

Protocol ITN095AI DESIGNATE**A T cell phenotype signature driven dose finding study with sipilizumab
in type 1 diabetes mellitus****Version 5.0 (April 21, 2025)****IND #154719****IND Sponsor:** Division of Allergy, Immunology, and Transplantation, National Institute of Allergy and Infectious Diseases (DAIT/NIAID)**NIAID Funding Mechanism:** Cooperative Agreement UM1AI109565**Consortium/Network:** Immune Tolerance Network (ITN)**Study Drug Manufacturer/Provider:** ITB-MED LLC

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SITE INVESTIGATOR SIGNATURE PAGE	
Protocol: ITN095AI DESIGNATE	Version/Date: 5.0 / April 21, 2025
Protocol Chair: Stephen E. Gitelman, MD	
Title: A T cell phenotype signature driven dose finding study with siplizumab in type 1 diabetes mellitus	
IND Sponsor: Division of Allergy, Immunology, and Transplantation, National Institute of Allergy and Infectious Diseases	
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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 United States 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 <i>Guidance for Industry: E6(R2) Good Clinical Practice: Integrated Addendum to ICH E6(R1)</i> dated March 2018. Further, I will conduct the study in keeping with local legal and regulatory requirements. As the site 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 the written permission of the IRB and DAIT/NIAID. *The site Principal Investigator should sign and date at the indicated location below. A written signature/date is acceptable (e.g., scanned and sent via email as a PDF version). An electronic signature is also acceptable (e.g., sent via email as a PDF version). Site Principal Investigator (Print) Site Principal Investigator (Signature) Date	

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PROTOCOL SYNOPSIS PART B

Title	A T cell phenotype signature driven dose finding study with siplizumab in type 1 diabetes mellitus
Short Title	Siplizumab in T1DM
Clinical Phase	Ib
Number of Sites	Approximately 20 US sites
IND Sponsor Number	DAIT/NIAID IND 154719
Study Objectives	The primary objective is to identify a safe, metabolically favorable, dosing regimen for siplizumab in patients with type 1 diabetes that induces changes in T cell phenotypes observed with alefacept therapy in new onset T1DM. The secondary objectives are to: 1. Assess the safety profile of siplizumab in recently diagnosed T1DM. 2. Assess the effects of siplizumab on residual beta cell function in recently diagnosed T1DM participants.
Study Design	Part B is a multi-center, open label, single ascending dose study testing three single doses of siplizumab (0.02 mg/kg, 0.04 mg/kg, and 0.06 mg/kg) in individuals aged 8-45 years with a T1DM diagnosis within 24 months of the baseline visit. Initially, an adult cohort will be studied. Five per-protocol sample (PP) adult participants will be treated in the lowest dosing arm. Safety data will be reviewed by the study team prior to treating five PP participants in the next highest dosing arm. In addition, the treatment of the first two participants in each dosing arm will be gated based on changes in the CD4 T cell counts observed at Week 2. Each dosing arm will be expanded to 15 total PP participants if 3 of the first 5 PP participants meet the primary endpoint at Week 8. Blood samples for mechanistic analyses will be obtained regularly through week 52. After the completion of treatment, participants will undergo follow-up visits with MMTT at weeks 12, 24, 36, and 52. If indicated, participants will enter long-term safety monitoring for up to an additional 48 weeks. An individual who achieves both primary endpoint T cell markers at any time point from Week 1 up to and including Week 8 is considered to have met the T cell phenotype signature criteria. In the adult age cohort, if dosing arms are closed for safety reasons or for failing to meet the primary endpoint, additional participants may be added to the remaining dosing arms so that a minimum of 30 adult participants across all dosing arms are treated. After the 30th adult participant reaches week 12, the Study Management Team (SMT), Data and Safety Monitoring Board (DSMB) and US Food and Drug Administration (FDA) will review the available data to weigh the potential risks as well as the prospect of direct benefit. If there are no concerns raised by the DSMB and FDA after a review of the safety and insulin secretory data from the siplizumab-treated adult participants, treatment in the pediatric cohort (8-17 years old) will be initiated.
Dosing Arms	Part B: 1. 0.02 mg/kg SQ 2. 0.04 mg/kg SQ 3. 0.06 mg/kg SQ

Primary Endpoint	1. Change from baseline of PD1+TIGIT+ frequency within circulating CD4 Tem cells during the first 8 weeks and 2. Change from baseline in the CD4 Treg/Tem ratio during the first 8 weeks in blood.
Secondary Endpoints	1. Frequency of AEs in all siplizumab dosing arms. 2. MMTT-stimulated mean 2-hour C-peptide AUC at Weeks 12, 24, 36 and 52. 3. Insulin use (U/kg/day) at Weeks 12, 24, 36 and 52.
Accrual Objective	Target treated population: 90
Study Duration	The enrollment duration is approximately 24 months. Participants will be followed for 52 weeks. If long-term safety monitoring is indicated, participants may be followed for up to an additional 48 weeks.
Treatment Description	Three single-dose regimens of siplizumab.
Inclusion Criteria	1. Ability to provide informed consent (parental permission and informed assent of minor, if applicable). 2. Male or female between 8 to 45 years of age. 3. Diagnosis of T1DM within 24 months (730 days) of V0. 4. Positive for at least one diabetes-related autoantibody, including: a. Glutamate decarboxylase (GAD-65), b. Insulin, if obtained within 10 days of the onset of exogenous insulin therapy, c. Insulinoma antigen-2 (IA-2), d. Zinc transporter-8 (ZnT8). 5. Peak stimulated C-peptide level > 0.1 nmol/L following a MMTT conducted $\geq$ 21 days from diagnosis and within 37 days of V0.
Exclusion Criteria	1. Use of investigational drugs within 24 weeks of participation. 2. Severe reaction or anaphylaxis to humanized monoclonal antibodies. 3. Inability to complete a mixed meal tolerance test: a. History of significant allergy (e.g., anaphylaxis) to milk or soy proteins, b. Inability to disable hybrid closed loop system. 4. History of recent (within 180 days of V0) or ongoing uncontrolled bacterial, viral, fungal or other opportunistic infections, including: a. Human immunodeficiency virus (HIV), b. Current or prior infection with hepatitis B (HBV), as indicated by positive HBsAg or positive HBcAb, c. Current or prior hepatitis C (HCV), unless treated with anti-viral therapy with achievement of a sustained virologic response (as indicated by undetectable viral load 12 weeks after cessation of therapy), d. Positive QuantiFERON-TB Gold or QuantiFERON-TB Gold Plus tests. Purified-Protein Derivative (PPD) or T-SPOT®TB may be substituted for the QuantiFERON-TB Gold or QuantiFERON-TB Gold Plus tests, e. Active infection with Epstein-Barr Virus (EBV) as detected by Polymerase Chain Reaction (PCR) or serology at the screening visit (V-1), f. Active infection with cytomegalovirus (CMV) as detected by PCR or serology at the screening visit (V-1). 5. Any of the following laboratory abnormalities, confirmed by repeat tests at least 1 week apart:

	a. White blood count (WBC) < 3 x 10³/μL, b. CD4 T cell count below the lower limit of normal, c. Platelet count < 150,000 /μL, d. Hemoglobin < 10 g/dL, e. ALT ≥ 2x upper limit of normal (ULN), f. AST ≥ 2x ULN, g. Serum creatinine >1.5x ULN in adults or >ULN in pediatrics, h. Absolute lymphocyte count (ALC) below the LLN. 6. Prior or current treatment that is known to alter the natural history of T1DM or immunologic status, including high dose inhaled, extensive topical or systemic glucocorticoids. 7. Current or prior (within last 14 days of the V-1 MMTT) use of any medication known to influence glucose tolerance (e.g., atypical antipsychotics, diphenylhydantoin, thiazide, or other potassium-depleting diuretics, β-adrenergic blockers, niacin). 8. Current or prior (within the last 30 days of the V-1 MMTT) use of non-insulin medications to treat insulin resistance or elevated glucose levels. 9. Previous or current diagnosis of malignancy. 10. History of bone marrow transplantation, solid organ transplantation, or primary immunodeficiencies. 11. History or diagnoses of other autoimmune diseases with the exception of stable thyroid or celiac disease. 12. History of significant cardiovascular disease. 13. Vaccination with a live attenuated vaccine (e.g., varicella, measles, mumps, rubella, cold-attenuated intranasal influenza vaccine, bacillus Calmette-Guérin, and smallpox) within 30 days of V0. 14. Women of child-bearing potential who are unwilling to use a medically acceptable form of contraception from 14 days prior to V0 until study Week 52. 15. Women who are pregnant, lactating, or planning on pregnancy during the study. 16. Current diagnosed mental illness (e.g., severe depression) or current diagnosed or self-reported drug or alcohol abuse that, in the opinion of the investigator, would interfere with the participant's ability to comply with study requirements. 17. Past or current medical problems or findings from physical examination or laboratory testing that are not listed above, which, in the opinion of the investigator, may pose additional risks from participation in the study, may interfere with the participant's ability to comply with study requirements, or may impact the quality or interpretation of the data obtained from the study.
Study Stopping Rules	1. Any death 2. Any Grade 4 AE except asymptomatic AE and those assessed as not related to sipilizumab treatment on review by the DAIT/NIAID Medical Monitor and the ITN Clinical Trial Physician. 3. An occurrence of Hy's Law, defined as elevations in the AST and/or ALT >3x ULN of the reference range, elevation in total bilirubin >2x ULN of the reference range, and no other cause of hepatic injury.
Cohort Dosing Arm Stopping Rules	1. Two of the first 5 treated participants in a dosing arm develop a dose limiting toxicity (DLT) as defined in Table 10. 2. Four of the 15 treated participants in a dosing arm develop a DLT as defined in Table 10.

GLOSSARY OF ABBREVIATIONS

Ab	Antibody
ADCC	Antibody-dependent cell-mediated cytotoxicity
ADCP	Antibody-dependent cellular phagocytosis
AD-SCCC	Autoimmune Disease Statistical and Clinical Coordinating Center
AE(s)	adverse event(s)
aGVHD	acute graft versus host disease
AIM	activation-induced marker
AKI	acute kidney injury
ALC	absolute lymphocyte count
ALP	alkaline phosphatase
ALT	alanine aminotransferase
AST	aspartate aminotransferase
APC(s)	antigen presenting cell(s)
AUC	area under the curve
ATG	anti-thymocyte globulin
BRI	Benaroya Research Institute
BUN	blood urea nitrogen
CBC	complete blood count
CD2	cluster of differentiation 2
CDSMC	[DAIT/NIAID] Clinical Data and Safety Management Center
CFR	Code of Federal Regulations
CGM	continuous glucose monitoring
cGMP	Current Good Manufacturing Practice
CMV	cytomegalovirus
CRP	C-reactive protein
DAIT	Division of Allergy, Immunology, and Transplantation

DLT	dose limiting toxicity
DSMB	Data and Safety Monitoring Board
CRP	C-reactive protein
DAIT	Division of Allergy, Immunology, and Transplantation
DSMB	Data and Safety Monitoring Board
EBV	Epstein-Barr virus
EBNA	Epstein-Barr nuclear antigen
EBV-LPD	Epstein-Barr virus-associated lymphoproliferative disorder
eCRF(s)	electronic case report form(s)
EDC	electronic data capture
ELISA	enzyme-linked immunosorbent assay
FcRn	Neonatal Fc receptor
FDA	US Food and Drug Administration
GAD-65	glutamate decarboxylase 65
GCP	good clinical practice
GLP	good laboratory practice
GLP-1R	glucagon-like peptide-1 receptor
GMP	good manufacturing practice
GVHD	graft versus host disease
HbA1c	hemoglobin A1c or glycated hemoglobin
HBsAg	hepatitis B surface antigen
hCG	human chorionic gonadotropin
HCV	hepatitis C virus
HIV	human immunodeficiency virus
IA-2	insulinoma antigen-2
IB	Investigator's Brochure
ICA	islet cell autoantibodies

ICH	International Conference on Harmonization
IEC	independent ethics committee
Ig	immunoglobulin
IgG	immunoglobulin G
IgM	immunoglobulin M
IM	intramuscular
IND	Investigational New Drug [application]
IP	investigational product
IRB(s)	institutional review board(s)
ITN	Immune Tolerance Network
IV	intravenous
LFA-3	lymphocyte function-associated antigen 3 (CD58)
LPD(s)	lymphoproliferative disorder(s)
LTFU	long term follow-up
mAUC	mean area under the curve
LPD(s)	lymphoproliferative disorder(s)
mAUC	mean area under the curve
MedDRA	Medical Dictionary for Regulatory Activities
MDS	myelodysplastic syndrome
mITT	modified intention to treat
MLR	mixed lymphocyte reaction [assay]
MMTT	mixed meal tolerance test
NAAT	nucleic acid amplification test
NCI-CTCAE	National Cancer Institute Common Terminology Criteria for Adverse Events, Version 5.0
NHP(s)	non-human primate(s)
NIAID	National Institute of Allergy and Infectious Diseases
NIDDK	National Institute of Diabetes and Digestive and Kidney Diseases

NIH	National Institutes of Health
NK	natural killer
NOD	non-obese diabetic
NSG	NOD SCID gamma [mouse model]
OHRP	Office for Human Research Protections
PBMC(s)	peripheral blood mononuclear cell(s)
PCR	polymerase chain reaction
PD	pharmacodynamic
PPD	purified protein derivative
PD1	programmed cell death protein 1
PHI	personal health identifiers
PK	pharmacokinetics
PTLD	post-transplant lymphoproliferative disorder
PoR	Pharmacist of Record
PP	per protocol
QC	quality control
QOL	quality of life
SAE(s)	serious adverse event(s)
SAR(s)	suspected adverse reaction(s)
SCID	severe combined immunodeficient
SCr	serum creatinine
SMT	Study Management Team
SNPs	single nucleotide polymorphisms
SOC	system organ classification
SOE	schedule of events
SOP	standard operating procedures
SQ	subcutaneous
SUSAR	serious and unexpected suspected adverse reaction

TB	tuberculosis
Tcm(s)	central memory T cell(s)
TEAE(s)	treatment-emergent adverse event(s)
Tem	effector memory T cells
TIGIT	T cell immune receptor with Ig and ITIM domains
T1DM	type 1 diabetes mellitus
Tn	T naïve cells
Treg(s)	regulatory T cell(s)
ULN	upper limit of normal
URI	upper respiratory [tract] infection
VCA	viral capsid antigen
WBC	white blood count
WD	withdrawal [visit]
ZnT8	zinc transporter-8

1. BACKGROUND AND RATIONALE

1.1 BACKGROUND AND SCIENTIFIC RATIONALE

Type 1 diabetes mellitus (T1DM) is a common, chronic autoimmune disease affecting both children and adults. The autoimmune process targets insulin secreting pancreatic beta cells, resulting in chronic hyperglycemia and predisposing patients to microvascular and macrovascular complications.^{1,2} Patients rely on parenteral insulin therapy, glucose monitoring (including continuous glucose sensing), and other medications (often used off-label) to improve metabolism and optimize glycemic control. Despite this armamentarium, the majority of patients do not reach target glycemic goals, experiencing ongoing risk for severe hypoglycemia and diabetic ketoacidosis as well as increased morbidity and mortality due to microvascular and macrovascular complications.³

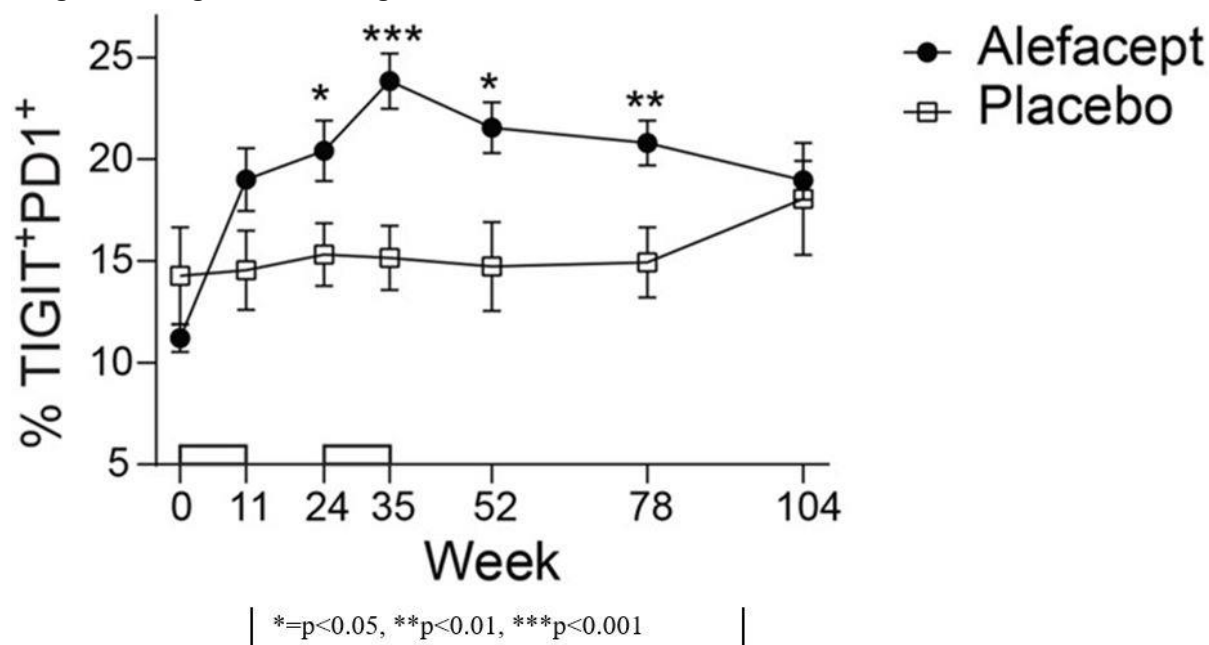
Given the limitations in current clinical tools to replace beta cells, research has focused on understanding the cause of beta cell destruction and finding safe and effective means to preserve residual beta cell function. A series of clinical studies have been conducted over the past two decades with the hope of slowing or reversing the autoimmune destruction of beta cells. Broad chronic immunosuppression with agents like cyclosporine will preserve beta cell function in some patients but there are associated toxicities and risks, particularly for infection and cancer.⁴ More focused targeting of the immune response has proven promising, including efforts with teplizumab (anti-CD3), rituximab (anti-CD20), abatacept (CTLA-4 Ig), anti-thymocyte globulin (ATG), and golimumab (anti-TNF), all of which have shown efficacy in Phase II studies in slowing the loss of residual beta cell function in newly diagnosed T1DM participants.⁵⁻⁹

The DAIT/NIAID-sponsored T1DAL study (Protocol ITN045AI conducted under investigational new drug (IND) #105308, NCT00965458) using Amevive® (alefacept), a fusion protein consisting of lymphocyte function-associated antigen 3 (LFA-3) linked to the Fc portion of IgG1, also showed significant retention of residual insulin secretion by blocking the CD2 co-stimulation pathway.^{10,11} In this randomized, placebo-controlled Phase II study, T1DM participants (12-35 years old) receiving two 12-week cycles of alefacept showed relative preservation in residual beta cell function compared to placebo. When evaluating loss of beta cell function with the 4 hour mixed meal tolerance test (MMTT) at week 104, alefacept-treated participants lost only 15% of pre-treatment insulin secretion while the placebo group lost 54% of pre-treatment insulin secretion.¹¹ The effect was significant because, unlike other T1DM clinical trials, the majority of participants were >18 years of age.¹¹ Age, however, appeared only to have a modest effect on C-peptide outcomes. When analyzing participants who partially responded (defined as participants with no more than a 50% loss in AUC) at month 24, 89% of alefacept treated participants between the ages of 12-21 met the partial response definition compared to only 29% of placebo participants between 12-21 years. This difference was statistically significant. The difference in partial responder frequency between the alefacept group and the placebo group in participants over the age of 21 was not statistically different.

T cell phenotyping suggested a possible mechanistic explanation for the clinical results. In ITN045AI T1DAL, the alefacept treated group had significant depletion of central (Tcm) and effector memory (Tem) T cells compared to the placebo group. In addition, alefacept therapy increased the frequency of CD4 Tem expressing inhibitory receptors such as programmed cell death protein 1 (PD1).¹¹ Expression of PD1 is associated with T cell exhaustion, a state of reduced functionality.¹² Alefacept therapy also spared CD4 T regulatory cells (Tregs) relative to CD4 Tem, resulting in an increased ratio of Treg:Tem. Altering the balance of regulatory T cell (Treg) to T effector cell numbers or function is one experimental approach to ameliorate autoimmune disease. Together, these findings suggested that alefacept therapy increases the expression of cell surface receptors consistent with T cell exhaustion while, at the same time, sparing Tregs from depletion. Both changes may have reduced the pathogenicity of islet autoreactive T cells and preserved residual beta cell function.

Further analysis of flow cytometry data from ITN045AI (T1DAL study) revealed that the frequency of CD4 Tem co-expressing PD1 and TIGIT (another inhibitory receptor associated with T cell exhaustion) increased significantly in active drug participants (Figure 1).¹³

Figure 1 Longitudinal Changes in TIGIT+PD1+ CD4 Tem in ITN045AI¹³



Most participants receiving alefacept showed these phenotypic changes described above. After twelve weekly doses of alefacept, 80% of treated participants demonstrated a 20% increase in frequency of PD1+ TIGIT+ cells within the CD4 Tem pool. At the same time point, 82% of alefacept treated participants had at least a 75% increase in Treg:Tem ratio compared to baseline. Only 8% of placebo participants had that degree of change. At week 11, 70% of participants receiving alefacept met both these criteria, increasing to 77% at week 24 after 12 weeks of observation without therapy. There was no difference in the age of

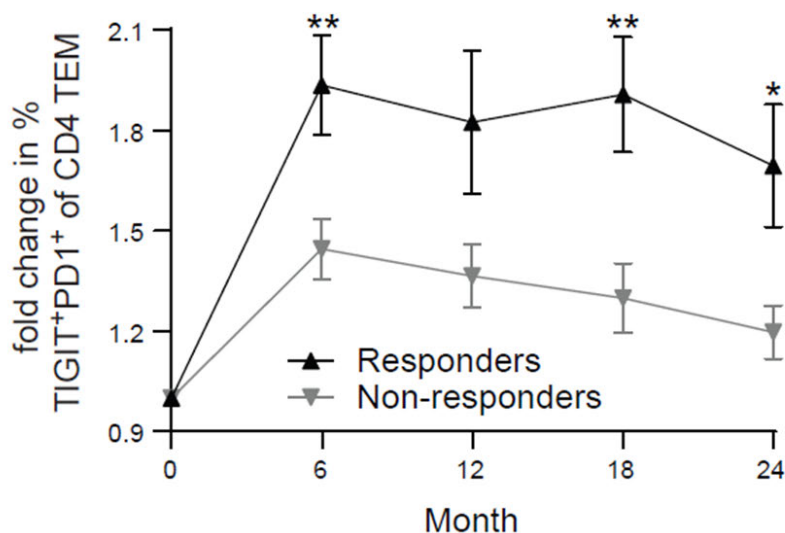
participants who had both T cell phenotype signatures present at week 11 or 24 and those who did not.

The frequency of PD1+TIGIT+ cells within the CD4 Tem subset clearly differentiated alefacept from placebo participants in ITN045AI T1DAL. Given the relatively few participants in this study (n=49), no correlation between T cell phenotypes and residual insulin was observed. However, when analyzing changes in this T cell subset across three DAIT/NIAID-sponsored ITN type 1 diabetes studies (ITN045AI T1DAL (IND #105308), ITN027AI AbATE (IND #100583) using the anti-CD3 molecule teplizumab, and ITN028AI START (IND #12693) using ATG), we noted differences between active therapy participants classified as complete responders (defined as those treated participants with no loss of insulin secretion measured by C-peptide at 2 years) and active therapy non-responders (defined as those with at least 40% loss of insulin secretion at 2 years)¹³. Across 2 years of follow-up, the fold change in the frequency of PD1+TIGIT+ cells within the peripheral pool of CD4 Tem was significantly increased from baseline in complete responders compared to non-responders (Table 1 and Figure 2). These data suggest that increases in the frequency of PD1+TIGIT+ cells within the CD4 Tem subset might be more than a pharmacodynamic (PD) response and may correlate with improved metabolic outcomes in T1DM.

ITN045AI T1DAL study enrollment was stopped early, and additional clinical studies with alefacept were not conducted since the manufacturer, Astellas Pharma, withdrew the drug from the market (not for safety reasons).¹⁴ However, agents that modulate the same pathway as alefacept (CD2-LFA-3) could be valuable therapeutics to use in T1DM given the favorable clinical outcomes, measurable immune modulatory effects, and acceptable safety profile observed in ITN045AI.

Table 1. Responses by Study in Treated Participants

Study	Complete Responders	Non-responders
ITN027AI AbATE	4	18
ITN028AI START	6	16
ITN045AI T1DAL	7	9
TOTAL	17	43

Figure 2. Longitudinal Changes in TIGIT+PD1+ CD4 Tem in ITN027AI, ITN028AI and ITN045AI¹³

1.2 RATIONALE FOR SELECTION OF INVESTIGATIONAL DRUG

A therapeutic agent under development by ITB-MED LLC, sipilizumab (TCD601, previously known as MEDI-507), targets the CD2-LFA-3 co-stimulatory pathway on memory T cells and may result in similar changes in the CD4 T cell population as observed with alefacept. Sipilizumab is a non-agonistic, humanized, anti-CD2 monoclonal antibody (Ab) of the IgG1 κ class. CD2 is an extracellular surface marker expressed on almost all mature human T-lymphocytes, as well as natural killer (NK) cells and thymocytes. The natural ligand of CD2 is LFA-3, or CD58. CD58 is a cell adhesion molecule expressed on T cells, NK cells, and antigen presenting cells (APCs). The CD2-CD58 complex stabilizes and enhances lymphocyte interactions and provides co-stimulation necessary for immune activation. CD2 is up-regulated on activated T cells and memory T cells, while CD58-ligation activates NK and dendritic cells, lowers the threshold for T cell activation, and enhances T cell responsiveness to pro-inflammatory cytokines.¹⁵ Sipilizumab binds to a unique epitope on human CD2, distinct from the CD58 binding site, with high affinity (K_d – 1 nM), inhibiting co-stimulation and T cell activation. In addition, the Fc portion of the sipilizumab Ab binds to Fc γ RIII receptors on NK cells, resulting in antibody-dependent cell mediated cytotoxicity (ADCC) and depletion of CD2+ lymphocytes. CD2 ligation of B-cell specific CD58 results in the upregulation of CD40 expression, suggesting that CD2 may also play a role in stimulation and/or delivery of T cell help to B cells, and thus sipilizumab may also impact this pathway.

