Immune
Tolerance
Network

INDUCING REMISSION IN NEW ONSET T1DM WITH ALEFACEPT (Amevive®)

Protocol ITN045AI

Version 10.0 (June 4, 2012)

IND # 105,308

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Protocol Approval

Trial ID: ITN045AI	**Protocol Version: 10.0**
	Dated: June 4, 2012
IND # 105,308	**Protocol Chair:** Mark Rigby, MD, PhD
Title: *Inducing Remission in New Onset T1DM with Alefacept (Amevive®)*	

I confirm that I have read the above protocol in the latest version. I understand it, and I will work according to the principles of good clinical practice (GCP) as described in the US Code of Federal Regulations (CFR)—45 CFR part 46 and 21 CFR parts 50, 56, and 312, and in the International Conference on Harmonization (ICH) document *Guidance for Industry: E6 Good Clinical Practice: Consolidated Guidance* dated April 1996. Further, I will conduct the study in keeping with local legal and regulatory requirements.

As the principal investigator, I agree to carry out the study by the criteria written in the protocol and understand that no changes can be made to this protocol without written permission of the NIAID.

_____

Principal Investigator (*Print*)

_____ _____

Principal Investigator (*Sign*) **Date**

Synopsis

Title	**Inducing Remission in New Onset T1DM with Alefacept (Amevive®)**
IND Sponsor	NIAID
Conducted by	Immune Tolerance Network
Protocol Chair	Mark R. Rigby, MD, PhD
Accrual Objective	Initial: 66; Revised to 50 upon the business decision made by Astellas Pharmaceuticals to discontinue manufacturing Amevive® (alefacept).
Study Treatment	Alefacept or placebo (saline)
Study Design	This trial will be conducted as a multi-center, prospective, double-blind, placebo-controlled, 50-patient, 2:1 randomized, phase II clinical trial for individuals with recent-onset T1DM aged 12−35 years. Participants will receive weekly IM injections of alefacept (15 mg) or placebo for 12 weeks, followed by a 12-week pause before resuming another 12 weeks of dosing, for a total course of 24 weeks of alefacept or placebo. Prior to initiating the study in the pediatric age group (12−15 years of age), the study drug will be given to an initial safety cohort of adult participants, defined as age 16–35 years old. When the 10th participant in the adult cohort has completed visit 11, or 6 months after the first participant is enrolled, the safety data will be reviewed by the study team and Data Safety and Monitoring Board (DSMB). Enrollment will continue in the adult cohort during this first safety review. Children (ages 12-15) may only be enrolled after completion of a satisfactory safety review of the first cohort of 10 adult participants (16-35) who have completed 12 weeks of treatment. This will require an additional safety review (review 1b) if the initial safety review does not achieve this milestone.
Study Duration	Total study duration will be approximately 188 weeks (3.5 years): • The enrollment phase will last up to 68-84 weeks (15-21 months). It is estimated that the first cohort of 10 adult participants (aged 16-35 years) will be enrolled within 6-9 months and the remaining 40 participants (aged 12-35 years) will be enrolled in the ensuing 9-12 months. • The study participation phase will be 104 weeks (2 years), which includes a treatment phase of 36 weeks and a follow-up phase of 68 weeks.
Primary Objective	The primary objective is to determine whether alefacept will slow the progression of the autoimmune destruction of β cells and lead to the preservation of C-peptide secretion in T1DM.
Primary Endpoint	The primary endpoint is a mixed-meal tolerance test (MMTT) stimulated 2-hour C-peptide AUC at week 52.
Secondary Endpoints	**Efficacy:** 1. MMTT-stimulated peak and 4-hour C-peptide AUC at weeks 52 and 104. 2. MMTT-stimulated 2-hour C-peptide AUC assessed longitudinally at weeks 24, 52 and 104. 3. Insulin use in units per kilogram body weight per day at weeks 52 and 104. 4. Major hypoglycemic events occurring from randomization to weeks 52 and 104. 5. HbA_{1C} levels at weeks 52 and 104.

Safety:

1. Rate of the following adverse events (AEs) in participants receiving alefacept or placebo:
   a. Injection reactions; defined as fever, chills, headache, nausea, vomiting, and injection-site pain.
   b. Hypersensitivity reactions; defined as signs and symptoms of anaphylaxis, wheezing, dyspnea, urticaria, and hypotension.
   c. Evidence of infection with EBV, CMV, or TB.
2. Frequency and severity of all AEs in participants receiving alefacept or placebo.

Exploratory Endpoints

Mechanistic:

1. Immunological assessments will be compared with clinical outcomes to determine whether there is evidence of immune tolerance after receiving alefacept treatment.
2. Immunological assessments will be compared with clinical outcomes to assess whether protective immune responses are affected after receiving alefacept treatment.
3. Immune response to neo-antigens and recall antigens will be assessed.

Metabolic:

1. Proportion of participants in each treatment arm who are exogenous insulin-free for at least 3 months with HbA_{1C} levels less than 6.5% at weeks 52 and 104.
2. Proportion of participants in each treatment arm who achieve a persistent reduction for at least 3 months in insulin dose to less than 0.5 units/kg at weeks 52 and 104.

Inclusion Criteria

Patients *must meet all* of the following criteria to be eligible for this study:

1. Males or females aged 12−35 years who meet the American Diabetes Association standard T1DM criteria.
2. Diagnosis of T1DM within 100 days of enrollment.
3. Positive for at least one diabetes-related autoantibody:
   a. Glutamate decarboxylase (GAD-65);
   b. Insulin, if obtained within 10 days of the onset of exogenous insulin therapy;
   c. IA-2;
   d. ZnT8; or
   e. ICA.
4. Peak stimulated C-peptide level > 0.2 pmol/mL following a mixed-meal tolerance test (MMTT).
5. Signed informed consent.

Exclusion Criteria

Patients who meet any of the following criteria will not be eligible for this study:

1. Severe reaction or anaphylaxis to human monoclonal antibodies.
2. History of malignancy or significant cardiovascular disease (including history of myocardial infarction, angina, use of anti-anginal medicines (e.g., nitroglycerin), or abnormal stress test).
3. History of recent or ongoing uncontrolled bacterial, viral, fungal, or other opportunistic infections.

4. Evidence of infection with HBV (as defined by hepatitis B surface antigen, HBsAg), HCV (anti-HCV antibodies), HIV or toxoplasmosis.
5. Positive tuberculin skin test (PPD).
6. Clinically active infection with EBV, CMV, or tuberculosis; or EBV viral load ≥ 10,000 copies per 10^6 PBMCs or CMV viral load ≥10,000 copies per mL whole blood.
7. Diagnosis of liver disease or hepatic enzymes, as defined by ALT and/or AST ≥ 2 times the upper limit of normal.
8. Prior or current treatment that is known to cause a significant, ongoing change in the course of T1DM or immunologic status, including high-dose inhaled, extensive topical or systemic glucocorticoids.
9. Current or prior (within the last 30 days) use of metformin, sulfonylureas, glinides, thiazolidinediones, exenatide, liraglutide, DPP-IV inhibitors or amylin.
10. Current use of any medication known to influence glucose tolerance (e.g., atypical antipsychotics, diphenylhydantoin, thiazide, or other potassium-depleting diuretics, β-adrenergic blockers, niacin).
11. Any of the following hematologic abnormalities, confirmed by repeat tests at least 1 week apart:
    a. White blood count <3500/μL or >14,000/μL;
    b. $CD4^+$ count below the lower limit of normal;
    c. Platelet count <150,000 /μL; *or*
    d. Hemoglobin <10 g/dL.
12. Females who are pregnant, lactating, or planning on pregnancy during the 2-year study period.
13. History of bone marrow transplantation, or autoimmune disease associated with lymphopenia.
14. Any medical condition that in the opinion of the principal investigator would interfere with safe completion of the trial.
15. Prior participation in a clinical trial that could potentially affect T1DM or immunologic status.
16. Receipt of a live vaccine (e.g., varicella, measles, mumps, rubella, cold-attenuated intranasal influenza vaccine, bacillus Calmette-Guérin, and smallpox) in the 6 weeks before enrollment.
17. Participation in an investigational clinical trial within the last six weeks.

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Abbreviations

ADA	American Diabetes Association
AE	adverse event
ALT	alanine aminotransferase
AST	aspartate aminotransferase
AUC	area under the curve
CBC	complete blood count
CFR	Code of Federal Regulations
CMV	cytomegalovirus
CRO	Contract Research Organization
DAIT	Division of Allergy, Immunology and Transplant
DCCT	Diabetes Control and Complications Trial
DSMB	Data and Safety Monitoring Board
EBV	Epstein-Barr virus
eCRF	electronic case report form
EDIC	Epidemiology of Diabetes Interventions and Complications Study
FDA	US Food and Drug Administration
GCP	Good Clinical Practice
HBV	hepatitis B virus
HCV	hepatitis C virus
HDL	high density lipoprotein
ICH	International Conference on Harmonization
IM	Intramuscular
IND	investigational new drug
IRB	Institutional Review Board
ITN	Immune Tolerance Network
ITT	intent to treat

IV	Intravenous
LCV	lymphocryptovirus
mAb	monoclonal antibody(ies)
MedDRA	Medical Dictionary for Regulatory Activities
MMTT	mixed-meal tolerance test
NCI-CTCAE	National Cancer Institute *Common Terminology Criteria for Adverse Events*
NIAID	National Institute of Allergy and Infectious Disease
NIH	National Institutes of Health
NK	natural killer
PASI	Psoriasis Area and Severity Index
PBMC	Peripheral Blood Mononuclear Cell
PGA	Physician Global Assessment
SAE	serious adverse event
T1DM	type 1 diabetes mellitus
TB	Tuberculosis
WHO	World Health Organization

1. BACKGROUND AND RATIONALE

1.1 BACKGROUND

Type 1 diabetes mellitus (T1DM) affects 1−2 million people in North America, with an incidence of approximately 30,000 new diagnoses per year.[1] This condition usually strikes in childhood, adolescence or young adulthood. Those with this condition are sentenced to multiple daily shots of exogenous insulin or therapy with continuous subcutaneous infusion by pump; daily pricks for glucose checks; life threatening hypoglycemic or diabetic ketoacidosis episodes; and, even with the best control, high risk for suffering nephropathy, blindness, stroke, heart disease, neuropathy, and shortened life expectancy. In addition to affecting patients and their families, treating diabetes and its side effects burdens society as a whole, with medical expenditures and loss of productivity valued at hundreds of millions of dollars annually.

As one of the most common chronic childhood diseases, T1DM is a particular burden to children and their families. While T1DM can occur into adulthood, the worldwide incidence of T1DM is increasing rapidly in children younger than 15 years, as recently documented by the WHO Diamond Project.[1] During the period 1995–1999, the global annual increase in childhood T1DM was 3.4% but was as high as 5.3% in North America.[1] In Europe, the EURODIAB study group has found the greatest rate of increase in the 0−4 years age group and is predicting a doubling in the number of new cases in children younger than 5 years in the next decade.[2] Overall, prevalence of T1DM in children under age 15 in Europe is predicted to rise from 94,000 in 2005 to 160,000 in 2020.[2] In contrast, the incidence of T1DM in young adults over age 15 is not increasing. For these reasons, there is considerable interest in identifying safe and effective interventions that can modify the course of T1DM in pediatric populations.

1.2 SCIENTIFIC RATIONALE

Despite progress towards understanding the genetic, environmental, and immunologic basis for T1DM, the prevention and cure of this condition remains elusive. The autoimmune pathogenesis of T1DM is well established, and several experimental strategies in animal models have focused preventing disease by an immunomodulatory intervention, specifically through targeting diabetogenic T cells.

The rationale for immune-intervention studies is the notion that at the time of clinical diagnosis a substantial number of β cells remain, perhaps 10−20% of baseline numbers. These residual cells are thought to be responsible for the temporary nadir in exogenous insulin requirements often experienced in the weeks to months following diagnosis, known as the “honeymoon” period. Non-selective immunosuppressive therapies have shown promise in the ability to protect β cells. For example, in the 1988 Bougneres et al. study, 27 of 40 (68%) children aged 7 to 15 years old with newly diagnosed T1DM treated with cyclosporine (CsA) did not require insulin at 4

months after CsA, and 12 of the 40 (30%) did not require insulin at 12 months.[3] Factors associated with remission included shorter duration of clinical symptoms prior to treatment and higher stimulated C-peptide response at diagnosis. Although the risks of nonspecific immunosuppressive approaches are greater than those of life with exogenous insulin, such studies were proof-of-concept that if the β-cell immune attack could be mitigated and, if sufficient β-cell mass was still present, patients could be rescued from diabetes.

Recent trials of a short course of non-stimulatory anti-CD3 antibodies[4-5] revealed that survival of functional β cells for a year or more following primary diagnosis is possible. Despite this success, there still remains a significant need to investigate alternative strategies to safely interrupt the anti-β-cell response, in order to induce long-term remission in this condition. At this point, clinical trials in T1DM using therapies directed at lymphocytes, and specifically T cells, have the greatest promise for interrupting diabetes autoimmunity, and thus inducing remission and re-establishing tolerance in T1DM. Successful preservation of β-cell function in pediatric populations has recently been reported for a variety of treatment modalities, including rituximab (age range: 8–45 years),[6] anti-CD3 monoclonal antibody (12–39 years), GAD-alum vaccine (10–18 years), and anti-TNFα (etanercept; 3–18 years).[4, 7-8] As such, identifying T cell-directed therapies that are not only effective but also safe and tolerable should be considered the most justifiable and promising agents to investigate in T1DM.

The basic concept of employing biologic agents such as anti-CD3 antibodies in T1DM to interfere with lymphocyte function has provided the rationale underlying other clinical trials in this disease that have tested the safety and efficacy of anti-thymocyte globulin, CTLA4Ig, rituximab, and daclizumab. In addition to anti-CD3, a number of other reagents have been used for the treatment of immune-mediated disease targeting pathways and molecules critical for T-cell activation and effector function. One example are the drugs interrupting the interaction between CD2 and the lymphocyte function-associated antigen-3 (LFA-3). CD2 is expressed on T cells and LFA-3 on antigen-presenting cells; following T cell receptor: MHC interaction, the CD2/LFA-3 interaction provides accessory stimulation for T cells. In addition, CD2 is expressed predominantly on memory T cells (i.e., $CD3^+$, $CD4^+$, $CD45RO^+$) and, by bridging these cells with natural killer (NK) cells, induces apoptosis and a reduction in circulating memory T cells. CD2 is also expressed at low levels on the surface of NK cells and certain bone marrow B lymphocytes.

While the primary ligand for CD2 in humans is CD58/LFA-3, in rodents the primary ligand is CD48.[9-11] In animal studies, disruption of the CD2:CD48 pathway can prevent diabetes autoimmunity, prolong allograft acceptance, and promote immune tolerance. In the BioBreeding rat model of T1DM, anti-CD2 treatment results in long-term protection from spontaneous disease in diabetes-prone rats, induction of diabetes in diabetes-resistant rats, and adoptive transfer of disease.[12] This protection appears to

be the result of a selective reduction in a CD4 T-cell subpopulation. In animal models of transplantation, blocking the CD2:CD48 pathway alone or in combination with other T-cell molecules delays rejection and in some instances results in immune tolerance. In studies of murine cardiac allografts, anti-CD2 therapy alone significantly prolongs graft survival; whereas, co-administration of anti-CD2 with anti-CD48, anti-CD3 or CTLA4Ig results in permanent acceptance of grafts in the absence of ongoing immune therapy.[13-18] In studies by Kapur et al., a brief course of anti-CD2 alone induced donor-specific tolerance in DBA/2 recipients of B6AF1 islet allografts.[19-20] Tolerance was associated with a specific reduction in the number of $CD2^+$ T cells and in the levels of intragraft granzyme B and IL-10.

