Protocol

This trial protocol has been provided by the authors to give readers additional information about their work.

Protocol for: Jason L. Gaglia, Heather L. Daley, Nora K. Bryant, et al. Novel Autologous Dendritic Cell Therapy AVT001 for Type 1 Diabetes. NEJM Evid. DOI: 10.1056/EVIDoa2300238.

Cover Page

This supplement contains the following items:

1. Original protocol, final protocol, summary of changes.

   More specifically, it contains:

   - Original Protocol for Avotres' T1D trial (AVT001-T1D-01), finalized on Jul 15th, 2013,
   - Latest version of Protocol (Amendment 3) for Avotres' T1D trial (AVT001-T1D-01) reported in the paper, finalized on Feb 1st, 2021,
   - Summary of Change, Protocol Amendment 3 vs 2, 2 vs 1 and 1 vs Original.

2. Original statistical analysis plan, final statistical analysis plan, summary of changes.

   More specifically, it contains:

   - Original Statistical Analysis Plan (SAP), finalized on Mar 28th, 2022,
   - Latest version of Statistical Analysis Plan (SAP) for the Day 360 analysis of Avotres' T1D trial (AVT001-T1D-01) reported in the paper, finalized on Dec 2nd, 2022,
   - Summary of Change, SAP v2.0 vs SAP v1.0.

A Phase I/II, Double-Blind, Placebo Controlled, Parallel Design of AVOTRES Cell-Based Therapy as a Treatment for Human Type 1 Diabetes

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Study Product:	Autologous dendritic cells loaded with Hsp60sp peptide (AVOTRES)
Protocol Number:	Not Yet Assigned
IND Number:	Not Yet Assigned
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Date:	July 14, 2013
Version:	Version 0.7
Amended:	N/A

Clinical Protocol Table of Contents

List of Abbreviations

A1c	Hemoglobin A1c
APC	Antigen Presenting Cells
ATG	Anti-thymocyte Globulin
CD4, CD8	Cluster of Differentiation
CFR	Code of Federal Regulations
CRF	Case Report Form
CTCAE	Common Terminology Criteria for Adverse Events
CTL	Cytotoxic T Cell
DC	Dendritic Cell
DFCI-CMCF	Dana Farber Cancer Institute, Cell Manipulation Core Facility
DMK	Dystrophia Myotonica Kinase
DSMB	Data Safety Monitoring Board
GAD	Glutamic Acid Decarboxylase
GCP	Good Clinical Practice
GFAP	Glial Fibrillary Acidic Protein
GM-CSF	Granulocyte Macrophage-Colony Stimulating Factor
HbA1c	Hemoglobin A1c
HBsAg	Hepatitis B Virus Surface Antigen
HBV	Hepatitis B Virus
HCV	Hepatitis C Virus
HIV	Human Immunodeficiency Virus
HLA	Human Leukocyte Antigen
Hsp60p	Heat Shock Protein 60 Signal Peptide
HTLV-1/2	Human T-lymphotropic virus Type I/2
ICA	Islet Cell Antibody
iDC	Immature Dendritic Cell
IFNγ	Interferon γ
IGRP	Islet-Specific Glucose-6-Phosphate Catalytic Subunit Related Protein
IL	Interleukin
IV	Intravenous
mcg	Micrograms
MHC	Major Histocompatability Complex
MMTT	Mixed Meal Tolerance Test
MRI	Magnetic Resonance Image
mRNA	Messanger Ribonucleic Acid
NOD	Non-Obese Diabetic (murine model)
ppIAPP	Pre-Pre-Islet Amyloid Polypeptide Protein
Qa-1	Murine MHC class I molecule
RNA	ribonucleic acid
s.c.	subcutaneous
T1D	Type 1 Diabetes
TNFα	Tumour Necrosis Factor α
Treg cells	Regulatory T Cells

Study Summary

Title	A Phase I/II Double-Blind, Placebo Controlled, Parallel Design of AVOTRES Cell-Based Therapy as a Treatment for Human Type 1 Diabetes
Short Title	AVOTRES: Autologous Dendritic Cells Loaded with hHsp60sp Peptide
Protocol Number	Not yet assigned
Clinical Phase	Phase I/II
IND Sponsor	Hong Jiang, MD, PhD Columbia University Medical Center PH - 8 East, Ste. 101, 622 West 168th St New York, NY 10032 Phone: 212-305-9984/201-944-3352 Fax: 212-305-6070 Email: hj4@columbia.edu
Principal Investigator	Daniel S. Donovan, Jr., MD, MS, CDE Columbia University Medical Center 161 Fort Washington Ave. Room 2-212 New York, NY 10032 Phone: 212-305-0504 Email: dsd8@columbia.edu
Study Duration	Treatment phase is 90 days; overall protocol period is 4 years with LTFU.
Study Center(s)	Columbia University Medical Center
Objectives	Primary Objectives are to determine the safety and tolerability of the AVOTRES reagent; and, to determine whether, among T1D patients with in vitro-reversible defects in the CD8+ T cell regulatory system, infusion of AVOTRES will lead to an in vivo reconstitution of this system. Secondary Objective is to determine whether, among T1D patients with in vitro-reversible defects in the CD8+ T cell regulatory system, infusion of AVOTRES will result in amelioration of β cell destruction.
Accrual Objective	Twenty-one (21)
Study Product, Dose, Route, Regimen	This is a double-blind, placebo controlled, parallel design of "AVOTRES" cell-based therapy as a treatment for human type I diabetes (T1D). The trial will involve 21 new onset T1D patients who have been identified (a), as having a defect in HLA-E-restricted CD8+ T cell function associated with β cell destruction and (b), as showing evidence that this defect of HLA-E-restricted CD8+ T cells can be corrected by *in vitro* retriggering with AVOTRES. The subjects will be randomly divided into two groups with 14 subjects in the treatment arm to receive AVOTRES, and 7 subjects in the placebo arm to receive with saline.

Inclusion Criteria	Patients who meet *all* of the following criteria are eligible for enrollment as study participants: – Diagnosis of Type 1 Diabetes by one or more of the following types of antibodies: anti-GAD65 (glutamic acid decarboxylase), anti–islet-cell antibody 512 (ICA512), anti-IA2 and anti-insulin antibody. – Age 18 or older and able to provide informed consent for participation. – Must have stimulated C-peptide levels ≥0.2 pmol/ml measured during a mixed meal tolerance test (MMTT) – Recent onset (diagnosis within the past 12 months) T1D patients who screen positive for a CD8+ T cell defect that is correctable *in vitro* by co-culture with immature dendritic cells loaded with self-peptide Hsp60sp. – All subjects will be using insulin at the time of enrollment. – If a participant is female with reproductive potential, she must be willing to avoid pregnancy and have a negative pregnancy test during the 12 months of treatment and for an additional 3 months. – Signed and dated written informed consent
Exclusion Criteria	Patients who meet *any* of the following criteria are *not* eligible for enrollment as study participants: – Uncontrolled diabetes (HbA1c >9.0%) – Hemoglobin <10.0 g/dL; leukocytes <3,000/uL; neutrophils <1,500/uL; lymphocytes <800/uL; platelets <100,000/uL – Current use of immunosuppressive or immunomodulatory therapies, including pharmacologic doses of systemic steroids – Coincident medical condition likely to require immunosuppressive or immunomodulatory therapies – Coincident medical condition likely to limit short term (5 year) life expectancy (malignancy, symptomatic coronary artery disease, recent stroke) – Prior radiation therapy, immunotherapy, or chemotherapy – Serologic evidence of current HIV-1 or HIV-2 infection – Evidence of current hepatitis B as demonstrated by HBSAg or circulating hepatitis B genomes – Serologic evidence of hepatitis C infection – Autoimmune disease aside from T1D – Pregnant or breastfeeding – Any chronic illness which, in the opinion of the principal investigator, should preclude participation in the trial – Adequate venous access to support leukapheresis – Urine Albumin Excretion < 300mg/gmCr – eGFR > 60 mL/min/1.73m^2

Statistical Considerations	Primary endpoint: To determine whether, among T1D patients with *in vitro*-reversible defects in the CD8+ T cell regulatory system, infusion of AVOTRES will lead to an *in vivo* reconstitution of this system. Analysis assumes that the proportion of subjects given placebo exhibiting reversal at 12 months will be 0 and that at least 60% of the subjects receiving AVOTRES will exhibit *in vivo* evidence of reversal of the defect. With a randomization of 2:1 subjects to Avotres or Placebo. N = 14 subjects randomized to Avotres and N = 7 subjects to placebo. There would be 94% power to detect this difference in proportions of 0.6 with a one sided $\alpha < 0.05$ using the Fisher's exact test. Allowing for up to 10% of subjects being unavailable for evaluation the total sample size is increased to N = 24. Secondary endpoint: The secondary outcome of each participant is the area under the stimulated C-peptide curve (AUC) over the first 2 hours of a mixed meal glucose tolerance test conducted at the one-year visit. The AUC is computed using the trapezoidal rule that is a weighted sum of the C-peptide values over the 120 minutes. The weighted mean C-peptide is simply AUC/120 (pmol/mL). Let represent the C-peptide value for study subjects receiving AVOTRES and those receiving placebo, respectively. The secondary analysis will be conducted on the transformed C-peptide using the log function: log(C-peptide +1). This provides better normal distributional behavior of the random variable. The comparison will be based on a t-test of treatment effect in an ANCOVA model adjusting for gender, baseline age, and baseline log (C-peptide +1). There will 90% power to detect a threefold increase in stimulated C-peptide with a one sided $\alpha < 0.05$ A log rank test of the difference in the C-peptide failure hazard function between groups (C-peptide failure is defined as the first occurrence at which the 2 hour peak C-peptide < 0.2 pmol/ml during a MMTT). Also, a proportional hazards model will be used to compare treatment groups while adjusting for baseline level of C-peptide, gender, and baseline age. Longitudinal analysis of HbA1c, Insulin dose (units/kg) and blood glucose by treatment group using normal variance-covariance structure. Number and severity of adverse events. Change in autoantibodies.

1 Introduction

This document is a protocol for a human research study. This study is to be conducted according to US and international standards of Good Clinical Practice (FDA Title 21 part 312 and International Conference on Harmonization guidelines), applicable government regulations and Institutional research policies and procedures.

1.1 Background

Type 1 diabetes (T1D) is a complex, chronic, T cell-mediated autoimmune disease in which the insulin producing β cells are destroyed. In T1D, there is a loss of tolerance to pancreatic β cells that results in a CD4 and CD8 T cell-dependent autoimmune process that culminates in complete destruction of insulin-producing β cells, leading to insulin deficiency and dysregulated glucose metabolism. Diabetic patients manage their hyperglycemia by daily insulin injections, however this treatment does not cure the disease or prevent the possibility of the disease's serious effects, which may include kidney failure, blindness, nerve damage, heart attack, stroke, and pregnancy complications (JDRF website). It is important then that other therapies to treat T1D, particularly target the primary event of the defect of the immune function that causes T1D, be identified and tested. As discussed below, immunotherapeutic approaches that could potentially treat and or prevent T1D have been moving into the clinic recently. Over the years, studies using the non-obese diabetic (NOD) mouse model have provided important insights into the immunology of T1D and have demonstrated the feasibility of preventing or curing T1D in an antigen-specific manner by specifically manipulating disease-relevant autoreactive T cell populations without compromising the immune system at large.

However, conceptually speaking, selective control of unwanted immune responses could be achieved by induction of "antigen-specific tolerance", provided that the specific antigens that elicit the unwanted immune responses have been identified. However, for decades, it has always been a tremendous challenge to identify the exact pathogenic peptide/s for any given human organ specific autoimmune diseases, or specific allo-antigen peptide/s responsible for graft rejection. In reality, no definitive answers have ever been obtained for any human autoimmune diseases yet. Furthermore, induction of "antigen specific tolerance" requires precise knowledge of the peptide associated with particular MHC molecules and highly polymorphic human HLA system inevitably increases the uncertainty of searching for such MHC/peptide complex in each patient. Thus, the necessity of identification of a precise MHC/peptide complex for each individual from an unknown and countless antigen specificity pool makes the induction of "antigen-specific tolerance" an unfeasible approach to specifically and effectively treat organ specific autoimmune diseases and/or control graft rejection in clinical immunology. In contrast, Qa-1/HLA-E restricted CD8+ T cells, by a simple and unified "class action" of self-nonself discrimination, control the unwanted immune responses, independent of the knowledge of the particular pathogenic peptides in autoimmune diseases and/or graft rejection for each patient, which are largely undetermined currently. (1, 2). AVOTRES, represents one such approach to manipulate autoreactive T-cell responses against all self antigens, including β-cell antigens (1, 2), that we would like to evaluate in this clinical protocol. Modulating the activity of self-reactive T cells to damper autoimmunity at a system level without damaging normal on-going anti-infection and anti-tumor immunity would be an ideal new approach to treat T1D.

1.2 Failure of self-nonself discrimination by the immune system results in pathogenesis of autotimmune disease such as T1D

The pioneering work of Burnet and Medawar clearly indicated that self-nonself discrimination is not achieved by recognizing the structural differences between self antigens and foreign antigens, because introducing a foreign allo transplantation antigen into the immune system early enough in life renders the recipient animals tolerant to that foreign antigen which is subsequently treated as if it were a self antigen (3, 4). It is well known now that thymic negative selection that deletes high avidity T cells specific to any antigens, self-antigens as natural cases, is the major mechanism of the specific tolerance established (5-8). However, as a biological consequence of natural thymic negative selection, self-reactive T cells with intermediate and low avidity escape into the periphery (9-11). The intermediate avidity self-reactive T cell pool in the periphery represent a potential danger of pathogenic autoimmunity (1, 12), which could be accidentally activated by a sufficiently high level of self-antigens to initiate organ-specific autoimmune diseases during infections or injuries (1, 11, 13-15) and must be effectively controlled. We have thus postulated that self-nonself discrimination must continue in the periphery after the thymic negative selection to maintain self-tolerance (1, 12). In this regard, it has been shown that Qa-1/HLA-E restricted CD8+ T cell mediated regulatory pathway is important in maintaining peripheral selftolerance (16-24). We have further proposed and tested an "Avidity Model Of Peripheral T Cell Regulation", in which Qa-1/HLA-E restricted CD8+ T cells, by selectively down-regulating the intermediate avidity T cell pool, play a central role in control of organ specific auto-immune diseases in the conventional soluble protein antigen system in both mice (19, 20, 22) and humans (23). The peripheral self-reactive T cell repertoire is devoid of high avidity T cells due to thymic negative selection. Therefore, selective down-regulation of the intermediate avidity T cell pool enables the selective suppression on the overall immune responses to self-antigens, mediated by the intermediate avidity T cells, but not on effective immune responses to foreign antigens dominated by the high avidity T cells. This way, the Qa-1/HLA-E restricted CD8+ T cells are able to specifically control organ-specific autoimmune diseases or establish donor specific transplantation tolerance without damaging on going effective anti-infection and anti-tumor immunity (2, 19, 20, 22, 23, 25). The selective down-regulation of the intermediate avidity T cell pool by the Qa-1/HLA-E restricted CD8+ T cells is accomplished by a specific recognition of the unique common target structure, the signal peptide of heat shock protein 60 (Hsp60sp) associated with the non-polymorphic MHC Class 1b molecule Qa-1/HLA-E, preferentially expressed on the intermediate avidity T cells (19, 20, 22, 23).