Sipilizumab has mainly been studied in adults with psoriasis, graft versus host disease (GVHD), kidney transplantation, and lymphoma and in ten pediatric participants, aged 5 to 16 years old, with acute graft versus host disease (aGVHD) (MI-CP049).¹⁶⁻²⁰ In these studies, lymphocyte depletion has been observed for at least 3 months following subcutaneous (SQ) or intravenous (IV) dosing. Anti-sipilizumab antibodies developed in approximately 70% of subcutaneously treated participants with psoriasis, although they were non-neutralizing; the

clinical significance of this finding is not clear.¹⁷ *In vitro* studies with sipilizumab have shown relative sparing of the CD4 Treg population but mechanistic studies with biospecimens from participants dosed with sipilizumab similar to those performed as part of the ITN045AI T1DAL study have not been conducted.²¹

Although no studies with sipilizumab have been completed in T1DM to date, it is reasonable to hypothesize that metabolic responses in sipilizumab may mimic those from alefacept therapy given that sipilizumab and alefacept target the same co-stimulatory pathway. However, given the lack of clinical or PD data to support using this agent in T1DM, a pilot study focused on mechanistic outcomes is proposed prior to studying sipilizumab in an adequately powered efficacy study. This rationale is based on the hypothesis that without a similar mechanistic effect on T cell modulation, sipilizumab might not demonstrate similar clinical effects as reported for alefacept.

1.3 PRECLINICAL EXPERIENCE

Sipilizumab binds with high specificity and affinity to the human CD2 receptor. The dissociation constants (Kd) for sipilizumab range from 1.0 nM when measured on human Jurkat T cell line, to 5 nM when used with soluble CD2. The EC50 was 32 pM when measured with immobilized CD2 in an enzyme-linked immunosorbent assay (ELISA) assay. Cell binding studies revealed no binding to platelets or hematopoietic precursor cells and tissue reactivity with normal human tissue was as expected, with T cell areas of lymphoid tissues being positive and no staining in non-lymphoid tissues. Sipilizumab cross-reacts with the CD2 of only one other primate species, the chimpanzee.²²

The mechanism of sipilizumab mediated T cell depletion was shown to be via ADCC and antibody-dependent cellular phagocytosis (ADCP) and not via complement dependent cytotoxicity mechanisms. Sipilizumab binds to the three human FcγRs (I, II and III) as well as the neonatal Fc receptor (FcRN) and is able to initiate ADCC mediated depletion of T- and NK-cells and IgG recycling via these pathways. ADCC lysis of normal human peripheral blood mononuclear cells (PBMCs) was observed with sipilizumab via NK cell activity. In addition, FcγR mediated cross linking of sipilizumab bound to CD2 at physiological temperature results in down-modulation of surface CD2 receptors on the Jurkat T cell line. The induction of apoptosis by sipilizumab bound to Jurkat T cells was observed in the absence of effector cells only when sipilizumab was cross-linked with an immobilized anti-idiotypic Ab (but not with soluble anti-idiotypic Ab).

The pharmacology of sipilizumab has been evaluated *in vitro* at concentrations of up to 50 µg/mL. In a mixed lymphocyte reaction assay (MLR), sipilizumab resulted in lymphocyte hypo-responsiveness and T- and NK-cell depletion at concentrations greater than 50 ng/mL.²³ Similarly, maximal depletion (ADCC) of CD2+ lymphocytes occurred with concentrations of 100 ng/mL. In HLA-mismatched allogeneic MLRs, sipilizumab predominantly reduced the percentage of CD4 and CD8 Tem, which express higher concentrations of CD2 than naïve T cells (Tn) or Tregs. Moreover, selective expansion of donor-reactive Tregs along with depletion of donor-reactive CD4 Tem was also noted at concentrations of 100 ng/mL and

higher. These results suggest that in addition to depletion of T- and NK-cells, sipilizumab exhibits immunomodulatory activity in the setting of an alloreactive (two-way) MLR.²¹

Lymphocyte depletion with sipilizumab has been demonstrated in vivo with a transgenic human CD2 (huCD2) murine model and in a humanized NOD SCID gamma (NSG) mouse model. In vivo activity was further evaluated in a human-xenomouse model. Weekly sipilizumab administration for 6 months increased survival of mice bearing a CD2⁺ human adult T cell leukemia cell line and FcγR receptors as compared to those expressing a double-knockout FcγR ^{-/-} genotype.

Toxicology studies with single and multiple doses of sipilizumab were conducted in non-human primate (NHP), rabbit, and rodent models. Studies in rodents were conducted to assess off-target pharmacologic toxicity. A repeat dose toxicity study in rats at 35 and 70 mg/kg revealed no significant findings in gross and microscopic pathology nor were there any laboratory or physiological indications of drug toxicity. The lack of cross-reactivity between rat and human CD2 means this model cannot detect the on-target pharmacologic activity expected to be exhibited in humans.

To further explore the safety and pharmacologic activity of sipilizumab, three repeated dose studies in chimpanzees were conducted. In the two initial NHP toxicology studies, six chimpanzees were administered IV infusions of varying drug doses (0.143, 0.43, 0.60, or 1.43 mg/kg). Two additional animals received saline control infusions, one of which received premedication with prednisolone as an additional control measure. The chimpanzees were sedated with anesthesia prior to receiving study drug and some animals required intubation as part of a general anesthesia procedure. Animals received up to three daily doses of sipilizumab or control saline under general anesthesia. Three of the animals had no acute toxic effects after receiving study drug. The other drug treated animals experienced a transient, acute, adverse event (AE) during the studies. Rapid infusion of the first dose of sipilizumab over 6 minutes was associated with brief (30 to 90 seconds) apneic episodes in two chimpanzees. One of the two chimpanzees who presented with apnea also had respiratory symptoms 1 to 2 hours after study drug administration. The third chimpanzee exhibited seizure activity after receiving the first dose of study drug; the onset closely followed a ketamine infusion, and causality could not be established. In evaluating changes in lymphocyte counts, the animal who received saline only had no change in cell counts, and the control animal receiving saline plus prednisolone had a prompt drop in absolute as well as CD2-bearing lymphocyte counts for 3 weeks. In chimpanzees treated with sipilizumab, lymphocytes were also depressed.

In the third repeat dose NHP toxicology study, two chimpanzees were dosed with 5 mg/kg sipilizumab daily for 3 days. Similar to the prior NHP studies, sipilizumab infusions were associated with symptoms of mild/moderate hypotension and diarrhea upon initial dosing. Symptoms in all animals resolved within a few hours and did not recur on subsequent administrations. A mild, transient decrease in platelet counts around the time of the first infusion was noted and attributed to activation of the reticuloendothelial system by large numbers of antibody-coated lymphocytes. In addition, a mild transient increase in serum alanine aminotransferase (ALT) and aspartate aminotransferase (AST) was noted on Day 7 and normalized by the next assessment on Day 42. One animal that received sipilizumab

developed suspected *Streptococcus pneumonia* in the right lung and died on study Day 29. Drug-related causality could not be excluded in this case. However, fatal pneumonia with human *S. pneumonia* isolates is known to occur in captive and wild chimpanzee populations.²⁴

Pharmacokinetics (PK) and PD activities were also assessed in the repeat dose NHP studies. Across all siplizumab treated animals, lymphocytes were depressed and recovery to pre-administration values occurred between 4-8 weeks after study drug administration. Lymph node biopsies conducted before and after drug administration indicated mild/moderate tissue-level depletion of T cells as compared to no change in the lymph node architecture in the saline-treated chimpanzees. Lymphocyte depletion in lymph nodes lasted up to 6 weeks in siplizumab treated animals. Formal PK/toxicokinetic assessments were not conducted; however, siplizumab concentrations were consistent with a 30-50 hour serum half-life.

Local tolerability studies conducted in rabbits with repeat dosing on Days 1, 4, and 8 with 0.12 mg/kg SQ or 0.48 mg/kg by the intramuscular (IM) route showed no toxicological effects in any animals at the Day 14 or Day 28 assessments at the site of injection nor any systemic effects.

Overall, the studies above demonstrate that siplizumab is a high affinity Ab against human CD2 with potent T cell depletion activity via the ADCC and ADCP mechanisms. Studies in primates and transgenic animals confirm this primary pharmacologic activity and do not reveal any significant toxicities or tolerability issues. However, siplizumab has not been evaluated in juvenile toxicology studies. These preclinical data support exploring the clinical use of siplizumab in adults with T1DM.

1.4 CLINICAL STUDIES

A total of 17 clinical studies with siplizumab have been completed to date in participants with CD2+ T cell malignancies, GVHD following hematopoietic stem cell transplantation, renal allograft transplantation, and psoriasis. Based on the current ITB-MED Investigator's Brochure (IB) for TCD601 (siplizumab), approximately 938 participants have participated in completed clinical studies to date with approximately 779 participants being exposed to siplizumab via IV or SQ routes. Almost all of the study participants (734 (94%)) received multiple doses, and 45 (6%) received a single dose. An additional 7 participants with aGVHD were treated under a compassionate use program, and 23 participants (mostly with a diagnosis of lymphoma) were enrolled in Phase I clinical studies conducted by a previous sponsor (BioTransplant Inc).

While no studies with siplizumab have been completed in participant with T1DM to date, we hypothesize that this antibody may mimic the clinical benefits of alefacept therapy in T1DM given that both molecules target the same pathway. However, given the lack of clinical or mechanistic data to support using siplizumab in T1DM, this Phase I study will determine if siplizumab results in T cell signatures similar to what has been observed in ITN045AI T1DAL.

1.4.1 Safety Data for Siplizumab

1.4.1.1 Common AEs

Across all completed clinical studies, the most common AEs reported following siplizumab administration included lymphopenia (Grade 1-3), which is expected based on its pharmacology, as well as a first dose infusion-related reaction (Grade 1-2; pyrexia, chills, nausea and fatigue). For participants with infusion reactions, the events were generally mild, transient, and resolved spontaneously or were managed with NSAIDs and antihistamines.

1.4.1.2 SAEs

Serious adverse events (SAEs) were reported more frequently in participants with lymphoma and GVHD, including 36 deaths related to complications of GVHD. In renal transplant and psoriasis participants, a total of 59 SAEs were reported in 47/651 participants. There was one death in a renal transplant participant reported as a complication of a post-transplant cardiac procedure not related to siplizumab administration (Study MI-CP027).²⁰ Also, one case of fatal multi-organ system failure associated with EBV reactivation was reported in an ongoing ITB-MED study for patients with amyotrophic lateral sclerosis (ALS) currently enrolling in Sweden (TCD601H101 / AURORA; NCT06453668). In that study, one seropositive individual received a cumulative dose of 30 mg/kg intravenously over 2 months prior to developing EBV reactivation and rapid progression to multi-organ system failure.

1.4.1.3 Safety Data in Psoriasis

The largest clinical studies of MEDI-507 (siplizumab) therapy have been performed in participants with psoriasis and include three double-blind, placebo-controlled Phase II clinical studies (MI-CP074, MI-CP076, and MI-CP082).^{17,19} Study MI-CP076 was a randomized, double-blind study conducted at 18 sites in Europe. A total of 121 participants were randomized in a 1:1:1:1 ratio to receive weekly SQ injections of siplizumab at doses of 3 mg for 12 weeks (n=28), 3 mg for 16 weeks (n=30), 5 mg for 12 weeks (n=32), or 5 mg for 16 weeks (n=31). All dosing regimens for siplizumab were well tolerated. Clinical events were typically mild and transient. For the 3 mg and 5 mg combined dose groups, there were statistically significant differences in the incidence of AEs in the metabolic and nutritional system organ classification (SOC) and respiratory SOCs. This difference was primarily due to three participants with an increased AST and/or ALT, two participants with peripheral edema, and one with cyanosis on Study Day 0. There were 17/58 (29%) and 31/63 (49%) participants with respiratory events in the 3 mg and 5 mg dose groups (p=0.028), respectively. This was due to an increased proportion of participants with bronchitis (8% vs. 2%), hyperventilation (21% vs. 12%) and upper respiratory tract infections (URI; 19% vs. 12%) in the 5 mg dose group compared to the 3 mg dose group. Overall, there was no apparent relationship between treatment groups and the incidence by preferred term of AEs, SAEs, related AEs, acute AEs (those which occurred after the injection on the day of injection or the following day), or potentially infection-related AEs. The most common (> 15%) AEs overall were lymphopenia (52%), hypertension (recorded during vital sign measurements post-injection; 38%), headache (28%), psoriasis (25%), chills (23%), bradycardia (23%), asthenia (19%), flu-like syndrome (17%), hyperventilation (tachypnea; 17%); injection site reaction (16%), URI (16%), arthralgia (15%), and fever (15%).

There were 19 severe AEs reported in MI-CP076. The most common were lymphopenia in 4% of participants and exacerbation of psoriasis in 2% of participants. All other severe AEs occurred in one participant each. The most common AEs overall judged by the investigator to be related to study drug were lymphopenia (51%), chills (21%), headache (17%), asthenia (17%), injection site reaction (15%), and fever (15%). The most common acute AEs overall were hypertension (37%), bradycardia (23%), chills (22%), hyperventilation (17%), asthenia (16%), injection site reaction (15%), and fever (15%). The incidence of acute adverse reactions was approximately twice as great after the first injection (ranging from a rate of 53% in the 3 mg for 16 week group to a rate of 68% in the 5 mg for 16 week group) as compared with each of the subsequent injections. Other than flu-like syndrome and fever, which were possibly directly related to study drug rather than infection, the most common event with potential to be infection related was URI (16%). There was no evidence of clinical immune suppression observed during the study. There were nine serious AEs reported by eight (7%) of the participants in the study. All of these events resolved by the end of the study, with the exception of one case of lymphopenia and one case of psoriasis. There were no deaths. Other than the effects of siplizumab on lymphocyte counts and lymphocyte subpopulations, there were no clinically meaningful changes from baseline in mean values of hematologic or chemistry parameters.

Two other large Phase II psoriasis studies (MI-CP074 and MI-CP082) have been conducted with siplizumab, one with IV dosing and one with SQ dosing, and the safety and efficacy in these two trials were reported in a combined data set.^{17,19} The age range was 18 to 65 for both studies. Study MI-CP074 involved a total of 124 psoriasis participants randomized 1:1:1 to receive 8 doses of siplizumab (0.012 mg/kg (n=40) or 0.04 mg/kg (n=42)) or placebo (n=42) by IV infusion every two weeks for 16 weeks.¹⁹ Overall, a decrease in mean ALC was observed in the siplizumab-treated groups but not in the placebo group. In general, both siplizumab treatment arms demonstrated an acceptable safety profile in this study with the majority of AEs being mild, transient, and acute (see Table 2). AEs occurred less frequently with subsequent doses. The most frequently reported AEs were lymphopenia, chills, and headache, and they occurred at a higher frequency in the siplizumab dosing arms than in the placebo. The most common acute AEs reported by >10% of all siplizumab-treated participants were chills, headache, and hypertension. A total of 7 SAEs were reported during this trial; one siplizumab-treated participant experienced 4 vascular SAEs of arteriosclerosis, cerebrovascular accident (2 episodes), and peripheral artery occlusive disease, and another siplizumab participant had cholecystitis. Two placebo participants each experienced 1 SAE of pneumonia and cholelithiasis. Only one of all the 7 SAEs reported (pneumonia, in a placebo-treated participant) was judged to be possibly related to study drug. All SAEs resolved, and there were no deaths or malignancies reported during the study.

Study MI-CP082 was a double-blind, randomized, placebo-controlled study conducted at 44 sites in the U.S. and Canada. A total of 420 participants between the ages of 18 to 65 were randomized in a 1:1:1:1 ratio to receive SQ siplizumab at 5 mg for 12 weeks (n=107), 5 mg for 6 weeks (n=101), 7 mg for 4 weeks (n=108), or placebo (n=104). In general, siplizumab therapy was well tolerated and clinical AEs were typically mild and transient (see Table 2 and Table 3). The majority of dosing interruptions or treatment discontinuations were protocol-

required due to reductions in lymphocyte count during sipilizumab treatment. Minimal local site of injection toxicity was noted. An increased incidence in the sipilizumab groups as compared to the placebo group was reported for the following AEs: chills, fever, injection site reaction, headache, nausea, diarrhea, lymphopenia, and leukopenia (see Table 3). An increased incidence of severe AEs was also reported in the sipilizumab participants compared to those receiving placebo (16% vs. 7%), mainly due to the higher incidence of lymphopenia graded as severe (defined per protocol toxicity table as $ALC < 0.50 \times 10^3$ cells/ μ L) in 6% of study drug-treated participants vs. 1% of placebo participants.

In MI-CP082 study, 10 SAEs were reported in 6/316 (2%) sipilizumab-treated and in 1/104 (1%) placebo-treated participants. All SAEs resolved, and there were no deaths or new malignancies reported during the study. In MI-CP085, a follow-up study on sipilizumab-treated psoriasis participants, there were 3 SAEs reported: cholecystitis, colitis and myelofibrosis, in sipilizumab treated participants originally enrolled in studies MI-CP081, MI-CP074, and MI-CP076 respectively. The 3 events were considered either to be unrelated or to have a remote possibility of a relationship to study drug. No deaths were reported in this follow-up study.

1.4.1.4 Safety Data in Other Indications

Among participants at a high risk of lymphoproliferative disorders (LPDs), namely those with CD2+ T cell lymphoma and GVHD following a bone marrow transplant, Epstein-Barr virus-associated lymphoproliferative disorder (EBV-LPD), post-transplant lymphoproliferative disorder (PTLD), and lymphoma have been reported in some participants (Table 4). In the T cell lymphoma studies (MI-CP099 and MI-CP107), EBV-LPD occurred in 5/51 (9.8%) participants with large granular lymphocytic leukemia (LGL; n=2), cutaneous T cell lymphoma (n=1) peripheral T cell lymphoma (n=1), and adult T cell lymphoma (n=1). One participant with LGL developed a secondary leukemia (acute myelomonocytic leukemia) one year after completing sipilizumab therapy. In the GVHD studies, EBV-LPD (n=1), PTLD (n=1), and EBV-related lymphoma (n=2) were reported in a total of 4/48 (8.3%) participants; a case of acute myeloid leukemia relapse was also reported in these trials. In addition, three cases of LPD have been reported in Investigator-initiated and compassionate use studies of sipilizumab in patients following bone marrow transplantation.

Table 2. Study MI-CP074 Adverse Events by Treatment Groups

(Source: IB edition 4.1)

Event	0.012 mg/kg N=40 N (%)	0.04 mg/kg N=42 N (%)	Placebo N=42 N (%)
≥1 Severe AE	6 (15)	5 (12)	1 (2)
≥1 Related AE	31 (78)	37 (88)	24 (57)
≥1 Acute AE	32 (80)	38 (90)	30 (71)
≥1 AE potentially infection related	20 (50)	23 (55)	19 (45)
≥1 SAE	2 (5)	0 (0)	2 (5)
Clinical AE resulting in DC	2 (5)	1 (2)	2 (5)
Lymphopenia resulting in DC	3 (8)	8 (19)	2 (5)
AEs >10% higher in any siplizumab group vs. placebo ¹			
Lymphopenia	12 (30)	13 (31)	4 (10)
Chills	17 (43)	28 (67)	5 (12)
Headache	15 (38)	15 (36)	10 (24)
Nausea	7 (18)	11 (26)	3 (7)
Fever	4 (10)	11 (26)	1 (2)
Tachycardia	5 (13)	8 (19)	0 (0)
Asthenia	5 (13)	7 (17)	2 (5)
Arthralgia	6 (15)	4 (10)	1 (2)
Dizziness	1 (3)	8 (19)	1 (2)
Myalgia	5 (13)	12 (29)	3 (7)

¹ Adverse events ordered by incidence in total siplizumab population**Table 3. Study MI-CP082 Adverse Events with Increased Incidence in SQ MEDI-507 Groups Compared to Placebo**

(Source: IB edition 4.1)

Preferred Term^a	Total (N=316) N (%)	5mg X 12 wks (N=107) N (%)	5 mg X 6 wks (N=101) N (%)	7 mg X 4 wks (N=108) n (%)	Placebo (N=104) n (%)
Lymphopenia	182 (58%)	61 (57%)	57 (56%)	64 (59%)	11 (11%)
Chills	105 (33%)	34 (32%)	33 (33%)	38 (35%)	8 (8%)
Headache	103 (33%)	34 (32%)	36 (36%)	33 (31%)	23 (22%)
Nausea	61 (19%)	22 (21%)	18 (18%)	21 (19%)	7 (7%)
Fever	48 (15%)	17 (16%)	9 (9%)	22 (20%)	5 (5%)
Leukopenia ^b	47 (15%)	16 (15%)	14 (14%)	17 (16%)	4 (4%)
Injection Site Reaction	45 (14%)	25 (23%)	11 (11%)	9 (8%)	5 (5%)
Diarrhea	31 (10%)	10 (9%)	12 (12%)	9 (8%)	4 (4%)

^a AEs ordered by incidence in all three MEDI-507 groups combined.^b Of the 51 patients with leukopenia, all but 8 (3, 2, 1, and 2 in the 5 mg for 12 week, 5 mg for 6 week, 7 mg for 4 week, and placebo groups, respectively) also had lymphopenia.

Table 4. Summary of Participants with LPD and PTLD in Clinical Study of MEDI-507

(Source: IB edition 5)

Protocol Number [Study Design/Population]	Event ID	Dose (Participant ID)	Event	Patient Outcome
Oncology Studies				
MI-CP099 [Phase I dose escalation / CD2+ LPD]	1	3.4 mg/kg (0681028)	EBV-LPD	Not recovered
	2	0.8 mg/kg (0681025)	EBV-LPD	Not recovered
	3	3.4 mg/kg (0681017)	EBV-LPD	Recovered
	4	3.4 mg/kg (0681012)	EBV-LPD	Death
MI-CP099 [Phase I dose escalation / CD2+ LPD]	5	4.8 mg/kg (1054016)	EBV-LPD	Death
GVHD Studies				
MI-CP042 [Phase I/ ≥Grade 2 steroid-resistant /GVHD (adult)]	6	0.12 mg/kg (0357-006)	PTLD	Death
MI-CP049 [Phase I/ ≥Grade 2 steroid-resistant /GVHD (pediatric)]	7	0.4 mg/kg (0016010)	EBV-Lymphoma	Unknown
	8	0.012 mg/kg (0150-002)	EBV-LPD	Death
	9	0.4 mg/kg (0116-007)	EBV-Lymphoma	Not recovered
Investigator-initiated and Compassionate Use Studies				
Investigator-initiated/Stem cell transplantation for hematologic malignancies	10	0.1 + 0.6 mg/kg (02-163)	PTLD	Recovered
Investigator-initiated/Allogeneic BMT	11	6mg (0.1 mg/kg); 36mg (0.6 mg/kg) X 2 (97-561)	PTLD	Death
Compassionate Use – aGVHD	12	0.12 mg/kg X 9	PTLD	Death

1.4.2 Clinical Efficacy Data

Siplizumab treatment has been investigated in four clinical settings involving a variety of trial participants: renal transplantation (n=25), CD2+ lymphoproliferative disorders (n=77), GVHD (n=68), and psoriasis (n=775).

1.4.2.1 Clinical Efficacy in Renal Transplantation

In the case of renal transplantation, four clinical studies (MI-CP027; ITN010ST; ITN036ST/NCT00801632; NCT01780454) have been conducted with MEDI-507 (siplizumab).²⁰ In the first in human trial, MI-CP027, 13 *de novo* renal transplant recipients were treated with one of 3 IV siplizumab doses (0.012, 0.06, or 0.12 mg/kg) for induction in combination with standard of care immunosuppression. There were 5 cases of acute rejection during the first year post-transplant. Of the twelve renal transplant recipients treated with siplizumab in combination with non-myeloablative conditioning and donor bone marrow infusion, eight were successfully weaned off immunosuppression for >5 years with maintenance of allograft function with good safety and tolerability. Six ITB-MED sponsored trials are currently evaluating the activity of TCD-601 (siplizumab) in renal transplant tolerance (n=2), liver transplant tolerance (n=1), and renal transplant induction (n=3).

1.4.2.2 Clinical Efficacy in Lymphoma and GVHD

For the lymphoma indication, three Phase I clinical studies (MI-CP099, MI-CP107 and NCT01445535 [NCI sponsored; combination therapy]) were conducted with sipilizumab doses up to 15 mg/kg (total number of participants n=66). Overall, it was concluded that the benefit/risk profile did not support continued dose escalation of sipilizumab as a single agent in T cell malignancies. Similarly, high-dose, short duration regimens (12 weeks) of sipilizumab were evaluated in three Phase I studies (MI-CP042, MI-CP046, and MI-CP049) for treatment of T cell mediated pathology in GvHD following hematopoietic stem cell transplantation (total number of participants n=48). Although a signal for mild activity was noted in a GVHD, there was no benefit on disease progression.

1.4.2.3 Clinical Efficacy in Psoriasis

Of most relevance to this study is the combined experience investigating sipilizumab therapy for the treatment of severe plaque psoriasis (total number of participants n=629). Extended dosing regimens (1-4 months) of MEDI-507 (sipilizumab) have been evaluated in four Phase I studies (MI-CP040, MI-CP061, MI-CP062, and MI-CP081) and in three Phase II studies (MI-CP074, MI-CP076, and MI-CP082) for this indication;^{17,19} a follow-up observational study (MI-CP085) in psoriasis was stopped prior to the event-driven, protocol-defined endpoint due to a lack of specific events (i.e., certain infections, malignancy, and autoimmune disease flare). Overall, the clinical response (PASI) in the Phase II clinical studies was moderate compared to standard of care therapy and did not justify continued development in this indication. However, post-hoc analysis suggested that participants receiving doses >0.6 mg/kg by SQ injection had better PASI scores (see Section 1.4.3). Doses ranged from 0.0004-0.04 mg/kg IV and 0.1-10 mg SQ and were administered to participants for treatment periods up to 16 weeks.

