_{reg} fitness. In excess, IL-2 can induce injection site reactions, changes in vascular permeability, localized inflammation, and when present in high concentrations, bind the intermediate affinity IL2R expressed on innate lymphoid cells, NK cells, and conventional and effector T cell subsets. TCR ligation, TGF-□1 signaling, and co-stimulation are known to upregulate CD25 on T cells and early IL-2 signaling is reported to suppress Th17 cell differentiation.

6.2 Potential Risks

Based on literature, hypothesized mechanism of action and the inherent potential for reactions when introducing foreign matter into the subcutis, the following risks are currently considered potential risks for human subjects.

6.2.1 Acceleration of Beta Cell Destruction

While the plasmid vector is aimed to provide antigen specific tolerogenic therapy, and no evidence of immune activation was seen in preclinical studies, unintentional acceleration of beta cell destruction due to immune activation is a potential risk, which will be monitored through scheduled assessments of insulin secretion by MMTT.

6.2.2 Hypersensitivity, Allergic, and Injection site reactions

Plasmid therapy is generally well tolerated and the DNA component is not considered to be immunogenic. Plasmid DNA vaccines have been evaluated in many clinical trials, and anaphylaxis has not been reported. The safety and tolerability of DNA plasmid vaccines has been evaluated in several clinical trials, both for autoimmune diseases where the plasmids expressed autoantigens (45) and for anti-viral responses (46), in both cases with repeated dosages. As summarized in a review article, multiple studies have reported the DNA platform to be well tolerated and to have a very good safety record (47,48).

It is noteworthy, that while anaphylactic reactions have been observed on occasion with subcutaneous or intradermal administration of peptides in mice as well as humans (49), such anaphylactic reactions have not been observed with DNA vaccines. This includes plasmid DNA administration in conjunction with cytokine response modifiers as well as other adjuvants for which, unlike peptide therapy, anaphylaxis has not been observed. Cytokine modulators have been analyzed in the past, and skewing of immune responses towards allergic pathways (but not anaphylaxis) can occur by using IL-4 and Il-5 (50). This is in contrast to the cytokines expressed by our plasmid, which were carefully chosen to induce tolerance in both TH1 (more the anti-viral and autoimmune effector responses) and TH2 (more the allergic responses) and thus not enhance any type of allergic (more TH2-like) response.

Further, an innate, immediate type 1 reaction should not be increased with this plasmid compared to others, and repeated administrations are not believed to be able to provoke such a response. In pre-clinical studies in mice and monkey with repeated dosing of the plasmid, importantly no anaphylactic or other allergic reactions have been demonstrated (see IB Appendix 1 for an overview of all non-clinical studies performed). The longest study conducted in monkeys with NNC0361-0041 is the 13 week GLP toxicity study. Monkeys were dosed once weekly with either vehicle, 4 or 20 mg NNC0361-0041 plasmid/animal (approximately 1 and 5 mg plasmid/kg) for 13 weeks followed by either 8 or 13 weeks recovery. Animals were observed after each dosing for clinical signs and no adverse reactions were observed. In particular, no anaphylactic reactions were observed. In the study, cytokines (IL-1β, IL-2, IL-4, IL-6, IL 8, IL-10, IL-12p40, IL-15, TGFβ-1, IFN-γ and TNF-α) were also measured (pre-study, 2 h post dose after first and 4th dose and at end of study) and no changes were observed. Mouse surrogate plasmid has been dosed thrice weekly and up to once daily in mice without any signs of anaphylaxis.

Evaluation of plasmid exposure in the 13 week toxicity study in non-human primates has demonstrated that plasmid exposure declines to levels below LLOQ within a week after dosing. Thus, with the planned weekly dosing, accumulation is not anticipated in this study, meaning that exposure will not increase as participants are dosed repeatedly for the 12 weeks.

Thus, while individuals may experience systemic or local adverse effects shortly after drug administration including hypersensitivity, or allergic reactions, this is not considered to be a likely effect of this therapy. Injection site reactions may also occur. All subjects will be closely monitored, and injection sites will be carefully assessed for local reaction and fibrosis at each study visit. Proinflammatory cytokines will be monitored in response to plasmid injection.

6.3 Protecting Against or Minimizing Risks.

6.3.1 Infectious Disease

The plasmid vector is aimed to provide antigen specific local tolerance and not broad immunosuppression. Thus, while the potential for infection will be carefully monitored with clinical and laboratory assessments, adverse events associated with infectious diseases are not expected.