More relevant, we have recently shown a defect in the T cell regulatory pathway which normally controls autoreactive T cells that attack the body's own tissues and organs (23). A majority of people with Type 1 diabetes who were tested in the study were found to have this newly-identified cellular/molecular defect on HLA-E restricted CD8+ T c ells, and we were able to successfully correct the defect in-vitro in most of the patients tested by retriggering the CD8+ T cells with Avotres in vitro made of the autologous DC loaded with self-peptide hHsp60sp (23).

Our preclinical human in vitro studies and murine in vivo studies (Please see details in Section 8) formed the scientific basis for this IND application for trying AVOTRES *in vivo.*

1.3 The Autoimmune Process of Type 1 Diabetes

Development of autoimmune disorders is a result of breakdown in mechanisms that maintain unresponsiveness to self. Specifically, T1D is an automimmune disorder in which T cells are self-reactive or activated T cells that are activated against major antigenic components of pancreatic β cells are out of

the control by the normal regulatory mechanisms. While these self-reactive T cells are under the control of peripheral regulatory mechanisms in healthy individuals, failure of control leads to the destruction of the β cells and consequent T1D. The autoimmune attack on pancreatic β cells is orchestrated by a variety of cells that produce cytokines and other toxic mediators. These self-reactive T cells work together with other lymphocytes and antigen-presenting cells to mediate this damage and have been shown in animal models to be important both in the early stages of diabetes development and in the final effector stages. They kill self-reactive T cells specific to self-antigens, which include β cell antigens, insulin and other key proteins associated with beta cell functions.

1.4 Autoreactive T cells in T1D

As reviewed in Wong 2008, many studies carried out in the NOD mouse model of T1D have indicated that both CD4+ and CD8+ T-cells inflict islet β–cell damage both at an early stage in diabetes development and at the final effector phase (26-28). There appears to be considerable similarity between the antigens recognized in humans and in the NOD animal models. In human studies, so far limited to testing peripheral blood lymphocytes, a variety of reactivities to putative autoantigens have been found, including proinsulin, GAD65, ppIAPP (pre-pro-islet amyloid polypeptide protein) and GFAP (glial fibrillary acidic protein). By the time diabetes occurs, it appears that there may be a broad spread of reactivity, which is different for each patient, and it will continue to be a major challenge to identify those antigenic epitopes that are important in the early phases of pathogenesis. Many of these antigenic epitopes represents attractive targets to go after for T1D therapeutic interventions.

Mechanisms by which T cells cause damage include production of cytotoxic cytokines such as IFN*γ* (interferon *γ*) and TNF*α* (tumour necrosis factor *α*), that have a direct cytotoxic action on the islets. In addition, these cytokines have an important role in up-regulation of MHC class I molecules, which could increase immune recognition of the cells. These T-cell-generated inflammatory cytokines can work together with cytokines produced by macrophages, such as IL (interleukin)-1, to have a synergistic damaging effect on islet β-cells. The CD8+ CTLs also exocytose granules from secretory lysosomes which contain cytotoxic proteins that include the pore-forming protein, perforin, and serine esterases, called granzymes. In summary, autoreactive T cells are considered as emerging targets for prospective antigen-specific therapeutic interventions.

1.5 Immunotherapeutic approaches Immunotherapeutic approaches to treat T1D

Many broad based immunosuppressive and antigen-specific immunoregulatory therapies have been and are currently being evaluated for their utility in the prevention and treatment of T1D. These may be administered as monotherapeis or in combination. Some of the below-mentioned have shown efficacy in initial clinical trials. Many tend to be small molecule inhibitors and monoclonal antibodies directed against cytokine and cytokine receptors, proteases, CD3 or CD20 receptors (i.e. Rituximab), GAD65, CTL14, heat shock proteins, or insulin-related molecules (29). Systemic non-antigen specific immunomodulator interventions include cycyclosporine A, azatiopirine, and anti-thymocyte globulin (ATG) plus prednisolone.

Other cell therapies using dendritic cells are also currently being tested as described briefly below and in Section 3.7. Additional examples of cell therapies include targeting regulatory T cells (Tregs). Novel approaches to isolate and expand polyclonal and antigen-specific Tregs *in vitro* have been developed for immunotherapy and a clinical trial to test this hypothesis is now underway in the US (Clinicaltrials.gov identifier NCT01210664).

Unlike those small molecule therapies and any currently tried cell-based therapies, Avotres provides for a new type of cell-based therapeutic approach to treat and/or prevent T1D. Avotres, used as a vaccine, activates and corrects the defective regulatory pathway mediated by HLA-E restricted CD8+ T cells in the identified patients that normally function to selectively control the pathogenic self-reactive T cells that destroy β cells without damaging the normal on going anti-infection and anti-tumor immunity, a process called self-nonself discrimination (19, 20, 22, 23).

1.6 Dendritic cell based therapies for T1D

Dendritic cells (DCs) were first described by Ralph Steinman (30) nearly forty years ago. is known that DCs existed in all lymphoid and most non-lymphoid tissues. DCs are antigen-presenting cells (APCs), which play a critical role in the regulation of the adaptive immune response. To perform this function, DCs are capable of capturing antigens, processing them, and presenting them on the cell surface along with appropriate costimulation molecules. The crucial function of DCs falls broadly into two categories, each of which involves antigen presentation. The first category of DCs function is antigen presentation and activation of T cells. The second category of DCs function has been suggested that a different class of DCs exist with the function of inducing and maintaining immune tolerance.

Cell therapy using dendritic cells use “immature” dendritic cells that are *in vitro* derived from monocyte precursors isolated from diabetic subjects and then either modified with and reinjected into the same individuals with the hope of resetting the immune system balance in the individual. There is a great deal of interest in how DCs might be exploited as a form of immunotherapy. DCs are being studied as adjuvants for vaccines or as a direct therapy to induce immunity against cancer. Human clinical trials are ongoing to use DCs to induce immunity to antigens against prostate cancer, lung cancer, melanoma and multiple myeloma etc. The first attempt to use DCs as cancer vaccines in humans was made by Edgar Engleman and Ronald Levy (31), who isolated DCs from patients with non-Hodgkin's lymphoma who had failed conventional chemotherapy, loaded the cells with immunoglobulin idiotype obtained from the patient's tumor, and re-injected the antigen-loaded cells back into the patients. Four patients involved in this pilot clinical trial and all treatments were well tolerated, no significant side effects were associated with the infusion of antigen-loaded DCs. Since then, pilot clinical trials of antigen-pulsed DCs have been conducted in various types of cancer, including melanoma, prostate cancer, multiple myeloma, and non-small cell lung cancer. DC-based therapy as immunotherapy is also tried in human type 1 diabetes and a clinical trial to test this hypothesis has finished its phase I study in the US (Clinical Trials. gov identifier NCT00445913) (32). In general, it is widely accepted that DC-based therapy is consider a very safe therapy (Please see Section 8.2.3)

1.7 Dosing Rationale

Our preclinical human *in vitro* studies and murine *in vivo* studies formed the scientific basis for this clinical application to assess AVOTRES *in vivo.* AVOTRES is the study product name for autologous immature dendritic cells loaded with self-peptide Hsp60sp. The study product represents an individualized preparation of autologous immature dendritic cells, derived by culture of the subject’s adherent primary monocytes with GM-CSF and IL-4 for 6 days, and loaded passively with the synthetic oligopeptide Hsp60sp (QMRPVSRVL). In this study design described in this protocol, three vaccines will be administered 30 days (+/- 2 days) apart.

We feel strongly that the selection of the proposed route of administration, dose levels, dosing regimen, timing of vaccination relative to disease state, etc. in our clinical trial should be more rely on the information from similar human clinical trials than animal studies. Particularly, it should not be heavily dependent on NOD/T1D model, which is a spontaneous disease animal model involving multiple mechanisms other than Qa-1 restricted CD8+ T cells and may not be exactly the same as human T1D patients that we plan to test. For example, the patients we propose to test are clearly defined to be the new onset T1D patients who have the defect of the HLA-E restricted CD8+ T cells and the defect can be corrected in vitro by retriggered with AVOTRES. This design is based on the studies from human T1D patients and its clinical regimen cannot be exactly mimicked and represented by the studies from NOD/T1D model. The best example in this case, which is almost identical to our circumstances, would be the recently completed clinical trial of an iDC based therapy on T1D patients (ClinicalTrials. gov identifier NCT00445913) (32) versus its preclinical studies on NOD/T1D model (33), in that injections of 10 x 10^6 iDC every 2 weeks for total four times in humans versus a single injection of 2-3× 10^6 iDC in NOD mice.

In NOD mice we have used 0.5-2 x 10^6/mouse without any abnormality in protected mice, which dose range would be proportionally about 50-100 x 10^6/person in humans. However, in search for the information in other similar human clinical trials, the dosage range used is between 2-50 x 10^6 /person every 2weeks for 2-13 times and showed no significant side effects (See Table below). Particularly, in one recently completed trial using iDC onT1D patients, 10 x 10^6 iDC every 2 weeks for total four times were used (32), which is very close to our proposal, eg, 10 x 10^6 iDC every 4 weeks for total three times will be used. Finally, from our own studies, HLA-E restricted CD8+ T cells represent a very potent subset of CD8+ T cells, probably account for 1-2% of total CD8+ T cells. We calculate if the iDCs needed for activate such CD8+ T cells is at a ratio of 1:50-100, 10 x 10^6 of iDC would activate 500-10,000 x 10^6 of HLA-E restricted CD8+ T cells, which is in the range of 1-2% of total 10x10^{12} CD8+ T cells.

The details on the rationale for the dosing regimen based on Clinical Trial (NCT00445913):
The product AVOTRES, autologous immature DC (iDC) loaded with self-peptide Hsp60sp, administered in our first-in-human safety study is, indeed, a first in human investigational product. We agree that the referenced trial is not the exact product, however represents the same class of immature dendritic cell product, and is the most similar investigational therapy that is currently in clinical testing. We therefore, are using this particular Clinical Trial (NCT00445913) as the basis for us to design the dosing regimen for AVOTRES for the following reasons:

a). Both products are autologous iDC treated, in vitro, with either antisense oligonucleotides (AS-ODN), or in our case self-peptide, Hsp60sp. Although, they are not exactly the same reagents and may work by different mechanisms, they are basically the same type of biomedical agents, which suggests that they are biologically and structurally very similar in their safety considerations.

b). Importantly, compared with iDC treated with AS-ODN in Clinical Trial (NCT00445913), AVOTRES even more closely mimics the natural form of iDC in vivo, because under certain circumstances iDC can present endogenous Hsp60sp. It could be a natural form of AVOTRES, except the conditions of how the process can be controlled is unknown and need to be thoroughly studied. AVOTRES is made under the condition that Hsp60sp was loaded at an excessive amount to iDC to make sure any other Qa-1/HLA-E binding peptides cannot compete with Hsp60sp. So, conceptually speaking, Avotres may be even safer than the product of iDC treated with AS-ODN in Clinical Trial (NCT00445913).

c). Both products were tried in preclinical studies in T1D/NOD mice model with almost identical dosing regimen and showed similar in vivo efficacy (Please see 8.2.2.2.2, Page172-175) (32,33).

d). In a dozen of DC-based therapies in anti-tumor clinical trials, the commonly used products are autologous DC loaded with different tumor antigen peptides with the dosing regimen in the range between 2-50 x 10^6 /person every 2 to 4 weeks for 2-13 times and showed no significant side effects, compared with which our dosing regimen of AVOTRES falls within the range (Please see 8.3.2, Page 177-178)

Table 1.7.1. Summary of dosage and route of administration of dendritic cell-base vaccines in completed human clinical trails, which have been concluded as safe therapies.

Therapy to	Dose/schedule	Route	No. Patients	Ref.
NHL (Non-Hodgkin's lymphoma)	2-17×10^6 / 4 weeks × 4	i.v.	35	(34)
	2-17×10^6 / 4 weeks × 4	i.v.	10	(31)
Melanoma	5-50×10^6 / 2 weeks × 4	i.v./i.d./i.n.	28	(35)
	5-50×10^6 / 2 weeks × 4	i.v.	14	(36)
	3×10^6+6/12×10^6 / 2 weeks × 5	i.d.+i.v.	13	(37)
Myeloma	0.5-11×10^6 / 4 weeks × 2	i.v.	12	(38)
	3.5-89×10^6 / 2 weeks × 3	i.v.	6	(39)
Prostate cancer	1-20×10^6 / 6-8 weeks × 4-5	i.v.	51	(40)
	10×10^6/ 2 weeks × 4	i.v.+i.d.	8	(41)
	0.3-40×10^6 / 4 weeks × 2	i.v./i.d./i.l.	21	(42)
	10×10^6/ 2 weeks × 3	i.v.+i.d.	13	(43)
	0.94-2.02×10^8/ 4 weeks × 3	i.v.	14	(44)
RCC (Renal cell carcinoma)	5-10×10^6 / 4 weeks × 3-13	i.v./i.d.	35	(45)
	10-50×10^6 +10×10^6 /2 weeks × 3	i.v.+i.d.	10	(46)
Type 1 Diabetes	10×10^6 / 2 weeks × 4	i.d.	10	(32)

Based on the information from our own studies and the published results from clinical trials on DC-based therapies we have chosen a relatively low dose and regimen of AVOTRES for safety consideration with a possible maximum efficacy in vivo in our proposal.

We choose to vaccinate newly diagnosed T1D patients that will be determined to have the defect of HLA-E restricted CD8+ T cells and the defect could be corrected by co-culture with AVOTRES in vitro.

2 Study Objectives

Our working model for maintenance of T cell self-tolerance holds that a subset of HLA-E restricted regulatory CD8+ T cells that specifically recognize the target structure of Hsp60sp associated with HLA-E expressed on the pathogenic self-reactive T cells and selectively down-regulate these pathogenic T cells

which are responsible for autoimmune diseases (19, 20, 22, 23). The failure of this HLA-E-restricted CD8+ T cell subset to recognize, or to suppress, its target population results in a permissive state in which organ-specific autoimmunity can emerge. We have identified defects in this CD8+ T cell system among nine of ten T1D subjects evaluated, and found the defect to be reversible in most of these *in vitro* by co-incubation with Hsp60sp-loaded autologous immature dendritic cells (AVOTRES).

2.1 Primary Objectives

- To determine the safety and to Tolerability of the AVOTRES reagent.
- To determine whether, among T1D patients with in vitro-reversible defects in the CD8+ T cell regulatory system, infusion of AVOTRES will lead to an in vivo reconstitution of this system.

2.2 Secondary Objectives

- To determine whether, among T1D patients with in vitro-reversible defects in the CD8+ T cell regulatory system, infusion of AVOTRES will result in amelioration of β cell destruction.