CD48 is structurally and phylogenetically related to CD58, and both are found on a variety of cell types, including antigen-presenting cells and endothelial cells. Due to species variation, biologic agents (i.e., antibodies or fusion proteins) that bind these molecules in rodents are not cross-reactive in primates and vice versa. However, we believe that the above preclinical evidence in diabetes and (islet) allograft models provides critical basic-science justification for investigating an approach which disrupts the CD2 pathway and/or $CD2^+$ cells in autoimmune diabetes, and one which has the potential to engender immune tolerance.

Alefacept (Amevive®, Astellas Pharma US, Inc.) is an immunosuppressive dimeric fusion protein that consists of the extracellular CD2-binding portion of human LFA-3 linked to the Fc (hinge, C_H2 and C_H3 domains) portion of human IgG1. Amevive® was FDA-approved in 2003 for the treatment of adult patients with chronic moderate to severe plaque psoriasis who are candidates for systemic therapy or phototherapy, but recently the company that makes alefacept decided to discontinue marketing the drug. This was for business reasons and not for safety concerns. As a result, fewer participants can be enrolled in the study. The purpose of the study has not changed, but the ability to detect a benefit of the treatment may be reduced.

Alefacept binds competitively to the CD2 receptor on the surface of T cells with the LFA-3 portion of the drug and thereby efficiently interferes with LFA-3/CD2 interactions and T-cell activation;[21] whereas, the Fc portion of alefacept engages the immunoglobulin receptor FcγRIII on the surface of NK cells, resulting in apoptosis of specific memory T-cell subsets.[22] Since CD2 expression is higher on memory than naïve T cells,[23] alefacept binds mainly to memory T cells and induces a selective reduction of specific T cell subtypes by apoptosis. This means that alefacept can inhibit T cell activation not only by antagonizing CD2-mediated signaling but also by selectively depleting memory T cells.[24] Consistent with its mechanism of action, alefacept reduces circulating memory T cells ($CD4^+CD45RO^+$ and $CD8^+CD45RO^+$), thereby decreasing the count of total lymphocytes, whereas the number of naïve T cells ($CD4^+CD45RA^+$ and $CD8^+CD45RA^+$) remains approximately normal.[25]

Alefacept does not have any functional effect on $CD19^+$ B cells or $CD16^+/CD56^+$ NK cells.[26-27] The selective effect of alefacept on effector-memory T cells is of particular

immunologic importance as these cells appear to be the primary mediators of target tissue destruction in autoimmune disorders. In psoriasis, the therapeutic effect of alefacept is associated with a reduction in the number of pathogenic effector-memory T cells.[27-28] Most importantly, in patients receiving multiple doses of alefacept, there is a sustained clinical effect from alefacept therapy that persists following the return of normal T cell numbers.[27, 29-30]

Although several approaches have been developed to prevent autoimmune diabetes during pre-diabetes or to induce remission soon after clinical disease in experimental models, attempts to translate these findings clinically have been only partly successful and are often fraught with unacceptable immune and non-immune side effects. Any novel approach to prevent or cure T1DM requires a careful risk-benefit analysis in the context of well-controlled disease. [31] Alefacept has shown significant efficacy in the T cell-mediated autoimmune disorder of plaque psoriasis. As detailed above, animal studies strongly suggest that the CD2 pathway is an integral component in diabetes autoimmunity. In addition to its proven clinical efficacy in psoriasis, a T cell-mediated disease, alefacept treatment has been well-tolerated without causing significantly increased risk for serious infection, making it an attractive drug for testing in T1DM.

1.3 PRECLINICAL AND CLINICAL EXPERIENCE

1.3.1 Preclinical Studies

1.3.1.1 Preclinical Safety Data

1.3.1.1.1 Overview

All preclinical safety data presented below was obtained from the Amevive® Package Insert (http://www.astellas.us/docs/amevive.pdf).

1.3.1.1.2 Toxicity Studies

In a chronic toxicity study, cynomolgus monkeys were dosed weekly for 52 weeks with intravenous alefacept at 1 mg/kg/dose or 20 mg/kg/dose. One animal in the high dose group developed a B-cell lymphoma that was detected after 28 weeks of dosing. Additional animals in both dose groups developed B-cell hyperplasia of the spleen and lymph nodes.

All animals in the study were positive for an endemic primate gamma herpes virus also known as lymphocryptovirus (LCV). Latent LCV infection is generally asymptomatic but can lead to B-cell lymphomas when animals are immune suppressed.

In a separate study, baboons given 3 doses of alefacept at 1 mg/kg every 8 weeks were found to have centroblast proliferation in B-cell dependent areas in the germinal centers of the spleen following a 116-day washout period.

The role of alefacept in the development of the lymphoid malignancy and the hyperplasia observed in non-human primates and the relevance to humans is unknown. Immunodeficiency-associated lymphocyte disorders (plasmatic hyperplasia, polymorphic proliferation, and B-cell lymphomas) occur in patients who have congenital or acquired immunodeficiencies including those resulting from immunosuppressive therapy.

1.3.1.1.3 Carcinogenicity and Fertility Studies

No carcinogenicity or fertility studies have been conducted.

1.3.1.1.4 Mutagenicity Studies

Mutagenicity studies were conducted in vitro and in vivo; no evidence of mutagenicity exists.

1.3.1.2 Preclinical Efficacy Data

Alefacept does not cross-react with rodent CD2. Because of the species difference in CD2 and its ligands and the unique mechanism of action of alefacept, the direct study of this agent is not possible in rodents, including the diabetes model of NOD mice. However, as discussed in section 1.2, disruption of CD2 with monoclonal antibodies in rodent models of diabetes and islet allografts has shown beneficial effects.

1.3.2 Clinical Studies

1.3.2.1 Clinical Pharmacology

Alefacept interferes with lymphocyte activation by specifically binding to the lymphocyte antigen, CD2, and inhibiting the LFA-3/CD2 interaction. Activation of T lymphocytes involving the interaction between LFA-3 on antigen-presenting cells and CD2 on T lymphocytes plays a role in the pathophysiology of chronic plaque psoriasis. The majority of T lymphocytes in psoriatic lesions are of the memory-effector phenotype characterized by the presence of the CD4RO marker,[32] express activation markers (e.g., CD25, CD69) and release inflammatory cytokines, such as interferon-γ. It is believed that a similar subpopulation of effector-memory T cells is responsible for the β-cell destruction in T1DM.

Alefacept also causes a reduction in subsets of $CD2^+$ T lymphocytes (primarily $CD45RO^+$), presumably by bridging between CD2 on target lymphocytes and immunoglobulin Fc receptors on cytotoxic cells, such as NK cells. Treatment with alefacept results in a reduction in circulating total $CD4^+$ and $CD8^+$ T lymphocyte counts. CD2 is also expressed at low levels on the surface of NK cells and certain bone marrow B lymphocytes. Therefore, the potential exists for alefacept to affect the activation and numbers of cells other than T lymphocytes. In clinical studies of alefacept, minor changes in the numbers of circulating cells other than T lymphocytes have been observed.

1.3.2.1.1 Pharmacokinetics

In patients with moderate to severe plaque psoriasis, following a 7.5 mg intravenous (IV) administration, the mean volume of distribution of alefacept was 94 mL/kg, the mean clearance was 0.25 mL/h/kg, and the mean elimination half-life was approximately 270 hours. Following an intramuscular (IM) injection, bioavailability was 63%.

The pharmacokinetics of alefacept in pediatric patients are currently under study in a population 12–17 years of age with psoriasis (ClinicalTrials.gov identifier NCT00808223). The effects of renal or hepatic impairment on the pharmacokinetics of alefacept have not been studied.

1.3.2.1.2 Pharmacodynamics

At doses tested in clinical trials, alefacept therapy resulted in a dose-dependent decrease in circulating total lymphocytes.[33] This reduction predominantly affected the memory effector subset of the $CD4^+$ and $CD8^+$ T lymphocyte compartments ($CD4^+CD45RO^+$ and $CD8^+CD45RO^+$), the predominant phenotype in psoriatic lesions. Circulating naïve T lymphocyte and NK cell counts appeared to be only minimally susceptible to alefacept treatment, while circulating B lymphocyte counts appeared not to be affected by alefacept.

1.3.2.2 Clinical Efficacy

1.3.2.2.1 Chronic Plaque Psoriasis in Adults

Alefacept has been approved by the FDA since 2003 for the treatment of adults with moderate to severe chronic plaque psoriasis who are candidates for systemic therapy or phototherapy. Alefacept was evaluated in two randomized, double-blind, placebo-controlled studies in adults with chronic (at least 1 year or more) plaque psoriasis who were candidates for or had previously received systemic therapy or phototherapy. Each course consisted of once-weekly administration for 12 weeks (IV for Study 1, IM for Study 2) of alefacept or placebo.

In Study 1, patients were randomized to receive one or two courses of alefacept 7.5 mg administered by IV bolus. The first and second courses in the two-course cohort were separated by an interval of 12 weeks or more. A total of 553 patients were randomized into three cohorts (see Table 1).

Table 1: Treatment group and number of patients dosed in psoriasis study 1

	Treatment cycle 1 (# patients)	Treatment cycle 2 (# of patients)
Cohort 1	Alefacept 7.5 mg (183)	Alefacept 7.5 mg (154)
Cohort 2	Alefacept 7.5 mg (184)	Placebo (142)
Cohort 3	Placebo (186)	Alefacept 7.5 mg (153)

Study 2 provided a basis for comparison of patients treated with either 10 mg or 15 mg alefacept IM. A total of 507 patients were randomized to receive a single course of one of the following: 10 mg of alefacept IM (n=173), 15 mg of alefacept IM (n=166), or placebo (n=168).

In Studies 1 and 2, 77% of patients had previously received systemic therapy and/or phototherapy for psoriasis. Of these, 23% and 19%, respectively, had failed to respond to at least one of these previous therapies.

Table 2 shows the treatment response in the first course of Study 1 and in Study 2. Response to treatment in both studies was defined as the proportion of patients with a reduction in score on the Psoriasis Area and Severity Index (PASI)[34] of at least 75% from baseline at two weeks following the 12-week treatment period. Other treatment responses included the proportion of patients who achieved a scoring of "clear" or "almost clear" by Physician Global Assessment (PGA) and the proportion of patients with a reduction in PASI of at least 50% from baseline two weeks after the 12-week treatment period.

Table 2: Percentage of patients responding to the first course of treatment in psoriasis Study 1 (7.5 mg IV) and psoriasis Study 2 (15 mg IM) two weeks post dosing

	Study 1			Study 2		
Response	**Placebo** (n=186)	**7.5 mg IV** (n=367*)	**Difference** (95% CI)	**Placebo** (n=168)	**15 mg IM** (n=166)	**Difference** (95% CI)
≥75% reduction in PASI	4%	14%	10** (6,15)	5%	21%	16** (9,23)
≥50% reduction in PASI	10%	38%	28** (22,35)	18%	42%	24** (14,33)
PGA "clear" or "almost clear"	4%	11%	7*** (3,12)	5%	14%	9**** (3,15)

*cohorts 1 and 2 are combined; **p values <0.001; ***p value 0.004; ****p value 0.006

In Study 2, the proportion of responders to the 10 mg IM dose was higher than placebo, but the difference was not statistically significant.

In both studies, onset of response to alefacept treatment (i.e., at least a 50% reduction of baseline PASI) began 60 days after the start of therapy.

With one course of therapy in Study 1 (IV route), the median duration of response (defined as maintenance of greater than or equal to 75% reduction in PASI) was 3.5 months for alefacept-treated patients and 1 month for placebo-treated patients. In Study 2 (IM route), the median duration of response was approximately 2 months for both alefacept-treated patients and placebo-treated patients. Most patients who had responded to either alefacept or placebo maintained a 50% or greater reduction in PASI through the 3-month observation period. The responders (n=52) in a subset of patients in Study 1 who crossed over to placebo for course 2 (cohort 2) maintained greater than or equal to 50% reduction in PASI for a median of 7 months.

Some patients achieved their maximal response beyond 2 weeks post-dosing. In Studies 1 and 2, an additional 11% (42/367) and 7% (12/166) of patients treated with alefacept, respectively, achieved a 75% reduction from baseline PASI score at one or more visits after the first 2 weeks of the follow-up period.

Patients in Studies 1 and 2 who had completed the first treatment course were eligible to receive a second treatment course if their psoriasis was less than "clear" by PGA and their $CD4^+$ T lymphocyte count was above the lower limit of normal. The level of response (decrease in median PASI score) over the two courses of IV treatment is shown below in Table 3. The median reduction in PASI score was greater in patients who received a second course of alefacept treatment (given IV or IM) compared to patients who received placebo. Of particular note is the significant prolongation of treatment response in patients who received a second course of treatment versus those who received a single course, as shown in Figure 1.[35]

Table 3: Comparison of overall response rates in patients receiving one or two courses of alefacept 7.5 mg/week IV or 15 mg/week IM with no other systemic therapy used between the two alefacept courses.

Route of Delivery	% with a ≥50% reduction in PASI	% with a ≥75% reduction in PASI	% with "clear"/"almost clear"
IV 1 course 7.5mg	56	28	23
IV 2 course 7.5mg	71	40	32
IV placebo	24	8	6
IM 1 course 15mg	57	33	24
IM 2 course 15mg	69	43	31
IM placebo	35	13	8

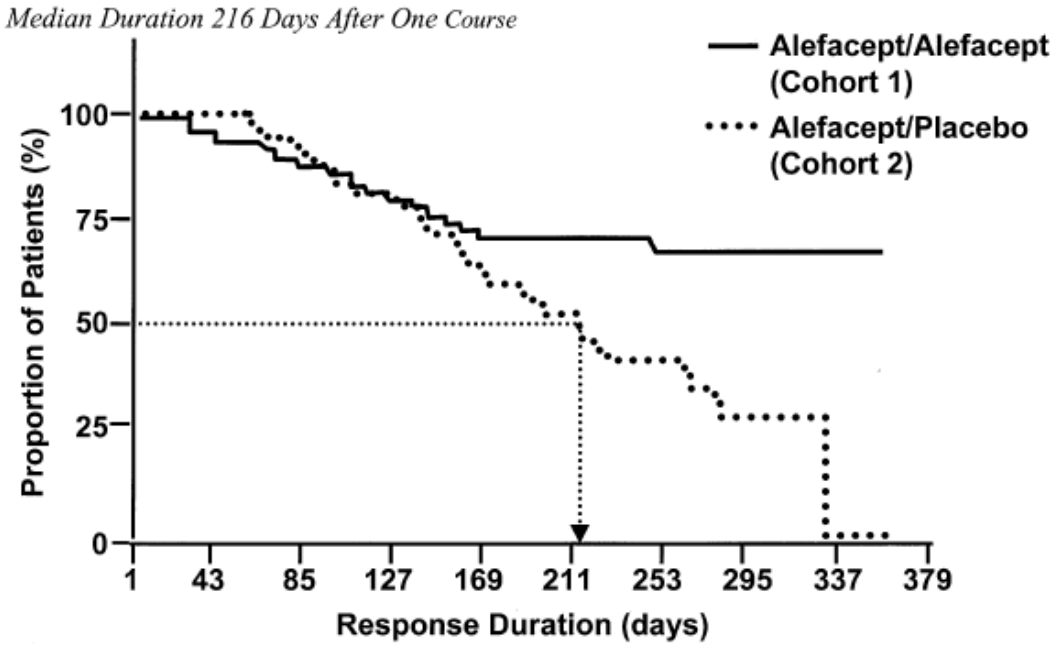

Figure 1. Duration of a ≥50% reduction in Psoriasis Area Severity Index (PASI) from baseline in patients who achieved a ≥75% reduction in PASI during or after treatment, plotted as a survival curve. Log-rank test: *P*=.019 for cohort 1 (2 courses of IV alefacept followed by placebo).