6.3.1.1 COVID-19

Risk of COVID-19 infection in relation to participation in trial:

Key risk factors for adverse outcomes of COVID-19 infection appear to be old age, particularly in combination with cardiovascular co-morbidity, obesity, cancer or compromised immune system.

The plasmid vector is not expected to increase the risk of COVID-19, but patients may be exposed to the risk of COVID-19 transmission and infection in relation to site visits if an outbreak is ongoing.

The risk of COVID-19 transmission in relation to site visits is overall considered to be low, however this may vary between geographical areas. For site visits and travel to sites, participants should follow local guidance and recommendations of regional health authorities, such as masking and social distancing, to minimize the risk of exposure to COVID-19.

6.3.1.2 COVID-19 Vaccination

The effect of study drug on efficacy of any vaccine is unknown. The aim of the study drug is to induce local, antigen specific tolerance to impact islet autoimmunity and beta cell destruction. The study drug is administered subcutaneously into the abdomen with effects in draining lymph nodes. There is no evidence of systemic tolerance from pre-clinical models of study drug. However, there is a theoretical possibility that simultaneous administration of a new antigen, such as COVID-19 vaccination could, through cross-presentation, lead to tolerization to that antigen (such as the COVID-19 spike protein). To mitigate this risk, study drug administration will be separated from COVID-19 vaccination both in space (subcutaneous injection in abdomen for study drug and deltoid for COVID-19 vaccine), and in time (as described below).

Similarly, the COVID-19 vaccine may impact the study by introducing AE's associated with COVID-19 vaccination rather than study medication and by impact on the immune measures tested in the study.

Participants will be instruction to inform the study team of plans to receive COVID-19 vaccination. To address both the potential risk of study drug on the COVID-19 vaccine and the impact of the COVID-19 vaccine on study drug:

1. Due to the potential severity of COVID-19 infection, COVID-19 vaccination will be required for all participants including booster vaccination if eligible. Should further future booster vaccination become available it will be recommended to all.
2. Documentation of COVID-19 vaccination will be requested from participants.
3. Participants are to complete the COVID-19 vaccine series or single dose vaccine and eligible boosters at least two weeks prior to randomization. If participants have access to both a mRNA based vaccine or viral vector vaccine, it is recommended the participant be administered mRNA COVID-19 vaccines.
4. Participants who screened for the trial prior to the requirement of COVID-19 vaccination (*Protocol V2.0 05Jan2021*) are able to receive the vaccine during the 12-week period of study drug administration but must wait 3 days from

last study drug dose before getting COVID-19 vaccination. They will receive no further study medication, but will continue on study for safety and mechanistic follow-up. Participants who have completed the 12-week course of study drug may receive vaccine 3 days after last dose of study drug. They will continue on study schedule.

5. If future recommendations are issued by the CDC regarding booster vaccinations, participants are able to receive the booster vaccination at least 3 days after their 12-week course of study drug. Participants who wish to obtain a future COVID-19 booster vaccination during the treatment phase will wait 3 days from last dose of study drug, will receive no further study medication but will continue on study for safety and mechanistic follow-up.

6.3.2 General precautions

Subjects will not be enrolled who have other serious active medical problems aside from type 1 diabetes. Frequent monitoring of subjects' medical history, physical examinations, and laboratory studies will allow for early identification of adverse events. All participants will be required to have adequate hemoglobin to allow safe frequent venipuncture. Every attempt will be made to minimize the number of venipunctures.

Subjects will be counseled about the need to report any change in health status between or at the time of visits. Directed questioning about concurrent illness will occur before each injection. No injection will occur in those with signs or symptoms indicative of active infection.

All initial study drug injections will take place in a facility that has resuscitation capabilities and subjects will be closely monitored during and after the injection.

6.4 Pregnancy

Male subjects who are sexually active will be instructed to use condoms from randomization until 3 months post last administration of study drug to assure safety. Female subjects with reproductive potential will be instructed to use highly effective means of birth control (which includes abstinence) from randomization until 3 months post last administration of study drug to assure safety. Female subjects will also be asked to avoid pregnancy until last scheduled study visit at 12 months post randomization. This is to assure accurate endpoint measurements. They will undergo urine pregnancy testing at the start of every study visit. All pregnancies that are identified during the study must be followed to conclusion and the outcome of each must be reported. The investigator should be informed immediately of any pregnancy occurring in a female participant. Pregnancy will be reported by sites to the TrialNet Coordinating Center, who will provide this information to the TrialNet Medical Monitor, DSMB, and Novo Nordisk Trial Safety Group. Monitoring of the participant should continue until the conclusion of the pregnancy. Subjects who are found to be pregnant while on this study shall have treatment withdrawn, but will still be followed for safety and other study measures as appropriate. Treatment will not be resumed. If a participant who was previously sexually inactive becomes sexually active, the participant will be counseled about the need to use a reliable form of birth control.