Study MI-CP076 was a randomized, double-blind study of participants with psoriasis randomized to receive weekly injections of sipilizumab at doses of 3 mg for 12 weeks (n=28), 3 mg for 16 weeks (n=30), 5 mg for 12 weeks (n=32), or 5 mg for 16 weeks (n=31). Treatment with sipilizumab at a dose of 5 mg for 12 or 16 weeks generally resulted in a greater, but not statistically significant, improvement in PASI scores compared to treatment with a dose of 3 mg for 12 or 16 weeks. The proportions of participants with a PASI75 response by combined dose groups at Study Day 135 were 6/52 (11.5%) and 12/54 (22.2%) for the 3 mg and 5 mg groups, respectively. Duration of treatment (12 vs. 16 weeks) did not appear to have any meaningful effect on response.

Two subsequent randomized, double-blind, placebo controlled Phase II trials studies of sipilizumab were conducted in adults with psoriasis enrolled from sites in the USA and Canada.^{17,19} One (MI-CP074) evaluated IV sipilizumab dosing, with 124 participants randomized 1:1:1 to 0.04 mg/kg, 0.012 mg/kg, or placebo infusions every other week for 8 doses. The other study (MI-CP082) was a double-blind, randomized, placebo-controlled study of 420 participants randomized in a 1:1:1:1 ratio to receive SQ sipilizumab at 5 mg for 12 weeks (n=107), 5 mg for 6 weeks (n=101), 7 mg for 4 weeks (n=108), or placebo (n=104).¹⁹ Compared to placebo, treatment with sipilizumab resulted in clinically meaningful, although not statistically significant, improvements in psoriasis, as measured by the PASI

score. In the SQ dosing study, the PASI50 and PASI75 response rates indicate that a weekly dose of 5 mg, given for 6 or 12 weeks, produced non-statistically significant differences compared to placebo. At Study Day 107, the PASI75 response rates were 13%, 12%, 8%, and 6% for the 5 mg for 12 weeks, 5 mg for 6 weeks, 7 mg for 4 weeks, and placebo groups, respectively. Continued improvement in clinical response to treatment with sipilizumab was noted during the 3-month follow-up period. Although no benefit was seen compared to placebo in the two large Phase 3 trials, MI-CO076 and MI-CP082, an analysis of weight-based sipilizumab dosing showed intriguing differences. In MI-CP082, participants receiving a “high” dose of sipilizumab (defined as a cumulative SQ dose exposure of > 0.6 mg/kg) showed statistically better PASI75 scores compared to placebo-treated participants at all-time points. This suggests it is possible a dose-response relationship exists in autoimmunity where a sufficient dose of sipilizumab is needed for immune regulation.

In summary, sipilizumab targets the CD2 pathway, similar to alefacept, and appears to be safe when administered as monotherapy in psoriasis. There is evidence that sipilizumab has some clinical benefit in autoimmunity that may be dose dependent requiring different dosing regimens than previously used. Therefore, sipilizumab might play an important role in the development of immunomodulatory therapy in T1DM.

1.4.3 Initial Experience with Sipilizumab in T1DM

1.4.3.1 ITN095AI (DESIGNATE): Part A

Protocol ITN095AI Part A was a multi-dose design testing four SQ dosing regimens (0.08 mg/kg/dose, 0.12 mg/kg/dose, 0.18 mg/kg/dose, and 0.22 mg/kg/dose). Each participant was to receive 12 weekly doses. All 7 treated participants in Part A were randomized under protocol v3.0. For additional details on the study design, please see Section 3.1.1.

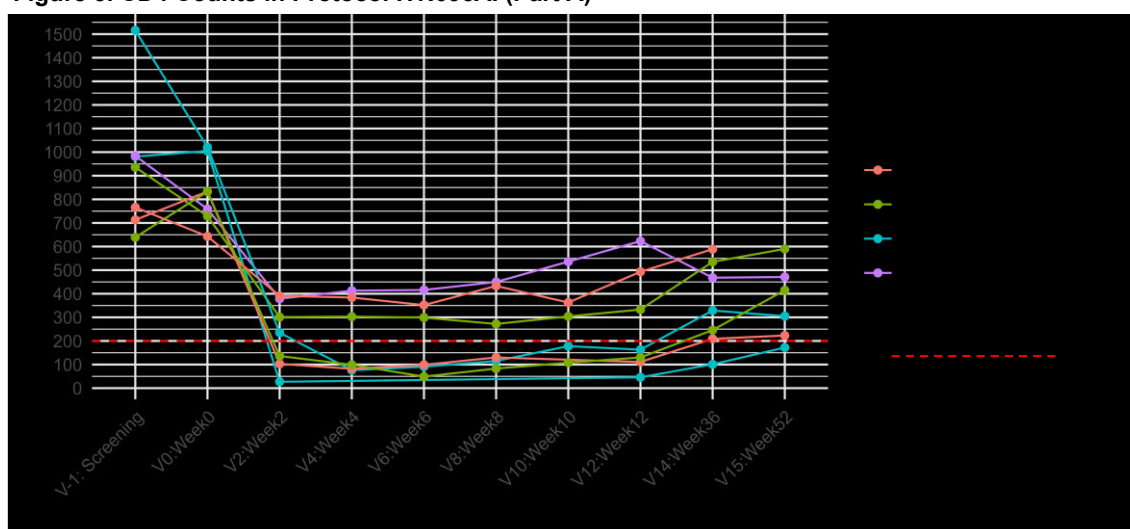
In Part A, each dosing arm had a planned initial enrollment of 5 adult participants. After the fifth per-protocol (PP) sample participant completed dosing (week 12), an interim analysis of the safety experience and the mechanistic changes in the T-cell population was planned. Part A’s schedule of events (see Appendix 1) and the EBV viral load management plan (see Appendix 2) are included for reference.

Of the 7 participants randomized and treated in Part A, four discontinued therapy due to excessive CD4 and/or lymphocyte depletion. No correlation between the dosing group and lymphodepletion was noted (Figure 3 and Table 5). There were no major infectious complications noted in any participant during the study. One participant had a symptomatic primary EBV infection, and another participant experienced a SARS-CoV-2 infection; both infections resolved without antiviral therapy.

Part A enrollment was suspended by the IND sponsor in January 2024 and placed on a Partial Clinical Hold (PCH) by the FDA in March 2024. Given the marked lymphodepletion, a long-term safety follow-up monitoring plan was added with protocol v4.0 (see Appendix 3).

Table 5. Nadir CD4 Counts in Protocol ITN095AI (Part A)

<u>Participant</u>	<u>Dosing Group (mg/kg/dose)</u>	<u>Completed Siplizumab Therapy</u>	<u>Nadir CD4 Count (cells/μL)</u>
1	0.08	Y	363
2	0.08	N	82
3	0.12	Y	272
4	0.12	N	49
5	0.18	N	<20
6	0.18	N	76
7	0.22	Y	379

Figure 3. CD4 Counts in Protocol ITN095AI (Part A)

1.4.3.2 ITB-MED STRIDE Study

The STRIDE study (NCT06025110) is a phase 2 randomized placebo-controlled study in adults with new onset T1DM (within 100 days of diagnosis) sponsored by ITB-MED. The primary outcome is the change from baseline in beta-cell function as compared to placebo at week 52 as measured by MMTT. A total of 96 adult participants are planned to be enrolled. After receiving a single IV loading dose of siplizumab (0.1 mg/kg), participants were randomized into one of three dosing groups: 0.12 mg/kg/dose, 0.24 mg/kg/dose, and 0.48 mg/kg/dose. Participants were expected to receive 12 weekly SQ doses. Enrollment was paused due to infusion reactions associated with the IV siplizumab loading dose, and the trial is currently being amended.

1.5 RATIONALE FOR DESIGNATE DOSING REGIMEN

1.5.1 Part A

The dosing rationale in Part A of Protocol ITN095AI was based on a post-hoc analysis of the Phase II psoriasis trial with weekly SQ MEDI-507 (siplizumab) administration, MI-CP082

(see Section 1.4.2). In this study, SQ doses of 5 mg and 7 mg for 4-12 weeks were studied in adult psoriasis patients. The trial did not meet its primary outcome, but a post-hoc, weight-based dosing analysis was performed. Doses were defined as high ($n=62$; ≥ 0.1 mg/kg based on the randomized unit dose or ≥ 0.6 mg/kg of the total drug received), low ($n=251$; participants receiving less than the high dose), or placebo ($n=104$). Significant improvement in clinical scores, as measured by mean percent reduction in PASI scores, were seen in the high mg/kg group vs. placebo at Study Days 91, 107, and 167: 36% vs. 23% ($p<0.05$), 36% vs. 18% ($p<0.01$), and 37% vs. 22% ($p<0.05$) respectively, suggesting a relationship between higher doses and clinical response¹⁴. The low dose of SQ siplizumab did not show significant differences from placebo (Figure 4). Importantly, recovery from lymphopenia did not differ between dosing groups (Figure 5). Based on this data, multiple weekly dosing regimens targeting cumulative doses >0.6 mg/kg were selected to evaluate the primary outcome.

Figure 4. Percent Reduction in PASI Scores in High vs. Low Subcutaneous Siplizumab Doses from MI-CP082¹⁷

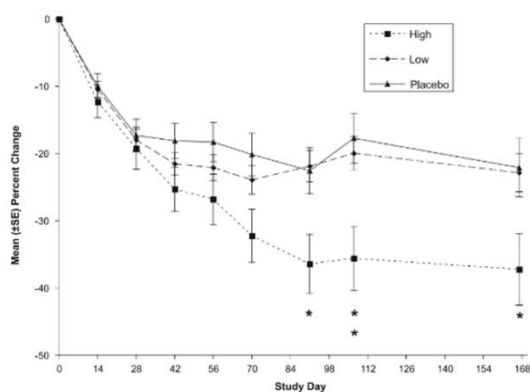
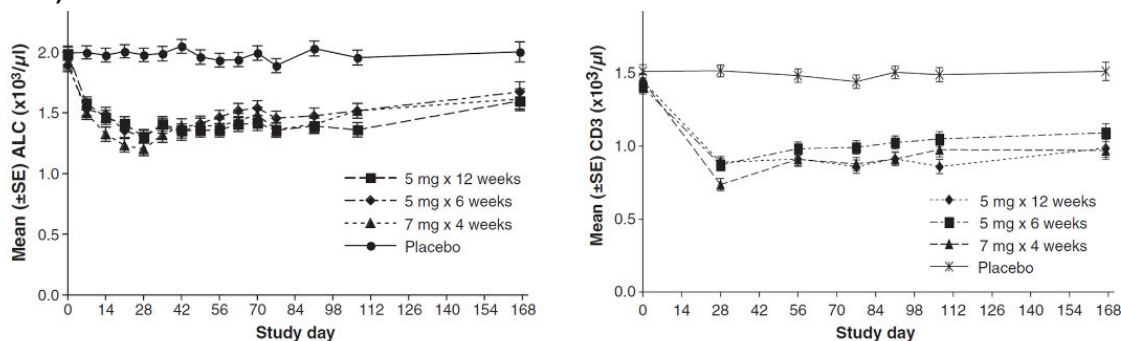


Figure 5. Changes in ALC and CD3 Cells with Subcutaneous Siplizumab (from Langley 2010 – MI-CP082)¹⁹



1.5.2 Part B

Given the magnitude of lymphodepletion observed in ITN095AI DESIGNATE Part A, Part B will employ a single ascending dose design to test lower doses of siplizumab (0.02 mg/kg, 0.04 mg/kg, and 0.06 mg/kg). In Part A, multiple doses ≥ 0.08 mg/kg/dose resulted in CD4 counts <200 cells/ μL in 4 of the 7 participants. Therefore, only single doses <0.08

mg/kg/dose are considered in Part B. The rationale for these doses comes from the observed tolerability of doses in two psoriasis studies using sipilizumab: MI-CP081 and MI-CP082.

In MI-CP081, participants received single SQ doses (3, 5, 7, and 10 mg) of sipilizumab. As described in the ITB-MED IB, lymphocyte nadirs were observed within 24 hours of dosing. These data, along with additional data extracted from the MedImmune final clinical study report for this study, are shown in Table 6.

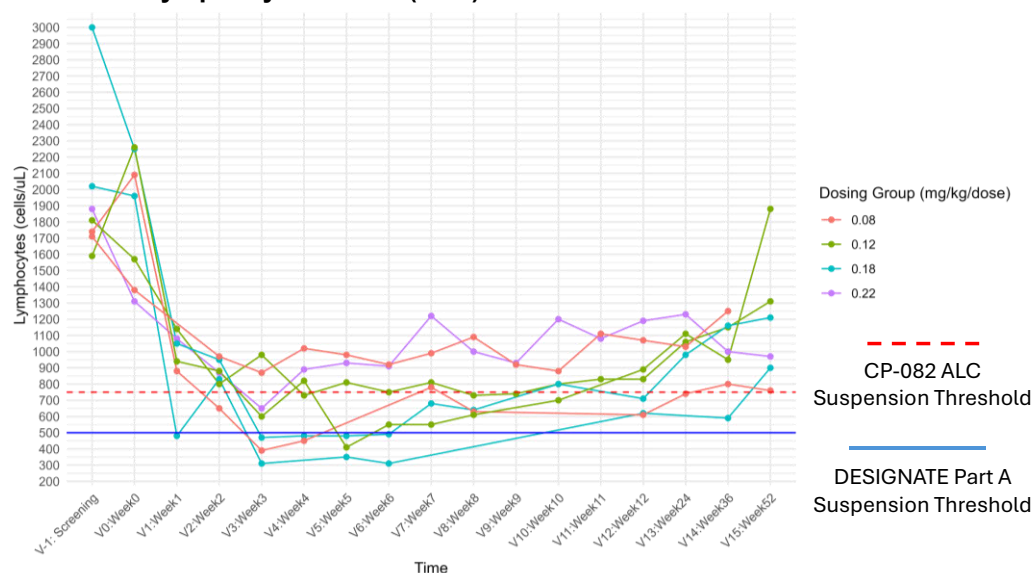
The doses of 3-10 mg used in MI-CP081 would have equated, in the first 7 randomized and treated participants in ITN095AI DESIGNATE Part A, to weight-based doses ranging from 0.04 to 0.16 mg/kg/dose. Together, these data suggest that single doses of sipilizumab do not result in deep and prolonged lymphodepletion.

Table 6. Changes in T-cells in Different Single-dose Dosing Groups in MI-CP081 (data derived from MI-CP081 CSR)

	3 mg	5 mg	7 mg	10 mg
Sample size	5	5	5	5
Mean percent decline in CD45+ at nadir (24 hours)	63	56	60	77
Mean number of days for CD45+ to return to 30% of baseline	5	9	9	30
Mean CD4 cell number at Day 30	833	661	889	629

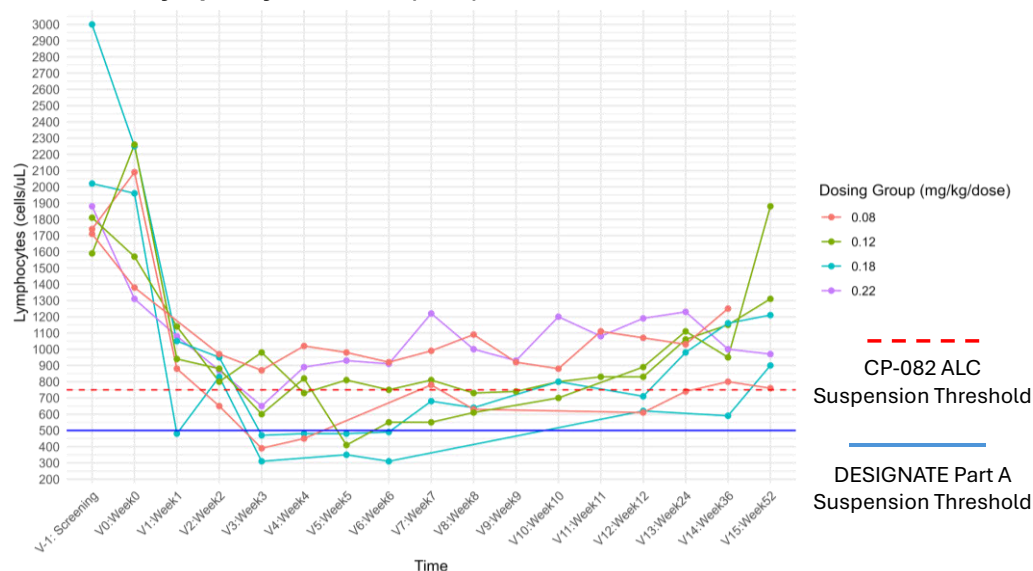
Second, an analysis was performed on weight data extracted from MedImmune's CSR of MI-CP082 (see Section 1.4.2). The three SQ sipilizumab doses in MI-CP082 were 5 mg for 12 weekly doses, 5 mg for 6 weekly doses, and 7 mg for 4 weekly doses, with discontinuation rates for lymphopenia of 17%, 11%, and 20%, respectively. The threshold for discontinuation for lymphopenia in MI-CP082 was an ALC <750 cells/ μ L. As shown in

Figure 6, six of the seven DESIGNATE Part A participants experienced ALCs $\leq$ 750 cells/ μ L at some point indicating a higher rate of discontinuation than MI-CP082 using similar criteria. The weight-based individual doses received by participants in MI-CP082 are shown in

Figure 6. Absolute Lymphocyte Counts (ALC) in DESIGNATE Part A

Given the data in MI-CP081 (demonstrating the lack of deep depletion with single doses of siplizumab) and in MI-CP082 (the range of individual doses tested in a multi-dose phase 2 study with discontinuation rates <20% started at 0.03 mg/kg/dose), a single ascending dose study design testing doses of 0.02, 0.04, and 0.06 mg/kg/dose was chosen.

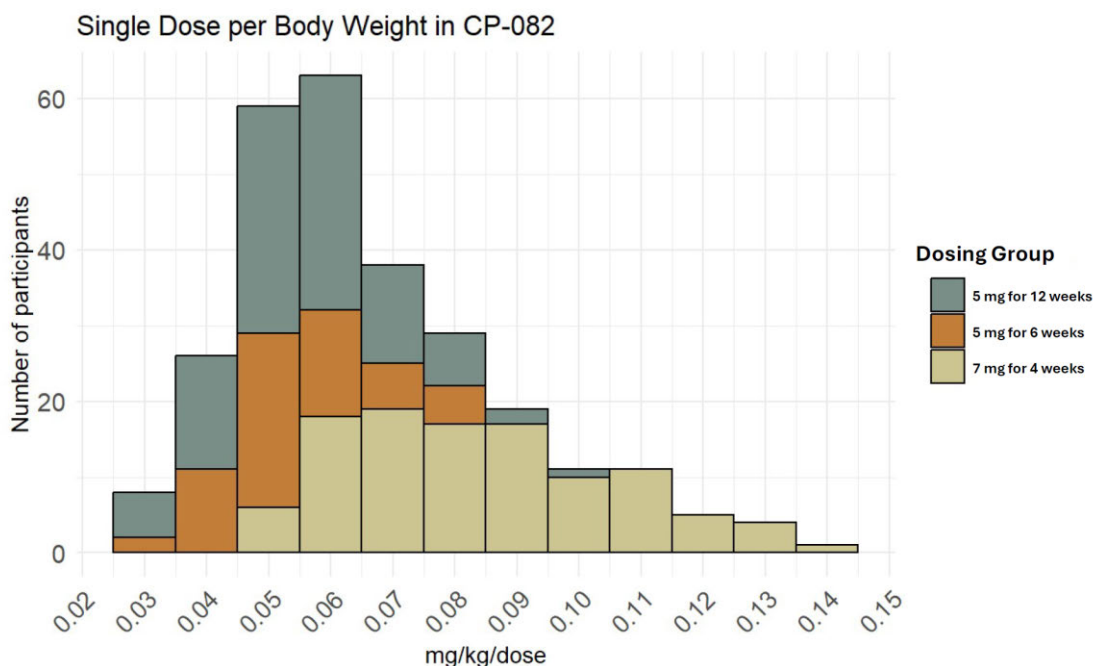
Figure 7. Participants in MI-CP082 received multiple individual doses of 0.08 and 0.12 mg/kg/dose, but doses as high as 0.18 mg/kg and 0.22 mg/kg (as used in Part A) were not tested.

Figure 6. Absolute Lymphocyte Counts (ALC) in DESIGNATE Part A

Given the data in MI-CP081 (demonstrating the lack of deep depletion with single doses of siplizumab) and in MI-CP082 (the range of individual doses tested in a multi-dose phase 2

study with discontinuation rates <20% started at 0.03 mg/kg/dose), a single ascending dose study design testing doses of 0.02, 0.04, and 0.06 mg/kg/dose was chosen.

Figure 7. Weight-based Dosing in MI-CP082 (data derived from MI-CP082 CSR)



2. STUDY HYPOTHESES / OBJECTIVES

2.1 HYPOTHESIS

The administration of sipilizumab to participants with recent-onset T1DM will increase the frequency of PD-1+TIGIT+ cells within the peripheral pool of CD4 Tem while increasing Treg/CD4 Tem ratios.

2.2 PRIMARY OBJECTIVE

To identify a safe, metabolically favorable dosing regimen for sipilizumab in participants with type 1 diabetes that induces changes in T cell phenotypes observed with alefacept therapy in new onset T1DM.

2.3 SECONDARY OBJECTIVES

1. To assess the safety profile of sipilizumab in recently diagnosed T1DM.
2. To assess the effects of sipilizumab on residual beta cell function in recently diagnosed T1DM participants.

2.4 EXPLORATORY OBJECTIVES

1. To assess the effects of sipilizumab on diabetes control.

2. Study how siplizumab administration alters immune cell function in peripheral blood.

3. STUDY DESIGN

3.1 DESCRIPTION OF THE STUDY DESIGN

Protocol ITN095AI consists of two parts, Part A and Part B.

Part A (see Section 1.4.3 and 3.1.1) was a multi-center, Phase Ib, randomized, open-label, dose finding study for individuals 8-45 years of age with T1DM diagnosed within 18 months of V0.

Part B (see Section 3.1.2) is a multi-center, Phase Ib, open-label, single ascending dose (SAD) finding study for individuals 8-45 years of age with T1DM diagnosed within 24 months of V0.

3.1.1 Part A

Participants were randomized 1:1:1:1 to one of four possible siplizumab dosing arms (Figure 8). The planned sample size for adult and pediatric cohorts is shown in

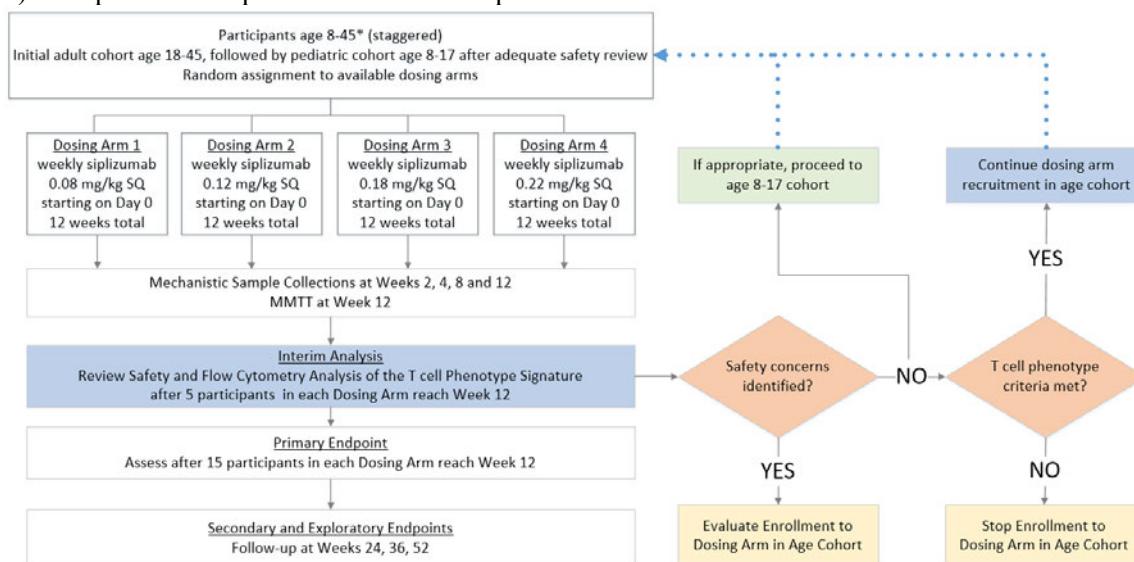
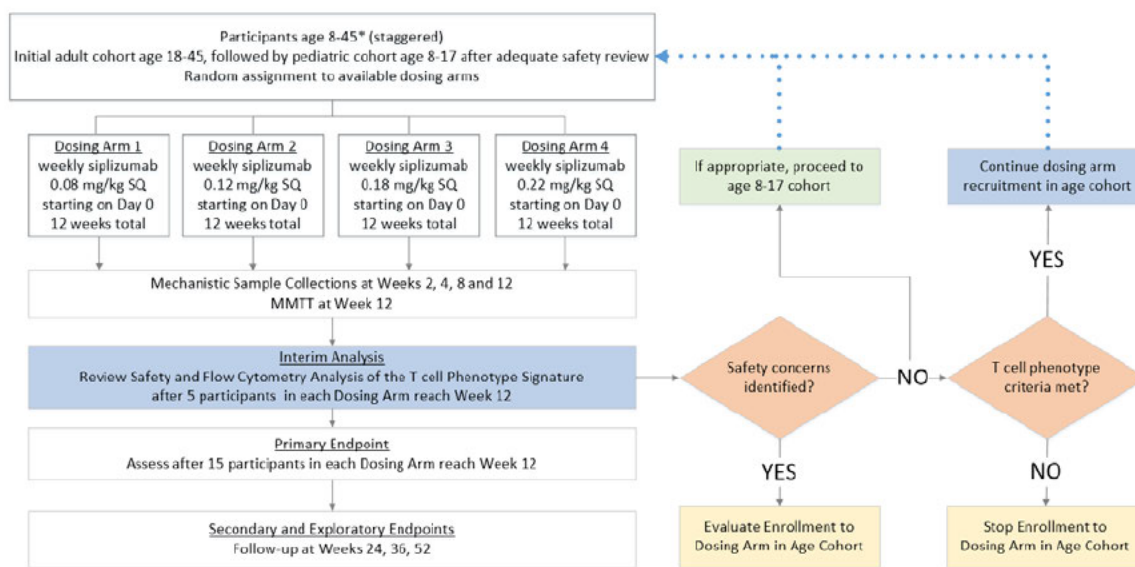


Table 7. Up to 120 participants were planned to be randomized across both adults and pediatric participants, with only adults between the ages of 18 and 45 years being enrolled initially. All dosing arms were scheduled to receive weekly SQ siplizumab doses for a total of 12 weeks. After the completion of siplizumab treatment, participants underwent follow-up visits at weeks 12, 24, 36, and 52, which included longitudinal MMTTs (Appendix 1). Due to prolonged CD4 cell depletion, some participants are followed in long-term safety monitoring for up to an additional 48 weeks (Section 6.4.3 and Appendix 3).