1.3.2.2.2 Case Studies of Alefacept Treatment

In addition to psoriasis, alefacept has been used with success and apparent safety in other dermatologic autoimmune conditions, systemic alloimmune conditions, and in patients less than 18 years of age. Some formal psoriasis trials include patients 16 years old and above.[9, 35] Additional reports include the successful treatment of psoriasis in a 10 year old girl and a 14 year old with alopecia areata, both of whom were treated with 15 mg IM dosing.[9, 35] Several case series by Shapira et al. have described successful approaches to treat severe refractory graft-vs.-host disease with regimens containing alefacept.[36-38] In these studies, several children up to age 14 years old (the youngest being 3 years old) received 15 mg IM dosing, whereas those older than 14 years received 30 mg. Alefacept is now being formally investigated for non-psoriatic dermatologic autoimmune conditions, graft-vs.-host disease, and renal transplantation.

1.4 SUMMARY OF KNOWN AND POTENTIAL RISKS AND BENEFITS FOR HUMAN PARTICIPANTS

1.4.1 Risks

1.4.1.1 Overview

The information provided here is summarized from the Amevive® package insert (http://www.astellas.us/docs/amevive.pdf), and is compiled from the clinical studies

conducted in patients 16 years of age and older with chronic plaque psoriasis. A number of adverse reactions have been reported with alefacept use. Some adverse reactions, mostly "constitutional" symptoms such as dizziness, chills, and nausea have occurred in up to 5% or more subjects treated with alefacept vs. placebo but did not result in discontinuation of treatment. Other adverse effects with potential significant sequelae have occurred in fewer than 2% of patients and are discussed below in further detail.

Commonly observed adverse events were: pharyngitis, dizziness, increased cough, nausea, pruritis, myalgia, chills, injection site pain, injection site inflammation and accidental injury. Less common events that were observed at a higher rate in alefacept-treated patients include rare cases of transaminase elevations of 5 to 10 times the upper limit of normal.

The most common events resulting in discontinuation of alefacept treatment were $CD4^+$ T lymphocyte counts below 250 cells/μL (2%), headache (0.2%), and nausea (0.2%).

Most adverse effects or severe adverse reactions occur within 6-8 weeks of initiating alefacept.

1.4.1.2 Lymphopenia

Alefacept induces dose-dependent reductions in circulating $CD4^+$ and $CD8^+$ T-cell counts. In the intramuscular study conducted in participants with chronic plaque psoriasis (see section 1.3.2.2.1), 4% of patients temporarily discontinued treatment and no patients permanently discontinued treatment due to $CD4^+$ T lymphocyte counts below the specified threshold of 250 cells/μL. In this same study, 10%, 28%, and 42% of patients had total lymphocyte, $CD4^+$, and $CD8^+$ T lymphocyte counts below normal, respectively. Twelve weeks after a course of therapy (12 weekly doses), 2%, 8%, and 21% of patients had total lymphocyte, $CD4^+$, and $CD8^+$ T cell counts below normal. In this trial, $CD4^+$ T-cell counts will be monitored every two weeks throughout the 12-week dosing courses, which follow the FDA-approved approach for psoriasis.

1.4.1.3 Malignancies

Immunosuppressive therapies, including alefacept, have been linked to increased risk of malignancies. In placebo-controlled studies in psoriasis, the incidence of malignancies was 1.3% for alefacept-treated patients, compared to 0.5% in the placebo group. The majority of malignancies were non-melanoma skin cancers. Other malignancies included melanoma, solid organ malignancies, and lymphomas. In other studies and analyses, there does not appear to be a statistically significant increase in malignancies as compared to those seen in patients with psoriasis. In a recent study, it was noted that patients with psoriasis and a history of Psoralen plus ultraviolet A (PUVA) exposure have an increased risk of non-melanoma skin cancers, and those with exposure to methotrexate have an increased risk for lymphomas. The

rate of malignancies observed with alefacept treatment appears to be within the expected incidence rates for patients with psoriasis.[39]

1.4.1.4 Infection

Alefacept has the potential to increase the risk of infection and reactivate latent, chronic infections. In the first 24 weeks of placebo-controlled studies, the proportion of patients with serious infections (infections requiring hospitalization) was 0.9% in the alefacept-treated group and 0.2% in the placebo group. Infections requiring hospitalization included cellulitis, abscesses, wound infections, toxic shock, pneumonia, appendicitis, cholecystitis, gastroenteritis, and herpes infections. Data from a meta-analysis of 13 clinical trials indicated that fewer than 1% of alefacept-treated patients developed a serious infection, and no opportunistic infections or infection-related deaths were reported.[40] In the current trial, participants will be monitored during the study for new infection or reactivation of infection with CMV, EBV, or TB.

1.4.1.5 Hypersensitivity Reactions

Hypersensitivity reactions, such as urticaria, have been associated with administration of alefacept. Angioedema was reported in 0.2% of patients in placebo-controlled studies. In the 24-week period constituting the first course of placebo-controlled studies, urticaria was reported in fewer than 1% of patients treated with alefacept vs. placebo. Urticaria resulted in discontinuation of therapy in one alefacept-treated patient.

1.4.1.6 Cardiovascular Events

The adverse reactions that most commonly resulted in clinical intervention were cardiovascular events including coronary artery disorder in fewer than 1% of patients and myocardial infarct in fewer than 1% of patients. These events were not observed in any of the placebo-treated patients. The total number of patients hospitalized for cardiovascular events in the alefacept-treated group was 1.2%. In a meta-analysis of 13 clinical trials, the combined incidence of myocardial infarction or clinical coronary artery disease was less than 1%. All cases occurred in subjects 44-76 years old, all but one were male, and the majority of patients had multiple cardiac risk factors.[40]

1.4.1.7 Hepatic Injury

In the 24-week period constituting the first course of placebo-controlled studies, 1.7% of patients who received alefacept and 1.2% of those receiving placebo experienced alanine aminotransferase (ALT) and/or aspartate aminotransferase (AST) elevations of at least 3 times the upper limit of normal. There have also been post-marketing case reports of asymptomatic transaminase elevation, fatty infiltration of the liver, hepatitis, and severe liver failure.

1.4.1.8 Immunogenicity

Approximately 3% of patients receiving alefacept developed low-titer antibodies to alefacept. No apparent correlation of antibody development and clinical response or adverse events was observed. The long-term immunogenicity of alefacept is unknown.

1.4.2 Intensive Diabetes Management

In this study, all participants will receive intensive diabetes management aimed at achieving near-normal metabolic control per the standard American Diabetes Association (ADA) guidelines.[41] Although intensive diabetes management is recommended for all patients with T1DM, it is not always available to all subjects in the community. The Diabetes Control and Complications Trial (DCCT) research group documented that improved metabolic control slows the onset of some long-term complications but does not eliminate them.[42] The means to achieve this improved control in the DCCT has become the idealized standard of care, with clinical management and education provided by a diabetes specialty team. It should be noted that such care may not necessarily be available to those outside the study, and those who are not seen by a diabetes specialty team may have worse outcomes over time.[43]

Unfortunately, in addition to potential benefits of intensive glycemic control in T1DM, the DCCT studies also showed a significant increased risk of severe, potentially life-threatening hypoglycemia with intensive glycemic control. As such, although there are important advantages of improvements in glycemic control by exogenous insulin, likely there will always continue to be risks associated with T1DM and its management no matter advances in technology.

Improved metabolic control early in the course of T1DM will have a long-standing effect on lowering the risk for long-term complications for many years to follow; i.e., there appears to be a "metabolic memory" that influences later risk. This effect has been documented in the Epidemiology of Diabetes Interventions and Complications (EDIC) study, the long-term follow-up of the DCCT cohort.[42, 44-48] Although the conventional group (with less stringent metabolic control) and the intensive group (with near-normal metabolic control) have had comparable HbA_{1C} levels since the end of the formal DCCT study, the intensive group continues to have significantly lower risk for complications 10 years later.

An additional benefit that may be realized by all participants is that maintaining near-normal glycemic control through intensive diabetes management may, in and of itself, lead to the preservation of ß-cell function.[49-52] The benefits of endogenous insulin secretion, even if one needs to continue exogenous insulin therapy, have been demonstrated in a number of studies, including the DCCT, where those subjects with residual C-peptide had improved metabolic control, with lower risk for severe hypoglycemia and less likelihood of microvascular complications. Finally, the treatment group may have significant benefits from participation even if alefacept

therapy has only modest effects on the preservation of endogenous insulin secretion, which was noted in the new-onset T1DM studies with anti-CD3 mAb therapy.[5, 53]

1.4.3 Potential Risks and Benefits of Trial Participation for Children

The safety and efficacy of alefacept in pediatric patients with moderate to severe psoriasis is currently under study (ClinicalTrials.gov identifier NCT00808223). As discussed in section 1.3.2.2.2, there are also ongoing clinical trials in psoriasis which include participants 16 years old and above, and case series and case reports of children as young as 3 years old, without obvious alefacept-specific adverse events.

There is considerable interest in identifying safe and effective interventions that can modify the course of T1DM in pediatric populations. As one of the most common chronic childhood diseases, T1DM is a particular burden to children and their families. While T1DM can occur into adulthood, the worldwide incidence of T1DM is increasing rapidly in children younger than 15 years, as recently documented by the WHO Diamond Project.[1] During the period 1995–1999, the global annual increase in childhood T1DM was 3.4% but was as high as 5.3% in North America.[1] In Europe, the EURODIAB study group has found the greatest rate of increase in the 0-4 years age group and is predicting a doubling in the number of new cases in children younger than 5 years in the next decade.[2] Overall, prevalence of T1DM in children under age 15 in Europe is predicted to rise from 94,000 in 2005 to 160,000 in 2020.[2] In contrast, the incidence of T1DM in young adults over age 15 is not increasing.[2] Successful preservation of β-cell function in pediatric populations has recently been reported for a variety of treatment modalities, including anti-CD3 antibody (age range: 12–39 years), GAD-alum vaccine (10–18 years), and anti-TNFα (etanercept; 3–18 years).[4, 7-8]

Also of note is that the evaluation of new interventions for T1DM in adults may not be informative about their success in children. It is known that the rate of β-cell decline is different in children versus adults and therefore lack of efficacy of a treatment in adults is not necessarily predictive of efficacy in children.[6] There are currently no approved interventions for new-onset T1DM. In contrast to many interventions currently being studied in the pediatric population, alefacept is an approved drug with an extensive clinical safety record. Alefacept is also unique among agents being studied for new-onset T1DM in that it is an intramuscular injection (as opposed to an intravenous infusion), which has special relevance in the pediatric age group.

2. OBJECTIVES

2.1 PRIMARY OBJECTIVE

The primary objective is to determine whether alefacept will slow the progression of the autoimmune destruction of β cells and lead to the preservation of C-peptide secretion in T1DM.

2.2 SECONDARY OBJECTIVES

Diabetes-related:

- Assess whether alefacept has prolonged clinical efficacy.
- Assess the effect of alefacept on selected secondary clinical outcomes.

Safety:

- Determine the safety of alefacept in participants with T1DM, especially with respect to lymphocyte depletion and opportunistic infections.

2.3 EXPLORATORY OBJECTIVES

Mechanistic:

- Characterize how alefacept alters general and diabetes-specific immune responses.
- Gain a better understanding of the mechanism of action for alefacept in the maintenance of β-cell function and determine whether this drug can reverse the loss of tolerance associated with this disease.

Metabolic:

- Determine whether treatment with alefacept improves glycemic control and reduces individual insulin requirements.

3. STUDY DESIGN

3.1 DESCRIPTION

This trial will be conducted as a multi-center, prospective, double-blind, placebo-controlled, 50-patient, 2:1 randomized, phase II clinical trial for individuals with recent-onset T1DM aged 12−35 years. Participants will receive weekly IM injections of alefacept (15 mg) or placebo for 12 weeks, followed by a 12-week pause before resuming another 12 weeks of dosing, for a total course of 24 weeks of alefacept or placebo.

Prior to initiating the study in the pediatric age group (12−15 years of age), the study drug will be given to an initial safety cohort of adult participants, defined as age 16–35 years old. When the 10th participant in the adult cohort has completed visit 11, or 6 months after the first participant is enrolled, the safety data will be reviewed by the study team and Data Safety and Monitoring Board (DSMB). Enrollment will continue in the adult cohort during this first safety review. Children (ages 12-15) may only be enrolled after completion of a satisfactory safety review of the first cohort of 10 adult participants (16-35) who have completed 12 weeks of treatment. This will require an additional safety review (review 1b) if the initial safety review does not achieve this milestone.

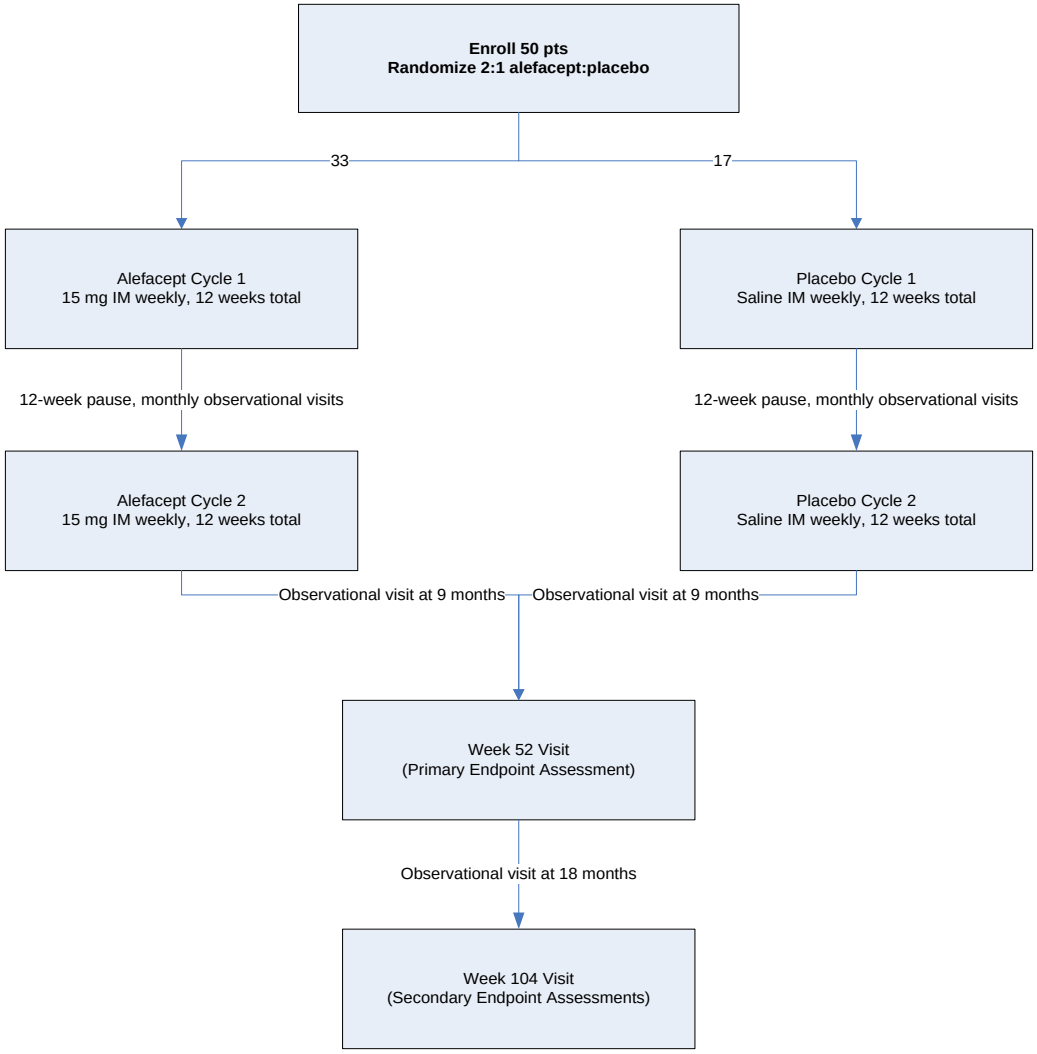

Figure 2. Study design for ITN045AI.