6.5 Hypersensitivity or Anaphylaxis

Individuals with a history of severe reactions to previous vaccinations will be excluded from the study. All visits will occur on a clinical research center or equivalent facility with equipment and staff trained to recognize and treat signs and symptoms of hypersensitivity or anaphylaxis reactions. All individuals will be monitored for reactions for 48 hours on-site after the first dose. The length of the observation time (48 hours) has been chosen based on the predicted T_{max} of less than 24 hours for the plasmid encoded proteins and it is anticipated that 48 hours is sufficiently long time to observe any acute adverse events possibly related to T_{max}. For the subsequent 4 doses, individuals will be monitored for 4 hours on-site, and for the remaining doses, for 2 hours on-site. Additionally, any individual with signs and symptoms of hypersensitivity or anaphylaxis reaction will be monitored for 8 hours and discharged only if stable with no further evidence of reactions. Individuals that are not stable after 8 hours will be admitted for appropriate medical care and further observation.

7 ADVERSE EVENT REPORTING AND SAFETY MONITORING

7.1 Adverse Event Definition

7.1.1 Adverse Event

In this clinical trial, an adverse event is any occurrence or worsening of an undesirable or unintended sign, symptom or disease whether or not associated with the treatment and study procedures.

From study start through visit 15, the investigator must record all adverse events which are Grade 1 or greater per the NCI CTCAE (see Section 7.1.5. Grading Event Severity below), except for events related to hypoglycemia or hyperglycemia. Hypoglycemia and hyperglycemia should be recorded as adverse events only in the case of requiring the assistance of others due to loss of consciousness or DKA. After visit 15 only grade two or higher AEs are to be recorded (visits 16 & 17).

All SAEs must be recorded throughout the study (from signing of informed consent through visit 17).

The investigator should treat participants with adverse events appropriately and observe them at suitable intervals until the events resolve or stabilize.

Adverse events may be discovered through:

- observation of the participant;
- questioning the participant;
- unsolicited complaint by the participant.

Questioning of the participant should be conducted in an objective manner.

7.1.2 Adverse Reaction

Adverse reactions are a subset of all suspected adverse events for which there is reason to conclude that the drug caused the event. Suspected adverse reaction means any adverse event for which there is a reasonable possibility that the drug caused the adverse event. For the purposes of safety reporting, “reasonable possibility” means there is evidence to suggest a causal

relationship between the drug and the adverse event. A suspected adverse reaction implies a lesser degree of certainty about causality than an adverse reaction, which means any adverse event caused by a drug. Examples of evidence that suggest a causal relationship (reasonable possibility) between the drug and the adverse event include:

- A single occurrence of an event that is uncommon and known to be strongly associated with drug exposure
- One or more occurrences of an event that is not commonly associated with drug exposure, but is otherwise uncommon in the populations exposed to the drug
- An aggregate analysis of specific events observed in a clinical trial (such as known consequences of the underlying disease or condition under investigation or other events that commonly occur in the study population independent of drug therapy) that indicates those events occur more frequently in the drug treatment group than in a concurrent or historical control group.

7.1.3 Serious Adverse Event/Reaction

A serious adverse event (SAE) or reaction is defined as "any adverse event occurring at any dose that suggests a significant hazard, contraindication, side effect, or precaution." An adverse event or suspected adverse reaction is considered "serious" if, in the view of either the investigator or sponsor, it results in any of the following outcomes:

1. Death. A death that occurs during the study or that comes to the attention of the investigator during the protocol-defined follow-up after the completion of therapy must be reported whether it is considered to be treatment related or not.
2. A life-threatening adverse event. A life-threatening event is any adverse therapy experience that, in the view of the investigator, places the participant at immediate risk of death from the reaction as it occurred.
3. Hospitalization or prolongation of existing hospitalization with the exception of hospitalization relating to initial diagnosis of type 1 diabetes.
    a) Note: In general, hospitalization signifies that the participant has been detained (usually involving at least an overnight stay) at the hospital or emergency ward for observation and/or treatment that would not have been appropriate in the physician's office or out-patient setting. Complications that occur during hospitalization are AEs. If a complication prolongs hospitalization or fulfils any other serious criteria, the AE is serious. When in doubt as to whether 'hospitalization' occurred or was necessary, the AE should be considered serious.
4. Persistent or significant incapacity or substantial disruption of the ability to conduct normal life functions
5. Congenital anomaly or birth defect.
6. Important medical events that may not result in death, be life-threatening, or require hospitalization may be considered serious when, based upon appropriate medical judgment, they may jeopardize the patient or subject and may require medical or surgical intervention to prevent one of the outcomes listed above.