The justification for the study design derives from the primary objective of the proposed trial to assess the safety and efficacy of this first-in-man therapy. The design exposes only small numbers of patients separated by defined intervals. The built-in delays in enrollment avoid exposing multiple individuals simultaneously as potential toxicities are assessed in a step-by-step fashion. The cohort size is chosen to allow detection of high frequency severe adverse events at the dose given and also the possible efficacy by this vaccine.

2.3 General Study Design

Participants will be randomized to receive AVOTRES or placebo (saline) by intravenous infusion. Administration of AVOTRES will be performed in a double-blinded fashion as follows: the dosage regimen will be 1 x 10^7 cells/infusion for three infusions 30 (+/- 2) days apart. Each infusion will be administered by qualified clinical personnel in an established clinical research facility. Patients will be monitored for physical complaints as well as for heart rate, blood pressure, and arterial oxygen saturation and changes in physical exam throughout the infusion procedure and for a minimum of 2 hours following the procedure. Although unlikely to be required, the clinical facility will include at the patient's side all medications and equipment necessary to treat acute anaphylactoid/infusion reactions, respiratory distress, and cardiac arrhythmia.

The saline placebo group was selected for following reasons:

(a) AVOTRES was well characterized in our preclinical human in vitro studies and three animal models for in vivo studies, where several control groups for AVOTRES were tested, including autologous DC loaded with B7 (human) or Qdm (mouse), and DC from Qa-1 KO mice loaded with Hsp60sp or Qdm (Please see Section 8 for details). The animal studies were performed in inbred mice of sufficiently large size of the experiments with statistically significant differences. Thus, to repeat such types of controls in human clinical trials is unnecessary and extremely costly and would not provide any more useful information.

(b) It is well know that DC is the most potent antigen presenting cells to evoke a verity of immune responses under different circumstances, including presenting self-peptide/s generated endogenously by the DC itself. For example, it can present endogenous Hsp60sp in certain conditions such as under stress. It could be a natural form of AVOTRES, except the conditions of how

the process can be controlled is unknown and need to be thoroughly studied. AVOTRES is made under the condition that Hsp60sp was loaded at an excessive amount to make sure any other Qa-1/HLA-E biding peptide cannot compete with Hsp60sp. Thus, only DC vaccine cannot be considered as a proper control for AVOTRES as placebo group either.

(c) For above reasons, untreated subjects with only infusion of saline would be an ideal control as placebo group that can provide clean background of evaluation of efficacy for AVOTRES treatment.

2.4 Study Duration

The AVOTRES study product administration will be completed on three separate visits within 60 days of the screen visit. Following completion of the three infusion procedures, all study participants will return at 60-90 day intervals for 12 months then every 6 months through month 24 (see ClinicalTrials.gov identifier NCT00445913) (32) for repeat evaluation of the HLA-E-restricted CD8+ T cell regulatory activity ("potency assay") as described in Section 5.4.

All study participants will have a repeat clinical evaluation of diabetes at 1 month, 3 months, 6 months, 12 months, 18 months and 24 months, including MMTT stimulated C-peptide, glucose tolerance, glycosylated hemoglobin, and autoantibody levels.

All patients will be contacted by the Principal Investigator or authorized representative via clinic visit or telephone every 6 months after the last vaccine for up to four years to determine survival status and disease status.

2.5 Study Endpoints

Primary Study Endpoints

(a) Safety and tolerability of the AVOTRES reagent. The primary analysis will describe the incidence of adverse events and laboratory abnormalities.
(b) Tolerability of the AVOTRES reagent.
(c) Activity of the HLA-E-restricted CD8+ T cell regulatory function over a 1-year period following treatment with AVOTRES.

Secondary Study Endpoints

(a) The change in the area under the curve (AUC) of the stimulated C-peptide levels over the first 2 hours of a mixed meal tolerance test (MMTT) 3 months, 6 months, 12 months, 18 months, and 24 months
(b) Change in A1c
(c) Change in total daily insulin dose
(d) Number of major hypoglycemic events (defined as loss of consciousness, seizure, or requiring assistance from another person because of altered state of consciousness)
(e) Reported hypoglycemic events confirmed with capillary blood glucose measurement less than 70 mg/dl.
(f) Autoantibody levels

3 Subject Selection and Withdrawal

3.1 Inclusion Criteria

Patients who meet *all* of the following criteria are eligible for enrollment as study participants:

(a) Diagnosis of Type 1 Diabetes by one or more of the following types of antibodies: anti-GAD65 (glutamic acid decarboxylase), anti–islet-cell antibody 512 (ICA512), anti-IA2 and anti-insulin antibody.
(b) Age 18 or older and able to provide informed consent for participation.
(c) Must have stimulated C-peptide levels ≥0.2 pmol/ml measured during a mixed meal tolerance test (MMTT)
(d) Recent onset (diagnosis within the past 12 months) T1D patients who screen positive for a CD8+ T cell defect that is correctable *in vitro* by co-culture with immature dendritic cells loaded with self-peptide Hsp60sp.
(e) All subjects will be using insulin at the time of enrollment.
(f) If a participant is female with reproductive potential, she must be willing to avoid pregnancy and have a negative pregnancy test during the 12 months of treatment and for an additional 3 months.
(g) Signed and dated written informed consent

3.2 Exclusion Criteria

Patients who meet *any* of the following criteria are *not* eligible for enrollment as study participants:

(a) Uncontrolled diabetes (HbA1c >9.0%)
(b) Hemoglobin <10.0 g/dL; leukocytes <3,000/uL; neutrophils <1,500/uL; lymphocytes <800/uL; platelets <100,000/uL
(c) Urine Albumin Excretion < 300mg/gmCr
(d) eGFR > 60 mL/min/1.73m^2
(e) ALT/AST < 3x normal
(f) Current use of immunosuppressive or immunomodulatory therapies, including pharmacologic doses of systemic steroids
(g) Coincident medical condition likely to require immunosuppressive or immunomodulatory therapies
(h) Coincident medical condition likely to limit short term (5 year) life expectancy (malignancy, symptomatic coronary artery disease, recent stroke)
(i) Prior radiation therapy, immunotherapy, or chemotherapy
(j) Serologic evidence of current HIV-1 or HIV-2 infection
(k) Evidence of current hepatitis B as demonstrated by HBSAg or circulating hepatitis B genomes
(l) Serologic evidence of hepatitis C infection
(m) Autoimmune disease aside from T1D
(n) Pregnant or breastfeeding
(o) Any chronic illness which, in the opinion of the principal investigator, should preclude participation in the trial
(p) Adequate venous access to support leukapheresis

3.3 Subject Recruitment and Screening

Subjects will be recruited for this study from the clinical practices of the Columbia University Medical Center and its referral sources. Subjects will be required to give written informed consent to participate in the study before any screening tests or evaluations are conducted.

3.4 Early Withdrawal of Subjects

Criteria for Premature Termination from the Study

Subjects may withdraw from the study prior to the expected completion date for the following reasons:

- Unacceptable toxicity and other safety reasons
- Progression or disease relapse
- Subject consent withdrawal
- Decision by the investigators that withdrawing is in the patient's best interest
- Death

Standard supportive therapy will be maintained for subjects withdrawn from active treatment.

Data Collection and Follow-up of Withdrawn Patients

Every effort will be made to collect toxicity information on withdrawn patients. Patients withdrawn prematurely from the trial will be followed-up for survival and disease status by the principal investigator or clinical investigators via clinic visit, telephone contact, or medical records review every 6 months for 2 years. Other methods that will be used for follow-up will include contacting next of kin, contacting referring or primary physicians, use of certified letters to the patient, and social security index.

4 Study Drug

4.1 AVOTRES Vaccine (Investigational)

Drug name and active ingredients: The study drug represents an individualized preparation of autologous immature dendritic cells, derived by culture of the subject's adherent primary monocytes with GM-CSF and IL-4 for 6 days, and loaded passively with the synthetic oligopeptide Hsp60sp (QMRPVSRVL).

Formulation of dosage: AVOTRES will be cryopreserved in infusible cryomedia in KryoSure® 20 mL bags. Each infusion bag will contain 1 x 10^7 cells, and each patient will have 3 such bags manufactured for infusion. The cryopreserved cells will be manufactured at the Dana Farber Cancer Institute (DFCI) Cell Manipulation Core Facility (CMCF; DFCI-CMCF) and will be shipped to Columbia for infusion.

Route of administration: The infusions will be administered intravenously.

Frequency of administration: Three (3) infusions will be administered 30 (+/- 2) days apart.

4.2 Preparation and Administration of Study Drug.

One dose of AVOTRES will be packed into a 20ml freezing bag (KryoSure® Cryopreservation Bags) and store in liquid nitrogen or -120 to -140 º C freezer. The bag has the port to be connected to the infusion

system, 20 minutes before administration, the bag will be thawed at room temperature and directly connected to the infusion tube that contains saline to the patient. The speed of infusion should be slow, <1mL/minute.

4.3 Receiving, Storage, Dispensing and Return

Receipt of Study Product: Study Drug will be shipped to the following address:

Stem Cell Processing Lab
NewYork-Presbyterian Healthcare Medical Centers
622 W 168th Street
Harkness Pavilion, HP4-418,
New York, NY 10032

Upon receipt of the of the study treatment supplies, an inventory will be performed and a drug receipt log filled out and signed by the person accepting the shipment. It is important that the designated study staff counts and verifies that the shipment contains all the items noted in the shipment inventory. Any damaged or unusable study drug in a given shipment (active drug or comparator) will be documented in the study files. The investigator must notify study sponsor of any damaged or unusable study treatments that were supplied to the investigator's site.

Storage: The vialed study product will be kept frozen at the aforementioned Stem Cell Processing Lab until immediately before thaw, reconstitution, and administration.

Dispensing of Study Product: The study product will be listed under each patient's own unique identifier. The date, patient identifier, and quantity used for each study product. Regular study product reconciliation will be performed to document study product consumed and study product remaining. This reconciliation will be logged on the study product reconciliation form, and signed and dated by the study team.

4.4 Return or Destruction of Study Product

At the completion of the study, there will be a final reconciliation of study product shipped, consumed, and remaining. This reconciliation will be logged on the study product reconciliation form, signed and dated. Any discrepancies noted will be investigated, resolved, and documented prior to return or destruction of unused study product. Study product destroyed on site will be documented in the study files.

5 Study Procedures

Study procedures are listed below and can also be found in the Schedule of Events (Section 15). No study procedures will be performed prior to obtaining informed consent.

5.1 Informed Consent

The consent process will be conducted by qualified study personnel (the Trial or Study Coordinator and/or Investigator or other designee). All participants (or their legally acceptable representative) must

read, sign and date a consent form prior to participation in the study, and/or undergoing any study-specific procedures.

The informed consent form must be updated or revised whenever important new safety information is available, when indicated for a protocol amendment, and/or whenever any new information becomes available that may affect a patient's participation in the study.

5.2 Screening

The following evaluation will be performed prior to enrollment to establish patient eligibility. All tests will be required within 30 days prior to treatment:

- Medical history including prior treatment, current medicines, and drug allergies
- Physical exam including blood pressure, pulse, temperature, weight, and retinal exam
- Laboratory studies comprising CBC/differential, creatinine, AST, ALT, and total bilirubin; including creatinine clearance and albuminuria
- Evaluation of pancreatic β cell function by established clinical tests: mixed meal stimulated C peptide measurement, glucose tolerance, glycosylated hemoglobin(A1c), insulin and GAD65 auto-antibodies
- Evaluation of HLA-E-restricted CD8+ T cells ("potency assay"), as described in Section 5.4.
- Pregnancy test if applicable

The following evaluations will be performed within 30 days prior to treatment:

- Viral studies comprising HIV-1, HIV-2, HTLV-1/2, and HCV serology and HBV surface antigen, surface antibody, and core antibody

5.3 Leukapheresis

Within 30 days of screening assessments, those patients qualified for enrollment will undergo leukapheresis at the Columbia University's Apheresis Unit according to standard clinical procedures. The apheresed product will be delivered to Dana Farber Center Cancer Institute (DFCI), Cell Manipulation Core Facility (CMCF) for processing to produce AVOTRES.

5.4 Immunoassessment "Potency" Assay

15-20% (about 40 mL) of the apheresis product will be delivered to Hong Jiang's laboratory at Columbia University and used for immunoassessment research assays ("potency assay"). The potency assay will be performed every 60-90 days after vaccination to evaluate the efficacy of AVOTRES, in improving the immune function, by taking a 75 mL peripheral blood sample/time point.

5.5 Treatment Regimen

All patients will receive the same regimen.

- On day 1, the patient will receive the AVOTRES infusion.
- The interval between injections will be 28 days for total three times.
- The staggering period between individuals is 4 weeks. We will monitor the first 9 patients to make sure that at least 6 subjects in the treatment group will be observed for 4 weeks for acute and subacute adverse events. The information obtained will allow the clinical PI and Sponsor to determine if the time interval of 2 weeks or 4 weeks is sufficient, we will consult with the FDA to lengthen or shorten the 4-week observation of patient staggering period in the clinical trial.

- After each Infusion, patients will be observed for at least 2 hour including measurement of vital signs prior to discharge.
- In subsequent Infusions, patients may be discharged immediately following injection.

Infusions will be given at the Irving Center for Clinical of the Columbia University Medical Center.

5.6 Baseline Clinical Assessments

Evaluation of HLA-E-restricted CD8+ T cells, as described in Section 5.4, will be performed within 30 days of the first vaccination, prior to infusion. The potency assay is performed in seven (see Section 14, Schedule of Events) circumstances during the overall study. (1) At the time of screening of potential participants. Eligibility to participate is based on the demonstration of a failure of the HLA-E-restricted T cell regulatory population and the ability to reverse this failure by co-incubation with AVOTRES (hHsp60sp-loaded iDC.). (2) To verify the functional quality of the iDC manufactured by DFCI which constitute the AVOTRES treatment, prior to infusion. (3) To monitor the in vivo efficacy every 60-90 days after the treatment with AVOTRES

The following baseline evaluations will be performed only if more than 2 months time has passed from date of screen visit:

- Medical history and physical exam, including vital signs
- Laboratory comprising CBC/diff, TSH, and CMV serology.
- Immunologic study specimens
- C-peptide responses, insulin, glucose, HbA1c levels

5.7 AVOTRES Vaccinations

AVOTRES will be transported at 2-8^{o}C to the patient bedside. Product administration training will be provided to each site. The KryoSure® Cryopreservation bag has the port to be connected to the infusion system. Twenty minutes before administration, the bag will be thawed at room temperature and directly connected to the infusion tube that contains at least 200mL of saline. The speed of infusion should be slow, <1mL/minute for the AVOTRES mixed with saline.

5.8 Interim Evaluations and Vaccinations

At the time of each subsequent vaccination, patient evaluation will include:

- Interim Medical history, including medication review
- Limited Physical exam, including vital signs and retinal exam.
- Laboratory studies as noted in Section 14, Schedule of Events
- Immune studies
- Adverse event assessment

5.9 Study Completion and Evaluation

The following clinical evaluation will be performed within 30 days after the last vaccination:

- Medical history and physical exam, including vital signs and performance status
- C-peptide responses, insulin, glucose, HbA1c levels
- Laboratory studies as noted in Appendix A

5.10 Subject Compliance Monitoring

The protocol requires frequent patient visits to the clinic for treatment and monitoring during participation in this trial. In addition, patients are followed-up by telephone contact between visits.