3.2 STUDY DURATION

Total study duration will be approximately 188 weeks (3.5 years):

- The enrollment phase will last up to 68 - 84 weeks (15 -21 months). It is estimated that the first cohort of 10 adult participants (aged 16-35 years) will be enrolled within 6-9 months and the remaining 40 participants (aged 12-35 years) will be enrolled in the ensuing 9-12 months.
- The study participation phase will be 104 weeks (2 years), which includes a treatment phase of 36 weeks and a follow-up phase of 68 weeks.

3.3 STUDY ENDPOINTS

3.3.1 Primary Endpoint

The primary endpoint is an MMTT-stimulated 2-hour C-peptide AUC at week 52.

3.3.2 Secondary Endpoints

Efficacy:

1. MMTT-stimulated peak and 4-hour C-peptide AUC at weeks 52 and 104.
2. MMTT-stimulated 2-hour C-peptide AUC assessed longitudinally at weeks 24, 52 and 104.
3. Insulin use in units per kilogram body weight per day at weeks 52 and 104.
4. Major hypoglycemic events occurring from randomization to weeks 52 and 104.
5. HbA_{1C} levels at weeks 52 and 104.

Safety:

1. Rate of the following adverse events (AEs) in participants receiving alefacept or placebo:
   a. Injection reactions; defined as fever, chills, headache, nausea, vomiting, and injection-site pain.
   b. Hypersensitivity reactions; defined as signs and symptoms of anaphylaxis, wheezing, dyspnea, urticaria, and hypotension.
   c. Evidence of infection with EBV, CMV, or TB*.
2. Frequency and severity of all AEs in participants receiving alefacept or placebo.

* Viral infection or reactivation of EBV or CMV is defined as viral load ≥ 10,000 copies per 10^6 PBMCs or ≥ 10,000 copies per mL whole blood, respectively.[54-55] TB infection will be assessed via tuberculin skin test (PPD).

3.3.3 Exploratory Endpoints

Mechanistic:

1. Immunological assessments will be compared with clinical outcomes to determine whether there is evidence of immune tolerance after receiving alefacept treatment.
2. Immunological assessments will be compared with clinical outcomes to assess whether protective immune responses are affected after receiving alefacept treatment.
3. Immune response to neo-antigens and recall antigens will be assessed.

Metabolic:

1. Proportion of participants in each treatment arm who are exogenous insulin-free for at least 3 months with HbA_{1C} levels less than 6.5% at weeks 52 and 104.
2. Proportion of participants in each treatment arm who achieve a persistent reduction for at least 3 months in insulin dose to less than 0.5 units/kg at weeks 52 and 104.

3.4 RATIONALE FOR SELECTION OF DRUG, ROUTE, DOSE, AND REGIMEN

The dose of 15 mg via the IM route is the FDA-approved adult dose for psoriasis. In addition, 15 mg has been used successfully in case series and case reports in populations less than 18 years of age without unexpected side effects.

The dosing frequency may be altered if an adverse event is encountered as outlined in section 5.2. The maximum allowed length of each treatment cycle will be 12 weeks, i.e., the length of a treatment cycle will not be extended to make up missed doses.

In order to preserve blinding of the study, participants receiving placebo will be given an IM injection of equal volume and appearance to treatment on the same schedule, formulated and prepared by each study site. Placebo will be a saline-containing solution.

3.5 STOPPING RULES

3.5.1 Ongoing Review

The progress of the study will be monitored by the NIAID Autoimmune DSMB, which will review safety data and make recommendations regarding continuation, termination, or modification of the study. Based on a 68-84 week enrollment period and an additional study period of 104 weeks, the DSMB will formally review the safety data at least yearly. The number of participants who discontinue study treatment will also be included in the reports prepared for the DSMB.

In addition, safety data will be reviewed by the DSMB when an event occurs that is of sufficient concern to the NIAID medical monitor, ITN physician, or protocol chair to

warrant review, or when an event occurs that contributes to a stopping rule listed in section 3.5.2.

Prior to opening enrollment to the pediatric population, the study drug will be given to an initial safety cohort of 10 adult participants, defined as age 16–35 years old. When the 10th enrolled participant has reached visit 11, or 6 months after the first participant is enrolled, the safety data will be reviewed by the study team and DSMB. Enrollment of the adult cohort will continue during this 1st safety review. Children (ages 12-15) may only be enrolled after completion of a satisfactory safety review of the first cohort of 10 adult participants (16-35) who have completed 12 weeks of treatment. This will require an additional safety review (review 1b) if the initial safety review does not achieve this milestone. A 2nd safety review will occur after the next 10 participants have enrolled and reached visit 11 or 6 months after the eleventh participant starts treatment, whichever comes first. If needed, review 2b will occur when all of these participants have reached visit 11. The 3rd cohort review will include the next cohort of 10 pediatric subjects (ages 12-15) who have completed 12 weeks of treatment, or at 6 months after the first pediatric subject start treatment, whichever comes first. Similar to the other cohorts, when this entire group of study participants has completed visit 11, a final safety review will be conducted (review 3b). Data on a total of 30 participants will be carefully examined during the course of these sequential reviews. Enrollment of both adult and pediatric participants will continue during these reviews.

Based on the safety data as it emerges and the team or the DSMB feel that it is warranted, further reviews of safety data may be conducted after the initial cohorts complete their second cycle of treatment.

3.5.2 Stopping Rule Guidance

If any of the following events occur, enrollment will be suspended and the DSMB chair will be notified such that a review of safety data will be conducted to determine if enrollment in the study will be stopped and/or administration of investigational study medication should be halted:

1. Any death.
2. One hospital admissions for an unexpected (i.e., not discussed in section 1.4.1) treatment-related adverse event, not related to glycemic event.
3. Three hospital admissions for a treatment-related adverse event, not related to glycemic event.
4. Three of the first 10 participants, or 30% thereafter, require discontinuation of study medication for the same or similar serious adverse event based on MedDRA preferred term or at the discretion of the DAIT Medical Monitor.
5. Five of the first 10 participants, or 50% thereafter, require discontinuation of study medication for any reason.

If considered necessary, the DSMB chair will request an ad-hoc review by the full Board.

4. ELIGIBILITY

4.1 INCLUSION CRITERIA

Patients must meet *all* of the following criteria to be eligible for this study:

1. Males or females aged 12−35 years who meet the ADA standard T1DM criteria.
2. Diagnosis of T1DM within 100 days of enrollment.
3. Positive for at least one diabetes-related autoantibody:
   a. Glutamate decarboxylase (GAD-65);
   b. Insulin, if obtained within 10 days of the onset of exogenous insulin therapy;
   c. IA-2;
   d. ZnT8; *or*
   e. ICA.
4. Peak stimulated C-peptide level > 0.2 pmol/mL following a mixed-meal tolerance test (MMTT).
5. Signed informed consent.

4.2 EXCLUSION CRITERIA

Patients who meet any of the following criteria will *not* be eligible for this study:

1. Severe reaction or anaphylaxis to human monoclonal antibodies.
2. History of malignancy or significant cardiovascular disease (including history of myocardial infarction, angina, use of anti-anginal medicines (e.g., nitroglycerin), or abnormal stress test).
3. History of recent or ongoing uncontrolled bacterial, viral, fungal, or other opportunistic infections.
4. Evidence of infection with HBV (as defined by hepatitis B surface antigen, HBsAg), HCV (anti-HCV antibodies), HIV or toxoplasmosis.
5. Positive tuberculin skin test (PPD).
6. Clinically active infection with EBV, CMV, or tuberculosis; or EBV viral load ≥ 10,000 copies per 10^6 PBMCs or CMV viral load ≥10,000 copies per mL whole blood.
7. Diagnosis of liver disease or hepatic enzymes, as defined by ALT and/or AST ≥ 2 times the upper limit of normal.
8. Prior or current treatment that is known to cause a significant, ongoing change in the course of T1DM or immunologic status, including high-dose inhaled, extensive topical or systemic glucocorticoids.

9. Current or prior (within the last 30 days) use of metformin, sulfonylureas, glinides, thiazolidinediones, exenatide, liraglutide, DPP-IV inhibitors or amylin.
10. Current use of any medication known to influence glucose tolerance (e.g., atypical antipsychotics, diphenylhydantoin, thiazide, or other potassium-depleting diuretics, β-adrenergic blockers, niacin).
11. Any of the following hematologic abnormalities, confirmed by repeat tests at least 1 week apart:
    a. White blood count <3500/μL or >14,000/μL;
    b. $CD4^+$ count below the lower limit of normal;
    c. Platelet count <150,000 /μL; *or*
    d. Hemoglobin <10 g/dL.
12. Females who are pregnant, lactating, or planning on pregnancy during the 2-year study period.
13. History of bone marrow transplantation, or autoimmune disease associated with lymphopenia.
14. Any medical condition that in the opinion of the principal investigator would interfere with safe completion of the trial.
15. Prior participation in a clinical trial that could potentially affect T1DM or immunologic status.
16. Receipt of a live vaccine (e.g., varicella, measles, mumps, rubella, cold-attenuated intranasal influenza vaccine, bacillus Calmette-Guérin, and smallpox) in the 6 weeks before enrollment
17. Participation in an investigational clinical trial within the last six weeks.

4.3 PREMATURE TERMINATION OF A PARTICIPANT FROM THE STUDY

Withdrawal of consent. Participants have a right to withdraw from the study.

Investigator decision. The principal investigator may choose to withdraw a participant from the study for any reason.

Failure to return. Participants who do not return for visits and who do not respond to repeated attempts by the site staff to have them return will be considered *lost to follow-up*.

Participants who prematurely terminate from the study for any reason will be asked to complete all of the assessments listed for the final study visit (visit 30) in Appendix A. Participants who prematurely terminate from the study will not be replaced.

5. STUDY MEDICATIONS

5.1 INVESTIGATIONAL MEDICATION: ALEFACEPT

5.1.1 Formulation and Packaging

Alefacept is supplied as a sterile, white-to-off-white, preservative-free, lyophilized powder for parenteral administration. After reconstitution with 0.6 mL of the supplied Sterile Water for Injection, USP, the solution of alefacept is clear, with a pH of approximately 6.9.

Alefacept is administered via a 0.5 mL intramuscular injection containing 15 mg alefacept. In addition to active drug, this formulation contains 12.5 mg sucrose, 5 mg glycine, 3.6 mg sodium citrate dihydrate, and 0.06 mg citric acid monohydrate.

Astellas Pharma is providing NIAID alefacept for the conduct of this trial. Packing and delivery of alefacept will be managed by a designated drug distributor under contract to NIAID. Alefacept placebo, which consists of saline, will be prepared by the site.

5.1.2 Reconstitution

Alefacept will be reconstituted and administered as per the Amevive® package insert.

5.1.3 Dosage, Preparation, and Administration

Study treatment will be administered for 2 cycles of 12 weeks each, separated by a 12-week pause in treatment. Participants randomized to alefacept will receive an intramuscular injection containing 15 mg of alefacept at each treatment visit, and participants randomized to placebo will receive a normal saline injection of equivalent volume.

Injection sites should be rotated (for example, the deltoid, vastus lateralis, or the gluteus medius muscles) and/or new injections should be given at least 1 inch from an old site avoiding areas where the skin is tender, bruised, red, or hard. The site of injection will be recorded on the eCRF.

Participants should remain at the site for post-injection observation for a minimum of 30 minutes after each of the first four study drug injections. After the fourth study drug injection, it is at the discretion of the investigator to determine whether post-injection observations are still necessary. Drug may be administered prior to other study procedures, except where noted in Appendix 1.

5.1.4 Recommended Storage Conditions

A vial will not be used beyond the expiration date stamped on the carton or vial label, or beyond 4 hours after reconstitution. Alefacept (lyophilized powder) will be refrigerated at 2˚C–8˚C (36˚F–46˚F). The vial will be protected from exposure to light and stored in original carton until time of use.

5.2 DOSE HOLDING AND MANAGEMENT OF ADVERSE EVENTS

Administration of study treatment will be modified as described below in Table 3 for the following adverse events:

Table 4: Management of alefacept-associated adverse events

Adverse event	Action
Lymphopenia $CD4^+$ T cell count < 250 cells/μL	Suspend administration of study treatment and recheck the participant at least weekly. If the $CD4^+$ count reaches ≥250 cells/ μL, dosing may resume at the next expected weekly interval. The overall course of study treatment will not be extended and missed doses will not be administered. If the adverse event does not resolve in 4 weeks, discontinue study treatment permanently. One suspension due to lymphopenia is allowed per cycle of study treatment.
Asymptomatic Hepatic injury ALT and/or AST elevations ≥2 times the upper limit of normal and ≤3 times the upper limit of normal (ULN) ALT and/or AST > 3 times upper limit of normal, with a confirmatory elevated ALT and/or AST assessment will result in permanent discontinuation of study treatment. See Section 5.3 below.	During a Treatment Cycle: Suspend administration of study treatment and recheck the participant within 72 hours, and at least weekly as needed thereafter. If the test value returns to less than 2 times the upper limit of normal, dosing may resume at the next expected weekly interval. The overall course of study treatment will not be extended and missed doses will not be administered. If the adverse event does not resolve in 4 weeks, discontinue study treatment permanently. During the Treatment Pause: Recheck the participant every 2 weeks. The test value must return to <2 times the upper limit of normal before Treatment Cycle 2 can begin. If the elevation/AE does not resolve in 4 weeks, discontinue study treatment permanently. One suspension from study treatment due to elevated ALT and/or AST is allowed during the entire treatment period. If a second elevation occurs that requires a dose to be held, then permanent discontinuation of study medication will occur. See Section 5.3 for further details.
Any adverse event Grade 3 or higher	Suspend administration of study treatment and recheck the participant at least weekly; repeat abnormal labs within 72 hours. If the adverse event reduces to grade 1 or less, dosing may resume at the next expected weekly interval. If the adverse event does not resolve in 4 weeks, discontinue study treatment permanently. The overall course of study treatment will not be extended and missed doses will not be administered. One suspension from study treatment due to a Grade 3 or higher adverse event is allowed during the entire treatment period. If an adverse event meeting this definition occurs during the first treatment cycle, the principal investigator should consult with the NIAID medical monitor, ITN clinical trial physician, and protocol chair prior to administering the second treatment cycle. If clinical and laboratory abnormalities are considered related to T1DM disease activity (e.g., hypoglycemia and hyperglycemia) and study treatment is not considered a contributing factor, administration may not need to be modified. The principal investigator should consult with the NIAID medical monitor, ITN clinical trial physician, and protocol chair if this is encountered.
Infection	Infection is defined as: • Any infection Grade 3 or higher per CTCAE criteria (see section 8.6.1), or, • Any infection less than Grade 3 but otherwise clinically significant per investigator's judgment, or, • Infection or reactivation of EBV or CMV as defined by clinical signs of infection; or if the EBV viral load is ≥ 10,000 copies per 10^6 PBMCs or the CMV viral load is ≥ 10,000 copies per mL whole blood.