An adverse event or suspected adverse reaction is considered "life-threatening" if, in the view of either the investigator or sponsor, its occurrence places the patient or subject at immediate risk of death. It does not include an adverse event or suspected adverse reaction that, had it occurred in a more severe form, might have caused death.

Regardless of the relationship of the adverse event to study drug, the event must be reported as a serious adverse event if it meets any of the above definitions.

7.1.4 Unexpected Adverse Event

An adverse event/reaction is considered unexpected when the nature (specificity) or severity of the event is not consistent with the risks described in the Investigator's Brochure. This includes events that occur more frequently than expected.

7.1.5 Grading Event Severity and Causality

TrialNet has adopted usage of the National Cancer Institute (NCI) Common Technology Criteria for Adverse Events (CTCAE) and/or study-specific criteria for classification to describe the severity of adverse events. TrialNet Investigators will also provide an assessment of relationship of AE to study drug as not, unlikely, possibly, probably, or definitely related.

The CTCAE v5.0 displays Grades 1 through 5 with unique clinical descriptions of severity of each AE based on this general guideline:

- Grade 1: Mild; asymptomatic or mild symptoms; clinical or diagnostic observations only; intervention not indicated.
- Grade 2: Moderate; minimal, local or noninvasive intervention indicated; limiting age appropriate instrumental activities of daily living (ADL)
- Grade 3: Severe or medically significant but not immediately life-threatening; hospitalization or prolongation of hospitalization indicated; disabling; limiting ADL
- Grade 4: Life-threatening consequences; urgent intervention indicated
- Grade 5: Death related to AE

A semicolon indicates "or" within the description of the grade

7.2 Adverse Event Reporting and Monitoring

Adverse events will be reported to the TrialNet Coordinating Center. The investigator will grade their severity according to common toxicity criteria or study-specific criteria and will make a determination of their relation to therapy. Events will be assessed and reported consistent with the ICH Guideline for Good Clinical Practice, 21 CFR 312.32 for expedited safety reporting, and per the guidance of the DHHS Office for Human Research Protections (OHRP).

The adverse event case report form for the protocol must be completed for all adverse events (AE). For all types of injection site reactions additional information about the objective findings and local symptoms must also be reported in the injection site reaction section of the case report form. For reporting serious adverse events (SAE), the MedWatch Form should also be completed and faxed to the TNCC *within 24 hours of when the site was notified of the event.* This will be reviewed by the TrialNet Medical Monitor, Novo Nordisk Trial Safety Group, and the DSMB as appropriate. Deaths must be reported immediately. Event outcome and other follow-up information regarding the treatment and resolution of the event will be obtained and reported when available, if not known at the time the event is initially reported. The follow-up information should contain sufficient detail to allow for a complete medical assessment of the case and an independent determination of possible causality.

Adverse events will be assessed by the TrialNet Medical Monitor. Formal safety evaluations will occur by TrialNet Medical Monitor, Novo Nordisk Trial Safety Group, and the DSMB as indicated

by enrolment and trial progress. Additional review will occur during regularly schedule DSMB meetings which occur approximately every six months.

For SAEs that are unexpected and considered possibly or probably drug related, the Medical Monitor will provide information on frequency of similar events, and generate FDA form 3500A reports (MedWatch form) for distribution to FDA, NIDDK, DSMB and site investigators. Expedited safety reports will be submitted to the IND by the NIDDK. The TNCC will also be responsible for sending required safety information to Novo Nordisk Safety.

7.2.1 Adverse Event Tabulations

All reported adverse events will be tabulated by system organ class (SOC) (e.g., cardiac disorders, endocrine disorders, infections and infestations, etc.), supra-ordinate term (e.g., palpitations, hyperthyroidism, appendicitis, etc.), severity, expectedness (per the investigational brochure and causality in relation to the study agent. Adverse events are reported separately for the number of study participants and the number of events should study participants experience the same type of event multiple times.

8 STATISTICAL CONSIDERATIONS AND ANALYSIS PLAN).

The primary objective of this study is to investigate the safety and tolerability of ascending subcutaneous weekly doses of NNC0361-0041 plasmid in patients with T1D. Secondary objectives are 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.