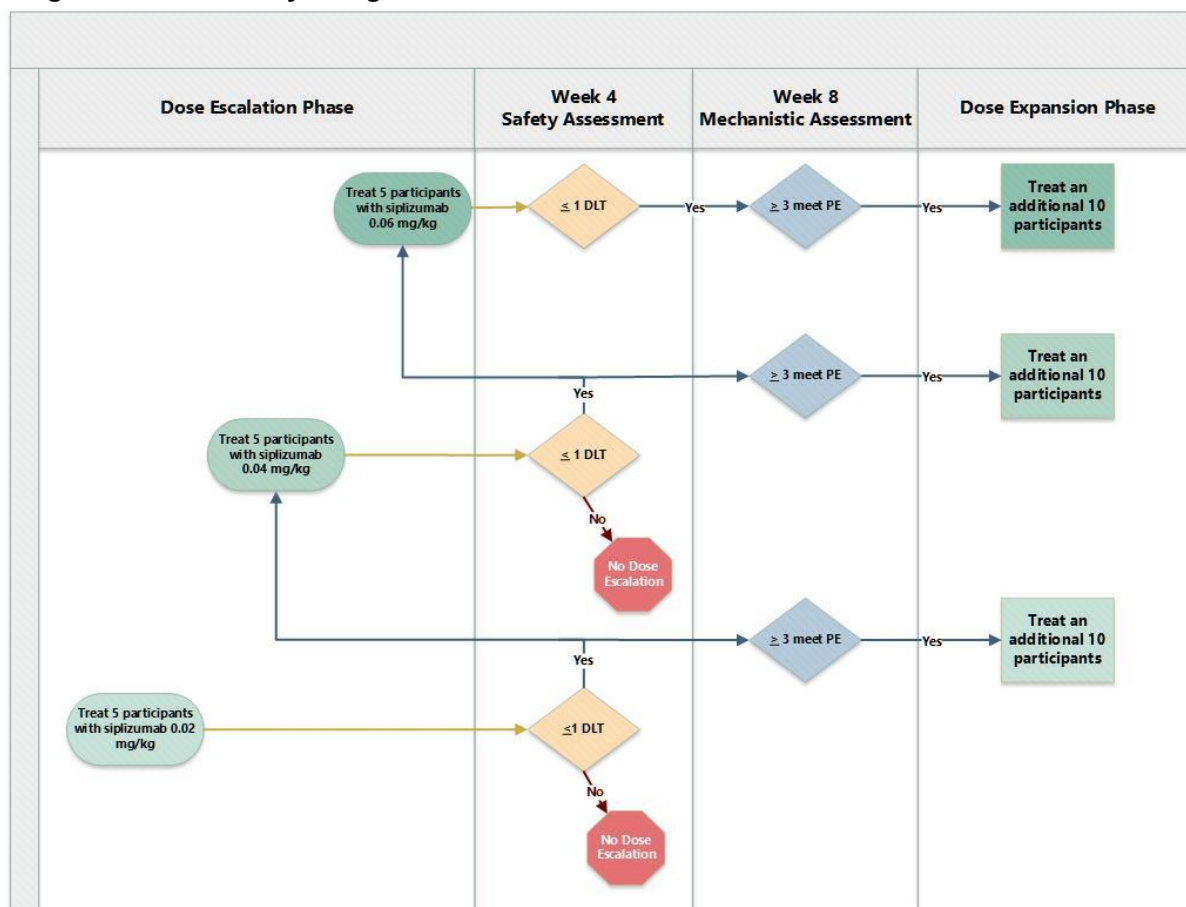
Figure 8. Part A Study Design**Table 7. Part A Adult and Pediatric Cohort Dosing Arms**

Dosing Arm	Target Number of Per-Protocol Participants Per Age Cohort	Siplizumab (SQ)
1	15	0.08 mg/kg/dose
2	15	0.12 mg/kg/dose
3	15	0.18 mg/kg/dose
4	15	0.22 mg/kg/dose
Total	60	

3.1.2 Part B

Two separate cohorts will be enrolled: an adult cohort (participants ages 18-45 years) and a pediatric cohort (participants ages 8-17 years). Three planned dosing arms will be tested in each cohort: 0.02 mg/kg, 0.04 mg/kg, and 0.06 mg/kg. Each participant will receive a single dose of the study drug. A total of 15 participants who meet the PP sample definition (see Section 13.4.1.2) are planned to be treated in each dosing arm. Five PP participants per dosing arm will be treated during dose escalation. Dosing arms will expand to 15 PP participants only if three of the first 5 PP participants meet the primary endpoint T-cell criteria (see Section 13.4.3) and no safety concerns are observed (see Table 10, Section 6.4.2). Treated participants who do not meet the PP definition will be replaced according to Section 11.3.

Treatment will occur in two distinct phases: dose escalation followed by dose expansion (Figure 9).

Figure 9. Part B Study Design

Participants in all dosing arms will receive one dose of SQ siplizumab at day 0 and have follow-up visits at weeks 1, 2, 4, 6, 8, 12, 24, 36, and 52. If indicated, participants will enter into long-term safety monitoring for up to an additional 48 weeks (Section 6.4.36.4.3 and Appendix 3).

Blood samples for mechanistic analyses will be obtained throughout the first 52 weeks. Endogenous insulin secretion will be assessed using the MMTT throughout the first 52 weeks (Appendix 4).

Dose Escalation Phase

Initially, adult participants will be treated in the 0.02 mg/kg arm and receive a single SQ dose at V0. After the 5th treated participant completes V3 (Week 4), a review of the safety data (inclusive of V3) will occur. If no more than one participant experiences a dose limiting toxicity (DLT) (Section 6.4.2, Table 10), treatment in the 0.04 mg/kg will proceed.

After the 5th treated participant in the 0.04 mg/kg arm completes V3 (Week 4), the safety data (inclusive of V3) will be reviewed. If no more than one participant experiences a DLT, treatment in the 0.06 mg/kg arm will proceed. Similar to the lower dosing arms, five participants will be treated in the 0.06 mg/kg/dose arm. See Section 11.6.2 if more than one participant in the first five experiences a DLT.

Additional participants may be treated if any participants need to be replaced (Section 11.3) in order to have 5 participants who meet the PP sample definition at Week 8. The lowest applicable dosing arm will be prioritized if participant replacement is needed. In the adult age cohort, if dosing arms are closed for safety reasons or after an interim analysis does not meet specific flow parameters, additional participants may be added to the remaining dosing arms so that a minimum of 30 participants across all dosing arms are treated.

In addition, a gated treatment strategy will be used (

Rationale for CD4 Thresholds During Gated Siplizumab Treatment

The CD4 thresholds used at Week 2 and Week 4 were selected based on analysis of data extracted from the MI-CP081 CSR. In that study, a total of four single doses was tested with 5 participants in each dosing group (3 mg, 5 mg, 7 mg, and 10 mg). Fourteen days after receiving siplizumab, 18 of the 20 participants had CD4 counts measured. Only 2 of the 18 participants had a CD4 count <300 cells/ μ L (one in the 5 mg group and one in the 10 mg group). Thirty days after receiving siplizumab, no participant had a CD4 count <300 cells/ μ L (see Section 1.5.2 and Table 6). In that study, lymphocyte counts were generally stable between day 14 and day 30.

In this protocol, the proposed thresholds at Week 2 and Week 4 are consistent with the data described above. If the Week 2 CD4 count is >300 cells/ μ L, that would indicate that the dose of siplizumab depleted lymphocytes similar to what was observed in MI-CP081. The proposed Week 4 CD4 count threshold of >200 cells/ μ L is also consistent with MI-CP081. As stated above, lymphocyte counts were stable between days 14 and 30 in MI-CP081. Therefore, a CD4 count >200 cells/ μ L at Week 4 (which would only be used if the Week 2 CD4 count was <300 cells/ μ L) would indicate that the depletion effect of siplizumab has stabilized (if the Week 2 CD4 count was between 200-300 cells/ μ L) or that lymphocytes are repopulating (if CD4 counts at Week 2 were <200 cells/ μ L).

The proposed gating strategy in each dosing arm will reduce the number of participants simultaneously exposed to siplizumab and allow the study team to detect if siplizumab depletes with a similar potency and duration as described above prior to treating more participants. It will also reveal if there is continued depletion of lymphocytes beyond 2 weeks after dosing.

Figure 10). In each dosing arm, the first participant's CD4 cell count at Week 2 must be ≥ 300 cells/ μ L in order to treat the second participant. If the second participant's CD4 cell count is also ≥ 300 cells/ μ L at Week 2, then the remaining three participants in that dosing arm will be treated without additional pauses. If either the first or second participant's CD4 cell count at Week 2 is <300 cells/ μ L, subsequent participants cannot be treated until after an evaluation

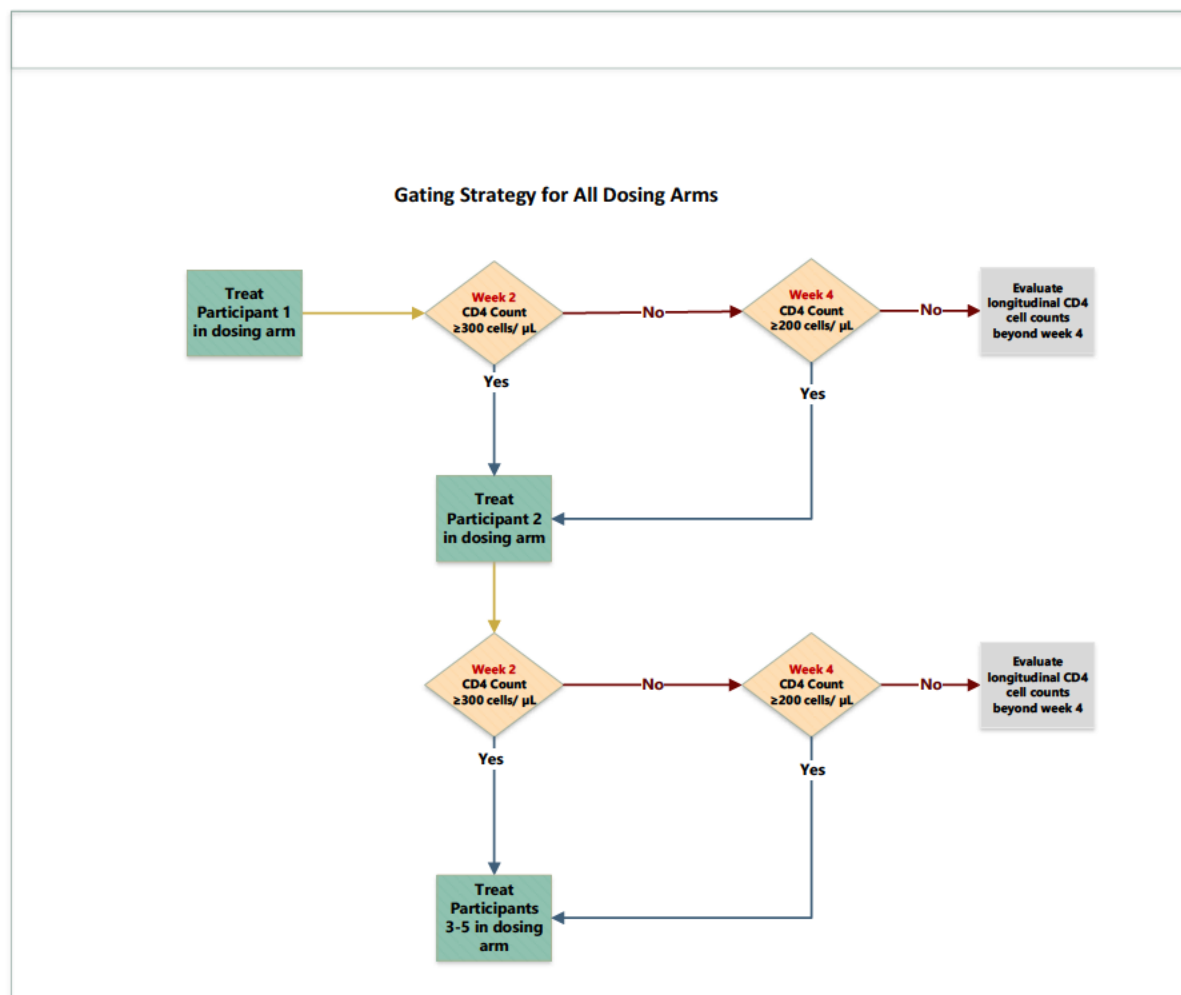
of that participant's Week 4 CD4 cell count. If the Week 4 CD4 cell count is ≥ 200 cells/ μ L, subsequent participants may be treated. If either of the first two participants has a Week 4 CD4 cell count < 200 cells/ μ L, further treatment will only occur after the DAIT/NIAID Medical Monitor, Protocol Chair, and ITN Clinical Trial Physician evaluate longitudinal CD4 cell counts in the treated participants beyond Week 4 and deem it safe to proceed.

Rationale for CD4 Thresholds During Gated Siplizumab Treatment

The CD4 thresholds used at Week 2 and Week 4 were selected based on analysis of data extracted from the MI-CP081 CSR. In that study, a total of four single doses was tested with 5 participants in each dosing group (3 mg, 5 mg, 7 mg, and 10 mg). Fourteen days after receiving siplizumab, 18 of the 20 participants had CD4 counts measured. Only 2 of the 18 participants had a CD4 count < 300 cells/ μ L (one in the 5 mg group and one in the 10 mg group). Thirty days after receiving siplizumab, no participant had a CD4 count < 300 cells/ μ L (see Section 1.5.2 and Table 6). In that study, lymphocyte counts were generally stable between day 14 and day 30.

In this protocol, the proposed thresholds at Week 2 and Week 4 are consistent with the data described above. If the Week 2 CD4 count is > 300 cells/ μ L, that would indicate that the dose of siplizumab depleted lymphocytes similar to what was observed in MI-CP081. The proposed Week 4 CD4 count threshold of > 200 cells/ μ L is also consistent with MI-CP081. As stated above, lymphocyte counts were stable between days 14 and 30 in MI-CP081. Therefore, a CD4 count > 200 cells/ μ L at Week 4 (which would only be used if the Week 2 CD4 count was < 300 cells/ μ L) would indicate that the depletion effect of siplizumab has stabilized (if the Week 2 CD 4 count was between 200-300 cells/ μ L) or that lymphocytes are repopulating (if CD4 counts at Week 2 were < 200 cells/ μ L).

The proposed gating strategy in each dosing arm will reduce the number of participants simultaneously exposed to siplizumab and allow the study team to detect if siplizumab depletes with a similar potency and duration as described above prior to treating more participants. It will also reveal if there is continued depletion of lymphocytes beyond 2 weeks after dosing.

Figure 10. Part B Gating Strategy for All Dosing Arms**Dose Expansion Phase**

Prior to expanding a dosing arm to 15 participants, an interim analysis will be performed after the 5th PP participant (see Section 13.4.1) in each of the three dosing arms completes V5 (Week 8). The interim analysis for one dosing arm may begin while participants are treated in a higher dosing arm. PBMC samples collected during the first 8 weeks (Weeks 0, 1, 2, 4, 6, and 8) will be analyzed by flow cytometry to determine the presence of the primary endpoint T cell phenotype signature:

1. A 75% increase or greater from baseline in the Treg/Tem ratio in blood and
2. A 20% increase or greater from baseline in the proportion of circulating PD1+TIGIT+ cells with the CD4 Tem population in blood

An individual who achieves both T cell markers at any time point from Week 1 up to and including Week 8 is considered to have met the T cell phenotype signature criteria. However,

both criteria do not need to be achieved at the same time point to be considered as meeting the definition.

An additional 10 participants per dosing arm will be treated if at least 3 of the 5 PP participants achieve the T-cell changes. Additional participants may be treated if any participants need to be replaced (Section 11.3) in order to achieve 15 participants per dosing arm who meet the PP sample definition at Week 8. Treatment in an expansion-eligible dosing arm will begin as soon as: 1) a review of the arm's interim flow data and safety data has been completed and 2) all higher dosing arms have 5 PP participants, assuming a safety signal does not preclude treating 5 PP participants. If multiple dosing arms are open simultaneously for expansion, participants will be randomized across the eligible dosing arms.

If dosing arms are closed for safety reasons (Section 11.6.2) or fail to meet the interim analysis criteria noted above, additional participants may be added to the remaining expansion-eligible dosing arms so that a minimum of 30 adult participants are treated in Part B.

Pediatric Cohort Considerations

After the 30th adult participant in Part B reaches V6 (Week 12), the SMT, Data and Safety Monitoring Board (DSMB) and FDA will review the available safety, mechanistic, and metabolic data. If, after a review of the safety, metabolic, and mechanistic data from the adult participants, the prospect of direct benefit is established, and the partial clinical hold on pediatric enrollment is lifted by the FDA, enrollment in the pediatric cohort (8-17 years old) will be initiated. Pediatric enrollment will follow the same strategy of treatment gating, dose escalation, and dose expansion with an interim analysis.

The prospect of direct benefit will be determined in part by comparing at least 3 months of insulin secretion data from at least 30 adult siplizumab-treated participants with that of historical data from participants in T1DM clinical studies, which have been conducted through DAIT NIAID-funded ITN and National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK)-funded TrialNet networks, as well as from other published studies, if available. At least 5 participants from the highest dosing arm in Part B will be included in the analysis if safety issues do not preclude treatment of 5 participants in that particular dosing arm. Comparisons of siplizumab-treated participant data with historical controls will be analyzed in multiple ways. A modified assessment of the previously published quantitative response (QR) may be used.²⁵

It is possible that siplizumab doses that do not elicit the desired T cell phenotype changes in adults may, in fact, be effective in achieving this mechanistic endpoint in children because of potential differences in PK between adults and children and potential differences in underlying disease mechanisms. Therefore, doses used in the adult cohort will be used in the pediatric cohort regardless of T cell changes observed in adults unless a dosing arm has been closed to further treatment due to meeting safety criteria or if, in the opinion of the SMT, FDA, or DSMB, the AE profile of a dosing strategy would increase the safety risk in the pediatric cohort. Like the adult cohort, an interim analysis of the T cell phenotype signature

will be conducted in the pediatric cohort for each dosing arm after the 5th participant who meets the PP sample definition in each dosing arm reaches Week 8.

3.2 PRIMARY ENDPOINT

3.2.1 Part A

1. Change from baseline in PD1+TIGIT+ frequency within circulating CD4 Tem cells during the first 12 weeks and
2. Change from baseline in the CD4 Treg/Tem ratio during the first 12 weeks in blood.

3.2.2 Part B

1. Change from baseline in PD1+TIGIT+ frequency within circulating CD4 Tem cells during the first 8 weeks and
2. Change from baseline in the CD4 Treg/Tem ratio during the first 8 weeks in blood.

3.3 SECONDARY ENDPOINTS

1. Frequency of AEs in all siplizumab dosing arms.
2. MMTT-stimulated mean 2-hour C-peptide AUC at Weeks 12, 24, 36 and 52. The mean 2-hour C-peptide AUC, measured in nmol/L, is computed by dividing the total AUC by the length of the MMTT in minutes.
3. Insulin use (U/kg/day) at Weeks 12, 24, 36 and 52.

3.4 EXPLORATORY ENDPOINTS

1. Glycemic control using continuous glucose monitoring data.
2. HbA1c over time.
3. Changes in phenotype, function, and epigenetic programming of immune cell subsets.

3.5 STRATIFICATION, RANDOMIZATION, AND BLINDING/MASKING

3.5.1 Part A

Participants who signed the informed consent/assent and met the eligibility criteria were randomly assigned to available siplizumab dosing arms.

3.5.2 Part B

During the dose escalation phase, participants who sign the informed consent/assent and meet the eligibility criteria will be treated according to the open dosing arm. During dose expansion, participants will be randomized evenly into open dosing arms if multiple dosing arms are open.

4. SELECTION OF PARTICIPANTS

4.1 RATIONALE FOR STUDY POPULATION

The study population will consist of recently diagnosed (within 24 months) participants with T1DM with significant residual beta cell function (peak C-peptide during an MMTT >0.1

nmol/L). A larger window for disease duration was chosen to facilitate recruitment. Importantly, to provide evidence for the prospect of benefit in pediatric participants, we will compare at least 3 months of insulin secretion data from at least 30 adult siplizumab-treated participants with that of historical data from participants in T1DM clinical studies, which have been conducted through DAIT NIAID-funded ITN and National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK)-funded TrialNet networks, as well as from other published studies, if available. The bulk of this historical data is collected from participants within 24 months of diagnosis. Therefore, the inclusion of participants up to 24 months from diagnosis improves recruitment while still allowing for the comparison of clinical and metabolic outcomes to duration-matched participant data.

A similar rationale is used for lowering the peak C-peptide on the screening MMTT to 0.1 nmol/L. The entry criteria of peak C-peptide of 0.1 nmol/L will improve recruitment while still allowing for a determination of whether siplizumab has adverse or beneficial effects on residual beta cell function.

4.1.1 Rationale for Use in Pediatrics

The number of patients being diagnosed with T1DM is increasing each year. While T1DM can occur at any age, the worldwide incidence of T1DM is increasing rapidly in children younger than 15 years, as documented by the WHO Diamond Project.²⁶ During the period 1995–1999, the global annual increase in childhood T1DM was 3.4% and as high as 5.3% in North America.²⁶ In Europe, the EURODIAB study group found the greatest rate of increase in the 0–4 years age group and predicted a doubling in the number of new cases in children younger than 5 years over the next decade.²⁷ Overall, prevalence of T1DM in children under age 15 in Europe is predicted to rise from 94,000 in 2005 to 160,000 in 2020.²⁷ In contrast, the incidence of T1DM is not increasing in young adults over age 15.²⁷ Similarly, the SEARCH study also noted upward trends in T1DM incidence in USA youth, with an annual increase of 1.4%/year from 2002–03 to 2011–12.²⁸ For these reasons, there is considerable interest in identifying safe and effective interventions that can modify the course of T1DM in pediatric populations.

Aside from asthma, T1DM is the most common chronic childhood disease and is a particular burden to children and their families. Short term complications include severe hyperglycemia and diabetic ketoacidosis (DKA), a life-threatening condition. As reported in the SEARCH study, almost one-third of individuals up to 19 years of age present with DKA at the time of clinical diagnosis. While the high prevalence of DKA has remained stable from 2002–2010 in the USA, it appears to be increasing in some states,²⁹ mirroring observations in other countries.³⁰ In addition to the cost of hospitalization, a rare but significant concern associated with DKA is cerebral edema, which is associated with a high risk for morbidity and mortality.³¹ Recurrent DKA after the new onset period remains a significant clinical problem, with 3% of T1D Exchange participants experiencing DKA in the prior 3 months,³² and with the highest frequency in those <26 years of age. Another significant acute risk is hypoglycemia, which can result in impaired cognition and, if severe, coma and death.³² Within the past 3 months, 5% of participants <18 years of age in the T1D Exchange registry reported a severe hypoglycemia event.³² Although continuous glucose monitoring is a means

to ameliorate risk for hypoglycemia, only 17% of adolescents in the T1D Exchange registry used this technology, the least of all age groups.³³

Regarding long term complications, the Diabetes Control and Complications study (DCCT) showed that microvascular complications could be reduced with near normal control of glucose levels but at the cost of an increased frequency of severe hypoglycemia.³⁴ One challenge found from analyzing results from the DCCT was that adolescent participants were not able to attain the same level of glycemic control as their adult counterparts, in both the intensive and conventional treatment arms. While there have been significant improvements in insulin analogs and insulin delivery systems since that time, such as continuous SQ insulin infusions with insulin pumps, continuous glucose monitoring, and hybrid closed loop systems, normal glucose control, particularly in children, is rarely achieved.³² Only 17% of children and adolescents <18 years of age met the American Diabetes Association's HbA1c targets. This is particularly true in the adolescent years, where the mean HbA1c in the T1D Exchange was reported in 2019 to be 9.3%. We note that there is no central registry for T1DM outcomes in the USA. The T1D Exchange registry enrolled ~33,000 participants followed at the top diabetes clinics around the country; thus, it offers what many consider to be the best in clinical practice T1DM metrics in the USA rather than a representative experience of outcomes from the overall T1DM community.