5.11 Prior and Concomitant Therapy

Study participants will continue all regularly prescribed medications, including those for glycemic control. The introduction of immunosuppressive agents, however, will constitute grounds for withdrawal from the study.

5.12 Long-term follow-up

All patients will be contacted by the Principal Investigator or authorized representative via clinic visit or telephone every 6 months after the last vaccine for up to four years to determine survival status and disease status.

6 Statistical Plan

6.1 Sample Size Determination and Methods

Primary endpoint: To determine whether, among T1D patients with *in vitro*-reversible defects in the CD8+ T cell regulatory system, infusion of AVOTRES will lead to an *in vivo* reconstitution of this system. Analysis assumes that the proportion of subjects given placebo exhibiting reversal at 12 months will be 0 and that at least 60% of the subjects receiving AVOTRES will exhibit *in vivo* evidence of reversal of the defect. With a randomization of 2:1 subjects to Avotres or Placebo. N = 14 subjects randomized to Avotres and N = 7 subjects to placebo. There would be 94% power to detect this difference in proportions of 0.6 with a one sided $\alpha < 0.05$ using the Fisher's exact test. Allowing for up to 10% of subjects being unavailable for evaluation the total sample size is increased to N = 24.

Secondary endpoint: The secondary outcome of each participant is the area under the stimulated C-peptide curve (AUC) over the first 2 hours of a mixed meal glucose tolerance test conducted at the one-year visit. The AUC is computed using the trapezoidal rule that is a weighted sum of the C-peptide values over the 120 minutes. The weighted mean C-peptide is simply AUC/120 (pmol/mL). Let represent the C-peptide value for study subjects receiving AVOTRES and those receiving placebo, respectively. The secondary analysis will be conducted on the transformed C-peptide using the log function: log(C-peptide +1). This provides better normal distributional behavior of the random variable. The comparison will be based on a t-test of treatment effect in an ANCOVA model adjusting for gender, baseline age, and baseline log (C-peptide +1). There will 90% power to detect a threefold increase in stimulated C-peptide with a one sided $\alpha < 0.05$

A log rank test of the difference in the C-peptide failure hazard function between groups (C-peptide failure is defined as the first occurrence at which the 2 hour peak C-peptide < 0.2 pmol/ml during a MMTT). Also, a proportional hazards model will be used to compare treatment groups while adjusting for baseline level of C-peptide, gender, and baseline age.

Longitudinal analysis of HbA1c, Insulin dose (units/kg) and blood glucose by treatment group using normal variance-covariance structure.

Number and severity of adverse events.

Change in autoantibodies.

7 Risks and Discomforts

7.1 Venipuncture

Patients may experience slight discomfort at the site and rarely develop an ecchymosis or superficial thrombosis.

7.2 Leukapheresis

The most common adverse reactions from leukapheresis are paraesthesias, dizziness, mild chest tightness, or cough related to the reinfusion of autologous red blood cells treated with citrate and ecchymoses at the catheter sites.

Potential adverse reactions related to leukapheresis include:

- Exacerbation of symptoms from an underlying medical condition including migraine headache, asthma/emphysema, or cardiovascular disease.
- Infection at catheter sites
- Hypotension, hypertension, or bradycardia from citrate toxicity
- Allergic reactions
- Anemia, if red blood cells cannot be reinfused

7.3 Response to AVOTRES

The response to AVOTRES would be to activate HLA-E restricted CD8+ T cells in vivo, which would in turn selectively down-regulate any anti-self responses to control autoimmune diseases. In animal toxicology study conducted by Covance, the data showed no adverse effect. In addition, in general DC-based therapies are considered safe therapies.

8 Safety and Adverse Events

Patients will be evaluated for evidence of toxicity from 0-4 years.

8.1 Definitions

Unanticipated Problems Involving Risk to Subjects

Any incident, experience, or outcome that meets all of the following criteria:

- Unexpected in nature, severity, or frequency (i.e. not described in study-related documents such as the IRB-approved protocol or consent form, the investigators brochure, etc)
- Related or possibly related to participation in the research (i.e. possibly related means there is a reasonable possibility that the incident experience, or outcome may have been caused by the procedures involved in the research)
- Suggests that the research places subjects or others at greater risk of harm (including physical, psychological, economic, or social harm).

Adverse Event

An *adverse event* (AE) is any symptom, sign, illness or experience that develops or worsens in severity during the course of the study. Intercurrent illnesses or injuries should be regarded as adverse events. Abnormal results of diagnostic procedures are considered to be adverse events if the abnormality:

- results in study withdrawal
- is associated with a serious adverse event
- is associated with clinical signs or symptoms
- leads to additional treatment or to further diagnostic tests
- is considered by the investigator to be of clinical significance

Serious Adverse Event

Adverse events are classified as serious or non-serious. A *serious adverse event* is any AE that is:

- fatal
- life-threatening
- requires or prolongs hospital stay
- results in persistent or significant disability or incapacity
- a congenital anomaly or birth defect
- an important medical event

Important Medical Events

Important medical events are those that may not be immediately life threatening, but are clearly of major clinical significance. They may jeopardize the subject, and may require intervention to prevent one of the other serious outcomes noted above. For example, drug overdose or abuse, a seizure that did not result in in-patient hospitalization, or intensive treatment of bronchospasm in an emergency department would typically be considered serious.

All adverse events that do not meet any of the criteria for serious event should be regarded as *non-serious adverse events*.

Adverse Event Reporting Period

The study period during which adverse events must be reported is normally defined as the period from the initiation of any study procedures (Day -1) to the end of the study treatment follow-up. For this study, the study treatment follow-up is defined as 30 days following the last administration of study treatment.

Preexisting Condition

A preexisting condition is one that is present at the start of the study. A preexisting condition should be recorded as an adverse event if the frequency, intensity, or the character of the condition worsens during the study period.

General Physical Examination Findings

At screening, any clinically significant abnormality should be recorded as a preexisting condition. At the end of the study, any new clinically significant findings/abnormalities that meet the definition of an adverse event must also be recorded and documented as an adverse event.

Post-study Adverse Event

All unresolved adverse events should be followed by the investigator until the events are resolved, the subject is lost to follow-up, or the adverse event is otherwise explained. At the last scheduled visit, the investigator should instruct each subject to report any subsequent event(s) that the subject, or the

subject's personal physician, believes might reasonably be related to participation in this study. The investigator should notify the study sponsor of any death or adverse event occurring at any time after a subject has discontinued or terminated study participation that may reasonably be related to this study. The sponsor should also be notified if the investigator should become aware of the development of cancer or of a congenital anomaly in a subsequently conceived offspring of a subject that has participated in this study.

Abnormal Laboratory Values

A clinical laboratory abnormality should be documented as an adverse event if any one of the following conditions is met:

- The laboratory abnormality is not otherwise refuted by a repeat test to confirm the abnormality
- The abnormality suggests a disease and/or organ toxicity
- The abnormality is of a degree that requires active management; e.g. change of dose, discontinuation of the drug, more frequent follow-up assessments, further diagnostic investigation, etc.

Hospitalization, Prolonged Hospitalization or Surgery

Any adverse event that results in hospitalization or prolonged hospitalization should be documented and reported as a serious adverse event unless specifically instructed otherwise in this protocol. Any condition responsible for surgery should be documented as an adverse event if the condition meets the criteria for an adverse event.

Neither the condition, hospitalization, prolonged hospitalization, nor surgery are reported as an adverse event in the following circumstances:

- Hospitalization or prolonged hospitalization for diagnostic or elective surgical procedures for a preexisting condition. Surgery should *not* be reported as an outcome of an adverse event if the purpose of the surgery was elective or diagnostic and the outcome was uneventful.
- Hospitalization or prolonged hospitalization required to allow efficacy measurement for the study.
- Hospitalization or prolonged hospitalization for therapy of the target disease of the study, unless it is a worsening or increase in frequency of hospital admissions as judged by the clinical investigator.

8.2 Recording of Adverse Events

At each contact with the subject, the investigator must seek information on adverse events by specific questioning and, as appropriate, by examination. Information on all adverse events should be recorded immediately in the source document, and also in the appropriate adverse event module of the case report form (CRF). All clearly related signs, symptoms, and abnormal diagnostic procedures results should recorded in the source document, though should be grouped under one diagnosis.

All adverse events occurring during the study period must be recorded. The clinical course of each event should be followed until resolution, stabilization, or until it has been determined that the study treatment or participation is not the cause. Serious adverse events that are still ongoing at the end of the study period must be followed up to determine the final outcome. Any serious adverse event that occurs after the study period and is considered to be possibly related to the study treatment or study participation should be recorded and reported immediately.

8.3 Reporting of Serious Adverse Events

Study Sponsor Notification by Investigator

A serious adverse event must be reported to the study sponsor by telephone within 24 hours of the event. A Serious Adverse Event (SAE) form must be completed by the investigator and faxed to the study sponsor within 24 hours. The investigator will keep a copy of this SAE form on file at the study site. Report serious adverse events by phone, facsimile or email to:

Name:	Hong Jiang, MD, PhD
Address:	Columbia University Medical Center
	PH - 8 East, Ste. 101,
	622 West 168th St
	New York, NY 10032
Phone:	212-305-9984/201-944-3352
Fax:	212-305-6070
Email:	hj4@columbia.edu

At the time of the initial report, provide as much of the following information as possible:

- Study identifier
- Study Center
- Subject number
- A description of the event
- Date of onset
- Current status
- Whether study treatment was discontinued
- The reason why the event is classified as serious
- Investigator assessment of the association and expectedness between the event and study treatment

Within the following 48 hours, the investigator must provide further information on the serious adverse event in the form of a written narrative. This should include a copy of the completed Serious Adverse Event form, and any other diagnostic information that will assist the understanding of the event. Significant new information on ongoing serious adverse events should be provided promptly to the study sponsor

IRB Notification by Investigator

This section describes the requirements for safety reporting by investigators who are faculty, affiliated with a research site, or otherwise responsible for safety reporting to the Columbia IRB. The Columbia requires expedited reporting of those events related to study participation that are unforeseen and indicate that participants or others are at increased risk of harm. The Columbia IRB will not acknowledge safety reports or bulk adverse event submissions that do not meet the criteria outlined below. The Columbia IRB requires researchers to submit reports of the following problems WITHIN 10 WORKING DAYS FROM THE TIME THE INVESTIGATOR BECOMES AWARE OF THE EVENT:

Any adverse event (regardless of whether the event is serious or non-serious, on-site or off-site) that occurs any time during or after the research study, which in the opinion of the principal investigator is:

- UNEXPECTED AND RELATED
    - Unexpected (An event is "unexpected" when its specificity and severity are not accurately reflected in the protocol-related documents, such as the IRB-approved research protocol, any applicable investigator brochure, and the current IRB-approved informed consent document and other relevant sources of information, such as product labeling and package inserts.)
    - Related to the research procedures (An event is "related to the research procedures" if in the opinion of the principal investigator or sponsor, the event was more likely than not to be caused by the research procedures.)

8.4 Reporting Process

Unanticipated problems posing risks to subjects or others as noted above will be reported to the IRB using the form: "Unanticipated Problems Posing Risks to Subjects or Others Including Reportable Adverse Events" or as a written report of the event (including a description of the event with information regarding its fulfillment of the above criteria, follow-up/resolution and need for revision to consent form and/or other study documentation).

Copies of each report and documentation of IRB notification and receipt will be kept in the Clinical Investigator's study file.

8.5 Other Reportable Events

For clinical drug trials, the following events are also reportable to the IRB:

- Any adverse experience that, even without detailed analysis, represents a serious unexpected adverse event that is rare in the absence of drug exposure (such as agranulocytosis, hepatic necrosis, Stevens-Johnson syndrome).
- Any adverse event that would cause the sponsor to modify the investigators brochure, protocol or informed consent form, or would prompt other action by the IRB to assure protection of human subjects.
- Information that indicates a change to the risks or potential benefits of the research, in terms of severity or frequency. For example:
    - An interim analysis indicates that participants have a lower rate of response to treatment than initially expected.
    - Safety monitoring indicates that a particular side effect is more severe, or more frequent than initially expected.
    - A paper is published from another study that shows that an arm of your research study is of no therapeutic value.
- Change in FDA safety labeling or withdrawal from marketing of a drug, device, or biologic used in a research protocol.
- Breach of confidentiality
- Change to the protocol taken without prior IRB review to eliminate apparent immediate hazard to a research participant.
- Incarceration of a participant when the research was not previously approved under Subpart C and the investigator believes it is in the best interest of the subject to remain on the study.
- Complaint of a participant when the complaint indicates unexpected risks or the complaint cannot be resolved by the research team.

- Protocol violation (meaning an accidental or unintentional deviation from the IRB approved protocol) that in the opinion of the investigator placed one or more participants at increased risk, or affects the rights or welfare of subjects.

FDA Notification by Sponsor

The study sponsor is required to report certain study events in an expedited fashion to the FDA. These written notifications of adverse events are referred to as IND safety reports. The following describes the safety reporting requirements by timeline for reporting and associated type of event:

- ***Within 7 calendar days***

  Any study event that is:
  - associated with the use of the study drug
  - unexpected,
  - fatal or life-threatening, and

- ***Within 15 calendar days***

  Any study event that is:
  - associated with the use of the study drug,
  - unexpected, and
  - serious, but not fatal or life-threatening

    -or-
  - a previous adverse event that was not initially deemed reportable but is later found to fit the criteria for reporting (reporting within 15 calendar days from when event was deemed reportable).

  Any finding from tests in laboratory animals that:
  - suggest a significant risk for human subjects including reports of mutagenicity, teratogenicity, or carcinogenicity.

8.6 Additional reporting requirements

Sponsors are also required to identify in IND safety reports all previous reports concerning similar adverse events and to analyze the significance of the current event in light of the previous reports.

DSMB Notification

Investigators will report all SAEs to the DSMB within 10 business days regardless of expectedness or attribution. SAE will be input into the CRF with follow up email to DSMB.

All Unexpected deaths or deaths related to the study agent must be reported within 24 hours. All other deaths should be reported within 30 days in the CRF.

Item	Report to DSMB	Do Not Report to DSMB
Expected, related, grade 1, 2 , 3 or 4	X	
Expected, related grade 1, 2, 3 or 4. >60 days since last subject treated		X
Expected, unrelated grade 1, 2 , 3 or 4	X	
1, 2, 3 or 4. >690 days since last subject treated		X

Item	Report to DSMB	Do Not Report to DSMB
Unexpected , related grade 1, 2 , 3 or 4	X	
Unexpected, related grade 1, 2, 3 or 4 >60 days since last subject treated		X
Unexpected , unrelated grade 1, 2 , 3 or 4	X	
Unexpected, unrelated grade 1, 2, 3 or 4. >60 days since last subject treated		X
Expected, related death	X	
Expected, unrelated death	X	
Unexpected, related death	X	
Unexpected, unrelated death	X	

8.7 Protocol Exceptions/Deviations

Exception: A one time, **intentional** action or process that departs from the IRB and CTSRMC approved study protocol, intended for **one** occurrence. If the action disrupts the study progress, such that the study design or outcome (endpoints) may be compromised, or the action compromises the safety and welfare of study subjects, **advance** documented IRB and DSMB approval is required.