Infection	Suspend administration of study treatment and recheck the participant at least weekly; repeat abnormal labs within 72 hours. If the adverse event reduces to grade 1 or less, dosing may resume at the next expected weekly interval. If the adverse event does not resolve in 4 weeks, discontinue study treatment permanently. The overall course of study treatment will not be extended and missed doses will not be administered. One suspension due to infection is allowed per cycle of study treatment.

The adverse events referenced above, if observed, will usually occur within 6–8 weeks of initiating alefacept.

5.3 DISCONTINUATION OF STUDY MEDICATION IN AN INDIVIDUAL PARTICIPANT

Administration of study medication according to study specifications will be *permanently* discontinued in an individual participant if *any* of the following events occur:

- Adverse events discussed in section 5.2 that do not resolve within 4 weeks after temporarily suspending study treatment or are recurrent on repeated administration of study drug.
- Infection requiring inpatient hospitalization or repeated doses of parenteral antibiotics.
- Severe hypersensitivity reactions, including anaphylaxis and angioedema.
- Clinically significant cardiovascular events, including myocardial infarction and coronary artery disorder.
- Asymptomatic hepatic injury, as defined by ALT and/or AST > 3 times upper limit of normal, with a confirmatory elevated ALT and/or AST assessment.
- Pregnancy.
- Other events that require discontinuation of study medication per the clinical judgment of the principal investigator.

If study treatment is discontinued, the NIAID medical monitor should be notified. Further care will be provided according to the judgment and practice of the principal investigator.

If a participant permanently discontinues study treatment, they will be asked to remain in the study and participate in follow-up through the remaining study visits. If the participant does not consent to follow-up visits, the final study visit (visit 30) should be completed.

5.4 ADDITIONAL STUDY MEDICATIONS

5.4.1 Hepatitis A vaccine

Participants who are shown to be serologically naïve at baseline (see section 6.7) will be given the full series of two IM injections of HAVRIX® at visits 27 and 29. Full prescribing information is available at http://us.gsk.com/products/assets/us_havrix.pdf

5.4.1.1 Formulation, Packaging, and Labeling

HAVRIX is a sterile suspension available in the following presentations:

- 0.5 mL single-dose vials and prefilled TIP-LOK® syringes.
- 1.0 mL single-dose vials and prefilled TIP-LOK® syringes.

Each 1-mL adult dose of vaccine contains 1440 EL.U. of viral antigen, adsorbed on 0.5 mg of aluminum as aluminum hydroxide.

Each 0.5-mL pediatric dose of vaccine contains 720 EL.U. of viral antigen, adsorbed onto 0.25 mg of aluminum as aluminum hydroxide.

The pediatric dose will be used for adolescents through age 18.

HAVRIX contains the following excipients: Amino acid supplement (0.3% w/v) in a phosphate-buffered saline solution and polysorbate 20 (0.05 mg/mL). From the manufacturing process, HAVRIX also contains residual MRC-5 cellular proteins (not more than 5 mcg/mL), formalin (not more than 0.1 mg/mL), and neomycin sulfate (not more than 40 ng/mL), an aminoglycoside antibiotic included in the cell growth media.

HAVRIX is formulated without preservatives.

HAVRIX is available in vials and 2 types of prefilled syringes. One type of prefilled syringe has a tip cap which may contain natural rubber latex. The other type has a tip cap and a rubber plunger which contain dry natural latex rubber. The vial stopper does not contain latex.

Store HAVRIX refrigerated between 2° and 8°C (36°and 46°F). Do not freeze. Discard if the vaccine has been frozen. Do not dilute to administer.

5.4.1.2 Preparation, Administration, and Dosage

Shake vial or syringe well before withdrawal and use. Parenteral drug products should be inspected visually for particulate matter and discoloration prior to administration, whenever solution and container permit. If either of these conditions exists, the vaccine should not be administered. With thorough agitation, HAVRIX is a homogenous, turbid, white suspension. Do not administer if it appears otherwise.

Severe allergic reaction (e.g., anaphylaxis) after a previous dose of any hepatitis A-containing vaccine, or to any component of HAVRIX, including neomycin, is a contraindication to administration of HAVRIX. Do not administer the vaccine if participants demonstrate a history of HAV exposure at baseline (see section 6.7).

5.4.2 Tetanus toxoid vaccine

Full prescribing information is available at
https://www.vaccineshoppe.com/image.cfm?doc_id=5976&image_type=product_pdf

5.4.2.1 Formulation, Packaging, and Labeling

Tetanus toxoid adsorbed USP, for intramuscular use, is a sterile suspension of alum-precipitated (aluminum potassium sulfate) toxoid in an isotonic sodium chloride solution containing sodium phosphate buffer to control pH. *Clostridium tetani* culture is grown in a peptone-based medium and detoxified with formaldehyde. The detoxified material is then purified by serial ammonium sulfate fractionation, followed by sterile filtration, and the toxoid is absorbed to aluminum potassium sulfate (alum). The absorbed toxoid is diluted with physiological saline solution (0.85%) and thimerosal (a mercury derivative) is added to a final concentration of 1:10,000. Each 0.5 mL dose is formulated to contain 5 Lf (flocculation units) of tetanus toxoid and not more than 0.25 mg of aluminum. The residual formaldehyde content, by assay, is less than 0.02%.

5.4.2.2 Preparation, Administration, and Dosage

Participants should receive a single IM injection in the deltoid muscle at visit 27 (see Appendix 1). The participant will be observed for 5 minutes after administration.

The tetanus toxoid vaccine should *not* be given to those who have had any of the following:

- A confirmed systemic or neurological reaction to a previous dose of a tetanus toxoid vaccine.
- A confirmed hypersensitivity reaction following a previous dose of a tetanus toxoid vaccine.
- Known hypersensitivity to any component of the tetanus toxoid vaccine.
- Previous splenectomy.
- Current treatment with warfarin.

5.5 CONCOMITANT MEDICATIONS

5.5.1 Required Medications

- Insulin preparations as advised by the principal investigator or the referring physician.

5.5.2 Permitted Medications

There are no medications contraindicated for use with alefacept, so participants may continue to receive medications as needed for standard care. Specific permitted medications that participants may need during the study include:

- Low-dose estrogen oral contraception.
- Acetaminophen and NSAIDs (e.g., ibuprofen and naproxen).
- Diphenhydramine (or equivalent antihistamines).

- Inactivated vaccines other than HAV and tetanus toxoid. HAV and tetanus toxoid is should be given per study schedule, unless deemed clinically necessary by the investigator. Off-schedule vaccination should be reported to the study team.

5.5.3 Prohibited Medications

- Agents that influence insulin sensitivity or secretion (sulfonylureas, metformin, diphenylhydantoin, thiazide, or other potassium-depleting diuretics, beta-adrenergic blockers, niacin).
- Live vaccines (e.g., varicella, measles, mumps, rubella, cold-attenuated intranasal influenza vaccine, bacillus Calmette-Guérin, and smallpox) in the 6 weeks before enrollment, during the treatment period, and within 3 months after completing study treatment. In addition, household contacts should be encouraged not received small pox or oral polio vaccine due to risk of viral shedding.[56]
- Agents that may result in immunosuppression or immunomodulation, including high-dose inhaled, extensive topical or systemic glucocorticoids. Note: A short course of steroids may be used for treatment of a transient condition.

If a participant receives, or if the principal investigator believes that a participant must receive, a prohibited medication, the case must be immediately discussed with the protocol chair, the NIAID medical monitor, and the ITN clinical trial physician. They will determine whether study treatment should continue or be prematurely discontinued. If study treatment is discontinued, the participant should be asked to remain in the trial for follow-up visits (see section 5.3).

The use of prohibited medications must be documented on the source document and eCRF, and a protocol deviation must be submitted for review for the participant to continue study treatment.

5.6 DRUG ACCOUNTABILITY

Under federal regulations (21CFR 312.62), a principal investigator is required to maintain adequate records of the disposition of the investigational product, including the date and quantity of drug that was received, the participants to whom drug was dispensed (participant by participant accounting), and an account of any drug accidentally or deliberately destroyed. The principal investigator will ensure that the investigational product supplies are stored as specified in the protocol and pharmacy manual in a secured area, with access limited to authorized study personnel as described in the clinical study agreement.

Records for receipt, storage, use, and disposition of all study drugs (alefacept or saline, HAV vaccine series, tetanus toxoid vaccine) will be maintained by the study sites. A drug-dispensing log will be kept current for each participant and will contain the identification of each participant and the date and quantity of drug dispensed. All remaining unused investigational product will be returned to the sponsor or sponsor's representative after study termination, or destroyed with the permission of the sponsor in accordance with applicable law and study site procedures. If investigational

product is to be destroyed locally, the principal investigator will provide documentation in accordance with sponsor's specifications.

All records regarding disposition of the investigational product will be available for inspection by the clinical trial monitor.

5.7 ASSESSMENT OF COMPLIANCE WITH STUDY MEDICATION

All study medications will be administered at sites by trained medical staff; compliance, therefore, will be monitored by the site and documented on the eCRF.

6. STUDY PROCEDURES

6.1 INTENSIVE DIABETES MANAGEMENT

During the study, all participants will receive "intensive" management of their diabetes, and HbA_{1C} will be assessed every 3 months (or as per schedule of events) to evaluate metabolic control. The goal of treatment will be to maintain the HbA_{1C} level as close to normal as possible, without frequent occurrence of hypoglycemia. All individuals should strive for targets in accordance with current ADA recommendations, with HbA_{1C} levels of less than 7% in individuals 18 years or age and older, and less than 7.5% in individuals aged 12–17 years; and with preprandial glucose levels of 90–130 mg/dL (plasma), postprandial levels of less than 180 mg/dL, and bedtime levels of 110–150 mg/dL.[57] All participants will be expected to take a sufficient number of daily insulin injections to meet the glycemic targets. In general, the expectation is that all participants will receive at least three injections of insulin daily, including short- and long-acting insulin preparations, or will utilize continuous subcutaneous insulin infusion (CSII insulin pump). Glucose levels should be checked at least four times daily. After reviewing these records, the diabetes management team will contact the treating physician about possible adjustments in the insulin regimen, referral to a registered dietitian, or other approaches that the diabetes management team believes would improve the glucose control if necessary. Records of glucose measurements and communication with the participant will be kept as source documentation. Participants will be contacted by the diabetes educator every 2 weeks between visits to assess their diabetes for the duration of the study (treatment and follow-up periods). In addition, insulin use and hypoglycemic events will be captured at each visit on the appropriate eCRFs. Participants will be required to record the type and amount of insulin they have used during the 5-day period immediately preceding each study visit. Insulin use logs will be provided to participants at each study visit and collected at the next visit. These logs will serve as the source documents.

6.2 VISIT WINDOWS

6.2.1 Scheduled Visits

Appendix A presents the schedule of events for this trial. Visit 0 must occur within 35 days (5 weeks) of visit –1. All other scheduled study visits must occur within the time limits specified below:

Visits 0 through 11 (treatment cycle 1):	±1 day
Visits 12 through 14 (treatment pause):	±3 days
Visits 15 through 26 (treatment cycle 2):	±1 day
Visits 27 through 30 (follow-up period):	±14 days

Target study visit dates should be calculated from the baseline visit (visit 0).

6.2.2 Unscheduled Visits

Unscheduled visits may be performed to follow adverse events as outlined in section 5.2.

6.3 RANDOMIZATION, BLINDING, AND UNBLINDING

6.3.1 Randomization

Participants who sign the informed consent and meet the eligibility criteria will be randomly assigned in a 2:1 ratio at visit 0 to treatment in either the alefacept or placebo group. A central automated system will be used for random assignment and to create a unique identifier for each new study participant. Random assignment will be stratified according to site.

6.3.2 Blinding

Blinding will be maintained for all study participants throughout the study.

For this study, routine lymphocyte subset counts will be reported from the central clinical laboratory in a blinded fashion, as it is expected that alefacept-associated lymphopenia can be unblinding to site staff. However, if the $CD4^+$ T cell count decreases below 250 cells/µL at any visit, this will be communicated to the site as an unblinded absolute value in order for the investigator to modify study treatment as described in section 5.2.

6.3.3 Unblinding

Unblinding before the study is completed will occur only if a participant's well-being is threatened and the principal investigator believes unblinding is necessary to protect the participant. It is not anticipated that emergency unblinding will be required in this trial, as there are no known therapies contraindicated for use with alefacept. If required, emergency management of a participant should be conducted assuming that the participant has been randomized to receive alefacept.

If a principal investigator determines a need where expedited unblinding of a participant is deemed necessary, he/she must confer with the NIAID medical monitor.

The principal investigator will notify the protocol chair of the unblinding event, and the medical monitor will notify the study management team (SMT).

Any unexpected and expedited unblinding will be recorded and reported to the DSMB. A full account of the event will be recorded, including the date, time, and reason for the decision to unblind, as well as the names of the medical monitor and others who were notified of the emergency. During site visits, the site monitor must verify that the medical monitor was notified and that a written account was completed. The reasons for unblinding of a participant's treatment will be included in the final study report.

ITN and NIAID approval is required for unblinding the treatment of an individual participant or subgroups of participants for unplanned interim analyses to support DSMB reviews and final analysis.

An exception to the above rule is that IND Safety Reports will be reported to the FDA, DSMB and IRBs in an unblinded fashion as requested by current FDA guidance.

6.4 GENERAL ASSESSMENTS

- Informed consent: Written informed consent will be obtained from the participant before any study assessments or procedures are performed.
- Eligibility criteria: Eligibility for study participation will be assessed during the screening period.
- Medical history: Medical history will be evaluated during screening to determine if there are any clinically significant diseases, other than the disease under study or medical procedures at baseline.
- Adverse events: Participants will be assessed at all visits for new or worsened events since the previous visit per section 8.5.
- Concomitant medications: All concomitant medications taken throughout the study will be recorded.
- Physical examination: A physical examination will be performed at all visits, including any body system with clinical signs or reported symptoms of adverse events.
- Vital signs: Weight, temperature, blood pressure, respiration, and pulse will be obtained at all visits.

6.5 CLINICAL LABORATORY ASSESSMENTS

6.5.1 Central clinical laboratory assessments:

- Hematology: Includes CBC with differential.
- Lymphocyte subset counts: CD4 and CD8.
- Coagulation tests: PT, PTT.

- Serum chemistry: Includes sodium, potassium, chloride, bicarbonate, BUN, creatinine, AST, ALT, alkaline phosphatase, total bilirubin, direct bilirubin, and glucose.
- Lipid panel: Total cholesterol, HDL, LDL, triglycerides.
- Urinalysis: Includes blood (RBCs), glucose, ketones, pH, protein and specific gravity.
- Infectious disease serology: Serology will be performed at screening for HBV, HCV, HIV, EBV, CMV and toxoplasmosis. Serology will be repeated after screening for CMV and EBV if participants show clinical signs of infection; *or* if the EBV viral load is ≥ 10,000 copies per 10^6 PBMCs or the CMV viral load is ≥ 10,000 copies per mL whole blood.
- Viral load testing: Viral load testing by PCR will be performed for CMV and EBV.
- $HbA_{1C.}$
- C-peptide (as part of MMTT, see section 6.6).
- Plasma glucose (as part of MMTT, see section 6.6).
- Insulin (as part of MMTT, see section 6.6).
- Serum hCG: Serum testing will be repeated after screening for participants with a positive urine hCG result at any study visit.
- Global immunity monitoring: Samples will be obtained to assess quantitative immunoglobulin, C-reactive protein, and complement levels (C3, C4 and CH50).