As a result of suboptimal glycemic control, while the frequency of long term complications, including end stage renal disease, is decreasing,³⁵ some degree of kidney disease is seen in virtually all those with long-standing T1DM,³⁶ and individuals with T1DM continue to have reduced life expectancy.^{3,37} In a recent study, 5.9% of SEARCH participants diagnosed at a mean age of 10 years had the co-occurrence of 2 or more complications after a mean duration of 7.8 years of disease.³⁸ Youth with T1DM remain at high risk for a number of other concerning outcomes, including increased risk for obesity and disordered eating;^{39,40} and mental health disorders, including depression, anxiety, and substance abuse.^{41,42} There have also been longstanding concerns about the impact of altered metabolism on the developing brain.⁴³ Recent reports highlight the vulnerability of children with T1DM to cognitive dysfunction, as well as structural CNS changes.⁴⁴⁻⁴⁶

Data from the NIH-sponsored TrialNet consortium has demonstrated that rate of decline in C-peptide post diagnosis is highly correlated with age, with a significantly more rapid rate of decline in those 21 years old and younger.⁴⁷ The reason why beta cell destruction proceeds faster in children than adults has not been fully delineated, although several lines of evidence suggests fundamental differences in the underlying genetic predisposition and autoimmune response across the age spectrum. Howson et al. ascribe these differences to a lower genetic load of genes predisposing to T1DM in adults than children.⁴⁸ Arif et al. have reported distinct immunological differences between younger children and adolescents who develop T1DM.^{49,50} In peripheral blood, younger children exhibit multiple autoantibodies with an IFN- γ dominated CD4 T cells response, as opposed to a more limited autoantibody response in the older group with an IL-10 dominated CD4 T cell response.^{49,50} Histopathological analyses of islets have revealed that younger patients show relatively heavy immune infiltration compared to older individuals and consist of greater numbers of CD20+ cells.⁵⁰ Another study of autoreactive T cell responses shortly after T1DM diagnosis found

significantly greater pro-inflammatory autoreactivity in younger participants, with preferential targeting of insulin and proinsulin peptides.⁴⁹ Employing whole blood RNA-seq from serial samples, Dufort et al. found a reciprocal correlation between age and higher levels of B cell gene expression, lower general neutrophil and neutrophil primary granule gene expression levels, and the rapidity of beta cell loss.⁵¹

Any intervention, that can stop or delay the progression to clinical disease or complete loss of functional residual β -cell mass, is significant as it may reduce the burden of living with clinical disease and/or provide protection against hypoglycemia and improved metabolic control, resulting in a delay in the micro and macro-vascular complications of diabetes. Indeed, children may stand the most to gain from interventions designed to preserve beta cell function, as they start with less beta cell mass at the time of diagnosis and decline more rapidly over time. Furthermore, the differences between children and adults in the underlying genetic predisposition and the autoimmune response of T1DM may have therapeutic implications, as various strategies may need to be taken depending on the age of onset of the disease. Although new onset T1DM immunotherapy trials are often not powered to determine the impact of age on response to therapy, there are several positive new onset studies in which outcomes appear to be largely driven by responses in the pediatric population, including trials with rituximab and abatacept.^{8,9,52} Given that children with T1DM face unique clinical challenges and appear to have disease mechanisms that can be distinguished from individuals whose disease develops at an older age, it is important in this initial study to investigate siplizumab therapy not only in a cohort of adults but also in children.

4.2 INCLUSION CRITERIA

Individuals must meet all of the following criteria to be eligible for treatment as study participants:

1. Ability to provide informed consent (parental permission and informed assent of minor, if applicable).
2. Male or female between 8 to 45 years of age.
3. Diagnosis of T1DM within 24 months (730 days) of V0.
4. Positive for at least one diabetes-related autoantibody, including:
 - a. Glutamate decarboxylase (GAD-65),
 - b. Insulin, if obtained within 10 days of the onset of exogenous insulin therapy,
 - c. Insulinoma antigen-2 (IA-2),
 - d. Zinc transporter-8 (ZnT8).
5. Peak stimulated C-peptide level > 0.1 nmol/L following a MMTT conducted ≥ 21 days from diagnosis and within 37 days of V0.

4.3 EXCLUSION CRITERIA

Individuals who meet any of the following criteria are not eligible for treatment as study participants:

1. Use of investigational drugs within 24 weeks of participation.

2. Severe reaction or anaphylaxis to humanized monoclonal antibodies.
3. Inability to complete a mixed meal tolerance test:
 - a. History of significant allergy (e.g., anaphylaxis) to milk or soy proteins,
 - b. Inability to disable hybrid closed loop system.
4. History of recent (within 180 days of V0) or ongoing uncontrolled bacterial, viral, fungal, or other opportunistic infections, including:
 - a. Human immunodeficiency virus (HIV),
 - b. Current or prior infection with hepatitis B (HBV), as indicated by positive HBsAg or positive HBcAb,
 - c. Current or prior hepatitis C (HCV), unless treated with anti-viral therapy with achievement of a sustained virologic response (as indicated by undetectable viral load 12 weeks after cessation of therapy),
 - d. Positive Quantiferon-TB Gold or Quantiferon-TB Gold Plus tests. PPD or T-SPOT®.TB may be substituted for the Quantiferon-TB Gold or Quantiferon-TB Gold Plus tests,
 - e. Active infection with EBV as detected by polymerase chain reaction (PCR) or serology at the screening visit (V-1),
 - f. Active infection with cytomegalovirus (CMV) as detected by PCR or serology at the screening visit (V-1).
5. Any of the following laboratory abnormalities, confirmed by repeat tests at least 1 week apart:
 - a. White blood count (WBC) $< 3 \times 10^3/\mu\text{L}$,
 - b. CD4 T cell count below the lower limit of normal (LLN),
 - c. Platelet count $< 150,000/\mu\text{L}$,
 - d. Hemoglobin $< 10 \text{ g/dL}$,
 - e. ALT $\geq 2\times$ upper limit of normal (ULN),
 - f. AST $\geq 2\times$ ULN,
 - g. Serum creatinine $>1.5\times$ ULN in adults or $>\text{ULN}$ in pediatrics,
 - h. Absolute lymphocyte count (ALC) below the LLN.
6. Prior or current treatment that is known to alter the natural history of T1DM or immunologic status, including high dose inhaled, extensive topical or systemic glucocorticoids.
7. Current or prior (within last 14 days of the V-1 MMTT) use of any medication known to influence glucose tolerance (e.g., atypical antipsychotics, diphenylhydantoin, thiazide, or other potassium-depleting diuretics, β -adrenergic blockers, niacin).
8. Current or prior (within the last 30 days of the V-1 MMTT) use of non-insulin medications to treat insulin resistance or elevated glucose levels.
9. Previous or current diagnosis of malignancy.

10. History of bone marrow transplantation, solid organ transplantation, or primary immunodeficiencies.
11. History or diagnoses of other autoimmune diseases with the exception of stable thyroid or celiac disease.
12. History of significant cardiovascular disease.
13. Vaccination with a live attenuated vaccine (e.g., varicella, measles, mumps, rubella, cold-attenuated intranasal influenza vaccine, bacillus Calmette-Guérin, and smallpox) within 30 days of V0.
14. Women of child-bearing potential who are unwilling to use a medically acceptable form of contraception from 14 days prior to V0 until study Week 52.
15. Women who are pregnant, lactating, or planning on pregnancy during the study.
16. Current diagnosed mental illness (e.g., severe depression) or current diagnosed or self-reported drug or alcohol abuse that, in the opinion of the investigator, would interfere with the participant's ability to comply with study requirements.
17. Past or current medical problems or findings from physical examination or laboratory testing that are not listed above, which, in the opinion of the investigator, may pose additional risks from participation in the study, may interfere with the participant's ability to comply with study requirements, or may impact the quality or interpretation of the data obtained from the study.

5. KNOWN AND POTENTIAL RISKS AND BENEFITS TO PARTICIPANTS

5.1 RISKS OF INVESTIGATIONAL DRUG AS CITED IN INVESTIGATOR'S BROCHURE

Siplizumab has been studied in 18 clinical studies with at least 779 participants exposed to the drug. Clinical studies have evaluated siplizumab in participants following renal transplantation as well as with lymphoma, aGVHD, and psoriasis. The largest clinical studies with the drug have been conducted in participants with psoriasis. No contraindications to the drug are listed in the current ITB-MED IB.

In the 4 completed (current version of ITB-MED IB) renal transplantation studies (total n=25), cytopenias were the most commonly reported AE. In MI-CP027, AEs reported as potentially related to siplizumab, other than those listed above, were anemia, chest pain, herpes esophagitis, and hypophosphatemia. There were 16 SAEs reported in 9 study participants. A single study participant experienced a basal cell carcinoma of the skin on an area of actinic exposure. Siplizumab administered in 1 to 2 doses from 0.012 mg/kg to 0.12 mg/kg was not associated with an unusual frequency of late infections, acute rejection, graft loss, malignancy, PTLT or death. In Protocol ITN010ST (BB-IND #101081 sponsored by MGH (David Sachs, MD), NCT00063817), where siplizumab was combined with non-myeloablative conditioning and a bone marrow cell infusion, one of the five participants developed a basal cell carcinoma on an underlying area of skin damage. A total of 89 AEs, 11 SAEs, and 3 infections were reported. Nine of these SAEs were considered 'possibly', 'probably', or 'definitely' related to study treatment. In Protocol ITN036ST (DAIT/NIAID-

sponsored IND #106802, NCT00801632), a study that used a similar regimen to ITN010ST with additional corticosteroids and B-cell depleting therapy (n=5), 40-60% of participants developed an infection (UTI or *C. difficile* colitis), 40% developed hypophosphatemia, and 40% developed transaminitis. SAEs were reported in 34.5% to 50% of renal transplant recipients in ITN036ST and ITN010ST.

The siplizumab experience in CD2 positive lymphoma is similar with a total of 66 participants with this condition treated. The two largest studies in CD2+ LPDs (MI-CP099 n=29 and MI-CP107 n=22) used escalating doses of weekly siplizumab up to 16 weeks. Both studies enrolled participants aged 18 and older. A total of 16 SAEs were reported in 10 participants in MI-CP099. The SAEs included EBV-associated lymphoproliferative disorder (4 participants, 13.8%); pyrexia (2 participants, 6.9%); and febrile neutropenia, thrombocytopenia, neutropenia, central line infection, catheter-related infection, acute myelomonocytic leukemia, malignant neoplasm, neoplasm progression, convulsion, and acute febrile neutrophilic dermatosis (1 participant each, 3.4%). SAEs occurred in 11 (50%) of the participants in MI-CP107, with 2 occurrences of pulmonary edema being the most common SAE reported; 4 (18%) participants had infections classified as SAEs, including sepsis, RSV, and Staphylococcal bacteremia.

The experience in GVHD (n=48) is difficult to interpret since the multiple toxicities reported in these studies were expected in post-HSCT participants suffering from GVHD. The largest study reported in the IB is MI-CP046 (n=34), a double-blind randomized placebo-controlled dose escalation study to compare concomitant conventional steroid administration with siplizumab vs. placebo in GVHD. The most common SAE was worsening of GVHD, reported by 6 (29%) siplizumab and 3 (23%) placebo participants. SAEs judged to be related to study drug were reported by 3 siplizumab participants (total of 6 events: viral infection, marrow depression (graft failure), multi-system organ failure, heart arrest, dizziness, and apnea) and 1 placebo participant (fungal infection). There were two cases of primary disease relapse, one AML relapse in the 0.4 mg/kg siplizumab group and one AML relapse in the placebo group. Infections were common in both siplizumab (19 [90%]), and placebo (12 [92%]) participants and the most common site of infection was the blood.

A total of 629 participants with psoriasis have been treated with siplizumab. The two largest placebo-controlled studies included participants receiving 8 doses of IV siplizumab (MI-CP074) every other week and participants receiving SQ siplizumab for 4-12 weeks (MI-CP082). These Phase II studies of siplizumab therapy for psoriasis did not demonstrate clinical efficacy.¹⁹ The most commonly reported AEs are listed in Table 2 and Table 3.

SAEs were reported in both MI-CP074 and MI-CP082. The most frequently reported adverse events in siplizumab treated participants were lymphopenia, chills, and headaches in MI-CP074. In MI-CP082, 6/316 participants (2%) of siplizumab and 1/104 (1%) of placebo participants experienced an SAE. In addition, 16% of siplizumab and 7% of placebo participants experienced a severe AE mostly secondary to lymphopenia. In this study, approximately 6% of participants in the siplizumab group developed an absolute lymphocyte count <500 cells/ μ L. Lymphopenia in these trials persisted beyond the dosing phase (see

Figure 5). Prolonged lymphopenia upon completion or sipilizumab withdrawal was also observed in ITN095AI (DESIGNATE) Part A (see Figure 3).

Siplizumab has been associated with LPDs/EBV reactivation. However, most reported cases have been observed in the context of unique study populations with a history of malignancies (i.e., CD2+ LPD and GVHD), and the rates are consistent with the background rates expected for those populations. Two participants participating in an ongoing ITB-MED sponsored clinical study in the EU (TCD601H101 / AURORA; NCT06453668) have presented with EBV associated SAEs / SUSARs. The trial is investigating the activity of sipilizumab in patients with amyotrophic lateral sclerosis (ALS) and has enrolled 7 patients at the time of writing. The first participant presented with a fatal multi-organ system failure associated with EBV reactivation. The second participant presented with multiple SAEs associated with EBV reactivation including EBV-retinitis as well as EBV DNAemia. The pan-uveitis resulted in a significant loss of visual acuity in the affected eye while the DNAmia has been partially responsive to anti-CD20 therapy and is ongoing. Both subjects were EBV-seropositive and received a cumulative dose of 30 mg/kg of sipilizumab intravenously over 2 months prior to presenting with EBV reactivation.

Siplizumab is a monoclonal antibody, and its administration may theoretically result in a cytokine release syndrome. To date, no cases of cytokine release syndrome have been noted with the use of sipilizumab by ITB-MED. Infusion reactions, as noted above, are mild-to-moderate and can be readily managed with acetaminophen, diphenhydramine and corticosteroids. Infections during sipilizumab treatment, while common in the GVHD and lymphoma studies, were not reported as an adverse event in the large psoriasis trials. Finally, two cases of malignancy have been observed in psoriasis studies (squamous cell carcinoma of the skin and MDS). Both cases occurred in participants with a prior history of these conditions and were not considered related to study drug.

Detailed information on the adverse events in various studies using sipilizumab is included in Section 1.4.

5.2 RISK OF INVESTIGATIONAL DRUG CITED IN MEDICAL LITERATURE

Aside from studies described above and listed in the current ITB-MED IB, the safety experience with sipilizumab has also been described in a study in 14 participants with T cell leukemia/lymphoma treated with an EPOCH-RS regimen.⁵³ All but one participant in the study had stage IV disease. There was no evidence of EBV reactivation reported in this study when sipilizumab was used in combination with Rituxan. There were 6 participants who suffered serious infections of at least grade 3 severity, including one participant who died from gram positive sepsis.

In addition, sipilizumab has been studied in 12 participants receiving bone marrow transplantation for hematological malignancy.⁵⁴ Aside from the development of GVHD, no other safety data were reported in the published article. In this study, two participants receiving sipilizumab therapy developed GVHD and one participant developed chronic GVHD.

5.3 RISK OF OTHER PROTOCOL SPECIFIED MEDICATIONS

Diphenhydramine may cause drowsiness, dizziness, headache, and dry mucous membranes.

5.4 RISKS OF STUDY PROCEDURES

The risks of the study procedures are as follows:

1. Study drug administration: Injection site reactions such as pain, redness, or swelling at the injection site are common.
2. Blood draw/IV-line insertion: Low risk of hemorrhage, hematoma, or infection at the venipuncture site. Dizziness and/or fainting can occur during the blood draw. Efforts will be made to minimize the discomfort by using well-trained personnel to perform this procedure.
3. MMTT: Low risk of nausea from the consumption of a liquid meal (i.e., Boost)

5.5 POTENTIAL BENEFITS

There may be no direct benefit to participants treated in Protocol ITN095AI. Siplizumab modulates the same pathway as alefacept used in Protocol ITN045AI T1DAL (conducted under DAIT/NIAID-sponsored IND 105,308). That study demonstrated a preservation of residual insulin secretion compared to placebo participants.¹¹ Although the current study is not designed to determine if siplizumab has similar metabolic effects as alefacept, there is potential that siplizumab may offer similar benefits in preserving residual insulin secretion, especially if siplizumab modulates T cell phenotypes similarly to alefacept. Retained residual insulin secretion benefits participants by reducing the probability of developing microvascular complications.⁵⁵ Therefore, a potential for benefit exists for participants treated with siplizumab based on this reasoning.

6. INVESTIGATIONAL AGENT

6.1 INVESTIGATIONAL PRODUCT

6.1.1 Siplizumab

Siplizumab is a recombinant monoclonal antibody with a human IgG1, kappa directed to CD2. Siplizumab is not approved in the United States or other countries for any indication and is under clinical development by ITB-MED, LLC. Under Current Good Manufacturing Practice (cGMP) conditions, siplizumab will be manufactured, packaged, labeled, and supplied to DAIT/NIAID for distribution to clinical sites.

6.1.2 Formulation, Packaging, and Labeling

Siplizumab (40 mg) is supplied as a clear to slightly opalescent, colorless to slightly brownish solution for injection and does not contain a preservative. The inactive ingredients include histidine HCl, sucrose, and polysorbate 80. It is supplied as a sterile solution in depyrogenized, 2-mL single-dose glass vials with 1 mL nominal fill at a concentration of 40 mg/mL and pH of 6 ± 0.05 . The vials should be stored refrigerated (2-8°C) with protection from light and freezing.

6.1.3 Siplizumab Dosage, Preparation, Administration

6.1.3.1 Dosage: Part A

Starting on Day 0, all participants were administered weekly SQ doses and received one of the four doses for 12 weeks based on the randomization assignment. Siplizumab dose was calculated using the participant's weight on Day 0. If the participant's body weight increased or decreased by >10% from baseline during the course of the treatment phase, the dosage was recalculated. Dose calculations based on the participant's body weight at the current visit were allowed.

6.1.3.2 Dosage: Part B

On Day 0, participants will receive a single SQ dose of siplizumab. (See Section 3.1.2 for doses). The siplizumab dose will be calculated using the participant's weight on Day 0.

6.1.3.3 Preparation

Siplizumab will be prepared by the site pharmacists using aseptic technique according to USP 797 guidelines for compounding of sterile products, local laws and regulations, the current IB, and study-specific pharmacy manual. Siplizumab will be diluted in saline prior to administration. Please refer to the Pharmacy Manual for details of dilution.

6.1.3.4 Administration

Screening and additional central and STAT local lab results (if indicated) must be reviewed, acknowledged, and signed by the site investigator before proceeding with siplizumab administration (see Section 6.4.2).

Prior to study drug administration, participants will receive 15 mg/kg of oral acetaminophen (up to a maximum dose of 650 mg) (or equivalent antipyretic, e.g., ibuprofen 10 mg/kg up to a maximum dose of 600 mg) and 25 mg of oral diphenhydramine (or equivalent antihistamine, e.g., hydroxyzine 25 mg), 15-60 minutes before receiving siplizumab. Please see Section 7.1.2 for additional details.

Siplizumab will be administered subcutaneously by trained medical study staff. Possible injection sites include the anterior abdominal wall, outer aspects of the upper arm, anterolateral aspects of the thighs, and the upper ventral gluteal area. Injection site selection will rely on the site investigator's discretion as well as participant preference.

Temperature, blood pressure, pulse, and respiration will be obtained immediately before and approximately every 15 minutes (+/-5 minutes) after study drug administration. Study participants will be monitored for at least 30 minutes after study drug administration to detect any possible allergic reactions (Section 6.4.2).

6.2 DRUG ACCOUNTABILITY

Under Title 21 of the Code of Federal Regulations (21CFR §312.62), the investigator bears the responsibility to maintain adequate investigational product (IP) disposition records (receipt, storage, dispense, return, and destruction). DAIT/NIAID requires the principal investigator(s) to delegate the IP disposition responsibility to a licensed/registered pharmacist

at a registered investigational pharmacy at a clinical research site. Detailed information on investigational pharmacy requirements is provided in the DAIT Pharmacy Guidelines. All IP inventory and dispensing will be documented using a DAIT/NIAID main accountability as well as participant-specific dispensing fillable PDF forms or electronic logs that are 21 CFR 11 compliant and approved by DAIT/NIAID. The participant dispensing log(s) will be kept current and will contain the identification of each participant as well as the date and quantity of each IP prepared and dispensed. Detailed IP shipment records to each clinical site's investigational pharmacy will be maintained by the DAIT/NIAID's Clinical Product Center (EMINENT Services Corporation). All records regarding IP disposition will be available for inspection. All remaining unused IP will be returned to the IND Sponsor (DAIT/NIAID) or sponsor's representative after study termination or destroyed with the prior approval from the sponsor in accordance with applicable federal and state laws and the study site procedures.

6.3 ASSESSMENT OF PARTICIPANT COMPLIANCE WITH INVESTIGATIONAL DRUG

Siplizumab treatment will be administered by trained medical study staff. Compliance, therefore, will be monitored by the site and documented on the electronic case report form (eCRF).

6.4 TOXICITY PREVENTION AND MANAGEMENT

6.4.1 Part A

Clinically significant laboratories were managed as outlined in Table 8 and Table 9. Missed doses were not administered to extend the overall course of siplizumab. Participants who discontinued siplizumab will continue to be followed for safety (see Section 6.4.3 for follow-up of participants who discontinue siplizumab).

Table 8. Part A Management of Siplizumab-associated AEs during Treatment *

*Study drug dosing based on the most recent laboratory result, either central or local, as applicable

Adverse Event	Action
Systemic Allergic Reaction	Permanently discontinue siplizumab if the participant develops a Grade 3 systemic allergic reaction as noted in Table 14 (Section 12.3.1).
Lymphopenia	Suspend siplizumab and repeat in 1 week if the ALC is <500 cells/ μ L. Resume siplizumab if the ALC is $\geq$ 800 cells/ μ L. Permanently discontinue siplizumab if the ALC does not increase to $\geq$ 800 cells/ μ L within 5 weeks of the initial lab result leading to dosing suspension.
CD4 T cell Decreased	Suspend siplizumab and repeat in 1 week if the CD4 T cell count is <200 cells/ μ L. Resume siplizumab if the CD4 T cell count is $\geq$ 250 cells/ μ L. Permanently discontinue siplizumab if the CD4 T cell count does not increase to $\geq$ 250 cells/ μ L within 5 weeks of the initial lab result leading to dosing suspension.
Thrombocytopenia	Suspend siplizumab and repeat in 1 week if the platelets count is <75,000 cells/ μ L. Resume siplizumab if the platelet count is $\geq$ 75,000 cells/ μ L. Permanently discontinue siplizumab if the platelet count does not increase to $\geq$ 75,000 cells/ μ L within 3 weeks from the initial lab result leading to dosing suspension.

Adverse Event	Action
Neutropenia	Suspend siplizumab and repeat in 1 week if the neutrophil count is <1000 cells/μL. Resume siplizumab if the neutrophil count is ≥ 1000 cells/μL. Permanently discontinue siplizumab if the neutrophil count does not increase to $\geq 1,000$ cells/μL within 3 weeks from the initial lab result leading to dosing suspension.
Anemia	Suspend siplizumab and repeat in 1 week if the hemoglobin level is <10.0 g/dL. Resume siplizumab if the hemoglobin level is ≥ 10.0 g/dL. Permanently discontinue siplizumab if the hemoglobin level does not increase to ≥ 10.0 g/dL within 3 weeks from the initial lab result leading to dosing suspension.
Asymptomatic Hepatic Injury	Suspend, resume, or permanently discontinue siplizumab based on Table 9.
Infection	Suspend siplizumab if the participant develops: - Grade 3 infection or other infectious event per NCI-CTCAE criteria, - Grade 2 infectious AE that the investigator judges to be significant, - SARS-CoV-2 infection confirmed by molecular diagnostic or antigen test (see Section 6.4.4), - Active EBV infection identified by PCR or serology (see Appendix 2), or - Active CMV infection identified by PCR or serology. Permanently discontinue siplizumab if the AE does not reduce to Grade 1 within 3 weeks from the date the initial AE began.
Any other AE Grade 3 or higher possibly related or related to siplizumab	Suspend siplizumab and monitor the participant at least weekly; repeat abnormal labs within a week. Resume siplizumab if the AE reduces to grade 2 or less. Permanently discontinue siplizumab if the AE does not reduce to Grade 2 or less within 3 weeks from the date of the initial AE began or if 3 consecutive doses are missed due to a Grade 3 or higher AE.

Table 9. Part A Dose Modification for Liver Chemistry Abnormalities

ALT/AST	Action
$\leq 3xULN$	Maintain dose
$>3xULN$ to $\leq 5xULN$ with serum total bilirubin $\leq 2xULN$	Suspend siplizumab Resume siplizumab at the next expected dose if ALT and AST decrease to $<2xULN$. If siplizumab is resumed, permanently discontinue siplizumab if ALT or AST increases to $>3xULN$ a second time.
$>3xULN$ with serum total bilirubin $>2xULN$ (Hy's Law)	Discontinue siplizumab
$> 5xULN$	Discontinue siplizumab

6.4.2 Part B

If the participant has an active systemic infection at V0, administration of sipilizumab will be deferred and the visit rescheduled. A repeat CBC with differential will be obtained (see Appendix 4) along with additional testing based on the evaluation of the site investigator (see Sections 8.3.2 and 8.3.3.). Sipilizumab may be administered when the participant is asymptomatic, and the CBC measurements do not meet exclusion criteria (Section 4.3).

If the participant has a resolved infection within 2 weeks prior to V0, a STAT local CBC with differential should be obtained at V0. If the participant is asymptomatic and the CBC measurements do not meet exclusion criteria (Section 4.3), sipilizumab may be administered.

It is recommended that all participants be up to date with age-appropriate vaccinations.⁵⁶

The definition and management of dose-limiting toxicities in Part B are shown in Table 10.

Table 10. Part B Definition and Management of Sipilizumab-associated DLTs

Adverse Event	Definition and Action
Systemic Allergic Reaction	A Grade 3 or higher systemic allergic reaction as noted in Table 14 (Section 12.3.1). Treatment will be provided per site investigator discretion.
Lymphopenia	ALC is <500 cells/ μ L at Week 4. Repeat CBC weekly until ALC >500 cells/ μ L.
CD4 T cell Decreased	CD4 T cell count is <200 cells/ μ L at Week 4. Repeat clinical lymphocytes subsets weekly until CD4 T cell count is >250 cells/ μ L.

6.4.3 Long-term Safety Monitoring Plan

Participants from Part A and Part B will enter long-term safety follow-up after Week 52 if they have a CD4 T cell count <400 cells/ μ L at Week 52 or if any other AE, that in the opinion of the DAIT/NIAID Medical Monitor, requires ongoing monitoring (see Appendix 3).

Participants with a CD4 T cell count <400 cells/ μ L at Week 52 will be followed until Week 100 (Visit 4SM) or until the CD4 T cell count is $\geq$ 400 cells/ μ L, whichever occurs first. The length of long-term safety monitoring for other AEs, besides CD4 T cell count, will be determined by the DAIT/NIAID Medical Monitor but will not extend beyond Week 100 (V4SM).