Deviation: A one time, **unintentional** action or process that departs from the IRB and DSMB approved study protocol, involving one incident and **identified retrospectively**, after the event occurred. If the impact on the protocol disrupts the study design, may affect the outcome (endpoints) or compromises the safety and welfare of the subjects, the deviation must be reported to the DSMB within 5 business days and the IRB within 10 business days.

Any departure from the protocol that meets the following criteria should be submitted to the Sponsor:

- May/can/have affects/affected subject safety.
- Violate eligibility
- Dose adjustment
- Stopping criteria
- Affect sample size (adding more subjects, decreasing number of subjects, changing the number of subject in a specific arm/cohort)

Other deviations should be explained in a memo to file (MTF; such as a subject missing a visit is not an issue unless a critical/important treatment or procedure was missed and must have been done at that specific time). The Principal Investigator should give the Sponsor at least 5 days advance notice of the deviation. Include the following information on the Sponsor supplied deviation form: Study identifier, description of deviation from protocol and necessity for deviation. Ensure all deviations are signed by the Principal Investigator (or co-investigator) and submitted to the Sponsor.

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The Protocol Monitor will submit the deviation to the Sponsor for review and approval. The approved deviation will be submitted by the Investigator to the IRB, DSMB as required.

8.8 Stopping Rules

Patients will be clinically evaluated for evidence of toxicity from ≥ grade 3 toxicity as determined by CTC, which is unmanageable, unexpected and unrelated, and attributable to protocol therapy. Termination of enrollment would be considered with 2 DLTs in the first 6 evaluable subjects and 3 in the first 10 evaluable subjects. With 10 evaluable patients enrolled, there is at least 90% power for detecting any previously unexpected toxicity whose prevalence in this population is at least 21%.

In the event of a DLT for a given patient, doses will be discontinued, the patient clinically evaluated, and taken off study. The study will be stopped for DLT resulting in death of a patient.

8.9 Medical Monitoring

It is the responsibility of the Principal Investigators to oversee the safety of the study at his site. This safety monitoring will include careful assessment and appropriate reporting of adverse events as noted above, as well as the construction and implementation of a site data and safety-monitoring plan. Medical monitoring will include a regular assessment of the number and type of serious adverse events.

8.10 Internal Data and Safety Monitoring Committee

The Data Safety and Monitoring Board (DSMB) will monitor the data quality and adherence to safety rules. Additionally, the DSMB will review all safety/toxicity data for the trial and recommend trial suspension or termination as needed. Specific details of monitoring and audit frequency will be included in the Monitoring Plan (see Appendix A) but will be at least every 6 months.

9 Data Handling and Record Keeping

9.1 Confidentiality

Information about study subjects will be kept confidential and managed according to the requirements of the Health Insurance Portability and Accountability Act (HIPAA). Those regulations require a signed subject authorization (see Appendix C) informing the subject of the following:

- What protected health information (PHI) will be collected from subjects in this study
- Who will have access to that information and why
- Who will use or disclose that information
- The rights of a research subject to revoke their authorization for use of their PHI.

In the event that a subject revokes authorization to collect or use PHI, the investigator, by regulation, retains the ability to use all information collected prior to the revocation of subject authorization. For subjects that have revoked authorization to collect or use PHI, attempts should be made to obtain permission to collect at least vital status (i.e. that the subject is alive) at the end of their scheduled study period.

9.2 Source Documents

Source data is all information, original records of clinical findings, observations, or other activities in a clinical trial necessary for the reconstruction and evaluation of the trial. Source data are contained in source documents Examples of these original documents, and data records include: hospital records, clinical and office charts, laboratory notes, memoranda, subjects' diaries or evaluation checklists, pharmacy dispensing records, recorded data from automated instruments, copies or transcriptions certified after verification as being accurate and complete, microfiches, photographic negatives, microfilm or magnetic media, x-rays, subject files, and records kept at the pharmacy, at the laboratories, and at medico-technical departments involved in the clinical trial.

9.3 Case Report Forms

The study case report form (CRF) is the primary data collection instrument for the study. All data requested on the CRF must be recorded. All missing data must be explained. If a space on the CRF is left blank because the procedure was not done or the question was not asked, write "N/D". If the item is not applicable to the individual case, write "N/A". All entries should be printed legibly in black ink. If any entry error has been made, to correct such an error, draw a single straight line through the incorrect entry and enter the correct data above it. All such changes must be initialed and dated. DO NOT ERASE OR WHITE OUT ERRORS. For clarification of illegible or uncertain entries, print the clarification above the item, then initial and date it.

9.4 Records Retention

It is the investigator's responsibility to retain study essential documents for at least 2 years after the last approval of a marketing application in their country and until there are no pending or contemplated marketing applications in their country or at least 2 years have elapsed since the formal discontinuation of clinical development of the investigational product. These documents should be retained for a longer period if required by an agreement with the sponsor. In such an instance, it is the responsibility of the sponsor to inform the investigator/institution as to when these documents no longer need to be retained.

10 Study Monitoring, Auditing, and Inspecting

10.1 Study Monitoring Plan

This study will be monitored according to the monitoring plan in Appendix A. The investigator will allocate adequate time for such monitoring activities. The Investigator will also ensure that the monitor or other compliance or quality assurance reviewer is given access to all the above noted study-related documents and study related facilities (e.g. pharmacy, diagnostic laboratory, etc.), and has adequate space to conduct the monitoring visit.

10.2 Auditing and Inspecting

The investigator will permit study-related monitoring, audits, and inspections by the EC/IRB, the sponsor, government regulatory bodies, and University compliance and quality assurance groups of all study related documents (e.g. source documents, regulatory documents, data collection instruments, study data etc.). The investigator will ensure the capability for inspections of applicable study-related facilities (e.g. pharmacy, diagnostic laboratory, etc.).

Participation as an investigator in this study implies acceptance of potential inspection by government regulatory authorities and applicable University compliance and quality assurance offices.

11 Ethical Considerations

This study is to be conducted according to US and international standards of Good Clinical Practice (FDA Title 21 part 312 and International Conference on Harmonization guidelines), applicable government regulations and Institutional research policies and procedures.

This protocol and any amendments will be submitted to a properly constituted independent Ethics Committee (EC) or Institutional Review Board (IRB), in agreement with local legal prescriptions, for formal approval of the study conduct. The decision of the EC/IRB concerning the conduct of the study will be made in writing to the investigator and a copy of this decision will be provided to the sponsor before commencement of this study. The investigator should provide a list of EC/IRB members and their affiliate to the sponsor.

All subjects for this study will be provided a consent form describing this study and providing sufficient information for subjects to make an informed decision about their participation in this study. See Appendix 4 for a copy of the Subject Informed Consent Form. This consent form will be submitted with the protocol for review and approval by the EC/IRB for the study. The formal consent of a subject, using the EC/IRB-approved consent form, must be obtained before that subject is submitted to any study procedure. This consent form must be signed by the subject or legally acceptable surrogate, and the investigator-designated research professional obtaining the consent.

12 Study Finances

12.1 Funding Source

This trial is funded by China Institute of Strategy and Management Lanmeng Investment Co., Ltd (CISM Lanmeng Investment Co., Ltd) ” in Beijing, China.

12.2 Conflict of Interest

The regulatory sponsor of this study (Dr. Hong Jiang), who is the person who reports to the Food and Drug Administration and to the University about the status and results of the study, invented the AVOTRES technology. She has not received money for her invention related to this technology; however, there is a potential to do so in the future. The AVOTRES technology has been licensed to a for-profit company. As a result, these investigators have benefited and it is anticipated that they will continue to benefit financially. In addition, the Columbia University has a financial interest in AVOTRES. As a result of the relationship with a commercial entity, Columbia University is expected to benefit financially in the future.

12.3 Subject Stipends or Payments

Patients will not be paid for participating in this clinical trial. However, assistance may be provided with parking and transportation and the costs of laboratory tests.

13 Publication Plan

Neither the complete nor any part of the results of the study carried out under this protocol, nor any of the information provided by the sponsor for the purposes of performing the study, will be published or passed on to any third party without the consent of the study sponsor. Any investigator involved with this study is obligated to provide the sponsor with complete test results and all data derived from the study.

14 Schedule of Events

VISIT NAME	SCREEN	APH	VISIT 1	VISIT 2	VISIT 3	VISIT 4	VISIT 5	VISIT 6	VISIT 8	VISIT 9	VISIT 10
PROCEDURES	**SCREEN**	**APH**	**VAC#1**	**VAC#2**	**VAC#3**	**F/U 1**	**F/U 2**	**F/U 3**	**F/U 4**	**F/U 5**	**F/U 6**
STUDY DAY	***-60***	***-30***	***0***	***30***	***60***	***90***	***120***	***180***	***365***	***---***	***---***
STUDY MONTH	***-1***	***0***	***---***	***1***	***2***	***3***	***4***	***6***	***12***	***18***	***24***
STUDY YEAR	***---***	***---***	***---***	***---***	***---***	***---***	***---***	***---***	***1***	***---***	***2***
History[1]	X					X					
Physical Exam[2]	X					X			X		X
Pancreatic β cell Function[3]	X		X	X	X	X		X	X	X	X
Laboratory Studies[4]	X		X	X	X	X			X		X
Adverse Event Assessment	X		X	X	X	X					
Viral Studies[5]	X										
Survival and Disease Status[6]											
Serum Pregnancy	X										
Leukapheresis		X									
Potency Assay[7]	X	X				X		X	X	X	X

VISIT NAME	VISIT 11	VISIT 12	VISIT 13	VISIT 14
PROCEDURES	**LTFU 1**	**LTFU 2**	**LTFU 3**	**LTFU 4**
STUDY DAY	***---***	***---***	***---***	***---***
STUDY MONTH	***30***	***36***	***42***	***48***
STUDY YEAR	***---***	***3***	***---***	***4***
History[1]				
Physical Exam[2]		X		X

Pancreatic β cell Function[3]		X		X
Laboratory Studies[4]		X		X
Adverse Event Assessment				
Viral Studies[5]				
Survival and Disease Status[6]	X	X	X	X
Serum Pregnancy				
Leukapheresis				
Potency Assay[7]				

1. Medical history including prior treatment, current medicines, and drug allergies
2. Physical exam including blood pressure, pulse, temperature, weight, and retinal exam
3. MMTT stimulated C-peptide, glucose tolerance, glycosylated hemoglobin (A1c), insulin, and GAD65 autoantibody levels
4. CBC/differential, creatinine, AST, ALT, and total bilirubin; including creatinine clearance and albuminuria
5. HIV-1, HIV-2, HTLV-1/2, and HCV serology and HBV surface antigen, surface antibody, and core antibody
6. Can be conducted either in the office or via telephone interview
7. Blood draw (60-75 mL; 7-8 BD Vacutainer Sodium Heparin (NH), 143 USP UNITS blood collection tube (10ml.16x100mm, REF 367874)) and sent to Jiang Lab

*** If patient is off study, end of study evaluations within 30 days from last vaccination

15 Appendices

15.1 Appendix A: Monitoring Plan

15.2 Appendix B: Data Safety Monitoring Board Charter

15.3 Appendix C: Combination Informed Consent and HIPPA Form

16 References

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DATA AND SAFETY MONITORING PLAN

A Phase I/II, Double-Blind, Placebo Controlled, Parallel Design of AVOTRES Cell-Based Therapy as a Treatment for Human Type 1 Diabetes

Principal Investigator:
Daniel S. Donovan, Jr., MD, MS, CDE

Sponsor:
Hong Jiang, MD, PhD
Columbia University

July 15, 2013

Table of Contents

1. Purpose and Assessment of Risk

The monitoring of a clinical trial is necessary to ensure the protection of the subject's rights, the safety of subjects enrolled in the trial and the integrity and quality of the resulting data on an on-going basis for the study duration. This monitoring plan details the Case Report Form (CRF) and source data verification of efficacy and safety parameters, the frequency of monitoring visits and reporting, regulatory document review, drug accountability and compliance review, and stopping rules for the study. This is a single site clinical trial with subjects being recruited at Columbia University Medical Center. Dr Hong Jiang the Regulatory Sponsor for this trial, and will be responsible for the conduct of the trial at the site in accordance with FDA requirements.

Monitoring will be conducted according to Good Clinical Practices, applicable federal regulations and the applicable Columbia University Standard Operating Procedures.

There is also a Data Safety and Monitoring Board (DSMB) which will monitor and audit the trial for compliance at the site. The Regulatory sponsor will also appoint an individual monitor for regulatory oversight of the entire trial, as per FDA guidelines for INDs.

2. Glossary of Terms

Adverse Event (AE)
Any untoward medical occurrence in a subject or clinical investigation subject administered a pharmaceutical product and that does not necessarily have a causal relationship with this treatment. An adverse event (AE) can therefore be any unfavorable and unintended sign (including an abnormal laboratory finding), symptom, or disease temporally associated with the use of a medicinal (investigational) product, whether or not related to the medicinal (investigational) product. (ICH)

Case Report Form (CRF)
A printed, optical, or electronic document designed to record all of the protocol-required information to be reported to the sponsor on each trial subject. (ICH)

Data Safety Monitoring Board (Monitoring Committee, Data Monitoring Committee)
A Data Safety Monitoring Board that will be established by the sponsor to assess at intervals the progress of a clinical trial, the safety data, and the critical efficacy endpoints, and to recommend to the sponsor whether to continue, modify, or stop a trial. (ICH)

Informed Consent
A **process** by which a subject voluntarily confirms his or her willingness to participate in a particular trial, after having been informed of all aspects of the trial that are relevant to the subject's decision to participate. Informed consent is documented by means of a written, signed, and dated informed consent form. (ICH) Under 21 CFR 50.20, no informed consent may include any "language through which the subject or the representative is made to waive or appear to waive any of the subject's

legal rights or releases or appears to release the investigator, the sponsor, the institution, or its agents from liability for negligence."