6.5.2 Local clinical laboratory assessments

- Urine hCG: Urine hCG will be measured to rule out pregnancy in female participants. During the treatment period, this should be performed before study drug administration.
- PPD: Exposure to tuberculosis will be assessed via tuberculin intradermal skin test. The test must be read within 48 to 72 hours after placement. The test will be performed at screening and prior to administration of the second treatment cycle.

6.6 DISEASE-SPECIFIC ASSESSMENTS

- MMTT: A 2-hour MMTT is to be performed at visits 15 and 29; a 4-hour MMTT is to be performed at visits –1 (screening), 28, and 30. See Appendix B for instructions.
- Blood glucose levels: Blood glucose levels will be recorded via glucometer readings (see section 6.1).
- Insulin use: Insulin use will be measured as U/kg body weight/day. Participants should record the type and amount of insulin they have used during the 5-day period immediately preceding each study visit (see section 6.1).

- Hypoglycemia events: Hypoglycemia will be monitored per section 8.2.1.

6.7 MECHANISTIC ASSESSMENTS

See section 7 for detailed discussion of additional mechanistic assays. Planned potential assays include:

- Peripheral blood monocyte (PBMC) assays.
- Flow cytometry panel staining.
- Serum T1DM auto-antibody analysis: Presence of autoantibodies to insulin, GAD65, IA-2, and ZnT8 will be measured throughout the trial. ICA will only be measured at screening for inclusionary purposes.
- Serum studies.
- Whole blood-gene expression profiling.
- DNA-HLA genotypes.
- Antigen recall response:
  - Neo-antigen: Participants who are shown to be serologically naïve at baseline to hepatitis A virus will be vaccinated at visits 27 and 29 with the complete HAVRIX series. Serum titers will be drawn prior to vaccine administration, and at weeks 52, 78 and 104. .
  - Recall antigen: Participants will receive a tetanus vaccination at visit 27. Serum titers will be drawn prior to vaccine administration and 3 months after the injection.

7. TOLERANCE ASSAYS

7.1 RATIONALE

Alefacept, a fusion protein comprised of LFA-3 linked to an Fc protein, binds competitively to the CD2 receptor on the surface of T cells with the LFA-3 portion of the drug and efficiently interferes with LFA-3/CD2 binding and thereby T cell activation. The Fc portion of alefacept engages the immunoglobulin receptor FcγRIII on the surface of natural killer (NK) cells. This bridging of lymphocytes to NK cells should theoretically activate the NK cell to release its cytotoxic granules leading to lymphocyte apoptosis. Since CD2 expression is higher on memory than on naïve T cells, alefacept binds mainly to memory T cells and induces a selective reduction of specific T cell subtypes by apoptosis. Alefacept treatment should therefore inhibit T cell activation while selectively depleting circulating memory T cells. This hypothesis can be tested by phenotyping T cells throughout alefacept treatment.

The selective effect of alefacept on effector-memory T cells is hypothesized to be beneficial for T1D therapy. In animal studies, disruption of the CD2:CD48 pathway can prevent diabetes autoimmunity, prolong allograft acceptance, and promote

immune tolerance.[9-11] (Although the primary ligand for CD2 in humans is CD58/LFA-3, in rodents the primary ligand is CD48.[9-11]) In the BioBreeding rat model of T1DM, anti-CD2 treatment results in long-term protection from spontaneous disease in diabetes-prone rats, induction of diabetes in diabetes-resistant rats, and adoptive transfer of disease.[12] This protection appears to be the result of a selective reduction in a CD4 T cell subpopulation.

As part of the mechanistic assessments, extensive flow panels will be used to determine how T cell sub-sets change with alefacept therapy and if these changes are predictive of improved β-cell function as measured by C-peptide levels. It is anticipated that consistent with the mechanism of action of alefacept, circulating memory T cells ($CD4^+CD45RO^+$ and $CD8^+CD45RO^+$) will be reduced during therapy, thereby decreasing the count of total lymphocytes, whereas the number of naïve T cells ($CD4^+CD45RA^+$ and $CD8^+CD45RA^+$) should remain approximately normal. Alefacept does not have any functional effect on $CD19^+$ B cells or $CD16^+/CD56^+$ NK cells.[26-27] However, NK cell types should be investigated in detail since there is some evidence that NK cell frequency is reduced in patients with recent-onset diabetes in comparison to age matched controls. Functional T and NK cell assays should also be considered since in patients receiving multiple doses of alefacept there is a broader and more sustained therapeutic effect without a cumulative effect on reduction of the total number of lymphocytes or the number of T-cell subsets.[27, 29-30]

7.2 RETENTION OF SAMPLES

Specimens collected in this trial may be used to reevaluate biologic responses as new research tools become available. The specimens will be stored at the ITN sample repository until the end of the ITN contract. Residual specimens may be used by the principal investigators for development of new immunologic assays or for cross trial comparisons. While specimens are described in this protocol in the context of assays to be performed, it should be noted that not necessarily all assays will be performed for all participants at each time point. Decisions to perform assays will be made according to statistical and scientific planning, questions being asked, and current technologies to be utilized. Finally, clinical outcomes will be taken into account to determine the potential value of the assays. For example, if a significant clinical effect fails to occur, the assays performed by the ITN may be minimal.

7.3 CELL ASSAYS

Blood samples will be collected at the sites and shipped to a central ITN core lab for PBMCs to be isolated and frozen prior to assay. Frozen cells will be stored in liquid nitrogen vapor at a central repository.

7.3.1 Functional Assays

The functional status of lymphocyte subsets, specifically T/B cells, as well as mast cells and monocytes, can be assessed in various cell-based assays. Inflammatory and TCR-driven autoantigen-specific stimulations need to be examined to fully evaluate

the effect of alefacept on immunological responses. T-cell assessments likely will include those measuring both the number and function of T cells. These measures may include the use of class I and class II tetramers to enumerate antigen-specific T cells. Additionally, in vitro cell culture techniques may be utilized to study T cell functionality. Readouts may include ELISPOT and intracellular staining technology for cytokine determination.

Additional studies may be done to study the function of regulatory T cells (Tregs) in these treated participants. Ag specific T cell proliferation could be measured using either carboxyfluorescein diacetate succinimidyl ester (CSFE) or ^{3}[H]-thymidine assays. Harvested blood cells could be separated by flow-based sorting using CD4, CD25 and CD127 antibodies (or magnetic bead separation) and assayed for their ability to suppress polyclonal T-cell responses. Follow-up experiments may include examination of Treg cell function as well as examination of responses to autoantigens such as GAD, proinsulin, IA-2 and insulin. Several assays can address responses to autoantigens implicated in T1DM. Secretion of various cytokines can be determined, thus enabling characterization of overall phenotype (Th1/Th17/Th2/Treg) of T-cell responses, which might indicate skewing towards a more regulatory phenotype during alefacept treatment.

These assays will be used to test the hypothesis that alefacept treatment tips the balance of T cell responses from effector-memory to regulatory responses, thus triggering beneficial changes in T cell-directed autoimmunity.

7.3.2 WHOLE BLOOD DNA METHYLATION STUDIES

Regulatory T cells play an important role in the control of autoimmunity and maintenance of transplantation tolerance. Tregs can be detected using flow cytometry with antibody markers for CD25 (part of the high-affinity IL-2 receptor) and intracellular staining for the transcription factor FOXP3 (a “master” regulator for the development and suppressor function of Tregs). However, CD25 by itself is a relatively non-specific activation marker and FOXP3 is transiently expressed in activated non-regulatory effector T cells. Therefore, using these 2 markers in flow cytometry detects a mixture of activated conventional T cells and natural Tregs. Recent publications have demonstrated that stable expression of FOXP3 in naturally occurring Tregs is controlled by an epigenetic mechanism, i.e., DNA methylation.[58] Demethylation at a highly conserved region within the FOXP3 gene was found to be restricted to natural Tregs when tested against all major peripheral blood types and a selection of non-blood cells.[58] For this study, we intend to use a commercial quantitative real-time PCR-based methylation assay to enable the specific and sensitive determination of Treg numbers by measuring demethylated FOXP3 (e.g. Epiontis GMBH, (www.epiontis.com). By using the same technology to quantify CD3 positive cells, it is possible to calculate Tregs as a percentage of total T cells.

7.3.3 Flow Cytometry

Flow cytometry will be performed on blood samples obtained throughout this trial. Multiple flow cytometry panels will be utilized to examine changes in expression of various molecules, both surface and intracellular, during patient treatment. Cell populations of interest include Treg cells expressing FOXP3 (data that will be complementary to FOXP3 methylation studies), CD4 and CD8 effector-memory T cells ($CD4^+CD45RO^+$ and $CD8^+CD45RO^+$) vs. naïve T cells ($CD45RA^+$). Circulating effector T cells may also be assessed. These flow assays can be combined with the use of Class I and Class II tetramers to enumerate T cells specific for antigens implicated in the pathogenesis of T1DM. For example, HLA-A2 tetramers that enable detection of CD8 cells specific for the following antigenic peptides could be used: GAD 65 114–123, preproinsulin 2-10, IGRP 228–236, insulin B chain 10-18, and insulin A chain 1-10. In combination with ex vivo polyclonal activation with agents like phorbol-12-myristate-13-acetate (PMA) and ionomycin, it would be possible to also address cytokine production profiles of these cells along with expression of costimulatory and activation molecules.

7.4 SERUM ASSAYS

7.4.1 Autoantibody Analysis

Key markers for the presence of the autoimmune processes directed against pancreatic islets include assessing the presence and titers of anti-GAD65, anti-insulin, anti-ICA512/IA-2, and anti-ICA autoantibodies. Detection of these autoantibody combinations has proven to be an accurate predictor of T1DM in several natural history studies. In the DPT-1 prevention study,[59] over half of the individuals who were positive for two, or more, of these antibodies progressed to full disease. Shifts in the titers of Ig isotypes may indicate a change in the type of T-helper cell responses to autoantigen. For example, increases in titers of anti-GAD IgE, IgG2, or IgG4 antibodies could indicate a shift to a more regulatory-type cytokine profile following drug administration. In summary, this study will test the hypothesis that successful treatment will be associated with a reduction in the titer or isotype of diabetes-related autoantibodies.

7.4.2 Serum Archive

Patient serum will be archived for future studies. These studies might include measurements of cytokines and subsequent correlation with induction of clinical tolerance. The archived serum samples could potentially be used for analysis of immune and inflammatory molecules at the proteome and transcriptome levels. In addition, expression of pro-inflammatory cytokines (IL-1β, IL-6, IL-12, TNFα), molecules that are induced by inflammation (SOCS1, 2, 3 and acute phase reactants (e.g., ceruloplasmin, SAA, and CRP) and anti-inflammatory cytokines (TGFß, IL-10) could also be assessed to determine the effect of alefacept treatment.

7.4.3 Serum Neoantigen & Recall Responses

The immune response to hepatitis A immunization in normal immunocompetent individuals is well characterized. Therefore, immunization with hepatitis A can be used to measure the responses of participants to neoantigens after administration of alefacept and compare their responses with those of normal individuals. If participants have not received the hepatitis A vaccine prior to study entry, the first vaccine dose will be administered after the end of alefacept treatment (9 months after visit 0) and serum will be collected for measuring titers of hepatitis A-specific antibodies prior to vaccine administration, and at weeks 52, 78 and 104.

As alefacept affects effector-memory T cell function and indirectly may affect B cell function, we will also evaluate the possible change over time in response to previous immunization with tetanus toxoid. Tetanus titers will be obtained at the end of alefacept treatment (9 months after visit 0) and a rechallenge will be administered. Antibody titers to the tetanus toxoid will be collected prior to vaccine administration and assessed three months later at week 52.

7.5 WHOLE BLOOD ASSAYS

7.5.1 Gene Expression Profiling

To further elucidate possible changes in cytokine and cellular profiles, gene-expression profiling analysis may be performed on RNA isolated from peripheral blood using microarray or high-throughput real-time polymerase chain reaction (RT-PCR) methods. RT-PCR, which is generally more sensitive than microarray analysis, could be used to compare the expression of genes previously reported to play a role in T1DM, such as IFN-γ, TGF-β, IL-4, t-bet, RORγt, FOXP3, STAT molecules, IL-2, IL-5, IL-13, IL-15, IL-21, IL-23 and IL-25. Microarrays have the advantage of allowing genome wide analysis, but may lack the sensitivity to identify gene changes in a small sub-set of cells without cell sorting or selection prior to RNA extraction. The goal of these assays is to identify differences between a tolerant versus non-tolerant state and to find new genes that could serve as potential markers of disease progression. These types of analyses may also explain why some individuals respond better to this treatment or elucidate mechanisms resulting in adverse responses to treatment. This assay has proven informative in characterizing unique genes that determine the clinical course in systemic lupus erythematosus, and preliminary studies with this technology have enabled us to determine a distinct subset of genes that are either up- or down-regulated in those with new-onset T1DM as opposed to controls in other studies.

7.5.2 DNA–HLA Genotypes

DNA collected from participants will be used to perform sequence-based HLA typing. A complete class I and class II haplotype will be performed, including fine typing of the DQB and DRB regions. Genotyping for single nucleotide polymorphisms (SNPs) in selected immune response genes may also be performed. The results of genotype

analyses might be used to correlate with disease progression and therapeutic responses (tolerance induction).

8. ADVERSE EVENTS

8.1 OVERVIEW

The principal investigator is responsible for the detection and documentation of events meeting the criteria and definition of an AE (adverse event) or SAE (serious adverse event) as described in sections 8.3 and 8.4 in this protocol. All AEs and SAEs will be recorded in the source documents and on the appropriate electronic CRF(s). All data will be reviewed periodically by the DSMB, which may provide recommendations to NIAID about withdrawing any participant and/or terminating the study because of safety concerns.

Adverse events that are classified as serious according to the definition of health authorities must be reported promptly and appropriately to the NIAID, ITN, principal investigators in the trial, IRBs, and health authorities. This section defines the types of AEs and outlines the procedures for appropriately collecting, grading, recording, and reporting them. Information in this section complies with 21CFR 312; ICH Guideline E2A*: Clinical Safety Data Management: Definitions and Standards for Expedited Reporting*; and ICH Guideline E-6: *Guidelines for Good Clinical Practice*; and applies the standards set forth in the National Cancer Institute (NCI), *Common Terminology Criteria for Adverse Events, Version 4.0* (May 28, 2009).

8.2 DEFINITIONS

8.2.1 Disease-Specific Adverse Event

For the purposes of this study, *major hypoglycemia events* will be recorded in the eCRF. Events are defined as:

- glucose concentration < 55 mg/dL (grades 2–5, NCI-CTCAE version 4.0), or
- clinically: involving seizure or loss of consciousness (coma), or requiring assistance from another individual in order to recover.

All episodes of hypoglycemia that require hospitalization and/or emergency care will be reported to the DSMB in an expedited manner as described in section 8.7.3.

8.3 ADVERSE EVENT

An AE is any occurrence or worsening of an undesirable or unintended sign, symptom, laboratory finding, or disease that occurs during participation in the trial. An AE will be followed until it resolves or until 30 days after a participant terminates from the study, whichever comes first. All AEs will be reported as specified in section 8.5.2.1 whether they are or are not related to disease progression or study participation.