Up to 12 patients will be randomized to treatment vs. placebo within each of the 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.

The following groups and periods are defined for analytic purposes:

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 for any

reason, including COVID-19 vaccination. Additional individuals may be enrolled upon agreement between TrialNet and Novo Nordisk.

All randomized subjects will be used for analyses of PK endpoints and all subjects receiving at least one dose of randomized treatment (partially treated and treated combined) will be used for analyses of the primary endpoint. Subjects receiving placebo treatment will be pooled across cohorts.

8.1 Primary Outcome and Analyses

The primary endpoint is the number of adverse events recorded during the on-treatment period. The primary endpoint will be presented 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. A summary table will also be made by system organ class and trial product.

Serious adverse events will be summarized over the entire trial (i.e., on-treatment and follow-up period).

A statistical analysis plan will be written in addition to the protocol including a more detailed and technical elaboration of the statistical analyses. The statistical analysis plan will be finalized before unblinding (database lock).

8.2 Secondary Outcome and Analyses

$\Delta AUC_{0\text{-}2h,C\text{-}peptide,MMTT}$; 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.

8.3 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.

The impact of COVID-19 vaccination and response to vaccination on clinical and immune responses to study drug may be evaluated, as needed.

8.4 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 (see 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 reduction in number (frequency), or change of phenotype, or function of antigen specific autoreactive T cells towards regulation. These studies will include, but not be limited to flow cytometry, measures of gene function, and evaluation of cytokine levels. 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.

8.5 Statistical Considerations for Assessment of Worsening of Insulin Secretion.

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 log-transformed area under the plasma C-peptide concentration-time curve from time 0 to 2 hours during MMTT obtained at the end-of dosing day 84 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 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 (51). 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%.

8.6 Interim Monitoring Plan

The study plan is to accrue 4 cohorts of 12 patients each, increasing the dose level with each cohort. Within cohort monitoring will follow section 4.9.1 and between cohort monitoring will follow section 4.9.2

9 ETHICAL CONSIDERATIONS AND COMPLIANCE WITH GOOD CLINICAL PRACTICE

9.1 Statement of Compliance

This study will be conducted in compliance with the protocol and consistent with current Good Clinical Practices (GCP), adopting the principles of the Declaration of Helsinki, and all applicable regulatory requirements (*ICH E6, 45CFR46, and FDA 21CFR sections 11, 50, 56, 312)*.

Prior to study initiation, the protocol and the informed consent documents will be reviewed and approved by an appropriate Independent Ethics Committee/Research Ethics Board (IEC/REB) or Institutional Review Board (IRB). Any amendments to the protocol or consent materials must also be approved before they are implemented.

9.2 Participating Centers

Participating TrialNet clinical sites must have an appropriate assurance, such as a Federal-wide Assurance (FWA) or an Unaffiliated Investigators Agreement (UIA), with the Office for Human Research Protections (OHRP), since they are actively engaged in research and provide informed consent. The protocol and consent forms will be approved by Institutional Review Boards or Ethics Committees/Research Ethics Boards at each of the participating clinical sites. The Health Insurance Portability and Accountability Act (HIPAA) and applicable local regulations will be followed by each participating institution in accordance with each institution's requirements. The participating international sites will obtain approval from their corresponding review boards in accordance with their local procedures and institutional requirements.

The investigator is required to keep accurate records to ensure the conduct of the study is fully documented. The investigator is required to ensure that all case report forms are legibly completed for every participant entered in the trial.

The investigational sites participating in this study will maintain the highest degree of confidentiality permitted for the clinical and research information obtained from participants participating in this study. When a subject participates in this study at more than one TrialNet site, sharing of this information is required. Sharing of information obtained during this study between TrialNet clinical centers and affiliates will be done to assure subject understanding and consent, safety, and adherence to protocol. Medical and research records will be maintained at each site in the strictest confidence. However, as a part of the quality assurance and legal responsibilities of an investigation, the investigational site must permit authorized representatives of the sponsor(s) and regulatory agencies to examine (and when required by applicable law, to copy) records for the purposes of quality assurance reviews, audits and evaluation of the study safety and progress. Unless required by the laws permitting copying of records, only the coded identity associated with documents or other participant data may be copied (obscuring any personally identifying information). Authorized representatives, as noted above, are bound to maintain the strict confidentiality of medical and research information that may be linked to identify individuals. The investigational site will normally be notified in advance of auditing visits.