6.4.4 EBV, CMV, SARS-CoV-2 Management

An EBV viral load management plan, based on serology status at screening and symptom status (see Section 8.3.1), is provided in Appendix 2.

CMV PCR will be collected at any study visit where CMV is suspected clinically based on symptoms (see Section 8.3.1) or if the participant's most recent CD4 T cell count is <200 cells/ μ L. Management and follow-up of a positive CMV PCR assay will be determined after discussion with the DAIT/NIAID Medical Monitor, Protocol Chair, and ITN Clinical Trial Physician.

In the case of SARS-CoV-2 infection, participants will follow current Centers for Disease Control and Prevention (CDC) guidelines for isolation during infection. If ALC is <500 cells/ μ L prior to a SARS-CoV-2 infection, the participant will follow the CDC isolation period for people who have weakened immune systems. The participant should follow the study site's guidance regarding isolation and testing before returning to clinic.

6.5 PREMATURE DISCONTINUATION OF INVESTIGATIONAL DRUG (PART A ONLY)

In Part A, study therapy was prematurely discontinued for any participant for any of the following reasons:

1. AEs described in Table 8 and Table 9.
2. Pregnancy.
3. Malignancy.
4. The participant, or participant's legal representative, requested that treatment be halted.
5. The investigator believed that the siplizumab treatment was no longer in the best interest of the participant.
6. The participant received a prohibited medication listed in Section 7.2.
7. SARS-CoV-2 symptoms recurred with resumption of siplizumab dosing following a SARS-CoV-2 infection (see Section 6.4).
8. A Grade 3 AE, that was possibly related or related to siplizumab, recurred.

Participants who discontinued siplizumab treatment were followed through week 52 for safety at visits with mechanistic assessments with PBMC collections as outlined in the Schedule of Events (Appendix 1). If indicated, participants entered into long-term safety monitoring for up to an additional 48 weeks (see Section 6.4.3 and Appendix 3).

If siplizumab treatment was discontinued, the DAIT/NIAID Medical Monitor was notified.

7. OTHER MEDICATIONS

7.1 CONCOMITANT MEDICATIONS

7.1.1 Protocol Mandated

There are no protocol mandated concomitant medications for this study.

7.1.2 Prophylactic Medications

Participants will receive 15 mg/kg of oral acetaminophen (up to a maximum dose of 650 mg) (or equivalent antipyretic, e.g., ibuprofen 10 mg/kg up to a maximum dose of 600 mg) and 25 mg of oral diphenhydramine (or equivalent antihistamine, e.g., hydroxyzine 25 mg) 15-60 minutes before receiving siplizumab.

Oral diphenhydramine (or an equivalent antihistamine) should be held on the day of the siplizumab injection if one or more of the following criteria are met:

- The participant is on a daily regular dose of long-acting antihistamine

- The participant has taken any dose of antihistamine on the day of the injection

If the participant held their regular daily dose of antihistamine, they may resume antihistamine use the day after the siplizumab injection.

7.1.3 Permitted Medications

1. Emergency use authorization or approved medications to treat or prevent SARS-CoV-2.
2. CDC recommended SARS-CoV-2 and seasonal influenza vaccinations.
3. Insulin per the investigator or referring physician.
4. Contraceptive medications.

Other medications, except over-the-counter medications, should be reviewed with the study management team prior to initiation.

7.2 PROHIBITED MEDICATIONS AND THERAPIES

Participants will be instructed not to use the following medications:

- Medications that can influence insulin sensitivity or secretion
- Non-insulin pharmaceuticals for glycemic control including, but not limited to, metformin, amylin, and GLP-1R agonists
- Live vaccine (varicella, measles, mumps, rubella, cold-attenuated intranasal influenza vaccine, bacillus Calmette-Guérin, and smallpox) 30 days before V0 and within 3 months after receiving study drug
- Agents that may result in immunosuppression or immunomodulation, including high-dose inhaled (defined as a total daily inhaled dose of 320 mcg of beclomethasone equivalent in participants 8-11 years of age and 480 mcg of beclomethasone equivalent in participants 12 and older), extensive topical, or systemic glucocorticoids

If a participant receives, or if the investigator believes that a participant must receive a prohibited medication, the case must be immediately discussed with the Protocol Chair, the DAIT/NIAID Medical Monitor, and the ITN Clinical Trial Physician.

The use of prohibited medications must be documented on the source document and eCRF.

7.3 CONTRACEPTION USE

All female participants of childbearing potential must not become pregnant during the course of the study and must either be sexually inactive by abstinence or use a highly effective, medically acceptable form of contraception for the duration of the study. Contraception must start 2 weeks before study drug administration and must continue until Week 52. Women who are abstinent must have complete abstinence from intercourse from 2 weeks prior to the administration of the study drug until Week 52. Periodic abstinence and withdrawal are not acceptable methods of contraception.

Medically acceptable forms of birth control include oral contraceptive pills (combined hormone or progestin alone), injectable or implantable progestogens, intrauterine devices, estrogen vaginal rings, male partner sterilization, or double barrier methods (e.g., condom and occlusive cap with spermicidal agent). If using male partner sterilization (vasectomy with documentation of azoospermia), sterilization must occur prior to the female participant's entry into the study and the male must be the sole partner for the participant. Documentation of male partner sterilization is required.

If participants withdraw prior to Week 52, they will be instructed to continue contraception for at least 16 weeks after study drug administration.

7.4 RESCUE MEDICATIONS

Not applicable.

8. STUDY PROCEDURES / ASSESSMENTS

8.1 ENROLLMENT

The research study will be explained in lay terms to each potential research participant. The potential participant will sign an informed consent form before undergoing any study procedures.

8.1.1 Part A

In Part A, participants who met the eligibility criteria were randomly assigned to a sipilizumab dosing arm. Enrollment was defined as the time of randomization.

8.1.2 Part B

Once the informed consent has been signed, the participant is considered enrolled in the study and will be assigned a unique participant identification number.

8.2 SCREENING/BASELINE VISIT

8.2.1 Pre-Screening

Potential participants who express interest in the study will be contacted over the phone to schedule a screening visit and provide visit instructions using an Institutional Review Board (IRB) approved Information Sheet. Protected Health Information (PHI) from potential participants who decline participation during the call will not be collected. PHI will be collected from potential participants interested in scheduling a screening visit.

8.2.2 Screening Visit

The purpose of the screening period is to confirm eligibility to continue in the study. Screening of study participants will take place at V-1. Participants will be assessed for the eligibility criteria listed in Sections 4.2 and 4.3.

8.2.3 Baseline Visit

The baseline visit is defined as Week 0 (V0).

8.3 STUDY ASSESSMENTS AND PROCEDURES

Appendix 1 includes Part A study assessments.

The study assessments to be performed at each study visit for Part B are listed in Appendix 3 and Appendix 4.

8.3.1 General Assessments

- Informed consent: Written informed consent (parental permission and informed assent of minor, if applicable) will be obtained before any study assessments or procedures are performed
- Eligibility criteria: Eligibility for study participation will be assessed during the screening period
- Randomization: May occur up to 1 business day prior to V0
- Demographics: Age, race, sex, and ethnicity
- Medical history: A history will be taken to determine if the participant has had any clinically significant diseases or medical procedures other than the disease under study
- T1DM history, including date of diagnosis and previous treatment
- AEs: Participants will be assessed for new or worsening AEs
- EBV-associated disease evaluation: Participants will be monitored for clinical symptoms/signs of EBV-associated disease including fever, lethargy, sore throat, lymphadenopathy, splenomegaly, infectious mononucleosis, and hepatitis at each study visit
- CMV-associated disease evaluation: Participants will be monitored for clinical symptoms/signs of CMV-associated disease including fever, lethargy, lymphadenopathy, splenomegaly, infectious mononucleosis, and hepatitis at each study visit
- Concomitant medications: All concomitant medications and their indications for use will be recorded
- Comprehensive physical examination includes the following body systems: Skin, respiratory, cardiovascular, gastrointestinal, neurologic, and renal/urinary
- Directed physical examination focuses on the body systems relevant to the participant's clinical complaints and clinical status at the study visit
- Vital signs: Height will be obtained at screening (V-1) for all participants. Participants who are less than 18 years old at screening will have their heights measured at the baseline visit, Week 24, and Week 52. Weight, temperature, pulse, blood pressure, and respiration will be obtained at all visits.

8.3.2 Central Laboratory Assessments

- Serum chemistry: Electrolytes (sodium, potassium, chloride, phosphate, total CO₂), blood urea nitrogen (BUN), creatinine (SCr), C-reactive protein (CRP), and hepatic

function panel (AST, ALT, alkaline phosphatase, bilirubin (direct and total), albumin, total protein)

- Hematology: Includes red blood cells count, hematocrit, hemoglobin, platelet count, WBC, and differential
- Lymphocyte subset count: CD4, CD8, B cells, and NK cells
- Viral load testing: Viral load testing by PCR will be performed for CMV and EBV
- HIV (Ab)
- Hepatitis B (HBsAg and total core Ab)
- Hepatitis C (IgG Ab with RNA confirmation if indicated)
- EBV Serology: VCA IgM Ab, VCA IgG Ab, EBNA, and EBV early antigen Ig Ab
- CMV Serology (IgG Ab)
- SARS-CoV-2 molecular test
- Serum pregnancy (for women of childbearing potential): Serum testing will be performed at screening to rule out pregnancy
 - Serum β human chorionic gonadotropin (hCG) will be repeated after screening for participants with a positive urine hCG result at any study visit
- HbA1c
- TSH

8.3.3 Local Laboratory Assessments

- Tuberculosis (TB) test: Exposure to TB will be assessed via QuantiFERON-TB Gold, QuantiFERON-TB Gold Plus, T-SPOT®.TB test or equivalent assay. A PPD tuberculin test may be substituted.
- Urine pregnancy (for females of childbearing potential)
- Serum chemistry: Electrolytes (sodium, potassium, chloride, phosphate, total CO₂), blood urea nitrogen (BUN), creatinine (SCr), C-reactive protein (CRP) and hepatic function panel (AST, ALT, alkaline phosphatase, bilirubin (direct and total), total protein, and albumin)
- Hematology: Includes red blood cells count, hematocrit, hemoglobin, platelet count, WBC, and differential
- Lymphocyte subset count: CD4, CD8, B cells, and NK cells
- STAT SARS-CoV-2 antigen test
- SARS-CoV-2 molecular test

8.3.4 Disease Specific Assessments

- 2-hour MMTT
- Plasma C-peptide

- Plasma glucose
- Diabetes-related autoantibodies
- Glucometer readings, if available
- Continuous Glucose Monitoring (CGM) readings, if available
- Insulin use: 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 the study visit.
- Hypoglycemic/hyperglycemic events

8.3.5 Study Drug Administration and Drug Related Assessments

- SQ injection of study treatment (siplizumab)
- Vital signs during study drug administration: Temperature, pulse, blood pressure, and respiration will be obtained prior to the start of study drug administration and after as outlined in Section 6.1.3.4
- PK
- Anti-siplizumab antibodies

8.3.6 Mechanistic Assessments

- PBMCs
- Serum
- Whole blood DNA
- Whole blood RNA

See Section 9 for a detailed discussion of mechanistic assays.

8.3.7 Intensive Diabetes Management

During the study period, all participants will receive “intensive” management of their diabetes to optimize metabolic control. The goal of treatment will be to maintain the HbA1c levels within the currently recommended ADA target range in the absence of significant or severe hypoglycemia. The primary responsibility for diabetes management will be with the treating or referring diabetes care provider; the research study team will provide close additional support through regular interaction.

Participants will check glucose levels frequently either via a CGM or a glucose meter. Participants will be encouraged to use a personal CGM during the study, but one will not be provided to them. Glucose levels from CGMs will be captured for analysis and may also be used for guidance by healthcare personnel. If appropriate, glucose meter readings should be collected when CGM is in use to ensure adequate calibration of the device. If participants choose not to use the CGM for glucose monitoring, glucose levels should be checked frequently and records communicated regularly to the study team. Records of glucose logs and communication with the participant will be kept as source documentation.

The general goal of glucose control is to target pre-prandial glucose levels of 90–130 mg/dL (plasma), postprandial levels of less than 180 mg/dL, and bedtime levels of 110–150 mg/dL. Participants who fail to achieve an HbA1c level of <7% will not be excluded from the study but additional measures will be instituted to improve the glycemic control. Any episodes of severe hypoglycemia (i.e., unconsciousness, seizure, or needing the assistance of another individual to correct the hypoglycemia) will prompt a review of the cause of the episode and adjustment of insulin dosing, diet, and exercise as deemed appropriate by the treating physician. All episodes of severe hypoglycemia that require hospitalization and/or emergency care will be reported to the DSMB. If adhering to these treatment goals results in an episode of severe hypoglycemia, the treatment goals may be relaxed to avoid a recurrent event.

8.4 WITHDRAWAL VISIT

Participants who withdraw from the study as described in Section 11.2 will be asked to complete the assessments outlined in the withdrawal (WD) visit in Appendix 4.

8.5 UNSCHEDULED VISITS

Unscheduled visits may be performed to evaluate concerns, including potential AEs. Assessments obtained during an unscheduled visit are outlined in Appendix 4. Some assessments for an unscheduled visit may be omitted at the discretion of the investigator if not indicated.

8.6 VISIT WINDOWS

Study visits for Part A took place within the time limits specified in Table 11.

Table 11. Part A Visit Windows

Visit	Time Point	Visit Window
Screening: Visit -1	Must occur no more than 37 days prior to V0	
Baseline: Visit 0	Day 0/Week 0	n/a
Visits 1 to 11	Every week through Week 11	+/- 1 day
Visit 12	Week 12	+/- 3 days
Visits 13 to 15	Weeks 24, 36, and 52	+/- 14 days
Visits 1SM to 4SM	Weeks 64, 76, 88, and 100	+/- 14 days

Study visits for Part B must occur within the time limits specified in Table 12.

Table 12. Part B Visit Windows

Visit	Time Point	Visit Window
Screening: Visit -1	Must occur no more than 37 days prior to V0	
Baseline: Visit 0	Day 0/Week 0	n/a
Visits 1 to 5	Weeks 1, 2, 4, 6, and 8	+/- 2 days
Visit 6	Week 12	+/- 3 days
Visits 7 to 9	Weeks 24, 36, and 52	+/- 14 days
Visits 1SM to 4SM	Weeks 64, 76, 88, and 100	+/- 14 days

8.6.1 Window for Administration of Investigational Drug

In Part A, siplizumab injections were given at least 5 days after the prior administration.

In Part B, there is no window for drug administration since participants will only receive one sipilizumab dose at V0.

9. MECHANISTIC ASSAYS

In Part B, PBMCs, plasma, and serum will be obtained at pre-specified time points (Appendix 4) for immunophenotyping and characterization of cellular subsets. Whole blood will be collected to support future genomic, epigenomic, and transcriptomic studies. Biospecimens used for mechanistic assays will be collected prior to drug administration unless otherwise noted.

9.1 PRIMARY MECHANISTIC HYPOTHESES

The hypothesis is that sipilizumab will result in changes in T cell phenotypes, similar to changes seen with alefacept in ITN045AI T1DAL. Therefore, the primary endpoint is mechanistic and will be based on the analysis of biospecimens. Please see Section 1.1 of this protocol for detail on the rationale for the selection of the primary endpoints. In this study, the two primary mechanistic questions to be addressed include:

1. Does the frequency of circulating PD1+TIGIT+ cells within the CD4 Tem pool increase during the first 8 weeks of treatment compared to baseline?
2. Does the ratio of CD4 Treg/Tem in blood increase during the first 8 weeks of treatment compared to baseline?

These two questions will be addressed in both the interim analysis of the first 5 PP participants in each dosing arm and in fully recruited dosing arms.

9.2 EXPLORATORY MECHANISTIC HYPOTHESES

Other exploratory hypotheses to be tested are detailed below. Sample availability and the current state of technology will guide the prioritization of hypothesis testing.

- Regulatory and exhaustion signatures previously reported in CD4 and CD8 T cells after alefacept therapy^{57,58} will be induced by sipilizumab therapy
- The developmental kinetics of exhausted CD4 and CD8 T cell phenotypes observed after sipilizumab therapy will resemble patterns observed after alefacept therapy⁵⁷
- Regulatory T cell phenotypes and frequencies observed with sipilizumab therapy will correlate with changes in C-peptide
- Changes in CD4 and CD8 exhausted T cells observed with sipilizumab therapy will correlate with changes in C-peptide

9.3 PROPOSED MECHANISTIC ASSAYS

9.3.1 PBMC-Based Assays

9.3.1.1 PBMC Preparation

All blood samples collected for PBMC isolation will be shipped from the clinical sites to the ITN core facility for processing using ITN standard operating procedures (SOPs) for PBMC

separation and freezing. This will ensure that standardized procedures are used and that high-quality biospecimens are obtained for testing. PBMCs will be stored in the vapor phase of liquid nitrogen until use.

9.3.1.2 Multi-parameter Immunoprofiling of PBMCs

Flow cytometry and/or mass cytometry will be done at an ITN designated laboratory, Human Immunophenotyping Core Laboratory (HIPc) at BRI (Benaroya Research Institute at Virginia Mason, Seattle, WA) to analyze the impact of treatment on the frequency and phenotype of specific immune cell populations in viably cryopreserved PBMC. Additional immunophenotyping may be carried out by ITN designated laboratories using new technology, such as CITE-seq, multi-omic profiling, and others, as they become available.

For the interim analysis and the primary endpoint, the change in frequency of PD1+TIGIT+ cells within the CD4 Tem pool and the change in the ratio of Treg (defined as CD3+CD56-CD8-CD4+CD127loFoxP3+) to CD4 Tem (defined as CD3+CD56-CD8-CD4+ Foxp3-CD45RA-CD45RO+CCR7-) will be monitored through multi-color flow cytometry. The flow panel used for this analysis (Table 13) has been tested and optimized by the HIPc and has been used in other DAIT/NIAID-sponsored ITN (Protocols ITN045AI, ITN027AI) and non-ITN studies (NCT02215200, NCT01030861) with minor modifications relevant to hypotheses in the individual studies.^{5,11,59-63} PBMC samples collected from participants during the first 8 weeks (see Appendix 4) will be stained and acquired through an LSR-Fortessa (BD Biosciences) cytometer as detailed in the supplementary HIPc SOP. All samples from the same participant will be batched and assayed the same day for interim analysis and all subsequent analyses, and an internal control arm from the same control participant will be assayed in each batch for performance comparison.

For this study, multiparameter profiling with flow cytometry will occur as part of the interim analysis to determine dosing arm expansion and again at the completion of the study during a comprehensive analysis of biospecimens collected during the study. The interim analysis for each dosing arm will occur once the 5th PP participant in a dosing arm completes the week 8 visit (see Appendix 4). All participant samples from a dosing arm (samples from 5 PP participants) will be assayed together in a batch on the same day and with the same set of controls. Laboratory personnel will be blinded to patient IDs and sample time points. Unblinding and statistical analysis will occur once the data has been gated, and the frequencies of PD1+TIGIT+ as % of CD4 Tem, total CD4 Tem, and total CD4 Treg as % total CD4 T cells determined and transferred to the ITN. Statistical analysis will be conducted as described in Section 13.

Additional mechanistic questions will be addressed during the study period as needed to guide decisions about dosing arms and at the completion of the study for exploratory questions. For these assessments, flow cytometry or mass cytometry will be used to evaluate the phenotype of relevant lymphocyte subsets, including CD4 and CD8 T cells and NK subsets, as well as functional outcomes (i.e., cytokine production and/or proliferation). Other methodologies to assess lymphocyte function may be used as new technologies become available, such as in vitro stimulation and activation-induced marker (AIM) assays, CITE-seq, and multi-omic analysis.

Table 13. Flow Panel for Primary Endpoint

Target	Format	Clone	Vendor
TIGIT	APC	MBSA43	eBio
CCR7	BV510	G043H7	BL
CD127	BV711	A019D5	BL
CD56	BUV395	NCAM16.2	BD
CD3	BV605	OKT3	BL
CD4	BB515	RPA-T4	BD
CD45RO	BV786	UCHL1	BD
CD45RA	BUV737	HI100	BD
CD8	Ax700	SK1	BL
PD-1	BV650	EH12.2H7	BL
FoxP3	PE-CF594	259D/C7	BD

9.3.1.3 Transcriptional Profiling of PBMCs

Bulk and/or single cell RNA-seq, CITE-seq, or single-cell multi-omics analysis may be performed on relevant cell subsets. Transcriptional analysis of T cells from participants in the ITN045AI T1DAL study demonstrated expression of gene modules associated with T cell exhaustion.

The goal of these assays is to identify transcriptional 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.

9.3.1.4 Epigenetic Profiling of PBMCs

Recent research using whole genome methylation analysis has shown that beta-cell specific T cells from T1DM participants have stem-like epigenetic programs (a hybrid of naïve and effector epigenetic programs), indicating preservation of a less differentiated state. Utilizing single cell or bulk ATAC-seq, the co-existence of both naïve and effector-associated epigenetic programs in individual beta cell-specific T cells was confirmed,⁶⁴ providing a mechanism to explain how the stem-like state of the cells can sustain the autoreactive immune response. In this study, we may assess if sipilizumab treatment can result in tolerization through an epigenetic reprogramming of T cell subsets, including the stem-like subset of autoreactive T cells.

9.3.1.5 Quantification and Functional Profiling of Autoreactive Lymphocytes

Functional profiling and quantification of autoreactive T cells can be identified by combining MHC class I and class II tetramers to enumerate islet antigen specific T cells implicated in the pathogenesis of T1DM with high-content single-cell mass cytometry, or “cytometry by time of flight” (CyTOF).⁶⁵ Through a collaborative project with BRI, the ITN identified islet specific T cells from PBMC samples from T1DM participants collected by BRI’s T1DM

Biorepository. We found that islet-specific T cells exhibited heterogeneous phenotypes within the same individual and identified three shared phenotypes shared across individuals. Two of the shared phenotypes were associated with disease progression rate in T1DM participants. An activated memory phenotype was more frequent among islet-specific T cells of rapid progressors, while an exhaustion phenotype was more prevalent among islet-specific T cells of slow progressors.⁶⁶ Additional methodologies for profiling of autoreactive lymphocytes may be implemented as they become available, including in vitro re-stimulation, AIM assays, and others.

9.3.2 Serum and Plasma Assays

Participant serum or plasma may be analyzed to investigate the impact of sipilizumab treatment on circulating levels of cytokines, autoantibodies, and inflammatory mediators. Longitudinal assessment of serum or plasma cytokines and inflammatory mediators may be performed using immunoassay or proteomics platforms and may include analysis of extracellular vesicles. Additionally, the impact of treatment on levels of cytokines and inflammatory mediators could be evaluated for correlations with induction of clinical tolerance, changes in immune cell populations and/or gene expression in peripheral blood.

9.3.3 Whole Blood Assays

9.3.3.1 Whole Blood DNA Genotyping

It is reasonable to assume that treatment may not be equally effective in all individuals and that genetic differences may, in part, determine response. DNA may be isolated from whole blood collected from all consenting participants, and the ITN may perform genotyping for HLA Class I/II alleles, single nucleotide polymorphisms (SNPs) in selected immune response genes, whole genome sequencing, or sequencing of other genetic factors of interest to investigate correlations with disease worsening and therapeutic response.

9.3.3.2 Gene Expression in Peripheral Blood

Gene expression profiling may be performed on RNA isolated from peripheral whole blood using RNAseq, Nanostring, RT-PCR, or other methods. The goal of these assays is to evaluate global changes in gene expression during and after treatment, to identify differences in transcriptional profiles between treatment arms and clinical outcome groups, and to monitor the durability of these potential changes after discontinuation of immunotherapy. These types of analyses may also explain why some individuals respond to treatment or may elucidate mechanisms resulting in adverse responses to treatment.

10. BIOSPECIMEN STORAGE

Informed consent will be obtained from all participants for their samples to be stored for use in future studies. Biological specimens collected in this study will be stored long-term to re-evaluate biological responses as new research tools to study tolerance become available. The specimens will, therefore, be stored in the ITN sample repository for a minimum of 10 years. Residual specimens may be used by the investigators for the development of new immunologic assays or for cross-trial study comparisons. Although specimens in this protocol are described 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 based on statistical and scientific planning, hypotheses to be tested, and technologies available. Finally, clinical outcomes will be considered to determine the potential value of the assays. The ITN sample sharing policy will apply to the provision of samples to study or outside investigators (www.immunetolerance.org).

11. CRITERIA FOR PARTICIPANT STUDY COMPLETION AND PREMATURE STUDY TERMINATION

11.1 PARTICIPANT COMPLETION

A participant is considered to have completed the study if s/he is enrolled and completes the Week 52 visit or, if indicated, completes long-term safety monitoring per Section 6.4.36.4.3.

11.2 PARTICIPANT WITHDRAWAL CRITERIA

Participants may be prematurely withdrawn from the study for the following reasons:

1. The participant elects to withdraw consent from all future study activities, including follow-up.
2. The participant is “lost to follow-up” (i.e., no further follow-up is possible because attempts to reestablish contact with the participant have failed).
3. The participant dies.
4. The Site Investigator believes that participation is no longer in the best interest of the participant.

11.3 PARTICIPANT REPLACEMENT

In Part B, treated participants will be replaced if either of the following conditions is met:

1. Do not have at least 1 viable pre-treatment PBMC sample (V-1 or V0)
2. Do not have viable PBMC samples available from at least 3 of 5 post-baseline visits (V1 through V5)

11.4 FOLLOW-UP AFTER STUDY WITHDRAWAL

If a participant withdraws from the study as described in Section 11.2, the participant will be asked to return for one final study visit where the assessments outlined in the WD visit in Appendix 4 will be performed. No additional follow-up will be requested from participants after early study withdrawal. Female participants who withdraw will be advised to continue using acceptable methods of contraception until at least 16 weeks after study drug administration (see Section 7.3).