8.3.1 Adverse Reaction and Suspected Adverse Reaction

An adverse reaction means any adverse event caused by a drug. Adverse reactions are a subset of all suspected adverse reactions for which there is reason to conclude that the drug caused the event.

Suspected adverse reaction (SAR) 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 adverse reaction, which means any adverse event caused by a drug (21 CFR 312.32(a)).

8.4 SERIOUS ADVERSE EVENT

An AE or SAR is considered "serious" if, in the view of either the investigator or DAIT/NIAID it results in any of the following outcomes (21 CFR 312.32(a)):

- Death: A death that occurs during the study or that comes to the attention of the principal investigator during the protocol-defined follow-up period must be reported whether it is considered treatment related or not.
- A life-threatening event: An AE or SAR is considered "life-threatening" if, in the view of either the investigator or DAIT/NIAID, its occurrence places the subject at immediate risk of death. It does not include an AE or SAR that, had it occurred in a more severe form, might have caused death.
- Inpatient hospitalization or prolongation of existing hospitalization.
- Persistent or significant incapacity or substantial disruption of the ability to conduct normal life functions
- An event that requires intervention to prevent permanent impairment or damage. An important medical event that may not result in death, be life threatening, or require hospitalization may be considered serious when, based upon appropriate medical judgment, it may jeopardize the participant and may require medical or surgical intervention to prevent one of the outcomes listed above.
- Congenital anomaly or birth defect.

8.4.1 Unexpected Adverse Reaction

A SAR is considered "unexpected" if it is not listed in the investigator brochure or is not listed at the specificity, or severity that has been observed (21 CFR 312.32(a)).

8.5 COLLECTING AND RECORDING ADVERSE EVENTS

8.5.1 Methods of Collection

Adverse events will be collected from the time the participant signs the informed consent until the time an event is resolved or until 30 days after the participant completes or terminates from the study, whichever comes first.

Adverse events may be collected as follows:

- Observing the participant.
- Questioning the participant in an objective manner.
- Receiving an unsolicited complaint from the participant.

An adverse event that is an asymptomatic abnormal value or result from a clinical or laboratory evaluation (e.g., a radiograph, an ultrasound, or an electrocardiogram) will be documented and maintained in the source records. With the exception of any asymptomatic elevation of AST or ALT $\geq$ 2 times the upper limit of normal which must be recorded on the appropriate AE form, other asymptomatic adverse events must be recorded on the appropriate AE form when they meet the criteria for a Grade 3 or greater AE per CTCAE criteria. The evaluation that produced the value or result should be repeated until that value or result returns to normal or can be explained and the participant's safety is not at risk.

8.5.2 Recording Method

8.5.2.1 Adverse Events

Throughout the study, the principal investigator will record AEs on the appropriate eCRF, except as noted in Section 8.5.1 above, regardless of their severity or relation to study participation. The principal investigator will treat participants experiencing AEs appropriately and observe them at suitable intervals until their symptoms resolve or their status stabilizes.

8.5.2.2 Serious Adverse Events

Serious AEs will be recorded on the AE eCRF and on the SAE eCRF, and health authorities will be notified as outlined in section 8.7.2.

8.6 GRADING AND ATTRIBUTION OF ADVERSE EVENTS

8.6.1 Grading Criteria

The study site will grade the severity of AEs experienced by study participants according to the criteria set forth in the National Cancer Institute's *Common Terminology Criteria for Adverse Events Version 4.0* (published May 28, 2009). This document (referred to herein as the "NCI-CTCAE manual") provides a common language to describe levels of severity, to analyze and interpret data, and to articulate the clinical significance of all AEs.

Adverse events will be graded on a scale from 1 to 5 according to the following standards in the NCI-CTCAE manual:

- Grade 1 = mild adverse event.
- Grade 2 = moderate adverse event.
- Grade 3 = severe and undesirable adverse event.
- Grade 4 = life-threatening or disabling adverse event.
- Grade 5 = death.

For additional information and a printable version of the NCI-CTCAE manual, go to http://ctep.cancer.gov/reporting/ctc.html.

8.6.2 Attribution Definitions

Adverse events will be categorized for their relation to alefacept. The principal investigator will do the initial determination of the relation, or attribution, of an AE to study participation and will record the initial determination on the appropriate eCRF and/or SAE reporting form. The relation of an AE to study participation will be determined using definitions in Table 5. Final determination of attribution for safety reporting will be decided by DAIT/NIAID.

Table 5. Attribution of adverse events

Code	Descriptor	Relationship (to primary investigational product and/or other concurrent mandated study therapy)
Unrelated Categories		
1	Unrelated	The adverse event is clearly not related.
2	Unlikely	The adverse event is unlikely related.
Related Categories		
3	Possible	The adverse event has a reasonable possibility to be related; there is evidence to suggest a causal relationship.
4	Probable	The adverse event is likely related.
5	Definite	The adverse event is clearly related.

8.7 REPORTING SERIOUS ADVERSE EVENTS

8.7.1 Reporting SAEs to the IND Sponsor

The following process for reporting an SAE ensures compliance with 21CFR 312 and ICH guidelines. After learning that a participant has experienced an SAE, the principal investigator or designee will report the SAE via the electronic SAE report form (SAE eCRF) within 24 hours of becoming aware of the event. Initial SAE eCRF

should include as much information as possible, but at a minimum must include the following:

- AE term.
- Study drug.
- Relationship to study medications.
- Reason why the event is serious.
- Supplementary eCRF pages that are current at the time of SAE reporting: medical history, concomitant medications, demographics, study drug administration, death.

As additional details become available, the SAE eCRF should be updated and submitted. Every time the SAE eCRF is submitted, it should be electronically signed by the principal investigator or subinvestigator.

For additional information regarding SAE reporting, contact Rho Product Safety:

Rho Product Safety
6330 Quadrangle Drive, Suite 500
Chapel Hill, NC 27517
Toll-free: 1-888-746-7231
SAE Fax Line: 1-888-746-3293
Email: rho_productsafety@rhoworld.com

8.7.2 Reporting SAEs to Health Authorities

After the SAE has been reported by the principal investigator and assessed by the IND sponsor, the IND sponsor must report an event to the appropriate health authorities using one of these two options:

- **Standard reporting (report in the IND annual report).** This option applies if the AE is classified as one of the following:
  - Serious, expected, suspected adverse reactions (see Section *8.3.1, Adverse and Suspected Adverse Reactions* and *Section 8.4.1, Unexpected Adverse Reaction).*
  - Serious and not a suspected adverse reaction (see *Section 8.3.1, Adverse and Suspected Adverse Reactions*).
- **Expedited Safety Report.** This option applies if the adverse event is classified as one of the following:
  - Serious and unexpected suspected adverse reaction (see *Section 8.3.1, Adverse and Suspected Adverse Reactions* and Section *8.4.1, Unexpected Adverse Reaction).*
  - Aggregate analysis of serious adverse events that suggests a causal relationship to the study drug.

- Any findings from clinical or epidemiological studies, analysis of data pooled across multiple studies, published or unpublished scientific papers, or from animal or in vitro testing that would result in a safety-related change in the protocol, informed consent, investigator brochure or other aspects of the overall conduct of the trial will be reported.

- Safety Reports must be reported by DAIT/NIAID to the appropriate health authorities within 15 calendar days; fatal or immediately life-threatening serious, unexpected, suspected adverse reactions must be reported within 7 calendar days.

All principal investigators must report SAEs to their respective IRBs as mandated by them.

8.7.3 Reporting SAEs to the DSMB

The NIAID and ITN will provide the DSMB with data for all SAEs on an ongoing basis and as indicated in section 3.5.2.

Major hypoglycemic events that require emergency care will also be reported to the DSMB in an expedited fashion regardless of whether they met the criteria for an SAE or not.

8.7.4 Reporting Pregnancy

The principal investigator should be informed immediately of any pregnancy and all available pregnancy information should be entered into the electronic data capture (EDC) system within 24 hours of becoming aware of the event. The principal investigator should counsel the participant and discuss the risks of continuing with the pregnancy and the possible effects on the fetus. Monitoring of the participant should continue until the conclusion of the pregnancy. Follow-up information detailing the outcome of the pregnancy should be entered into the EDC system as it becomes available. Any premature termination of the pregnancy will be reported.

Pregnancies are tracked as SAEs for tracking purposes only. Any pregnancy complication or elective termination of a pregnancy for medical reasons will be recorded as an AE or SAE as described in sections 8.3 and 8.4, respectively.

9. STATISTICAL CONSIDERATIONS AND ANALYTICAL PLAN

9.1 ANALYSIS SAMPLES

Intent to treat (ITT) sample will include all randomized participants who received any dose of study treatment. The efficacy analyses will be based on ITT sample according to the group to which the participants are assigned.

Per protocol (PP) samples will be defined as all participants who are part of the ITT sample, completed at least 18 of the 24 (75%) expected weekly injections of study treatment, adhered to major protocol requirements, and completed the 52-week visit.

Safety sample (SS) will be defined as all participants who received any degree of study treatment. Safety analyses will be based on actual treatment the participants receive.

9.2 ANALYSIS OF ENDPOINTS

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

Primary analysis of treatment effect will be conducted under the ITT principle of eligible patients, whereby outcome data from all patients in the ITT population will be included regardless of treatment compliance or duration of study participation.

9.2.1 Primary Endpoint

The primary analysis of the primary endpoint, MMTT-stimulated 2-hour C-peptide AUC at 52 weeks, will test the null hypothesis of "no treatment group difference" versus the two-sided alternative using the ITT sample. The hypothesis test and baseline-adjusted treatment group estimates will be derived from an ANCOVA model fit to the transformed 2-hour C-peptide AUC response at 52 weeks, ln ($AUC_{2hr,\ 52wk}$ $^{+}$1), with fixed effects for treatment group and baseline transformed 2-hour C-peptide, ln ($AUC_{2hr,\ baseline}$ $^{+}$1).

For a participant who misses the entire week 52 MMTT assessment, we will impute the missing AUC values under a worst-case scenario for the primary ITT analysis using the results from the participant's randomized treatment group. For each participant with both baseline and week 52 data, we will calculate the percent change from baseline for AUC. To impute missing week 52 AUC values for participants in the active therapy group, we will assume a worst-case scenario where percent change from baseline is taken as the greatest observed percent decrease from baseline (GPD) among all subjects in the active therapy group who have available data. Then, the imputed AUC for participants in the active therapy group is given as

$$AUC_{52wk\_i,\ imputed} = AUC_{\_baseline,i} * (100^{+} GPD_{active\ arm})/100$$

To impute missing week 52 AUC values for participants in the placebo group, we will assume a worst case scenario where percent change from baseline is taken as the smallest observed percent decrease from baseline (SPD) among all participants in the placebo arm who have available data. Then, the imputed AUC for participants in the placebo group is given as

$$AUC_{52wk\_i,\ imputed} = AUC_{\_baseline,i} * (100^{+} SPD_{placebo\ arm})/100$$

Therefore, the imputed AUC value for a participant who misses the entire week 52 MMTT assessment will be the value that results in a percent change from baseline

equivalent to the worst-case scenario in their treatment group. Sensitivity analyses will be included as secondary analyses. These will include:

- Using imputed data as described above but using "best cases" scenario values for missing week 52 MMTT assessments. That is, for participants in the alefacept group, values will be imputed using the smallest observed percent decrease; for participants in the placebo group, values will be imputed using the greatest percent decrease.
- Using imputed data as described above but using "best guess" scenario values for missing week 52 MMTT assessments. That is, for each treatment group, values will be imputed using the median within-group percent change.
- Using observed data only (i.e. without imputing any missing data)

Note that, we do not plan to use the last-observation-carried-forward approach for imputing missing week 52 values. Since endogenous insulin production is known to decrease over the first year in patients with newly diagnosed T1DM, carrying the last observation forward is inconsistent with known biology in the placebo group and could overestimate the treatment effect in the alefacept group.

Additional analyses on the primary endpoint will include a sensitivity analysis to assess the impact of unblinding due to low CD4 counts, an analogous ANCOVA model fit to the PP sample, as well as separate ANCOVA models including covariates such as age, sex, baseline HbA_{1C}, and the baseline mean 2-hour C-peptide $\ln(AUC_{2hr,\ baseline}{}^{+}1)$.

Statistical presentation and analysis of all patient data will be further defined in the statistical analysis plan (SAP).

9.2.2 Secondary Endpoints

The null hypothesis proposes that there is no difference in the secondary endpoint (measured either as means or proportions) between study groups. The alternative hypothesis proposes the opposite; that there is a difference in the secondary endpoints between the treatment and control groups.

- An analysis similar to the analysis of the primary endpoint will be performed on the corresponding peak and 4-hour C-peptide AUC at weeks 52 and 104.
- A linear mixed model analysis will be used to evaluate treatment group differences over time for 2-hour C-peptide AUC from baseline through weeks 24, 52, and 104.
- ANCOVA model adjusted for baseline will be used to analyze insulin use and HbA_{1C} level at weeks 52 and 104.
- Fisher's exact test will be used to compare the proportion of participants with a major hypoglycemic event between the two groups at weeks 52 and 104.

9.2.3 Exploratory Endpoints

- A linear mixed model analysis will be used to evaluate the impact of total dose on treatment group differences over time for 2-hour C-peptide AUC from baseline through week 104.
- A mixed effects models will be used to compare the treatment group and the control group for C-peptide in response to MMTT (4 hour AUC, peak), HbA1C, required dose of insulin and major hypoglycemia events over time from baseline through week 104.
- Fisher's exact test will be used to compare proportions of participants who are exogenous insulin free with HbA_{1C} less than or equal to 6.5 between the two groups at weeks 52 and 104.
- Fisher's exact test will be used to compare proportions of participants who achieve a persistent reduction for at least 3 months in insulin dose to less than 0.5 units/kg between the two groups at weeks 52 and 104.

9.2.4 Safety Analysis

The safety analyses will be performed using the safety sample. Safety parameters presented for each treatment group will include summaries of AEs, changes in vital signs, and changes in laboratory values between baseline and end of study.

Adverse events will be coded using the MedDRA dictionary. The number of events and number (%) of participants experiencing AEs will be summarized by system organ class and preferred term for each treatment group and overall. In addition, AEs will also be summarized by a maximum severity and relationship to study drug for each treatment group, system organ class, and preferred term.

Separate summaries will be provided for SAEs and AEs leading to study discontinuation.

9.2.5 Medical History

Medical history within the past 12 months—including the existence of current signs and symptoms—will be collected for each body system.

9.2.6 Use of Medications

All medications taken by or administered to study participants beginning 30 days before enrollment and continuing throughout the study will be collected. All medications used will be coded according to the WHO drug dictionary. The number and percentage of participants receiving prior and concomitant medications/therapies will be presented overall and by medication class.

9.3 SAMPLE SIZE

To derive the sample size for this study, we used the methods and control group estimates for 2-hr C-peptide AUC at 12 months presented in a white paper written by Lachin and McGee, who are members of the TrialNet Coordinating Center.[60] They

derived control group estimates from two new-onset T1DM studies with primary endpoints and populations similar to those proposed for this study.

Based on these studies, the 52 week geometric mean 2-hour C-peptide AUC (pmol/mL/min) in the control group is assumed to be 0.384. For analysis, the transformation $\ln(AUC^{+}1)$ is applied to allow for log transformation of C-peptide AUC values close to 0. After transformation, the $\ln(AUC^{+}1)$ value in the control group is $\ln(0.384^{+}1) = 0.325$ with root mean square error (RMSE) = 0.154.