9.3 Informed Consent

The process of assuring that individuals are making an informed decision about participating in this study includes both verbal and written communication. Written materials include a Volunteer Handbook, Volunteer Understanding Assessment, and written consent forms. The consent forms will be reviewed with participants and the participant will be given time to review the written consent form and ask questions.

As part of the informed consent process, the participant will also be required to complete a short, written Volunteer Understanding Assessment that is designed to ensure that the subject understands the study, as well as what is being asked of him/her. The participant will be given a copy of their consent forms.

The consent process will be conducted by qualified study personnel (the Trial Investigator or Study Coordinator and/or another designee). All participants (or their legally acceptable representative) must read, sign and date a consent form prior to participation in the study, and/or undergoing any study-specific procedures.

The informed consent form must be updated or revised whenever there is new, clinically significant information applicable to the safety of the participants, when indicated for a protocol amendment, and/or whenever any new information becomes available that may affect a subject's participation in the study.

9.4 Study Subject Confidentiality

Study subject data, which is for reporting purposes, will be stored at the TrialNet Coordinating Center. Data sent to the Coordinating Center will identify participants by the unique TrialNet Identification Number. The data entry system at the Coordinating Center is a secured, password protected computer system. At the end of the study, all study databases will be archived at the Coordinating Center for long-term storage.

Stored samples including genetic samples could be utilized to learn more about causes of type 1 diabetes, its complications (such as eye, nerve, and kidney damage) and other conditions for which individuals with diabetes are at increased risk, and how to improve treatment. The results of these future analyses, and any mechanistic studies will not be made known to the participant.

9.5 Risks and Benefits

The risks of this study are presented in this protocol, and informed consent form. As a phase 1 study, no benefit is likely for study participants but there is benefit for society if the study leads to therapy to halt type 1 diabetes disease progression.

10 STUDY ADMINISTRATION

10.1 Organizational Structure

This study is part of Type 1 Diabetes TrialNet, which is funded by the National Institutes of Health.

10.2 Role of Industry

This is a TrialNet protocol. TrialNet is funded by NIDDK. For this protocol, Novo Nordisk provides investigational product, supports clinical activities associated with the study, and participates in the safety monitoring of the study. The protocol and immune biomarker plan, execution, and analyses has been developed in collaboration between the TrialNet Investigators and Novo Nordisk. Novo Nordisk investigators will be involved in discussions about additional use of samples.

10.3 Groups and Committees

10.3.1 TN27 Study Chair Committee

The Study Chair Committee will receive periodic reports from the TNCC on the progress of the study. These will include accrual status for each cohort and the overall study. As appropriate, abstracts and manuscripts dealing with the progress of the trial shall be directed by the TN27 Study Chair Committee.

10.3.2 TrialNet Chairman's Office and TNCC

The TrialNet Chairman's Office and TNCC will work together in providing leadership to the TrialNet study group to include protocol and manual preparation, training for clinical sites, development of statistical design for each study, and analysis of study results. The TNCC will also coordinate

interactions among the participating TrialNet Clinical Centers, test laboratories including TrialNet Core Laboratories and other subcontract laboratories, NIDDK, and other sponsoring agencies.

10.3.3 Clinical Sites

Principal Investigators at each participating TrialNet clinical site will oversee all operations at that site. The clinical sites will forward all laboratory and data collection form information to the TNCC for analysis. Direct communication and site visits, as needed, will facilitate evaluation of the trial management.

10.3.4 Medical Monitor

TrialNet has established policies and procedures for assurance of safety monitoring of TrialNet protocols and of TrialNet subjects.

All adverse events will be recorded on the adverse event forms, which will be sent to the local IRBs/REBs, per their reporting requirements, and to the Coordinating Center.

An independent physician will serve as the medical monitor for this study who will maintain regular contact with the study and the Study Chair. The Medical Monitor will review all AEs, masked to treatment, and involve other TrialNet members as appropriate.

10.3.5 Clinical Site Monitoring

In order to conduct this study with established research principles, site visits will be conducted during the study to evaluate study conduct and ensure subject safety. All sites will be monitored by the TNCC and appropriate TrialNet committees for patient enrollment, compliance with protocol procedures, completeness and accuracy of data entry, the occurrence and reporting of adverse events (AEs) and serious adverse events (SAEs), site pharmacy accountability/operations and to confirm the presence of appropriate IRB/REB regulatory approvals/documents.

A blinded monitor and unblinded monitor will be utilized in the evaluation of the aforementioned regulatory requirements and protocol procedures. An unblinded monitor will visit the sites to ensure product handling procedures are adhered to and verify study agent accountability.