11.5 STUDY STOPPING RULES

11.5.1 Part A

In Part A, an ad hoc DSMB review would have been convened for the following reasons:

1. Any death or Grade 4 AE except asymptomatic AE and those assessed as not related to siplizumab treatment on review by the DAIT/NIAID Medical Monitor and the ITN Clinical Trial Physician.
2. An occurrence of Hy's Law defined as elevations in the AST and/or ALT $>3\times$ ULN of the reference range, elevation in total bilirubin $>2\times$ ULN of the reference range, and no other cause of hepatic injury.
3. Three of the first 10 participants cumulatively across all dosing arms, or 30% of all participants thereafter, required discontinuation of siplizumab treatment for the same or similar serious AE based on MedDRA preferred term or at the discretion of the DAIT/NIAID Medical Monitor, except for lymphopenia and low CD4 T cell counts. This rule was to be monitored within and across age cohorts.
4. Five of the first 10 participants cumulatively across all dosing arms, or 50% of all participants thereafter, required discontinuation of siplizumab treatment for AEs, except for lymphopenia and low CD4 T cell counts. This rule was to be monitored within and across age cohorts.

In case a study stopping rule was met, as determined by the ITN Clinical Trial Physician, Protocol Chair, and DAIT/NIAID Medical Monitor, siplizumab treatment and enrollment/randomization for all dosing cohorts would have been suspended. Screening activities would have been allowed to proceed. As outlined in Section 12.8.2.2, an *ad hoc* DSMB meeting would have been scheduled where all pertinent information would be discussed. The DSMB's recommendations as to whether dosing may or may not be resumed would have been further discussed with DAIT/NIAID and communicated to sites.

11.5.2 Part B

In Part B, an *ad hoc* DSMB review will be convened for the following reasons:

1. Any death
2. Any Grade 4 AE except asymptomatic AE and those assessed as not related to siplizumab treatment on review by the DAIT/NIAID Medical Monitor and the ITN Clinical Trial Physician.
3. An occurrence of Hy's Law defined as elevations in the AST and/or ALT $>3\times$ ULN of the reference range, elevation in total bilirubin $>2\times$ ULN of the reference range, and no other cause of hepatic injury.

In case a study stopping rule is met, as determined by the ITN Clinical Trial Physician, Protocol Chair, and DAIT/NIAID Medical Monitor, treatment will be suspended. Screening activities will be allowed to proceed. As outlined in Section 12.8.2.2, an *ad hoc* DSMB meeting will be scheduled where all pertinent information will be discussed. The DSMB's recommendations as to whether treatment may or may not be resumed will be further discussed with DAIT/NIAID and communicated to sites

11.6 DOSING ARM STOPPING RULES

11.6.1 Part A

A dosing arm would have been prematurely terminated for the following reasons (see Section 6.4.1):

1. Two of the first 5 participants in a dosing arm develop a Grade 3 or higher adverse event, except for low ALC or low CD4 T cell count, that is at least possibly related to siplizumab treatment.
2. Two of the first 5 participants in a dosing arm permanently discontinue siplizumab treatment due to low ALC or low CD4 T cell count.
3. Four out of the 15 planned participants in a dosing arm develop a Grade 3 or higher adverse event, except for low ALC or low CD4 T cell count, that is at least possibly related to siplizumab treatment.
4. Four out of the 15 planned participants in a dosing arm permanently discontinue siplizumab treatment due to low ALC or low CD4 T cell count.

In case a cohort dosing arm stopping rule is met, as determined by the ITN Clinical Trial Physician, Protocol Chair, and DAIT/NIAID Medical Monitor, an immediate enrollment suspension in that dosing arm and in dosing arms with higher doses would have been implemented. As outlined in Section 12.8.2.2, an *ad hoc* DSMB meeting would have been scheduled where all pertinent information would be discussed. The DSMB's recommendations as to whether enrollment may or may not be resumed would have been further discussed with DAIT/NIAID and communicated to sites.

11.6.2 Part B

In Part B, a dosing arm in either the adult or pediatric cohort may be prematurely terminated for the following reasons (Section 6.4.2):

1. Two of the first 5 treated participants in a dosing arm develop a DLT as defined in Table 10.
2. Four of the 15 treated participants in a dosing arm develop a DLT as defined in Table 10.

If the ITN Clinical Trial Physician, Protocol Chair, and DAIT/NIAID Medical Monitor determine that a dosing arm stopping rule is met, an immediate treatment suspension in that dosing arm and in dosing arms with higher doses will be implemented. Screening activities will be allowed to proceed. As outlined in Section 12.8.2.2, an *ad hoc* DSMB meeting will be scheduled where all pertinent information will be discussed. The DSMB's recommendations as to whether treatment may or may not be resumed will be further discussed with DAIT/NIAID and communicated to sites. AEs experienced by participants enrolled in Part A during follow-up will not contribute to a dosing arm suspension in Part B.

12. SAFETY MONITORING AND REPORTING

12.1 OVERVIEW

This section defines the types of safety data that will be collected under this protocol and outlines the procedures for appropriately collecting, grading, recording, and reporting those data. Adverse events that are classified as serious according to the definition of health authorities must be reported promptly (Protocol Section 12.5) to the IND Sponsor (DAIT/NIAID). Appropriate notifications will also be made to the Institutional Review Boards (IRBs) and health authorities.

Information in this section complies with *ICH Guideline E2A: Clinical Safety Data Management: Definitions and Standards for Expedited Reporting*, *ICH Guideline E6(R2) Good Clinical Practice: Integrated Addendum to ICH E6(R1)*, 21CFR Parts 312 and 320, and applies the standards set forth in the National Cancer Institute (NCI), Common Terminology Criteria for Adverse Events (NCI-CTCAE), Version 5.0:
https://ctep.cancer.gov/protocolDevelopment/electronic_applications/docs/CTCAE_v5_Quick_Reference_5x7.pdf.

12.2 DEFINITIONS

12.2.1 Adverse Event

An AE is any untoward medical occurrence associated with the use of an intervention in humans, whether or not considered intervention-related (21 CFR 312.32(a)).

For this study, an AE will include any untoward medical occurrence associated with siplizumab administration or any study mandated procedure (Section 8.3).

12.2.2 Suspected Adverse Reaction

Any AE for which there is a reasonable possibility that the investigational study drug caused the AE. For the purposes of safety reporting, 'reasonable possibility' means there is evidence to suggest a causal relationship between the drug and the AE. A suspected adverse reaction (SAR) 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)).

12.2.3 Unexpected Adverse Event

An AE or SAR is considered "unexpected" if it is not consistent with the risk information described in the ITB-MED IB for TCD601 (siplizumab) and the general investigational plan. The IND Sponsor will be responsible for determining whether an AE is expected or unexpected.

12.2.4 Serious Adverse Event

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

1. Death.

2. A life-threatening event: An AE or SAR is considered “life-threatening” if, in the view of either the investigator or IND Sponsor (DAIT/NIAID), its occurrence places the participant 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.
3. Inpatient hospitalization or prolongation of existing hospitalization.
4. Persistent or significant incapacity or substantial disruption of the ability to conduct normal life functions.
5. Congenital anomaly or birth defect.
6. Important medical events that may not result in death, be life threatening, or require hospitalization may be considered serious when, based upon appropriate medical judgment, they may jeopardize the participant and may require medical or surgical intervention to prevent one of the outcomes listed above.

Elective hospitalizations are not to be reported as an SAE unless hospitalization is prolonged due to complications.

12.2.5 Adverse Events of Special Interest

Any occurrence of opportunistic infection, lymphoproliferative disorders, allergic reactions (if the reactions are at least possibly related to study drug administration), and Grade 2 or higher injection site reactions to study drug will be considered an Adverse Event of Special Interest (AESI) and must be reported to the IND Sponsor within 24 hours, according to the procedure for SAEs described in Section 12.5.1.

Injection site reactions will be defined as those events that meet Grade 2 or higher as defined in the NCI-CTCAE V 5.0.

In Part A of the study, any AE leading to discontinuation of study drug was also considered an AESI.

12.3 GRADING AND ATTRIBUTION OF ADVERSE EVENTS

12.3.1 Grading of Allergic Reactions

For this study, systemic allergic reactions will be graded according to Table 14. The final grade of the reaction will not be determined until the event is over.

Non-systemic allergic reactions should be graded according to the appropriate NCI-CTCAE term.

Table 14. Grading of Systemic Allergic Reactions

Modified from World Allergy Organization Subcutaneous Immunotherapy Systemic Reaction Grading System.⁶⁷

Grade 1	Grade 2	Grade 3	Grade 4	Grade 5
<i>Symptom(s)/ sign(s) of one organ system present</i> <u>Cutaneous</u> Generalized pruritus, urticaria, flushing or sensation of heat or warmth or Angioedema (not laryngeal, tongue or uvular)	<i>Symptom(s)/ sign(s) of more than one Grade 1 organ system present</i> or <u>Lower respiratory</u> Asthma: cough, wheezing, shortness of breath (e.g.,	<u>Lower respiratory</u> Asthma (e.g., 40% PEF or FEV1 drop, NOT responding to an inhaled bronchodilator) or <u>Upper respiratory</u>	<u>Lower or Upper respiratory</u> Respiratory failure with or without loss of consciousness or <u>Cardiovascular</u>	Death

Grade 1	Grade 2	Grade 3	Grade 4	Grade 5
or <u>Upper respiratory</u> Rhinitis (e.g., sneezing, rhinorrhea, nasal pruritus and/or nasal congestion) or Throat-clearing (itchy throat) or Cough perceived to come from the upper airway, not the lung, larynx, or trachea or <u>Conjunctival</u> Conjunctival erythema, pruritus or tearing or <u>Other</u> Nausea, metallic taste, or headache	less than 40% PEF or FEV1 drop, responding to an inhaled bronchodilator) or <u>Gastrointestinal</u> Abdominal cramps, vomiting, or diarrhea or <u>Other</u> Uterine cramps	Laryngeal, uvula or tongue edema with or without stridor	Hypotension with or without loss of consciousness	

12.3.2 Grading of Hepatic Function Abnormalities

For this study, hepatic function abnormalities will be graded using protocol specific criteria, and are defined relative to the ULN as follows:

- AST increased
 - Grade 1: > ULN - 3.0x ULN
 - Grade 2: > 3.0x ULN - 5.0x ULN
 - Grade 3: > 5.0x ULN - 20.0x ULN
 - Grade 4: > 20.0x ULN
- ALT increased
 - Grade 1: > ULN - 3.0x ULN
 - Grade 2: > 3.0x ULN - 5.0x ULN
 - Grade 3: > 5.0x ULN - 20.0x ULN
 - Grade 4: > 20.0x ULN
- Alkaline phosphatase (ALP) increased
 - Grade 1: > ULN - 2.5x ULN
 - Grade 2: > 2.5x ULN - 5.0x ULN
 - Grade 3: > 5.0x ULN - 20.0x ULN
 - Grade 4: > 20.0x ULN
- Blood bilirubin increased
 - Grade 1: > ULN - 1.5x ULN
 - Grade 2: > 1.5x ULN - 3.0x ULN
 - Grade 3: > 3.0x ULN - 10.0x ULN
 - Grade 4: > 10.0x ULN

12.3.3 Grading of Glycemic Events

For this study of participants with T1DM, only major hypoglycemic and hyperglycemic events will be reported as AEs.

Major hypoglycemic events will be defined as those requiring the assistance of other individuals to recover and/or resulting in loss of consciousness.

Major hyperglycemic events will be defined and graded as follows:

- Grade 3 = only if hospitalization indicated
- Grade 4 = coma or life-threatening event.
- Grade 5 = death.

For this study significant diabetic ketoacidosis (DKA) will be reported together with grade 4 hyperglycemia (as a single AE) resulting in hospitalization and life-threatening consequences.

12.3.4 Grading Criteria

For all other AEs not related to systemic allergic reactions, liver chemistry abnormalities or glycemic events, the study site will grade the severity of AEs experienced by the study participants according to the criteria set forth in the NCI-CTCAE (version 5.0, November 27, 2017). 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. The NCI-CTCAE has been reviewed by the Protocol Chair, ITN Clinical Trial Physician and DAIT/NIAID Medical Monitor and has been deemed appropriate for the participant population to be studied in this protocol.

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

Grade 1 = mild AE.

Grade 2 = moderate AE.

Grade 3 = severe or medically significant AE.

Grade 4 = life-threatening or urgent intervention needed AE.

Grade 5 = death.

For grading an abnormal value or result of a clinical or laboratory evaluation (including, but not limited to, a radiograph, an ultrasound, an electrocardiogram etc.), a treatment-emergent adverse event (TEAE) is defined as an increase in grade from baseline or from the last post-baseline value that doesn't meet grading criteria. Changes in grade from screening to baseline will also be recorded as adverse events if related to a study-mandated procedure or if they are deemed serious. If a specific event or result from a given clinical or laboratory evaluation is not included in the NCI-CTCAE manual, then an abnormal result would be considered an AE if changes in therapy or monitoring are implemented as a result of the event/result.

For additional information and a printable version of the NCI-CTCAE manual, consult the NCI-CTCAE web site:

https://ctep.cancer.gov/protocoldevelopment/electronic_applications/ctc.htm#ctc_50.

12.3.5 Attribution Definitions

The relationship, or attribution, of an AE to the study drug or study procedure(s) will initially be determined by the site investigator and recorded on the appropriate AE/SAE eCRF. The final determination of attribution for safety reporting will be determined by the IND Sponsor (DAIT/NIAID). The relationship of an AE to study drug or procedures will be determined using the descriptors and definitions provided in Table 15. The relationship of an AE related to the administration of diphenhydramine (or equivalent antihistamine) and acetaminophen (or equivalent antipyretic), blood draw, IV line insertion, and MMTT will be assessed if it occurs within 24 hours of the administration of those.

Table 15. Attribution of Adverse Events

Code	Descriptor	Relationship (to primary investigational drug and/or other concurrent mandated study drug or study procedure)
Unrelated Category		
1	Not Related	The AE is clearly not related: there is insufficient evidence to suggest a causal relationship.
Related Categories		
2	Possibly Related	The AE has a reasonable possibility to be related; there is evidence to suggest a causal relationship.
3	Related	The AE is clearly related.

12.4 COLLECTION AND RECORDING OF ADVERSE EVENTS

12.4.1 Collection Period

All AEs will be collected from the time the participant signs the informed consent until the participant completes study participation (including LTFU, if applicable) or until 30 days after he/she prematurely withdraws (without withdrawing consent) or is withdrawn from the study.

12.4.2 Collecting Adverse Events

Details regarding all AEs and SAEs will be collected in the source documents. AEs (including SAEs) may be discovered through any of these methods:

- Observing the participant
- Interviewing the participant
- Receiving an unsolicited complaint from the participant
- In addition, an abnormal value or result from a clinical or laboratory evaluation can also indicate an AE, as defined in Section 12.3

12.4.3 Recording Adverse Events

All symptomatic AEs and $\geq$ Grade 2 asymptomatic AEs, except for non-major glycemic events (Section 12.3.3), will be recorded on the appropriate eCRF. Confirmed SARS-CoV-2

infection will also be recorded regardless of grade. The investigator will record these AEs and SAEs (Section 12.2) on the appropriate AE/SAE eCRF regardless of the relationship to siplizumab treatment or study procedure.

Once recorded, an AE/SAE will be followed until it resolves with or without sequelae, or until the end of study participation, or until 30 days after the participant prematurely withdraws (without withdrawing consent) or is withdrawn from the study, whichever occurs first.

12.5 REPORTING OF SERIOUS ADVERSE EVENTS AND ADVERSE EVENTS

12.5.1 Reporting of Serious Adverse Events to the Investigational New Drug Sponsor

This section describes the responsibilities of the site investigator to report SAEs to the IND Sponsor (DAIT/NIAID) via the eCRF. Timely reporting of AEs is required by 21 CFR and ICH E6(R2) guidelines.

Site investigators will report all SAEs (Section 12.2.4), regardless of relationship or expectedness within 24 hours of awareness of the event. Any AESI (Section 12.2.5) must also be reported within 24 hours of awareness.

For SAEs and AESIs, all requested information on the AE/SAE eCRF will be provided. However, unavailable details of the event will not delay submission of the known information. As additional details become available, the AE/SAE eCRF should be updated and submitted.

For additional information regarding SAE/AESI reporting, contact the DAIT/NIAID Clinical Data and Safety Management Center (CDSMC) at:

[REDACTED]

12.5.2 Reporting to Health Authority

After an AE requiring 24-hour reporting (Section 12.5.1) is submitted by the site investigator and assessed by the IND Sponsor (DAIT/NIAID), there are two options for DAIT/NIAID to report the AE to the appropriate health authorities:

12.5.2.1 Annual Reporting

DAIT/NIAID will include in the IND Annual Report to health authorities all AEs classified as:

- Serious, expected, SAR (Section 12.2.2)
- Serious and not a SAR (Section 12.2.3)

- Pregnancies

Note that all AEs (not just those requiring 24-hour reporting) will be reported in the Annual IND Report.

12.5.2.2 Expedited Safety Reporting

This option, with 2 possible categories, applies if the AE is classified as one of the following:

Category 1: Serious and unexpected suspected adverse reaction (SUSAR) (see Section 12.2.2, Section 12.2.3, and 21 CFR 312.32(c)(1)(i)).

The IND Sponsor shall report any SAR that is both serious and unexpected. The IND Sponsor shall report an AE as a SAR only if there is evidence to suggest a causal relationship between the study drug and the AE, such as:

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

Category 2: Any findings from studies that suggests a significant human risk

The IND Sponsor shall report any findings from other epidemiological studies, analyses of AEs within the current study or pooled analysis across clinical studies or animal or *in vitro* testing (e.g., mutagenicity, teratogenicity, carcinogenicity) that suggest a significant risk in humans exposed to the drug that would result in a safety-related change in the protocol, informed consent, current IB or other aspects of the overall conduct of the study.

DAIT/NIAID shall notify the FDA and all participating investigators of expedited Safety Report within 15 calendar days; unexpected fatal or immediately life-threatening SAR(s) shall be reported as soon as possible or within 7 calendar days.

12.5.3 Reporting of Adverse Events to Institutional Review Boards

All investigators shall report AEs and SAEs, including expedited reports, in a timely fashion to the single IRB of record (Advarra) and their respective local institutional review board(s) (IRB(s)) in accordance with applicable regulations and guidelines. All safety reports to the FDA shall be distributed by the Sponsor (DAIT/NIAID) or designee to the single IRB and all participating institutions for local IRB submission if required.

12.6 PREGNANCY REPORTING

The investigator shall be informed immediately if a female study participant becomes pregnant. A pregnant participant shall be instructed to stop taking study medication. The

investigator will provide counseling for participants and discuss the risks of continuing with the pregnancy and the possible effects on the fetus. Monitoring of a pregnant participant shall continue until the conclusion of the pregnancy.

The investigator shall report to DAIT/NIAID all pregnancies that occur in female study participants within 1 business day of becoming aware of the event using the Pregnancy eCRF. All female participant pregnancies identified during the study shall be followed to conclusion and the outcome of each must be reported. The Pregnancy eCRF shall be updated and submitted to the CDSMC when details about the outcome are available.

Information requested about the delivery shall include:

- Gestational age at delivery
- Any abnormalities

For all pregnancy complications that result in a congenital abnormality, birth defect, miscarriage, and medically indicated abortion, an SAE shall be submitted to DAIT/NIAID using the SAE reporting procedures described above.

12.7 REPORTING OF OTHER SAFETY INFORMATION

An investigator shall promptly notify their local and central IRB, in accordance with applicable regulations and guidelines, as well as the CDSMC and DAIT/NIAID via email when an “unanticipated problem involving risks to participants or others” is identified, which is not otherwise reportable as an AE.

12.8 REVIEW OF SAFETY INFORMATION

12.8.1 Medical Monitor Review

The DAIT/NIAID Medical Monitor shall receive monthly reports from the Autoimmune Diseases Statistical and Clinical Coordinating Center (AD-SCCC) compiling new and accumulating information on AEs, SAEs, and pregnancies recorded by the study site(s) on appropriate eCRFs.

In addition, the Medical Monitor shall review and make decisions on the disposition of the SAE (Section 12.5.1) and pregnancy reports (Section 12.6) received by the CDSMC.

12.8.2 Data and Safety Monitoring Board Review

12.8.2.1 Planned Data and Safety Monitoring Board Reviews

The joint NIDDK and NIAID ACE/ITN/TrialNet DSMB will review safety data and make recommendations regarding the continuation, termination, or modification of the study. The DSMB shall review safety data at least yearly during planned DSMB Meetings. Data for the planned safety reviews will include, at a minimum, a listing of all reported AEs and SAEs. The DSMB will also review C-peptide results at Week 12 by dosing arm and age cohort to monitor for an unexpected drug-induced worsening of T1DM.

The DSMB will be informed of an Expedited Safety Report in a timely manner.

12.8.2.2 Ad hoc Data and Safety Monitoring Board Reviews

In addition to the pre-scheduled data reviews and planned safety monitoring, the DSMB may be called upon for *ad hoc* reviews. The DSMB will review any event that potentially impacts safety at the request of the Protocol Chair or the IND Sponsor (DAIT/NIAID). In addition, the following events will trigger an *ad hoc* comprehensive DSMB Safety Review:

- Any Study Stopping Rules (Section 11.5) or Dosing Arm Stopping Rules (Section 11.6)
- If there is an unexpected decline in C-peptide mAUC observed at the week 12 visit. Within each age cohort, across both parts of the study, once 10 participants have been treated, mean 2-hour C-peptide AUC will be monitored on an ongoing basis. The DSMB will be notified if 70% or more of the participants show a >50% drop in mean 2-hour C-peptide AUC by week 12.

The DSMB will review the qualifying events and make recommendations. Additionally, an *ad hoc* DSMB Safety Review will occur with:

- The occurrence of a Grade 3 or higher SAE related to siplizumab treatment that is also a SUSAR in one or more of the study participants who have received siplizumab treatment
- Any occurrence of a Grade 3 or higher system allergic reaction (Table 14).

For these additional events, the DSMB will review the data within 2 weeks of the event being identified. If the DSMB has not reviewed the data within 2 weeks, study enrollment, as defined in Section 8.1, will be suspended and no additional participants will sign consent. Participants already in screening may continue with screening assessments, and participants already enrolled in the study will continue as planned, pending completion of the DSMB *ad hoc* review. After review of the data, the DSMB will make recommendations regarding study conduct and/or continuation.

13. STATISTICAL CONSIDERATIONS AND ANALYTICAL PLAN

13.1 OVERVIEW

Protocol ITN095AI has two parts. Part A was a randomized open label study of multiple doses of siplizumab in participants with T1DM. Part B is an open-label single ascending dose study. The primary objective is to identify safe dosing regimens of siplizumab that result in changes in T cell phenotypes. The study also aims to evaluate the safety of siplizumab and its impact on exploratory T1DM metabolic endpoints over 52 weeks.

13.2 ENDPOINTS

Primary, secondary, and exploratory endpoints are listed in Sections 3.2, 3.3, and 3.4.

13.3 MEASURES TO MINIMIZE BIAS

This study has well-defined entry criteria. The study is open label, but site personnel will not have access to any mechanistic data until completion of the study. Also, key endpoints are objectively measured and not impacted by investigator judgement.

13.4 ANALYSIS PLAN

13.4.1 Analysis Populations

To meet the primary dose-finding objective of this study, we must be able to observe changes in the T cell phenotypes at early time points in participants with exposure to sipilizumab (see per protocol sample definition below). Missing phenotype data will not be imputed. As such, the following PP sample will be used for evaluating the primary objective. Safety and mITT samples are included for evaluation of safety and exploratory endpoints.

13.4.1.1 Part A

Per protocol (PP) sample: All randomized participants who have viable PBMC samples available following two consecutive doses of sipilizumab. Per protocol analyses will be based on the actual dose the participants receive.

Safety sample (SS): All participants who receive any amount of sipilizumab. The safety analyses will be based on the actual treatment the participants receive.

Modified intent to treat (mITT) sample: All randomized participants who receive any amount of sipilizumab. The impact of sipilizumab on T1DM metabolic endpoints will be based on the mITT sample according to the group to which the participants are assigned.

13.4.1.2 Part B

Per protocol (PP) sample: All participants who receive sipilizumab and have viable PBMC samples available from V-1 or V0 and from at least 3 of the 5 subsequent visits (V1 through V5). Per protocol analyses will be based on the dose the participants receive.

Safety sample (SS): All participants who receive sipilizumab. The safety analyses will be based on the dose the participants receive.

Modified intent to treat (mITT) sample: All participants who receive sipilizumab. The impact of sipilizumab on T1DM metabolic endpoints will be based on the mITT sample according to the group to which the participants are assigned.

13.4.2 Analysis of Primary Endpoint (Part A)

Dosing regimens in Part A were evaluated for a T cell phenotype signature based on the following two criteria:

1. A 75% increase or greater from baseline in the CD4 Treg/Tem ratio in blood and
2. A 20% increase or greater from baseline in PD1+TIGIT+ frequency within circulating CD4 Tem.

The two criteria will be evaluated at weeks 2, 4, 8, and 12 on participants in the PP sample for Part A. An individual who achieves both signature criteria at any time point after Day 0 and on or before week 12 is counted as having the signature response. Both criteria do not need to be achieved at the same time point.

The T cell phenotype signature response will be listed for each participant in Part A.

13.4.3 Analysis of Primary Endpoint (Part B)

The primary analysis in Part B will identify siplizumab doses with acceptable safety profiles and favorable changes in the T cell phenotype in each age cohort. The primary assessments will be made during the first 8 weeks in Part B with an interim analysis for futility after 5 PP individuals per dosing arm reach Week 8 in each age cohort.

For both the adult and pediatric cohorts, we are evaluating different dose levels of a single dose of siplizumab for a T cell phenotype signature based on the following two criteria:

1. A 75% increase or greater from baseline in the CD4 Treg/Tem ratio in blood and
2. A 20% increase or greater from baseline in PD1+TIGIT+ frequency within circulating CD4 Tem.

The two criteria will be evaluated at weeks 1, 2, 4, 6, and 8. An individual who achieves both signature criteria at any time point after Week 0 and on or before week 8 is counted as having the signature response. Both criteria do not need to be achieved at the same time point.

The goal is to identify dosing arms where at least 80% (12 of 15) PP participants in an age cohort achieve the T cell phenotype signature with no safety concerns. The 80% goal is based on the mechanistic response observed in ITN045AI T1DAL (Section 1.1).