Using a method similar to that used by Lachin et al,[61] if the 52 week C-peptide result is 50% better in the alefacept group, then the AUC for the alefacept group would be $1.50 \times 0.384 = 0.576$. Then on the transformed scale, the $\ln(AUC^{+}1)$ value in the alefacept group is given by $(\ln[0.576^{+}1]) = 0.455$.

The study was originally designed to detect a 50% improvement in the alefacept group compared to controls. With 2:1 alefacept versus placebo random assignment and a two-sided t-test with significance level of 5%, a sample size of 66 provided 85% power allowing for a 10% dropout rate. As noted in Section 1.2, however, enrollment for this trial has been reduced to 50. At this reduced size, the study will still have at least 80% power if % improvement in the alefacept group is at least 55% (allowing for 10% dropout).

9.4 INTERIM ANALYSIS

The DSMB will receive periodic safety reports on enrolled participants along with all study treatment discontinuation cases. The DSMB may request modifications to the protocol based on its review of the findings.

No formal interim analyses are planned for efficacy or futility. However, the primary efficacy analysis of 2-hour C-peptide AUC in response to an MMTT at week 52 will be performed upon completion of data collection and verification for all week 52 assessments (prior to end of study). Because the primary efficacy analyses will be performed only once, no adjustment to account for multiple comparisons is required. Treatment assignments for individual subjects will not be unmasked at the time of the primary analysis. Because the secondary HbA1c and C-peptide endpoints are derived from laboratory results rather than subjective clinical assessments, the release of the primary endpoint results prior to completion of the study is expected to have minimal impact on the validity of the secondary endpoint analyses.

9.5 REPORTING DEVIATIONS FROM THE ORIGINAL STATISTICAL PLAN

The principal features of both the study design and the plan for statistical data analysis are outlined in this protocol and in the statistical analysis plan (SAP). Any change in these features requires either a protocol or an SAP amendment, which is subject to review by the DSMB, the study sponsor(s), and the health authorities. These changes will be described in the final study report as appropriate.

10. ACCESS TO SOURCE DATA/DOCUMENTS

The investigational sites participating in this study will maintain the highest degree of confidentiality permitted for the clinical and research information obtained from participants in this clinical trial. Medical and research records should 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 sites must permit authorized representatives of the ITN, sponsor, and health authorities to examine (and to copy when required by applicable law) clinical 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 (and any personally identifying information must be obscured). Authorized representatives as noted above are bound to maintain the strict confidentiality of medical and research information that may be linked to identified individuals. The investigational sites will normally be notified in advance of auditing visits.

11. QUALITY CONTROL AND QUALITY ASSURANCE

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

The sponsor is responsible for regularly reviewing the conduct of the trial, for verifying adherence to the protocol, and for confirming the completeness, consistency, and accuracy of all documented data.

The eCRFs will be completed online via a web-based electronic data capture (EDC). Some data requirements will be addressed outside the EDC using SAS® software. Data queries will be issued and resolved within the EDC system or SAS®.

Study staff will enter data from a study visit on the relevant eCRFs within 3 days following the visit or the time when data become available.

12. ETHICAL CONSIDERATIONS AND COMPLIANCE WITH GOOD CLINICAL PRACTICE

12.1 STATEMENT OF COMPLIANCE

This trial will be conducted in compliance with the protocol, current Good Clinical Practice (GCP) guidelines—adopting the principles of the Declaration of Helsinki—and all applicable regulatory requirements.

Prior to study initiation, the protocol and the informed consent documents will be reviewed and approved by the sponsor and an appropriate ethics review committee or institutional review board (IRB). Any amendments to the protocol or consent materials must also be approved by the Sponsor, the IRB and submitted to FDA before they are implemented.

12.2 INFORMED CONSENT

The informed consent form is a means of providing information about the trial to a prospective participant and allows for an informed decision about participation in the study. All participants (or their legally acceptable representative) must read, sign, and date a consent form before participating in the study, taking the study drug, and/or undergoing any study-specific procedures. If a participant does not speak and read English, the consent materials must be translated into the appropriate language.

The informed consent form must be updated or revised whenever important new safety information is available, whenever the protocol is amended, and/or whenever any new information becomes available that may affect participation in the trial.

A copy of the informed consent will be given to a prospective participant for review. A study investigator, in the presence of a witness, will review the consent and answer questions. The participant will be informed that participation is voluntary and that he/she may withdraw from the study at any time, for any reason.

12.3 PRIVACY AND CONFIDENTIALITY

A participant's privacy and confidentiality will be respected throughout the study. Each participant will be assigned a sequential identification number. This number, rather than the participant's name, will be used to collect, store, and report participant information.

13. PUBLICATION POLICY

The ITN policy on publication of study results will apply to this study. Authorized participants may find details regarding the policy statement on the ITN internet website at http://www.immunetolerance.org.

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Appendix 1. Schedule of Events

		Treatment Cycle 1												Treatment Pause			Treatment Cycle 2													Follow-Up Period			
Week	**≤-5**	**0**	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**15**	**19**	**23**	**24**	**25**	**26**	**27**	**28**	**29**	**30**	**31**	**32**	**33**	**34**	**35**	**40**	**52**	**78**	**104**
Visit	**-1**	**0**	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**	**16**	**17**	**18**	**19**	**20**	**21**	**22**	**23**	**24**	**25**	**26**	**27**	**28**	**29**	**30**[1]
GENERAL ASSESSMENTS																																
Informed consent	x																															
Eligibility criteria	x																															
Randomization		x																														
Medical history	x																															
Adverse events	x	x	x	x	x	x	x	x	x	x	x	x	x	x	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	x	x	x	x	x	x	x	x	x	x	x	x	x
Physical examination	x	x	x	x	x	x	x	x	x	x	x	x	x	x	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	x	x	x	x	x	x	x	x	x	x	x	x	x
CLINICAL LABORATORY ASSESSMENTS																																
CBC with differential[2]	x	x	x		x		x		x		x		x	x	x	x	x	x		x		x		x		x		x	x	x	x	x
Lymphocyte subset counts2	x	x	x		x		x		x		x		x	x	x	x	x	x		x		x		x		x		x	x	x	x	x
Coagulation tests	x	x											x				x												x		x	x	x
Serum chemistry2	x	x	x		x		x		x		x		x	x	x	x	x	x		x		x		x		x		x	x	x	x	x
Lipid panel		x											x				x												x		x	x	x
Urinalysis	x	x											x				x												x		x	x	x
Infectious disease serology[3]	x																															
Viral load testing (PCR)	x																x														x	x	x
HbA_{1c}	x	x			x				x					x	x		x			x				x					x	x	x	x	x
C-peptide	x																x														x	x	x
Plasma glucose	x																x														x	x	x
Serum hCG[4]	x																															
Urine hCG	x	x	x		x		x		x		x		x	x	x	x	x	x		x		x		x		x		x	x	x	x	x
PPD	x															x																
Global immunity monitoring	x																x														x	x	x

[1] Participants who terminate early from the study should complete all procedures listed for this visit (see sections 4.3 and 5.3).

[2] May be increased to at least weekly if study drug administration is paused (see section 5.2).

[3] Serology will be repeated after screening for CMV and EBV if participants show clinical signs of infection; *or* if viral load testing shows that the EBV viral load is ≥ 10,000 copies per 10^6 PBMCs or the CMV viral load is ≥ 10,000 copies per mL whole blood.

[4] Additional serum testing will be performed for participants with a positive urine HCG result after screening.

		Treatment Cycle 1												Treatment Pause			Treatment Cycle 2													Follow-Up Period			
Week	**≤-5**	**0**	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**15**	**19**	**23**	**24**	**25**	**26**	**27**	**28**	**29**	**30**	**31**	**32**	**33**	**34**	**35**	**40**	**52**	**78**	**104**
Visit	**-1**	**0**	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**	**16**	**17**	**18**	**19**	**20**	**21**	**22**	**23**	**24**	**25**	**26**	**27**	**28**	**29**	**30**[1]
ADMINISTRATION OF STUDY MEDICATION																																
Alefacept or saline injection		x	x	x	x	x	x	x	x	x	x	x	x				x	x	x	x	x	x	x	x	x	x	x	x				
HAV vaccine administration[5]																													x		x	
Tetanus toxoid vaccine administration																													x			
DISEASE-SPECIFIC ASSESSMENTS																																
Mixed-meal tolerance test (MMTT)[6]	x																x[7]													x	x	x
Glucometer readings	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x
Insulin use reporting	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x
Hypoglycemia assessment	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x
MECHANISTIC ASSESSMENTS																																
PBMC T-cell assays		x											x				x												x		x	x	x
Serum-autoantibody analysis	x												x				x												x		x	x	x
Serum archive		x											x				x												x		x	x	x
Whole blood-gene expression profiling		x											x				x												x		x	x	x
Whole blood DNA-HLA genotypes		x																															
Whole blood DNA Methylation		x											x				x												x		x	x	
Tetanus toxoid antibody titer assessments																													x[8]	x			
HAV antibody titer assessments		x																											x 8	x	x 8	x	x

[5] Vaccine should only be administered to participants who do not show previous history of exposure at Visit 0.

[6] 2-hour MMTT to be performed at visits 15 and 29; 4-hour MMTT to be performed at visits -1 (screening), 28 and 30. Refer to Appendix B for procedure instructions.

[7] MMTT should be done prior to study drug injection.

[8] Samples to be drawn prior to vaccine administration.

Appendix 2. Procedures for Performing the Mixed-Meal Tolerance Test

The mixed-meal tolerance test (MMTT) is performed in the morning (between 7:00 a.m. and 10:00 a.m.), which means that administration must begin within this time. It is recommended that the tests be scheduled early in the morning (7:00–7:30 am) because blood glucose will be more likely to be within the target range at that time.

The mixed meal used in this protocol will be the Boost High Protein Nutritional Energy Drink® (Mead-Johnson). If a participant has a known food allergy to one or more components of Boost, an equivalent substitution may be used contingent upon agreement with the study team. The 4-hour MMTT should take 250 minutes to perform, and the 2-hour MMTT should take 130 minutes.

Dietary Guidelines and Pretest Instructions

Carbohydrates (CHO) should not be restricted from the diet before the test. A general guideline is that preadolescent participants should consume at least 25 kcal (6.25 g) CHO/kg/day and adolescent and adult participants should consume at least 15 kcal (3.75 g) CHO/kg/day for 3 days before the test. These are minimum amounts of CHO; most diets will include greater amounts of CHO. There is no need to alter the participant's diet unless he or she has been on a CHO-restricted diet.

In preparation for the visit, each participant should:

- Fast for at least 10 hours (but not more than 16 hours) before the test. Fasting should start the night before the test, and should continue up until the start of the test. Participants should not eat or drink anything except water. This means no coffee, tea, soda, cigarettes, alcohol, or chewing gum during the fasting period.
- Refrain from vigorous exercise during the fasting period.
- Refrain from working the night before the morning of the test.
- Discontinue taking any prescription medications that must be taken daily.

Glucose and Insulin Before the Test

- Short-acting insulin analogues (such as lispro or l-aspart) may be administered up to 2 hours before the test.
- Regular insulin may be administered up to 6 hours before the test.
- Intermediate-acting insulin (such as NPH) may be administered on the evening before the MMTT, but not on the morning of the test. Participants managed with intermediate-acting insulin (NPH or Lente) should administer their usual dose on the evening before the MMTT, but not on the morning of the test.
- Long-acting basal (such as glargine) insulin or continuous subcutaneous insulin infusion may be administered before, during and after the test as usual. Participants on glargine may take their usual injection at the appropriate time, and

those on continuous subcutaneous insulin infusion may continue with their usual basal settings.

Target Glucose Level at the Start of Test

The target glucose level at the start of the test is between 70 and 200 mg/dL. Regular insulin or short acting insulin analogues may be used up to 6 and 2 hours before the test, respectively, to achieve the desired glucose level. The principal investigator and the study participant should discuss the individual situation for insulin administration to attain the goal of meter capillary glucose values within the range of 70–200 mg/dL at the start of the test. For example, as a practical matter, participants may be instructed to check their blood glucose by meter at home 2 hours before the start of the test so that marked hyperglycemia can be treated with a short-acting insulin analogue. Alternatively, participants who arrive at the research unit with elevated blood glucose can receive additional short-acting insulin analogues at the time of their arrival, if the test itself does not start until at least 2 hours after insulin administration and occurs before 10 a.m.

If a subject's blood glucose is below the limit (70 mg/dL) prior to performing the MMTT, the participant should be treated according to local practice, and the MMTT should be rescheduled.

IV Placement During the Test

- The IV should be in place for the duration of the test and must be flushed after each draw with saline solution or heparin flush.
- The participant should remain sitting or resting in bed quietly throughout the test and until the test is completed. However, he or she may engage in quiet, non-strenuous activities, such as reading, playing cards, or watching TV. The participant may walk to the bathroom between blood draws if necessary.

Testing Instructions:

Time Point −10 minutes

- The first sample should be taken at least 10 minutes after establishing the line(s) and when the participant is calm and relaxed (if possible, depending on age) — this is the "−10 minute" sample.
- Draw one 2-mL sample into the purple-top tube for C-peptide and insulin. After each vacutainer is collected the tube must be inverted gently at least 8 to 10 times. Chill sample in a bucket of crushed ice or in a refrigerator set at 4˚C for 20 to 30 minutes. At the laboratory, spin the tube in a tabletop centrifuge (1000–1300 g, ~3000 RPMRPMRPM) for 10 minutes. Tubes must be spun within 30 to 60 minutes from blood draw. Freeze the sample at -80°C.

- Draw one 2-mL sample into the gray-top tube for glucose. Invert tube gently 8 to 10 times. If it is not possible to centrifuge the sample immediately post collection, chill sample in a bucket of crushed ice or in a refrigerator set at 4˚C for 20 to 30 minutes. Refrigerate the sample no longer than one hour prior to centrifugation.

Time Point 0 minutes

- The second sample should be taken just before the participant drinks the Boost; this is the "0- minute" sample.
- Then the MMTT dose should be given with 6 kcal/kg @ 1 kcal/mL of mixed meal, to a maximum of 360 mL. The participant should consume the MMTT dose in no more than 5 minutes.

Time Points 15, 30, 60, 90, 120, 150, 180, 210, and 240 minutes

- Draw one 2-mL sample into the gray-top tube for glucose at each of the time points specified.
- Draw one 2-mL sample into the purple-top tube for C-peptide and insulin levels.
- Invert all tubes gently 8 to 10 times after collection. If it is not possible to centrifuge the sample immediately post collection, chill sample in a bucket of crushed ice or in a refrigerator set at 4˚C for 20 to 30 minutes. Refrigerate the sample no longer than one hour prior to centrifugation. Please check blood glucose by meter.
- At the conclusion of the test, please check blood glucose by glucometer, and administer insulin as per participant's standard insulin plan.

Tube-Processing Instructions

- Spin the gray- and purple---top tubes. Then transfer the plasma into individual vials. Please make sure that each vial is properly identified with a label that indicates the time point.
- Freeze the samples for C-peptide levels. The glucose samples are shipped ambient.
- Ship the specimens to the laboratory where the assays will be performed.

A clogged line, missed sample, or other deviation from the protocol must be noted on the Comments section of the MMTT specimen transmittal form.

Sample

Time (minutes)	Glucose Sample Taken	C-peptide/Insulin Sample Taken
–10	x	x
0	x	x
Participant drinks Boost		
15	x	x
30	x	x
60	x	x
90	x	x
120	x	x
150	x	x
180	x	x
210	x	x
240	x	x