10.3.6 Data Safety and Monitoring Board (DSMB)

NIDDK has appointed individuals outside of TrialNet to serve as the DSMB for this study. This DSMB will provide monitoring for this study as described.

10.3.7 The Novo Nordisk Trial Safety Group

The purpose of the Novo Nordisk trial safety group is to review and evaluate the safety data prior to each dose escalation. The Novo Nordisk trial safety group has the mandate to accept the progression to next dose level, accept reduction to lower dose level or stop dosing according to the stopping rules. If safety signals and/or issues are identified indicating a need for implementation of actions not covered by the trial protocol, this must be escalated to the Novo Nordisk safety Committee for an agreement on actions needed. Members of the trial safety group include:

- CVP of Clinical Pharmacology and Translational Medicine (Chair)
- Medical specialist

- Clinical Pharmacology Scientist
- Chairperson from safety Committee or delegate
- Ad Hoc person

10.4 Sample and Data Storage

Samples to be stored for research purposes will be located at the NIDDK Repository and at TrialNet Laboratory Sites. While TrialNet is active, the use of the samples will be restricted to TrialNet researchers unless researchers from outside of TrialNet obtain approval from the TrialNet Steering Committee and the NIDDK to utilize the samples. All samples will be coded with unique study numbers, but TrialNet researchers will be able to identify samples if it is necessary to contact participants for reasons of health or for notification to them about future studies. Approval from the TrialNet Steering Committee and the NIDDK would be required before such linkage could occur. Researchers from outside of TrialNet will not be permitted to identify samples.

Data collected for this study will be sent to the TNCC. De-identified data will be stored at the NIDDK Repository, under the supervision of the NIDDK/NIH, for use by researchers including those outside of TrialNet.

With permission of the subject, when TrialNet is completed, samples will continue to be stored at the NIDDK Repository. Since the stored data will be fully de-identified, it will no longer be possible to identify samples. Thus, whereas a sample can be destroyed upon a participant's request during the existence of TrialNet, it can no longer be destroyed once TrialNet is completed. However, there will still be the potential to link data derived from the samples with data that had been derived from TrialNet studies. Once TrialNet is completed, researchers will only obtain access to samples through grant proposals approved by the NIDDK. The NIDDK will convene an external panel of experts to review requests for access to samples.

10.5 Preservation of the Integrity of the Study

The scientific integrity of the trial dictates that results be reported on a study-wide basis; thus, an individual Center will not report the data collected from its site alone. All presentations and publications using TrialNet trial data must protect the main objectives of the study. Data that could be perceived as threatening the study outcome will not be presented prior to release of the primary study outcomes. Approval as to the timing of presentations of data and the meetings at which they might be presented will be granted by the TrialNet Steering Committee. Study results should be discussed with the news media only upon authorization of the Steering Committee, and never before the results are presented. Any written statements about this study that are shared with national media must be approved by TrialNet before release.

10.6 Participant Reimbursement and Compensation

Participants will be compensated for study participation. In compliance with ICH Guidance E6, the amount and method of payments to subjects shall be designed to avoid coercion or undue influence on the study subjects. Payments to subjects will be prorated and not wholly contingent on completion of the trial by the subject.