In the adult cohort, an interim analysis will occur once the first 5 adult PP participants in a dosing arm have reached Week 8. If at least 3 of the first 5 PP adults in a dosing arm achieve the T cell phenotype signature (Section 3.1.2), recruitment in that dosing arm may be expanded to recruit 15 total adult PP participants per arm, unless safety concerns are identified. Otherwise, if <3 adult PP participants achieve the signature, no additional adult participants will be recruited to the dosing arm. Treated adults in a closed dosing arm will have scheduled visits per the SOE.

In the pediatric cohort, an interim analysis will occur once the first 5 pediatric PP participants in a dosing arm have reached Week 8. If at least 3 of the first 5 PP pediatric participants in a dosing arm achieve the T cell phenotype signature (Section 3.1.2), recruitment in that dosing arm may be expanded to recruit 15 total pediatric PP participants, unless safety concerns are identified. Otherwise, if <3 pediatric PP participants achieve the signature, no additional pediatric participants will be recruited to the dosing arm. Pediatric participants enrolled in a closed dosing arm will continue to have scheduled visits per the SOE.

The safety data will be reviewed by the DSMB for each age cohort. The DSMB may recommend changes to the above pre-specified decisions, rules, or criteria for when pediatric enrollment may begin.

13.4.4 Analyses of Secondary Endpoints

AEs and SAEs will be tabulated by MedDRA Preferred Term and System Organ Class for all dosing arms in Part A and Part B using the safety samples. The incidence rate will also be provided. C-peptide mAUC and insulin use will be summarized by visit, dosing regimen, and

age cohort. Summary statistics for the change from baseline will also be provided. Metabolic data will be summarized using the PP and mITT samples for Part A and Part B.

The current study is not powered to show a difference in insulin secretory data, but the current sample size should be sufficient to demonstrate trends in C-peptide that support the prospect of clinical benefit to enroll pediatric participants.

13.4.5 Analyses of Exploratory Endpoints

In general, descriptive statistics and/or data plots will be used to gain an understanding of the nature of exploratory endpoints over 52 weeks. Additional exploratory analyses may also be considered based on results of planned analyses.

13.4.6 Descriptive Analyses

Descriptive statistics will be provided by each of the dosing arms and age cohorts for participant disposition, baseline and demographic characteristics, and use of concomitant medications. Continuous measures will be summarized using n, mean, standard deviation (SD), median, minimum, and maximum. Categorical variables will be summarized using counts and percentages.

13.5 INTERIM ANALYSES

Interim futility analyses for dose-finding are discussed in Section 13.4.3.

13.6 STATISTICAL HYPOTHESES

The main purpose of this study is to identify appropriate doses for future studies. No statistical hypotheses are pre-specified.

13.7 SAMPLE SIZE CONSIDERATIONS

To determine if a particular dose is sufficiently promising for future study, we are implementing a two-stage design.⁶⁸ In order to reach the goal of 15 PP participants in each dosing regimen per age cohort in Part B, it is anticipated that 50 adults and 50 pediatric participants will be treated to account for replacement of participants who do not meet the criteria for inclusion in the PP population. Fifteen participants per dosing arm were selected as a number sufficient for identifying gross safety concerns. Fewer than 15 participants will be treated in a dosing arm in an age cohort if the threshold for continuing treatment in the dosing arm is not met during the interim review of the first 5 PP participants. All dosing arms used in the Part B adult cohort will be used in the pediatric cohort unless determined otherwise during the safety review of at least 30 adult Part B participants. If an event occurs, the SMT and DSMB will determine if it is appropriate to enroll pediatric participants in that dosing arm. The goal is to identify dosing arms where at least 80% (12 of 15) PP participants in the age cohort achieve the T cell phenotype signature (Protocol Section 13.4.3) with no safety concerns. The T cell phenotype signature and 80% target were based on mechanistic data from ITN045AI T1DAL.

The T cell phenotype signature response probability (p) for any given dosing regimen is unknown. Table 16 provides the parameters assumed for finding the 15-person design that

minimizes the “expected sample size” when using the 80% threshold rule for success.⁶⁸ If the unknown signature response probability of a dosing regimen is $p \leq 0.60$ (i.e., not promising), then the design has at least a 91% chance of rejecting it, which includes at least a 32% chance of stopping after the first 5 participants. The chance of rejecting a desirable dose with a signature response probability $p \geq 0.88$ is no more than 10%. If fewer than 15 PP are enrolled in a dosing arm, the probability for error will be greater. However, this is acceptable for this stage of clinical development.

Table 16. Parameter Definitions and Notation for Optimizing the Simon Two-stage Design

Symbol	Definition	Value
<i>Pre-Specified Parameters</i>		
n	The maximum number in the dosing group cohort.	15
r	Stage 2 cutoff for rejecting the dose.	11
p	The true response rate (unknown)	
p0	If the $p \leq p0$, then the dosing level is not promising.	0.60
p1	If the $p \geq p1$, then the dosing level is promising.	0.88
beta	Maximum acceptable Pr (rejecting dose $p \geq p1$)	0.10
alpha	Maximum acceptable Pr (not rejecting dose $p \leq p0$)	0.20
<i>Selected Design Parameters</i>		
<i>Based on a grid search of all possible values for n1, r1 given pre-specified parameters. Optimized at minimum EN.</i>		
n1	The number in cohort at the time of the stage 1 look	5
r1	Stage 1 cutoff for rejecting the dose early.	2
R_p1	Probability of rejecting a dose when the $p \geq p1$ n, n1, r, r1, p1	0.10
R_p0	Probability of rejecting a dose when the $p \leq p0$ n, n1, r, r1, p0	0.91
PET	Probability of early termination given $p = p0$	0.32
EN	“expected sample size” given $p = p0$	11.8

Note: the same sample size approach was utilized in Part A prior to enrollment being terminated early.

14. IDENTIFICATION AND ACCESS TO SOURCE DATA

14.1 SOURCE DATA

Source documents and source data are considered to be the original documentation where participant information, visits, consultations, examinations and other information are recorded. Documentation of source data is necessary for the reconstruction, evaluation and validation of clinical findings, observations and other activities during a clinical trial.

14.2 ACCESS TO SOURCE DATA

The site investigators and site staff will make all source data available to the IND Sponsor (DAIT/NIAID), ITN, and relevant Health Authorities. Authorized representatives as noted above are bound to maintain the strict confidentiality of medical and research information that may be linked to identified individuals.

15. QUALITY ASSURANCE AND QUALITY CONTROL

Each clinical site will perform internal quality management of study conduct, data collection, documentation and completion. An individualized quality management plan will be developed to describe a site's quality management. Quality control (QC) procedures will be implemented beginning with the data entry system and data QC checks that will be run on the database will be generated. The site PI is required to keep accurate records to ensure that the conduct of the study is fully documented. The site PI is required to ensure that all eCRFs are completed for every participant entered in the trial. Any missing data or data anomalies will be communicated to the site(s) for clarification/resolution.

Following written SOPs, the monitors will verify that the clinical study is conducted, and data are generated, documented (recorded), and reported in compliance with the protocol, good clinical practice (GCP), and the applicable regulatory requirements (e.g., good laboratory practices (GLP), good manufacturing practices (GMP)).

The investigational site will provide direct access to all study related sites, source data/documents, and reports for the purpose of monitoring and auditing by the IND Sponsor, and inspection by local and regulatory authorities.

16. PROTOCOL DEVIATIONS

16.1 PROTOCOL DEVIATION DEFINITIONS

Protocol Deviation – The investigators and site staff will conduct the study in accordance with the protocol; no deviations from the protocol are permitted. Any change, divergence, or departure from the study design or procedures constitutes a protocol deviation. As a result of any deviation, corrective actions will be developed by the site and implemented promptly.

Major Protocol Deviation (Protocol Violation) – A Protocol Violation is a deviation from the IRB approved protocol that may affect the participant's rights, safety, or well-being and/or the completeness, accuracy and reliability of the study data. In addition, protocol violations include willful or knowing breaches of human participant protection regulations, or policies, any action that is inconsistent with the NIH Human Research Protection Program's research, medical, and ethical principles, and a serious or continuing noncompliance with federal, state, local or institutional human participant protection regulations, policies, or procedures.

Non-Major Protocol Deviation – A non-major protocol deviation is any change, divergence, or departure from the study design or procedures of a research protocol that does not have a major impact on the participant's rights, safety or well-being, or the completeness, accuracy and reliability of the study data.

16.2 REPORTING AND MANAGING PROTOCOL DEVIATIONS

The study site principal investigator has the responsibility to identify, document and report protocol deviations as directed by the IND Sponsor (DAIT/NIAID). However, protocol deviations may also be identified during site monitoring visits or during other forms of study

conduct review. Instructions for reporting deviations are in the Manual of Operations for Protocol ITN095AI.

17. ETHICAL CONSIDERATIONS AND COMPLIANCE WITH GOOD CLINICAL PRACTICE

17.1 STATEMENT OF COMPLIANCE

This clinical study will be conducted using GCP, as delineated in *Guidance for Industry: E6(R2) Good Clinical Practice: Integrated Addendum to ICH E6(R1)*, and according to the criteria specified in this study protocol. Before study initiation, the protocol and the informed consent documents will be reviewed and approved by the single IRB of record. Any amendments to the protocol or to the consent materials will also be approved by the IRB before they are implemented.

17.2 INFORMED CONSENT PROCESS

The consent process will provide information about the study to a prospective participant and will allow adequate time for review and discussion prior to his/her decision. The principal investigator or licensed physician designee listed on the Form FDA 1572 and the Delegation of Duties log will review the consent and answer questions. The prospective participant will be told that being in the study is voluntary and that he or she may withdraw from the study at any time, for any reason. All participants will read, sign, and date a consent form before undergoing any study procedures. Consent materials will be presented in participants' primary language. A copy of the signed consent form will be given to the participant. Consent/Assent process will be documented in the research or medical records.

A separate assent form will be utilized to obtain assent from children aged 7-13 and participants aged 14 year and older will sign on the assent line on the main consent form. Consent will be obtained from participants who reach legal age of consent per state laws during participation in the trial.

The consent process will be ongoing. The consent form will be revised when important new safety information is available, the protocol is amended, and/or new information becomes available that may affect participation in the study.

17.3 PRIVACY AND CONFIDENTIALITY

A participant's privacy and confidentiality will be respected throughout the study. Each participant will be assigned a unique identification number and these numbers rather than names will be used to collect, store, and report participant information. Site personnel will not transmit documents containing personal health identifiers (PHI) to the IND Sponsor or their representatives.

18. 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/>.

19. REFERENCES

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APPENDIX 1. SCHEDULE OF EVENTS – PART A

PHASE OF TRIAL	SCR	DOSING												FOLLOW-UP				WD	U
DAY	--	0	7	14	21	28	35	42	49	56	63	70	77	84	168	252	364	WD	U
WEEK	--	0	1	2	3	4	5	6	7	8	9	10	11	12	24	36	52	WD	U
VISIT WINDOW (DAYS)	37	+/-1												+/-3	+/-14				
VISIT	V-1	V0	V1	V2	V3	V4	V5	V6	V7	V8	V9	V10	V11	V12	V13	V14	V15	WD	U ¹
GENERAL ASSESSMENTS																			
Informed consent/assent	X																		
Eligibility criteria	X																		
Randomization ²		X																	
Demographics	X																		
Medical history	X																		
T1DM history	X																		
Adverse events	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
EBV-associated disease evaluation ³	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
CMV-associated disease evaluation ⁴	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
Comprehensive physical examination ⁵	X					X				X				X	X	X	X	X	
Directed physical examination ⁵		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
CENTRAL LABORATORY ASSESSMENTS ⁶																			
Serum Chemistries	X	X		X		X		X		X		X		X	X	X	X	X	X
Hematology	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Lymphocyte subset counts ⁷	X	X		X		X		X		X		X		X	X	X	X	X	X
Viral load CMV (PCR) ⁴	X																	X	X
Viral load EBV (PCR) ³ for seropositive	X	X				X				X				X ⁸	X ⁸	X ⁸	X ⁸	X	X
Viral load EBV (PCR) ³ for seronegative	X	X		X		X		X		X		X		X ⁸	X ⁸	X ⁸	X ⁸	X	X
Infectious disease serology ^{3,4}	X																		X
SARS-CoV-2 molecular test	X	X ⁹																	
SARS-CoV-2 antibody test		X												X					
Serum BhCG ¹⁰	X																		
TSH	X																		
HbA1c	X													X	X	X	X	X	
LOCAL ASSESSMENTS																			
Urine hCG ¹⁰		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Tuberculosis test ¹¹	X																		

¹ Unscheduled assessments are at the discretion of the site investigator but should include glucose meter and CGM downloads if available.

² May occur up to 1 business day prior to V0.

³ Symptoms of EBV are listed in Protocol Section 8.3.1. After the screening visit, EBV serology will be repeated if participants show clinical signs of infection. See Appendix 2 for additional details of EBV monitoring.

⁴ After the screening visit, CMV PCR and serology will only be collected at study visits where CMV is suspected clinically based on symptoms in Protocol Section 8.3.1 or if the participant's most recent CD4 T cell count is <200 cells/ μ L. Management and follow-up of a positive CMV PCR assay will be determined after discussion with the DAIT/NIAD Medical Monitor, Protocol Chair, and ITN Clinical Trial Physician.

⁵ During the dosing phase, physical exams must be conducted prior to study drug administration.

⁶ Verify prior visit's lab values before study drug administration. The PoR must review ALC and CD4 T cell count prior to dispensing study drug. Notify study team if prior visit's central lab values are not available.

⁷ May be increased to at least weekly if study drug administration is paused. See Appendix 3 if CD4 T cell count is <400 cells/ μ L at V15 (week 52).

⁸ See Appendix 2 for additional details of EBV monitoring after the dosing period.

⁹ SARS-CoV-2 molecular test results must be available prior to V0. If run locally, performed within 3 days of V0. If run centrally, performed within 5 days of V0.

¹⁰ Female participants with reproductive capacity will be monitored for pregnancy.

¹¹ QuantiFERON-TB Gold, QuantiFERON-TB Gold Plus, T-SPOT®.TB test or equivalent assay. A PPD tuberculin test may substitute.

PHASE OF TRIAL	SCR	DOSING												FOLLOW-UP				WD	U
DAY	--	0	7	14	21	28	35	42	49	56	63	70	77	84	168	252	364	WD	U
WEEK	--	0	1	2	3	4	5	6	7	8	9	10	11	12	24	36	52	WD	U
VISIT WINDOW (DAYS)	37		+/-1												+/-3	+/-14			
VISIT	V-1	V0	V1	V2	V3	V4	V5	V6	V7	V8	V9	V10	V11	V12	V13	V14	V15	WD	U ¹
STAT CBC with differential ¹²			X	X	X	X*	X*	X*	X*	X*	X*	X*	X*						
STAT SARS-CoV-2 antigen test ¹³			X	X	X	X	X	X	X	X	X	X	X						
SARS-CoV-2 molecular test		X ⁹																	
DISEASE SPECIFIC ASSESSMENTS																			
2-hour MMTT (C-peptide & glucose)	X													X	X	X	X	X	
Diabetes-related autoantibodies	X																		
Glucometer data review ¹⁴	X	X				X				X				X	X	X	X	X	X
CGM data review ¹⁵	X	X				X				X				X	X	X	X	X	X
Insulin use	X	X						X						X	X	X	X	X	X
Hypoglycemic/hyperglycemic events	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
STUDY DRUG ADMINISTRATION AND DRUG RELATED ASSESSMENTS																			
SQ Study drug administration ¹⁶		X	X	X	X	X	X	X	X	X	X	X	X						
Pre- and post-administration vitals		X	X	X	X	X	X	X	X	X	X	X	X						
PK ¹⁷		X		X		X		X		X		X		X	X	X	X		
Anti-siplizumab antibodies ¹⁷		X		X		X				X				X	X	X	X		
MECHANISTIC ASSESSMENTS¹⁸																			
Serum ¹⁹	X	X		X		X		X		X		X		X	X	X	X	X	
Cellular Assays (PBMCs)	X	X		X		X				X				X	X	X	X	X	
Gene Expression (whole blood)		X		X		X				X				X	X	X	X	X	
DNA (whole blood) ²⁰		X																	

¹² Required within 24 hours of V1, V2, and V3. *Only to be done if the prior week's ALC is <800 cells/mL or if participant has recovered or is recovering from a SARS-CoV-2 infection.

¹³ Only if participant has recovered from a SARS-CoV-2 infection. See Protocol Section 6.4.4 for additional details.

¹⁴ If available, reviewed by site staff for diabetes management. Data is not sent to CDSMC.

¹⁵ If available, reviewed by site staff for diabetes management. Data is uploaded by the site and sent to CDSMC.

¹⁶ Siplizumab treatment must be suspended if the participant develops signs or symptoms that suggest an EBV or CMV infection (see Protocol Section 8.3.1), has a high risk exposure to SARS-CoV-2, or has a confirmed SARS-CoV-2 infection. Additional guidance on suspending siplizumab is provided in Table 8 and Table 9.

¹⁷ During the dosing phase (V0, V2, V4, V6, V8 and V10), PK and anti-siplizumab antibodies samples are collected before SQ administration. For the follow-up phase (V12-15), these samples are collected before the MMTT administration.

¹⁸ The schedule for these assessments may vary as appropriate. At no time will the blood draw volume exceed what is allowable according to the participant's age and body weight per country specific requirements.

¹⁹ Includes diabetes-related autoantibodies, PK and anti-siplizumab antibodies samples.

²⁰ If there are blood volume limits, this specimen may be collected at another time point (e.g., V12).

APPENDIX 2. EBV VIRAL LOAD MANAGEMENT PLAN – PART A AND PART B

EBV Serology Status at Screening	Symptom Status	EBV Viral Load	Part A Action	Part B Action
Sero-positive	Symptomatic	≥ 200 IU/mL	Hold siplizumab; repeat EBV within 3 business days	Repeat EBV within 3 business days
Sero-positive	Asymptomatic	$<2K$ IU/mL	Continue siplizumab; repeat EBV within 3 business days and hold siplizumab if >5 fold increase	Repeat EBV within 3 business days
Sero-positive	Asymptomatic	$\geq 2K$ and $<10K$ IU/mL	Continue siplizumab; repeat EBV within 3 business days, hold siplizumab if EBV repeat is $\geq 2K$ IU/mL	Repeat EBV within 3 business days
Sero-positive	Asymptomatic	$\geq 10K$ IU/mL	Hold siplizumab; repeat EBV within 3 business days	Repeat EBV within 3 business days
Sero-negative	Symptomatic	≥ 200 IU/mL	Hold siplizumab; repeat EBV within 3 business days	Repeat EBV within 3 business days
Sero-negative	Asymptomatic	≥ 200 IU/mL	Hold siplizumab; repeat EBV within 3 business days	Repeat EBV within 3 business days

Additional Guidance for Part A:

For participants who tested negative for EBV during siplizumab dosing (and who are negative at Visit 12 (Day 86)), no further visits or blood tests will be performed until Visit 13 (Day 168).

For participants who tested positive for EBV during dosing or at Visit 12 (Day 86), follow-up between Visit 12 (Day 86) and Visit 13 (Day 168) will be handled based on the presence of symptoms:

- **Asymptomatic participants** - Phone follow-up by the site at 2 and 4 weeks following Visit 12. If the participant develops symptoms they should be evaluated by a physician and repeat EBV PCR and CBC with differential should be performed.
- **Symptomatic participants** - The participant should be evaluated by a physician and repeat EBV PCR and CBC with differential should be performed.

Additional SMT consultation is required for any circumstances not covered above.

Additional Guidance for Part B:

Follow-up for positive EBV participants will be made in consultation with the Protocol Chair, ITN Clinical Trial Physician, and DAIT/NIAID Medical Monitor.

APPENDIX 3. SCHEDULE OF EVENTS – LONG-TERM SAFETY MONITORING

PHASE OF TRIAL	LONG-TERM SAFETY MONITORING ¹				
DAY	448	532	616	700	U
WEEK	64	76	88	100	U
VISIT WINDOW (DAYS)	+/- 14 DAYS				
VISIT	1SM	2SM	3SM	4SM	U
GENERAL ASSESSMENTS					
Adverse events	X	X	X	X	X
EBV-associated disease evaluation ²	X	X	X	X	X
CMV-associated disease evaluation ³	X	X	X	X	X
Concomitant medications	X	X	X	X	X
Directed physical examination	X	X	X	X	X
Vital signs	X	X	X	X	X
LABORATORY ASSESSMENTS⁴					
Hepatic function panel	X	X	X	X	X
Hematology	X	X	X	X	X
Lymphocyte subset count	X	X	X	X	X

¹ If the participant is unable to return for a visit, telephone follow-up and remote assessments will be performed.

² EBV PCR and serology will be collected when EBV is suspected clinically based on symptoms listed in Section 8.3.1. Management and follow-up of a positive EBV PCR assay will be determined after discussion with the DAIT/NIAID Medical Monitor, Protocol Chair, and ITN Clinical Trial Physician.

³ CMV PCR and serology will be collected when CMV is suspected clinically based on symptoms in Section 8.3.1. Management and follow-up of a positive CMV PCR assay will be determined after discussion with the DAIT/NIAID Medical Monitor, Protocol Chair, and ITN Clinical Trial Physician.

⁴ The list of laboratory assessments is for participants with CD4 T cell counts <400 cells/μL; it may be modified for other AEs by the DAIT/NIAID Medical Monitor.

APPENDIX 4. SCHEDULE OF EVENTS – PART B

WEEK		0	1	2	4	6	8	12	24	36	52	WD	UN
VISIT WINDOW (DAYS)	-37		±2	±2	±2	±2	±2	±3	±14	±14	±14	WD	UN
VISIT	V-1	V0	V1	V2	V3	V4	V5	V6	V7	V8	V9	WD	UN
GENERAL ASSESSMENTS													
Informed consent/assent	X												
Eligibility criteria	X	X											
Randomization ¹		X											
Demographics	X												
Medical history	X												
T1DM history	X												
Adverse events	X	X	X	X	X	X	X	X	X	X	X	X	X
EBV-associated disease evaluation ²	X	X	X	X	X	X	X	X	X	X	X	X	X
CMV-associated disease evaluation ²	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
Comprehensive physical examination	X				X		X	X	X	X	X	X	
Directed physical examination		X	X	X		X							X
Vital signs	X	X	X	X	X	X	X	X	X	X	X	X	X
CENTRAL LABORATORY ASSESSMENTS													
Serum Chemistries	X	X	X	X	X	X	X	X	X	X	X	X	X
Hematology	X	X	X	X	X	X	X	X	X	X	X	X	X
Lymphocyte subset counts	X	X	X	X	X	X	X	X	X	X	X	X	X
Viral load CMV (PCR) ³	X		X	X	X	X	X	X	X	X	X	X	X
Viral load EBV (PCR)	X		X	X	X	X	X	X	X	X	X	X	X
HIV (Ab)	X												
Hep B (HBsAg and total core)	X												
Hep C (IgG Ab and RNA if positive)	X												
EBV Serology (VCA IgM Ab, VCA IgG Ab, EBNA, and EBV early antigen IgG Ab) ⁴	X												X
CMV Serology (CMV IgG Ab) ⁴	X												X
SARS-CoV-2 molecular test ⁵													X
Serum BbCG ⁶	X												
TSH	X												
HbA1c	X							X	X	X	X	X	
LOCAL ASSESSMENTS													
Urine hCG ⁶		X	X	X	X	X	X	X	X	X	X	X	X
Tuberculosis test	X												
STAT SARS-CoV-2 antigen test ⁵													X
SARS-CoV-2 molecular test ⁵													X
Hematology ⁷		X											
DISEASE SPECIFIC ASSESSMENTS													
2-hour MMTT (C-peptide & glucose)	X							X	X	X	X	X	
Diabetes-related autoantibodies	X												
Glucometer data review ⁸	X	X			X		X	X	X	X	X	X	X

¹ Only applies to expansion phase if multiple dosing arms dosing are open for dosing (see Section 3.1.2).

² Symptoms of EBV and CMV are listed in Protocol Section 8.3.1.

³ After the screening visit, CMV PCR will only be collected at study visits where CMV is suspected clinically based on symptoms in Section 8.3.1 or if the participant's most recent CD4 T cell count is <200 cells/μL. Management and follow-up of a positive CMV PCR assay will be determined after discussion with the DAIT/NIAID Medical Monitor, Protocol Chair, and ITN Clinical Trial Physician.

⁴ After the screening visit, EBV and CMV serologies are only performed if the respective PCR test is positive.

⁵ SARS-CoV-2 tests are only performed if clinically indicated.

⁶ Female participants with reproductive capacity will be monitored for pregnancy. The V0 urine sample must be collected and reviewed prior to study drug administration.

⁷ A local STAT CBC with differential should be performed at V0 before sipilizumab administration if the participant has experienced an infectious illness within 2 weeks prior to V0. A routine local CBC with differential will be ordered in the event of an active systemic infection at V0.

⁸ If available, reviewed by site staff for diabetes management. Data is not sent to CDSMC.

WEEK		0	1	2	4	6	8	12	24	36	52	WD	UN
VISIT WINDOW (DAYS)	-37		±2	±2	±2	±2	±2	±3	±14	±14	±14	WD	UN
VISIT	V-1	V0	V1	V2	V3	V4	V5	V6	V7	V8	V9	WD	UN
CGM data review ⁹	X	X			X		X	X	X	X	X	X	X
Insulin use	X	X						X	X	X	X	X	X
Hypoglycemic/hyperglycemic events	X	X	X	X	X	X	X	X	X	X	X	X	X
STUDY DRUG ADMINISTRATION AND DRUG RELATED ASSESSMENTS													
SQ Study drug administration ⁷		X											
Pre- and post-administration vitals		X											
MECHANISTIC ASSESSMENTS													
Serum ¹⁰	X	X		X	X	X	X	X	X	X	X	X	
Cellular Assays (PBMCs)	X	X	X	X	X	X	X	X	X	X	X	X	
Gene Expression (whole blood)		X		X	X		X	X	X	X	X	X	
DNA (whole blood)		X											

⁹ If available, reviewed by site staff for diabetes management. Data is uploaded by the site and sent to CDSMC.

¹⁰ Includes diabetes-related autoantibodies, PK and anti-siplizumab antibodies samples.