Monitoring
The act of overseeing the progress of a clinical trial, and of ensuring that it is conducted, recorded, and reported in accordance with the protocol, standard operating procedures (SOPs), Good Clinical Practice (GCP), and the applicable regulatory requirement(s). (ICH)

Monitoring Report
A written report from the monitor to the sponsor after each site visit and/or other trial-related communication according to the sponsor's SOPs. (ICH)

Serious Adverse Event (SAE)
Any untoward medical occurrence that at any dose: results in death, is life-threatening, requires subject hospitalization or prolongation of existing hospitalization, results in persistent or significant disability/incapacity, or is a congenital anomaly/birth defect. (ICH)

Source Data
All information, original records and certified copies of original records of clinical findings, observations, or other activities in a clinical trial necessary for the reconstruction and evaluation of the trial. Source data are contained in source documents (original records of certified copies). (ICH)

Source Documents
Original documents, data, and records (e.g., hospital records, clinical and office charts, laboratory notes, memoranda, subjects' diaries or evaluation checklists, pharmacy dispensing records, recorded data from automated instruments, copies or transcriptions certified after verification as being accurate and complete, microfiches, photographic negatives, microfilm or magnetic media, x-rays, subject files, and records kept at the pharmacy, at the laboratories, and at medico-technical departments involved in the clinical trial). (ICH)

3. Protocol Summary

Type 1 diabetes (T1D) is a complex, chronic, T cell-mediated autoimmune disease in which the insulin producing β cells are destroyed. In T1D, there is a loss of tolerance to pancreatic β cells that results in a CD4 and CD8 T cell-dependent autoimmune process that culminates in complete destruction of insulin-producing β cells, leading to insulin deficiency and dysregulated glucose metabolism. Diabetic patients manage their hyperglycemia by daily insulin injections, however this treatment does not cure the disease or prevent the possibility of the disease's serious effects, which may include kidney failure, blindness, nerve damage, heart attack, stroke, and pregnancy complications (JDRF website). It is important then that other therapies to treat T1D be identified and tested. As discussed below, immunotherapeutic approaches that effectively prevent and or treat T1D have been moving into the clinic recently. Over the years, studies using the non-obese diabetic (NOD) mouse model have provided important insights into the immunology of T1D and have demonstrated the feasibility of preventing or curing T1D in an antigen-specific manner by specifically manipulating disease-relevant autoreactive T cell populations without compromising the immune system at large. Avotres, represents one such approach to manipulate autoreactive T-cell

responses against beta-cell antigens, that we would like to test under this new IND. Modulating the activity of self-reactive CD8+ T cells to damper the activation level may be one approach to treat T1D.

Our working model for maintenance of T cell self-tolerance holds that a subset of HLA-E restricted regulatory CD8+ T cells that specifically recognize the target structure of hHsp60sp associated with HLA-E expressed on the pathogenic self-reactive T cells and selectively down-regulate these pathogenic T cells which are responsible for autoimmune diseases. The failure of this HLA-E-restricted CD8+ T cell subset to recognize, or to suppress, its target population results in a permissive state in which organ-specific autoimmunity can emerge. We have identified defects in this CD8+ T cell system among nine of ten T1D subjects evaluated, and found the defect to be reversible in most of these *in vitro* by co-incubation with hHsp60sp-loaded autologous immature dendritic cells (AVOTRES).

4. Monitor Selection and Training

The sponsor will designate a qualified individual monitor for regulatory oversight of the entire trial, as per FDA guidelines for INDs. One monitor will be assigned for this trial and will be responsible to complete the monitoring process. The monitor will be selected by the sponsor based on experience and qualifications. The monitor will be qualified both by education and understanding of Good Clinical Practice requirements as will as regulatory compliance.

A CV for the monitor will be obtained and updated annually. The CV will be kept on file in the Sponsor section of the Regulatory Binder to document the qualifications of the monitor.

Prior to study initiation, the monitor will be trained on the protocol and case report forms.

5. Monitoring Process

5.1 Frequency

Monitoring will be conducted on the first three subjects enrolled. The frequency of monitoring additional subjects will be determined after the review of the first three subjects. The Principal Investigator or a member of the study staff must inform the monitor when a subject is enrolled.

5.2 Monitoring Visits

Informed consent, enrollment logs, regulatory documentation, subject source data, and case report forms will be reviewed 100% for all subjects. This will be conducted in accordance with applicable regulations and University policies

The monitor will perform 100% drug accountability in an ongoing manner at the time of each monitoring visit. The monitor will confirm the receipt, use and return or disposal of drug.

5.3 Anticipated Adverse Events

Side effects of the investigational agent is not completely known. However, based on previous clinical studies of vaccines in T1D patients, vaccines are well tolerated with little to no aide-effects.

Adverse effects associated with the blood draws include: (common, greater than 10%) local pain or swelling; (uncommon, 1-10%) vein damage or irritation/phlebitis, excessive bleeding or bruising, feeling faint, infections.

5.4 Monitoring Reports

Monitoring reports will be provided to the Regulatory Sponsor within two weeks of completion of monitoring visit. The Principal Investigator will take action as required.

5.5 Reporting of AEs/SAEs

The Sponsor will act as the central reporting entity to the FDA. The PI will be responsible for contacting the Sponsor within 24 hours of an SAE, and completing both a SAE form and a MedWatch Form documenting the event within the following 48 hours. Copies of these forms will be kept on file at Columbia University. The Sponsor will submit to the FDA a 7-day report for any unexpected fatal or life-threatening experience associated with use of the investigational agents or a 15 day report for AEs that are serious and unexpected associated with the use of the investigational agent under Dr. Jiang's IND. On an annual basis, a summary of all AEs will be provided to the FDA.

5.6 Corrective action

If non-compliance is determined, the PI will be notified in writing. This notification will include a statement of the corrective action that is required to resolve the situation and the consequences to be taken or contemplated should the action not be implemented. The PI will have 10 business days to respond to the notification, at which time, no additional subjects can be enrolled.

6. Data Management

The PI and Research Coordinator will be responsible for CRFs. Clinical and laboratory data will be entered directly into the CRF by the Research Coordinator. The monitor will perform data queries and ensure database corrections.

CRFs are maintained and stored according to GCP. The clinical records are stored according to policies and procedures established Columbia University.

7. Drug Accountability

Sponsor and Investigator will maintain drug accountability in accordance with General Responsibilities of Sponsors (21 CFR 312.50) and Investigators (21 CFR 312.60). The monitor will perform 100% drug accountability in an ongoing manner at the time of each monitoring visit. The monitor will confirm the receipt, use and return or disposal of drug.

8. Close Out Visit

The monitor will conduct the Close Out Visit within 1 month after the last subject has completed the study.

The following activities will be completed by the monitor to close out the study:

- Ensure all data has been reviewed and collected;
- Ensure all outstanding queries are answered;
- Confirm all Serious Adverse Events, MedWatch Reports and IND Safety Reports, if applicable, have been reported to the IRB(s), Sponsor and/or FDA;
- Review the Regulatory documentation and Subject Files for completeness and compliance with GCP and all applicable federal regulations;
- Ensure initial and revised 1572 forms were submitted to the IRB (if applicable);
- Ensure all protocol violations were submitted to the IRB;
- Ensure that all continuing review reports were submitted to the IRB;
- Perform drug accountability;
- Review requirements for record retention with the investigator and the clinical staff.

A copy of the report will be sent to the Regulatory Sponsor.

A Phase I/II, Double-Blind, Placebo Controlled, Parallel Design of AVOTRES Cell-Based Therapy as a Treatment for Human Type 1 Diabetes

Principal Investigators: Daniel S. Donovan Jr.

This Data and Safety Monitoring Board (DSMB) Charter describes the Board responsibilities, membership, meeting processes, reports and reporting process. The DSMB will act in an advisory capacity to the study sponsor (Hong Jiang, MD, PhD.) and Columbia University Medical Center, to monitor patient safety of the intervention of the above-noted study. Dr. Daniel Donovan is the investigator conducting the above-noted clinical trial.

Reports will be provided to the DSMB by the investigative staff.

DSMB RESPONSIBILTIES

The initial responsibility of the DSMB will be to review the research protocol, informed consent documents and plans for data safety and monitoring, and to approve the initiation of this clinical trial if all the above are satisfactory. After this approval, and at periodic intervals (described below) during the course of the trial, the DSMB responsibilities are to:

- evaluate the progress of the trial, including periodic assessments of data quality and timeliness, participant recruitment, accrual and retention, participant risk versus benefit, aspects of trial conduct, and other factors that can affect study outcome;
- consider factors external to the study when relevant information becomes available, such as scientific or therapeutic developments that may have an impact on the safety of the participants or the scientific value or ethics of the trial;
- ensure that the safety of the study participants is not being compromised;
- make recommendations to the sponsor, the PI, the Safety Advisor, the institution, and Institutional Review Board (IRB) concerning continuation, termination or other modifications of the trial based on the observed beneficial or adverse effects of the treatment under study
- consider whether the data exceed any early stopping boundaries which have been clearly defined in advance of data analysis;
- maintain the confidentiality of the trial data and the results of monitoring; and,
- assist the sponsor by making recommendations regarding any problems with study conduct, enrollment, and sample size and/or data collection.

MEMBERSHIP

- The DSMB will consist of five voting members. The DSMB voting members have no financial, scientific, or other conflict of interest with the trial. The DSMB coordinator is [TBD] and is employed by [TBD]. The role of the DSMB coordinator is to schedule and coordinate the meetings, assure a quorum is constituted and provide minutes of the meeting. The members have been selected by Dr. Jiang, the study sponsor. The DSMB includes members with expertise

in the clinical area under study and/or clinical trial methodology. All voting members will provide written documentation regarding absence of any disqualifying conflicts of interest.

[TBD] has been selected by the sponsor to serve as the Chairperson. S/he is responsible for overseeing the meetings and developing the agenda in consultation with the non-voting coordinating member and the PI. [TBD] will serve as the liaison between the study sponsor and investigator, and the DSMB, and will provide the logistical management for and support of the DSMB.

Contact Information

Name	Role	Contact Information
Hong Jiang, MD, PhD	Sponsor	Columbia University Medical Center PH - 8 East, Ste. 101, 622 West 168th St - New York, NY 10032 Phone: 212-305-9984/201-944-3352 Fax: 212-305-6070 Email: hj4@columbia.edu
Daniel Donovan, MD, MS	Principal Investigator	Columbia University Medical Center 161 Fort Washington Ave. Room 2-212 Phone: 212-305-0504 Email: dsd8@columbia.edu
TBD	Clinical Trials Coordinator	TBD
TBD	DSMB Coordinator	TBD
TBD	Chair, and Voting DSMB Member	TBD
TBD	Voting DSMB Member	TBD
TBD	Voting DSMB Member	TBD
TBD	Voting DSMB Member	TBD
TBD	Voting DSMB Member	TBD
Rajasekhar Ramakrishnan, Eng.Sc.D.	Study Statistician	Director, Biomathematics Division Department of Pediatrics Address: 601 West 168 Street #54 New York NY 10032 Phone: 212 305 7989 Fax: 212 305 5834 Email: rr6@columbia.edu

BOARD PROCESS

The first meeting of the DSMB will be devoted to a discussion of the protocol and associated documents. The DSMB will consider whether any modifications of the trial would be desirable, discuss guidelines for monitoring the study, and discuss, finalize and approve the DSMB charter. The Sponsor (or designated representative), will prepare the agenda to address the review of the manual of operating procedures,

modification of the study design, initiation of the trial, monitoring roles, reporting of adverse events, stopping rules, and interim analysis plan.

The DSMB meeting schedule will be based on safety events and study milestones. The DSMB will have an initial meeting prior to study initiation. Subsequent meetings may occur via teleconference after the first three participants complete his/her three vaccinations. If necessary, additional meetings of the Board may be held if there are safety issues between scheduled meetings. Meetings will also be held at a minimum of every six months. Meetings may be closed at the committee's request. The initial and subsequent meetings may occur either via teleconference or face-to-face.

The DSMB will convene at any time during the study in the event of a protocol specific, study related serious adverse event that is considered to be possibly, likely or definitely related to treatment.

Meetings shall be closed to the public because discussions may address confidential patient data. The principal investigator and members of his staff may attend portions of the meeting as appropriate, but the DSMB may conduct some sessions without presence of the sponsor or study personnel.

MEETING FORMAT AND FREQUENCY

An appropriate format for DSMB meetings consists of an open and a closed session. The open sessions may be attended by the principal investigator(s), and institution staff. Issues discussed at open sessions will include conduct and progress of the study, including patient accrual, compliance with protocol, and problems encountered. Closed sessions will be attended only by voting members and will include discussion of safety data. The DSMB will vote on the continuation or termination of the study during the closed session.

At any time, the committee may request an executive closed session.

A minimum of four (4) voting members of the DSMB will be required to determine continuance or discontinuance of the study. In the event of a split vote (to be defined by the DSMB), study enrollment will be interrupted but participants already enrolled and dosed will continue to be closely followed. The sponsor will immediately notify OHR, and the IRB with copies to the FDA. In such a case, the sponsor, and FDA will need to decide the final disposition of the study

The DSMB will provide advice to the investigator, and will consult with Sponsor as necessary. The initial responsibility of the DSMB will be to approve the initiation of this clinical trial. At later meetings, the DSMB will evaluate patient-subject safety and vote on continuation of the study at the following study milestones:

- After the first three participants receive AVOTRES and complete the three vaccinations; or after 3 months, whichever is first.
- After the second set of three participants receive AVOTRES and complete the three vaccinations, including follow-up data from previous participants; or after three months, whichever is first.
- At a minimum of every 6 months thereafter.

If necessary, additional meeting of the DSMB may be held if safety issues arise between scheduled meetings. The DSMB's findings and recommendations regarding patient safety and continuation or termination of the trial will be shared with the Sponsor, Columbia University IRB, and FDA.

PROTOCOL STOPPING RULES (same for all protocols)
The study will be stopped if:

- Premature study termination may occur if the Investigator, Study Funder, Sponsor, DSMB or any independent review board or regulatory body decides for any reason that subject safety may be compromised by continuing the study.
- Premature study termination may occur if the Sponsor or Study Funder decides to discontinue the development of the intervention to be used in this study.

The protocol will also be stopped if a third or more of the patients have ≥ grade 3 toxicity as determined by CTC, which is unmanageable, unexpected and unrelated, and attributable to protocol therapy. Depending on the nature of the toxicities, the protocol may be amended to lower the AVOTRES dose or make other modifications that would limit toxicities observed. Dose limiting toxicity (DLT) is defined as a ≥ grade 3 toxicity as determined by CTC, which is unmanageable, unexpected and unrelated to chemotherapy and attributable to protocol therapy.

The safety assessment will be made after completion of the first three subjects enrolled and again after the second three (subjects 4-6) subjects are enrolled. In the event of DLT in the first 6 subjects, the study will continue or be stopped in accordance with the following:

1. The study will continue if < 2 out of the first 3 patients experience DLT; the study will stop if 2 or more in the first 3 patients experience DLT. Based on this rule, if 1 of 3 subjects experiences a DLT, the 95% CI for the true toxicity rate is 0.8% to 91%. The probability to observed 1 of 3 subjects experiences a DLT is <44% if the true DLT rate is >33%, and

2. The study will continue if <3 out of the first 6 patients experience DLT; the study will stop if 3 or more in the first six patients experience a DLT. Based on this rule, if 2 of 6 subjects experience a DLT, the 95% CI for the true toxicity rate is 4% to 78%. The probability to observed 2 of 6 subjects experiences a DLT is <33% if the true DLT rate is >33%.

If the study continues enrollment after 6 subjects (i.e. DLT is observed in fewer than 3 of 6 subjects), the study will be stopped in the event that a total of three subjects in the study have experienced a DLT.

The study will be paused if:

- The protocol will be paused pending review by IRB, FDA and the DSMB if any patient experiences any of the following events within two weeks of the CART-19 infusion
    - respiratory failure requiring mechanical ventilation;
    - any adverse event, exclusive of elective surgical procedures, requiring admission to an intensive care unit for management;
    - life-threatening (grade 4) toxicity attributable to protocol therapy; or
    - death.