Schedule of Assessments

	Screening	Treatment														Follow-up			
Visit number	**-1**	**0**	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**	**16**	**17**
Visit Day	**(-37)**	**0**	**1**	**2**	**7**	**14**	**21**	**28**	**35**	**42**	**49**	**56**	**63**	**70**	**77**	**84**	**120**	**180**	**365**
Visit (approximate months)																**3**	**4**	**6**	**12**
Visit (weeks)		**0**			**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**16**	**24**	**52**
Visit Window (days)			**+/- 1**	**+/- 1**	**+/- 1**	**+/- 1**	**+/- 1**	**+/- 1**	**+/- 1**	**+/- 1**	**+/- 1**	**+/- 1**	**+/- 1**	**+/- 1**	**+/- 1**	**+/-1**	**+/-3**	**+/- 14**	**+/- 14**
Visit Type	outpatient	inpatient			outpatient														
Procedure	Screening[1]	Dose 1	Observe[A]		Dose 2[B]	Dose 3[B]	Dose 4[B]	Dose 5[B]	Dose 6[C]	Dose 7[C]	Dose 8[C]	Dose 9[C]	Dose 10[C]	Dose 11[C]	Dose 12[C]	Follow-up	Follow-up	Follow-up	Follow-up
Informed consent	X																		
Inclusion/exclusion review	X	X																	
Medical history	X	X														X	X	X	X
Physical exam[2]	X	X		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Vital signs	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Adverse Event Assessment[3]		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Concomitant Medications	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Covid-19 Assessment[4]	X	X															X	X	X
Study Agent/Placebo Administration																			
Randomization		X																	
Study Agent/Placebo Administration		X			X	X	X	X	X	X	X	X	X	X	X				
Study Agent/Placebo Accountability		X			X	X	X	X	X	X	X	X	X	X	X				
Laboratory Tests																			
CBC with differential and platelets	X	X	X		X	X		X		X	X		X			X	X	X	X
Chemistry and Liver function tests	X	X	X		X	X		X		X			X			X	X		X
HIV/Hepatitis B and C	X																		X
T1D autoantibodies	X	X									X					X		X	X
TSH	X																X		X
Coagulation panel	X	X	X		X	X		X		X			X			X	X		X
Urinalysis/dip	X	X														X			
Urine pregnancy test	X	X			X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
IGRA/PPD	X																		
Metabolic Assessments																			
HbA1c	X	X														X		X	X
MMTT	X							X								X		X	X
Insulin use[5]	X	X			X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Safety Studies																			
EKG	X	X	X	X												X			
EBV/CMV viral load[6]	X	X			X	X		X		X	X		X			X	X		
EBV/CMV serology	X																X		
Anti-dsDNA[7]		X						X					X			X		X	X
Drug-induced antibodies (DIA)[7]		X						X					X			X		X	X
Circulating Plasmid (DNA/RNA)[8]		X	X	X	X			X			X					X	X	X	X
Cytokines[9]		X	X	X	X			X			X					X			X
Mechanistic Studies[10]		X	X		X			X			X					X	X	X	X

A= Individuals will be monitored on-site for 48 hours after dosing with discharge at that time only if stable.
B= Individuals will be monitored on-site for 4 hours after dosing with discharge at that time only if stable. Individuals with any signs or symptoms of hypersensitivity or anaphylaxis will be monitored a minimum of 8 hours with discharge at that time only if stable
C= Individuals will be monitored on-site for 2 hours after dosing with discharge at that time only if stable. Individuals with any signs or symptoms of hypersensitivity or anaphylaxis will be monitored a minimum of 8 hours with discharge at that time only if stable

1 = Screening: May occur over multiple days as needed
2 = Physical Exam: Full exam at screening, and 3 months (visit 14). Directed exam as indicated by medical history or symptoms at other visits as indicted.
3 = For visits 16 & 17: Only required to collect SAEs and any grade two or higher AEs; All other visits will report all AEs grade 1 or higher.
4 = Covid-19 Assessments will be completed PRN during visits 3-13 based on exposure and symptoms.
5 = CGM data from participants who currently use these devices as a part of their standard clinic care will be asked to provide this data to assess their diabetes management and insulin use.
6 = Viral PCR: All subjects will have serology and PCR for EBV/CMV at screening and visit 15. Samples for viral load will be obtained at other time points to be tested in symptomatic individuals.
7 = The following drug-induced antibodies (DIA) will be measured: anti-dsDNA, anti-IL2, anti-IL10, anti-TGFβ. Anti-dsDNA will be measured real-time and collected separately from the other DIAs, which will be tested in batch. Samples collected at dosing visits will be collected pre-dose.
8 = Plasmid DNA/RNA samples will be collected at visits 0,1,2,3,6,9,14,15,16,17. At visits 0-2, samples will be collected at the following time points related to Dose 1: Pre-dose and then post-dose at 1h, 8h +/-1h, 12h +/-1h, 24h +/-4h, 48h +/-6h. Samples at V3 and later dosing visits will be collected pre-dose.
9 = Cytokine samples to assess safety and mechanism of action will be collected at Visits 0,1,2,3,6,9,14,17. At Visits 0-2, samples will be collected at the following time points related to Dose 1: Pre-dose and then post-dose at 8h +/-1h, 24h +/-4h, 48h +/-6h. Samples at V3 and later doing visits will be collected pre-dose.
10 = Mechanistic studies: Will include samples for RNA, plasma, serum, DNA, measures of T and B cell number and function. The schedule for these assessments may vary as appropriate. At no time will the blood draw volume exceed what is allowable according to the participant's age and body weight. At dosing visits, mechanistic samples will be collected pre-dose. Visit 1 samples will be collected 24h +/-4h after first dose.

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