REPORTS

1. **Immediate response**
   In the event of a decision to close the study, the Principal Investigator, study sponsor, and IRB will be immediately notified. FDA will be subsequently notified in writing as soon as possible.

2. **Serious Adverse Event Reporting**
   In the event of a Serious Adverse Event as defined in the protocol Section 10 that is considered to be possibly, likely or definitely related to treatment, the following process will occur. The principal investigator will notify the IRB and the study sponsor. The research coordinator/nurse will fax a completed Serious Adverse Event form to the Sponsor. The IRB will be notified via RASCAL. A phone call and/or e-mail will also be sent to the Sponsor. The sponsor will notify the FDA. The DSMB Coordinator will notify the DSMB and convene an immediate teleconference with the chair and board. The outcome of the DSMB review and discussion of the SAE will be provided to the sponsor who will notify the FDA, and will assure that the IRB is notified of the outcome.

3. **Interim Reports:** Interim reports will be prepared and distributed to the DSMB at least 10 working days prior to the scheduled meeting if at all possible. These interim reports are numbered and provided by email unless otherwise instructed by the DSMB Chair. The format and content of the report will be proposed by the sponsor and may be modified by request of the DSMB.

Proposed data to be included in the report are as follows:

- **Summary of Previous Meeting**
  - Provided by DSMB Coordinator
- **Executive Summary**
  - Enrollment and important findings as determined by Sponsor and PI
- **Demographics, Study Status and Manufacturing Summary** (Includes data on all enrolled subjects and any available data on screened but not enrolled subjects)
  - Enrollment (total screened, total enrolled, pending enrollment, total active)
  - Demographics by subject, no totals or ranges
  - Overview of subjects enrolled (last visit, date of visit, infusion date, all protocol deviations, AEs including laboratory AEs [yes/no])
  - Cell manufacturing and release by subject All Protocol Deviations by subject (approved by sponsor)
- **Data Summaries** (The following tables include data from enrolled subjects only)
  - Overview and table/figure of potency assay
  - Overview and table/figure of patient response
  - Summary of Adverse Events (includes laboratory toxicities)
    - Table of adverse events by body system and severity
    - Table of adverse events by body system and relatedness
    - Listings of adverse events by subject (includes AE term, severity and relationship)
  - Table of other concomitant medications by patient with start/stop dates

4. **Reports from the DSMB:**
   As previously stated, the formal DSMB report will conclude with a recommendation to continue or to terminate or pause the study. A termination or pause recommendation may be made by the DSMB at any time. The DSMB Chair is responsible for notifying the sponsor, and the PI if the decision is to terminate or pause. In the event of a split vote in favor of continuation, a report

will be sent to the IRB with copies to the FDA. In such a case, the sponsor and FDA will need to decide the final disposition of the study.

5. A formal report containing the meeting minutes and any recommendations for continuation or modifications of the study will be prepared by the DSMB Coordinator in consultation with the DSMB Chairperson. As noted above, sponsor will have communicated immediate meeting outcome to required entities. This report will be sent to the full DSMB within 2 weeks of the meeting. Once approved by the DSMB, the OHR representative will forward the formal DSMB recommendation report to the sponsor and PI within 3 weeks of each meeting. It is the responsibility of the sponsor and PI to distribute the formal DSMB recommendation report to the UPENN IRB.

CONFIDENTIALITY

All materials, discussions and proceedings of the DSMB are completely confidential. Members and other participants in DSMB meetings are expected to maintain confidentiality.

COLUMBIA UNIVERSITY RESEARCH SUBJECT INFORMED CONSENT AND HIPAA AUTHORIZATION FORM

Protocol Title: A Phase I/II Double-Blind, Placebo Controlled, Parallel Design of AVOTRES Cell-Based Therapy as a Treatment for Human Type 1 Diabetes

Principal Investigator:
Daniel S. Donovan, Jr., MD, MS, CDE
Columbia University Medical Center
161 Fort Washington Ave. Room 2-212
New York, NY 10032
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Name: Leonard Chess MD
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Email: lc19@columbia.edu

Why am I being asked to volunteer?

You are being invited to participate in a research study. Your participation is voluntary which means you can choose whether or not you want to participate. If you choose not to participate, there will be no loss of benefits to which you are otherwise entitled. Before you can make your decision, you will need to know what the study is about, the possible risks and benefits of being in this study, and what you will have to do in this study. The research team is going to talk to you about the research study, and they will give you this consent form to read. You may also decide to discuss it with your family, friends, or family doctor. You may find some of the medical language difficult to understand. Please ask the study doctor and/or the research team about this form. If you decide to participate, you will be asked to sign this form.

What is the purpose of this research study?

We are doing this research study to find out if AVOTRES works as a vaccine and can correct Type 1 diabetes.

AVOTRES is an investigational drug. This means that the drug has not been approved by the Food and Drug Administration (FDA) for medical use in patients, but has only been approved for use in research.

You are being asked to take part in this study because you have type 1 diabetes. There are 21 people who will be enrolled in this study.

How long will I be in the study?

This study takes a total of four (4) years to finish. You will need to schedule a visit with the study physician to see if you can be on this study. The study outlines special circumstances, called Inclusion and Exclusion Criteria, that help the doctor determine if you can be on the study. In order for the doctor to determine if you can be on the study, the doctor will need your consent to draw blood and conduct a physical and retinal exam.

If you can be included in this study, then you will need to come to the doctor's office many times over the next 6 months. After 6 months, the visits slow down. After two years, you can speak to your doctor (or another person from the study team) over the telephone.

What am I being asked to do?

You are being asked to participate in a study which will see if the AVOTRES vaccine can reverse type 1 diabetes.

Fourteen (14) patients will receive the vaccine. Seven (7) patients will receive saline (type of water) only. This is called randomization, where the patient is randomly given AVOTRES or saline. You will not know which you are getting.

What are the possible risks or discomforts?

WE do not know of any risks associated with AVOTRES. Other studies that looked at something similar to AVOTRES had no complications.

You will have a procedure done called APHERESIS, where your blood is taken from one arm, filtered, and then put back in your other arm at the same time. The most common side effects are dizziness, mild chest tightness, or cough related to the reinfusion of your blood.

Reproductive risks: Because of the effects of this drug/device, there could be harm to unborn children or children who are breast-feeding. These effects could also harm the mother. It is also possible that harmful side effects that are not yet known could happen to both the mother and unborn or breast-feeding child. If you are currently pregnant, it is important that you inform the investigator because you will not be able participate in the study. If you are able to become pregnant, you will be given a serum pregnancy test before entry into the study. You are asked to use a medically accepted method of birth control while you participate in the study. You should not become pregnant while you are taking AVOTRES. If you do become pregnant, you must tell the investigator and consult an obstetrician or maternal-fetal specialist.

What if new information becomes available about the study?

During the course of this study, we may find more information that could be important to you. This includes information that, once learned, might cause you to change your mind about being in the study. We will notify you as soon as possible if such information becomes available.

What are the possible benefits of the study?

This is the first time that AVOTRES is being tested in human beings. However, because this is the first time AVOTRES is being tested in humans, there is a chance you may not get any benefit from being in this research study.

What other choices do I have if I do not participate?

You may investigate other ongoing studies and you can discuss alternatives with your physician.

Will I be paid for being in this study?

You will not be paid for being in this study.

Will I have to pay for anything?

You are still responsible for any deductibles or applicable co-pays for routine office visits, scans and blood work. Please talk to your doctor and study team about putting you in touch with a financial counselor to determine exactly what the deductible and co-pay will be for you; this is highly variable depending on your type of insurance.

What happens if I am injured from being in the study?

We will offer you the care needed to treat injuries directly resulting from taking part in this research. We may bill your insurance company or other third parties, if appropriate, for the costs of the care you get for the injury, but you may also be responsible for some of them.

There are no plans for the Columbia University to pay you or give you other compensation for the injury. You do not give up your legal rights by signing this form.

If you think you have been injured as a result of taking part in this research study, tell the person in charge of the research study as soon as possible. The researcher's name and phone number are listed in the consent form.

When is the Study over? Can I leave the Study before it ends?

This study is expected to end after all participants have completed all visits, and all information has been collected. This study may also be stopped at any time by your physician, the study Sponsor, or the Food and Drug Administration (FDA) without your consent because:

- The Primary Investigator feels it is necessary for your health or safety. Such an action would not require your consent, but you will be informed if such a decision is made and the reason for this decision.
- You have not followed study instructions.
- The Sponsor, the study Principal Investigator, or the Food and Drug Administration (FDA) has decided to stop the study.

If you decide to participate, you are free to leave the study at anytime. Withdrawal will not interfere with your future care.

What information about me may be collected, used or shared with others?

You will be assigned a unique number that tells the physician and the study team who you are, but no one else will know who you are.

Why is my information being used?

Your information is used by the research team to contact you during the study. Your information and results of tests and procedures are used to:

- do the research
- oversee the research
- to see if the research was done right.

Who may use and share information about me?

The following individuals may use or share your information for this research study:

- The investigator for the study and the study team
- Other authorized personnel at Columbia University

Who, outside of Columbia University, might receive my information?

- Those working under the direction of the investigator for the study, (e.g. under subcontracts).
- The funding sponsor and organizations supporting the sponsor

Oversight organizations:

- The Food and Drug Administration
- The Office of Human Research Protections
- The study data and safety monitoring board

The Principal Investigator or study staff will inform you if there are any additions to the list above during your active participation in the trial. Any additions will be subject to Columbia University procedures developed to protect your privacy.

How long may Columbia University use or disclose my personal health information?

Your authorization for use of your personal health information for this specific study does not expire.

Your information may be held in a research database. However, the School of Medicine may not re-use or re-disclose information collected in this study for a purpose other than this study unless:

- You have given written authorization
- The Columbia University's Institutional Review Board grants permission
- As permitted by law

Can I change my mind about giving permission for use of my information?

Yes. You may withdraw or take away your permission to use and disclose your health information at any time. You do this by sending written notice to the investigator for the study. If you withdraw your permission, you will not be able to stay in this study.

What if I decide not to give permission to use and give out my health information?

Then you will not be able to be in this research study.

You will be given a copy of this Research Subject HIPAA Authorization describing your confidentiality and privacy rights for this study.

By signing this document you are permitting the School of Medicine to use and disclose personal health information collected about you for research purposes as described above.

Who can see or use my information? How will my personal information be protected?

We will do our best to make sure that the personal information obtained during the course of this research study will be kept private. However, we cannot guarantee total privacy. Your personal information may be given out if required by law. If information from this study is published or presented at scientific meetings, your name and other personal information will not be used. If this study is being overseen by the Food and Drug Administration (FDA), they may review your research records.

Who can I call with questions, complaints or if I'm concerned about my rights as a research subject?

If you have questions, concerns or complaints regarding your participation in this research study or if you have any questions about your rights as a research subject, you should speak with the Principal Investigator listed on page one of this form.

When you sign this form, you are agreeing to take part in this research study. This means that you have read the consent form, your questions have been answered, and you have decided to volunteer. Your signature also means that you are permitting the Columbia University to use your personal health information collected about you for research purposes within our institution. You are also allowing the Columbia University to disclose that personal health information to outside organizations or people involved with the operations of this study.

A copy of this consent form will be given to you.

_____	_____	
Name of Subject (Please Print)	Signature of Subject	Date

_____	_____	
Name of Person Obtaining Consent (Please Print)	Signature	Date

Investigational Product:
AVT001 Suspension for Intravenous Infusion
Hsp60sp-loaded Autologous Immature Dendritic Cells
($7x10^6$-$10x10^6$ cells/dose)

Study Title:

A Phase 1 / 2 Double-Blind, Randomized, Placebo Controlled Study of Safety, Tolerability and Potential Efficacy of AVOTRES Cell-Based Therapy (AVT001) in Patients with Type 1 Diabetes

Sponsor: Avotres, Inc.
140 East Hanover Avenue
Cedar Knolls, NJ 07927
Phone: 973-487-6972

Protocol Number: AVT001-T1D-01

Initial protocol version: July 15, 2013
Amendment 1: February 14, 2019
Amendment 2: January 22, 2020
Amendment 3: February 1, 2021

1 Table of Contents

2 List of Abbreviations

Abbreviation	Definition
A1c	Hemoglobin A1c
APC	Antigen Presenting Cells
ATG	Anti-thymocyte Globulin
CBC	Complete Blood Count
CD4, CD8	Cluster of Differentiation
CFR	Code of Federal Regulations
CMP	Complete Metabolic Panel
CRF	Case Report Form
CTCAE	Common Terminology Criteria for Adverse Events
CTL	Cytotoxic T Cell
DC	Dendritic Cell
DFCI-CMCF	Dana Farber Cancer Institute, Cell Manipulation Core Facility
DIFF	Differential
DMSO	Dimethyl sulfoxide
DMK	Dystrophia Myotonica Kinase
DLT	Dose Limiting Toxicity
DSMB	Data Safety Monitoring Board
GAD	Glutamic Acid Decarboxylase
GCP	Good Clinical Practice
GFAP	Glial Fibrillary Acidic Protein
GM-CSF	Granulocyte Macrophage-Colony Stimulating Factor
HbA1c	Hemoglobin A1c
HBsAg	Hepatitis B Virus Surface Antigen
HBV	Hepatitis B Virus
HCV	Hepatitis C Virus
HIV	Human Immunodeficiency Virus
HLA	Human Leukocyte Antigen
Hsp60sp	Heat Shock Protein 60 Signal Peptide
ICA	Islet Cell Antibody
iDC	Immature Dendritic Cell
IFNγ	Interferon γ
IGRP	Islet-Specific Glucose-6-Phosphate Catalytic Subunit Related Protein

IL	Interleukin
IP	Investigational Product
i.v.	Intravenous
mcg	Micrograms
MHC	Major Histocompatability Complex
MMRM	Mixed model for repeated measures
MMTT	Mixed Meal Tolerance Test
MOP	Manual of Operating Procedures
MRI	Magnetic Resonance Image
mRNA	Messanger Ribonucleic Acid
NOD	Non-Obese Diabetic (murine model)
ppIAPP	Pre-Pre-Islet Amyloid Polypeptide Protein
Qa-1	Murine MHC class I molecule
RNA	ribonucleic acid
SAP	Statistical analysis plan
s.c.	Subcutaneous
T1D	Type 1 diabetes
TNFα	Tumour Necrosis Factor α
Treg cells	Regulatory T Cells

3 Synopsis

Name of Sponsor/Company:
Avotres, Inc.

Name of Investigational Product (IP):
AVT001 suspension for intravenous infusion (AVT001)

Name of Active Ingredient:
The study drug represents an individualized preparation of autologous immature dendritic cells, derived by culture from the subject's adherent primary monocytes with GM-CSF and IL-4 for 6 days, and loaded passively with a synthetic oligopeptide from Hsp60sp.

Title of Study:
A Phase 1 / 2 Double-Blind, Randomized, Placebo Controlled Study of Safety, Tolerability and Potential Efficacy of AVOTRES Cell-Based Therapy (AVT001) in Patients with Type 1 diabetes

Study center(s): This study is planned as a single-center study, at the Joslin Diabetes Center. However, an additional center may be added as enrollment and accrual needs dictate.

Principal Investigator:
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Studied period (years):	**Phase of development:**
Duration of the study is approximately 3 years, from enrollment of the first subject until last subject completes 2 years of follow up.	Phase 1 / 2

Objectives:

Primary Objectives:

- To determine the safety and tolerability profile of three doses of AVT001 (as compared to placebo) in T1D subjects with *in vitro*-reversible defects in the CD8+ T cell regulatory system

Secondary Objectives

- To determine whether, among T1D subjects with *in vitro*-reversible defects in the CD8+ T cell regulatory system, infusion of AVT001 will lead to an *in vivo* reconstitution of this system.
- To determine whether, among T1D subjects with *in vitro*-reversible defects in the CD8+ T cell regulatory system, infusion of AVT001 will result in β cell preservation assessed by stimulated C-peptide.

Methodology:
This is a double-blind, randomized, placebo-controlled study to evaluate the safety and tolerability of AVT001, and to assess AVT001 as a potential treatment for type 1 diabetes (T1D). The trial will involve approximately 24 new-onset T1D subjects who have been identified as having: (a) a defect in

HLA-E-restricted CD8+ T cell function associated with pancreatic β cell destruction; and (b) evidence that this defect of HLA-E-restricted CD8+ T cells can be corrected by *in vitro* exposure to the proprietary AVT001 procedure.

Each subject will be randomized to one of two treatment groups:

- AVT001: 16 subjects to receive AVT001 through i.v. administration.
- Placebo control: 8 subjects to receive placebo infusion solution only through i.v. administration.

Subjects will be treated with 3 consecutive treatments, administered with one month (+/-7 days) intervals between doses. Infusions must be at least 21 days apart. The primary time point for assessment and statistical analysis will be 3 months post-last dose (Month 5), with longer-term follow-up through 22 months post-last dose (Month 24).

This is a first-in-man study, and therefore, exposure will be managed using a staggered enrollment that will allow for review of each subject's outcomes following the initial i.v. adminstration. This staggered enrollment and dosing will be in-place for the first 6 subjects treated. The staggering will be performed as follows:

- The second subject will be treated (dosed) only after the first subject has completed the Month 1 safety visit and the Investigator and Medical Monitor have reviewed those data.
- Treatment (dosing) of subsequent subjects will be managed in a similar fashion, with subjects treated at a rate of one subject per month, with Investigator and Medical Monitor review of the Month 1 safety and tolerability outcomes for each subject before treatment of the subsequent subject is performed.

This will continue through the 6th subject. Assuming an acceptable safety and tolerability profile is observed, enrollment of the remaining subjects will be determined by the site's accrual rate.

Data Safety Monitoring Board (DSMB)

A DSMB will be established to monitor subject safety. The DSMB will initially meet after 3 subjects and then after 6 subjects and then again after 15 subjects have received at least one dose of double-blind study medication and have completed their in-clinic visit through at least one month post-dose. The DSMB will review *unblinded* data to assess the safety and tolerability of the treatments given. The DSMB will, following each of these meetings, provide the Sponsor with a summary of their findings and recommendations (if any) for protocol mediation/amendment.

The DSMB Charter will describe the full scope of DSMB activities, roles and responsibilities.

Number of subjects (planned):

Up to approximately 24 subjects will be randomized (in a 2:1 ratio, AVT001:Placebo), and thus up to approximately 16 subjects will be treated with AVT001 and 8 subjects will be treated with placebo.

Diagnosis and main criteria for inclusion:

Inclusion Criteria

Subjects who meet *all* of the following criteria are eligible for enrollment as study participants:

1. Diagnosis of type 1 diabetes, within 12 months of first dosing, confirmed by positive lab result for one or more of the following types of autoantibodies:
   - Glutamic acid decarboxylase (GAD65)
   - Insulinoma associated protein 2 (IA-2, also known as ICA-512)
   - Zinc transporter 8 (ZnT8)

2. Age 16 or older and able to provide informed consent/assent.
3. Has a CD8+ T cell defect that is correctable *in vitro* by co-culture with immature dendritic cells loaded with a synthetic oligopeptide from Hsp60sp.
4. If a participant is female with reproductive potential, willing to avoid pregnancy through the duration of the trial.
5. Signed and dated written informed consent/assent.

Exclusion Criteria

Subjects who meet *any* of the following criteria are *not* eligible for enrollment as study participants:

1. Poorly controlled diabetes despite insulin therapy, who in the opinion of the investigator would not be a good candidate for participation in a clinical trial.
2. Screening hemoglobin <10.0 g/dL; leukocytes <3,000/uL; neutrophils <1,500/uL; lymphocytes <800/uL; platelets <100,000/uL
3. Screening Urine Albumin Excretion > 300mg/gmCr
4. Screening eGFR < 60 mL/min/1.73m^2
5. Screening ALT or AST > 1.5x upper limit of normal (ULN)
6. Screening bilirubin > 2.0 mg / dL, or > 3.0 mg / dL for participants with Gilbert's Syndrome
7. Current use of immunosuppressive or immunomodulatory therapies, including pharmacologic doses of systemic steroids (topical steroidal creams and inhaled steroids without large systemic absorption are allowed).
8. Coincident medical condition likely to require immunosuppressive or immunomodulatory therapies
9. Coincident medical condition likely to limit short term (5 year) life expectancy (malignancy, symptomatic coronary artery disease, recent stroke)
10. Prior radiation therapy, immunotherapy (within 1 year of screening), or chemotherapy
11. Serologic evidence of current HIV-1or HIV-2 infection
12. Serologic evidence of hepatitis C infection
13. Serologic evidence of acute or chronic active hepatitis B as measured by Core Ab positive and / or Surface Antibody antigen positive
14. Subjects with other autoimmune conditions (except compensated or treated autoimmune thyroid, celiac, alopecia, or vitiligo diseases)
15. Women who are pregnant (pregnancy testing during screening), breastfeeding, or planning pregnancy during the study period.
16. Inadequate venous access to support leukapheresis
17. Any condition that in the opinion of the investigator would preclude the subject from participating in a clinical trial.
18. Abnormal screening ECG that in the opinion of the investigator or sponsor would pose a safety risk

Investigational product, dosage and mode of administration:

Formulation of dosage: AVT001 will be cryopreserved in infusible cryomedia in cryopreservation 20 mL bags. Each infusion bag will contain between 7 x 10^6 and 10 x 10^6 cells, and each subject randomized to AVT001 will have 3 such bags manufactured for infusion. The cryopreserved cells will be manufactured at the Dana Farber Cancer Institute Cell Manipulation Core Facility (DFCI-CMCF) and will be transported to the site for infusion.

Route of administration: The infusions will be administered intravenously.

Frequency of administration: Three (3) infusions will be administered approximately 30 (+/- 7) days apart. Infusions must be at least 21 days apart.

Duration of treatment:

There are three pre-defined periods in this study.

- Screening and cell collection period will last up to 3 months.
- Treatment period will consist of 3 doses, each ~30 days apart (Baseline, Month 1, Month 2) and a 1-month post-last dose assessment period (through Month 3). Thus, the treatment period is defined to be 3 months in duration.
- Post-Treatment Follow-up Period will extend approximately 21 additional months (and thus through Month 24 of the study).

Reference therapy, dosage and mode of administration:

Matching placebo will have similiar intravenous infusion

Criteria for evaluation:

Safety (Primary Endpoint):

Consistent with the study objectives, the primary endpoints will focus on the safety and tolerability of AVT001 versus placebo.

The primary endpoints are:

(a) The incidence of treatment-emergent adverse events (TEAEs).
(b) Clinically significant changes from baseline in clinical laboratory parameters
(c) The incidence and severity of local i.v.-site reactions
(d) Changes from baseline in vital signs and electrocardiograms
(e) Incidence of severe hypoglycemic events

Efficacy (Secondary/Exploratory Endpoints):

Secondary objectives focus on the CD8+ T-cell regulatory system and therapeutic outcomes associated with T1D. These pharmacodynamic effects of AVT001 will be assessed in an exploratory manner over the course of the study period.

Secondary endpoints will include:

(a) Assessment of the HLA-E-restricted CD8+ T cell regulatory activity ("potency assay")
(b) Changes from baseline in the area under the curve (AUC) of the stimulated C-peptide levels over a 4-hour mixed meal tolerance test (MMTT)
(c) Changes from baseline in HbA1c
(d) Change from baseline in insulin usage
(e) Changes from baseline in autoantibody levels

Statistical methods:

Sample Size

The primary objective of this study is to assess the safety and tolerability of AVT001, as compared to placebo, in subjects with type 1 diabetes. A sample size of 14 subjects treated with AVT001 will provide at least 80% power to observe at least one occurrence of treatment-emergent adverse events with a true underlying incidence of 12%. The sample size provides the same power to detect at least one occurrence of local tolerability events relating to the i.v. administration of study drug.

Secondary objectives of this study include assessment of changes in pharmacodynamic markers, including increases of the function in the CD8+ T-cell regulatory system and improvements of C-peptide levels, and HbA1c. The sample size of 14 subjects treated with AVT001 and 7 subjects treated with placebo will provide estimates of the mean and standard deviation of the treatment benefit of AVT001 as compared to placebo in these endpoints.

To account for an estimated 10% potential dropout prior to the completion of the Month 5 safety/efficacy assessments, a total sample size of approximately 24 subjects will be randomized, in a 2:1 ratio of AVT001:Placebo.

Analysis Timing

The primary analysis for this study will be performed after the last subject has completed the Month 5 study visit, at which time the study database will be locked and the treatment allocation codes unblinded for analysis. This analysis will form the primary basis for the assessment of the study objectives.

Continuing data collection through the long-term follow-up will be performed through Month 24. While Investigators and subjects will remain blinded to the treatment allocations, assessment and analysis of outcomes will be performed by the Sponsor in an on-going manner, to better understand the longer-term effects (on both safety and efficacy) of the therapy.

Safety Assessment

Adverse events will be reported using the MedDRA coding dictionary. Tabulations will include an overall incidence of at least one adverse event, incidence within body system, and incidence by preferred term. Each subject may only contribute once (ie, first occurrence) to each of the incidence rates, regardless of the number of occurrences. The incidence of adverse events will be presented as follows:

- The incidence of treatment-emergent adverse events (TEAEs), ie, those events that start on or after the first dose of study medication, will be tabulated by treatment for the Treatment Phase. This will include events starting within 30 days following the last dose.
- The incidence of post-treatment events (those events that start more than 30 days following the last dose) will be tabulated.

Subjects with serious adverse events (including deaths) and subjects who discontinue due to adverse events will be listed. Subjects who have other significant serious adverse events deemed to be of special interest because of clinical importance will also be listed.

Laboratory, vital signs, and ECG outcomes will be assessed for changes over time as well as clinically significant shifts from pre-treatment. The incidence of severe hypoglycemic events will be tabulated.

Efficacy Assessment

Changes over time in C-peptide levels, HbA1C, total daily insulin dose and antibody values will be assessed. For purposes of data presentation, a mixed model for repeated measures (MMRM) will be used to present changes in these parameters over time. Details regarding this MMRM will be provided in the SAP. The SAP will also detail the HLA-E restricted CD8+ regulatory T cell function analysis.

4 Introduction

This document is a protocol for a human research study to be conducted according to US and international standards of Good Clinical Practice (FDA Title 21 part 312 and International Conference on Harmonization guidelines), applicable government regulations and Institutional research policies and procedures.

4.1 Background

Type 1 diabetes (T1D) is one of the most common chronic diseases of childhood. It accounts for approximately two-thirds of all cases of diabetes in subjects ≤19 years of age. According to statistics reported by the Juvenile Diabetes Research Foundation (JDRF), it is estimated that as many as 3.0 million subjects have T1DM in the United States, with more than 15,000 children and 15,000 adults diagnosed each year.

T1D is a complex, chronic, T cell-mediated autoimmune disease in which the insulin producing pancreatic β cells are destroyed. In T1D, there is a loss of tolerance to β cells that results in a CD4 and CD8 T cell- dependent autoimmune process that culminates in complete destruction of insulin-producing β cells, leading to insulin deficiency and dysregulated glucose metabolism. Diabetic subjects manage their hyperglycemia by daily insulin injections, however this treatment does not cure the disease or prevent the possibility of the disease's serious effects, which may include kidney failure, blindness, nerve damage, heart attack, stroke, and pregnancy complications (JDRF website). It is important to develop other therapies able to target the primary defect of the immune function that causes T1D. As discussed below, immunotherapeutic approaches that could potentially treat and or prevent T1D have been moving into the clinic recently. Over the years, studies using the non-obese diabetic (NOD) mouse model have provided important insights into the immunology of T1D and have demonstrated the feasibility of preventing or curing T1D in an antigen-specific manner by specifically manipulating disease-relevant autoreactive T cell populations without compromising the immune system at large.

Conceptually speaking, selective control of unwanted immune responses could be achieved by induction of "antigen-specific tolerance", provided that the specific antigens that elicit the unwanted immune responses have been identified. However, for decades, it has always been a tremendous challenge to identify the exact pathogenic peptide/s for any given human organ specific autoimmune diseases, or specific allo-antigen peptide/s responsible for graft rejection. In reality, no definitive answers have ever been obtained for any human autoimmune diseases yet. Furthermore, induction of "antigen specific tolerance" requires precise knowledge of the peptide associated with particular MHC molecules and highly polymorphic human HLA system inevitably increases the uncertainty of searching for such MHC/peptide complex in each subject. Thus, the necessity of identification of a precise MHC/peptide complex for each individual from an unknown and countless antigen specificity pool makes the induction of "antigen-specific tolerance" an unfeasible approach to specifically and effectively treat organ specific autoimmune diseases and/or control graft rejection in clinical immunology. In contrast, Qa-1/HLA-E restricted CD8+ T cells, provide a simple and unified "class action" of self-nonself discrimination, regulating the unwanted immune responses, independent of the knowledge of the particular pathogenic peptides in autoimmune diseases and/or graft rejection for each subject, currently largely undetermined. (1, 2). Avotres has developed a novel approach to manipulate autoreactive T-cell responses against all self antigens, including β-cell antigens (1, 2), that we would like to evaluate in this clinical protocol. Modulating the activity of self-reactive T cells to damper autoimmunity at a system level without damaging normal on-going anti-infection and anti-tumor immunity represents an ideal new

4.2 Failure of self-nonself discrimination by the immune system results in pathogenesis of autotimmune disease such as T1D

The pioneering work of Burnet and Medawar clearly indicated that self-nonself discrimination is not achieved by recognizing the structural differences between self antigens and foreign antigens, because introducing a foreign allo transplantation antigen into the immune system early enough in life renders the recipient animals tolerant to that foreign antigen which is subsequently treated as if it were a self antigen (3, 4). It is well known now that thymic negative selection that deletes high avidity T cells specific to any antigen, self-antigens as natural cases, is the major mechanism of the specific tolerance established (5-8). However, as a biological consequence of natural thymic negative selection, self-reactive T cells with intermediate and low avidity escape into the periphery (9-11). The intermediate avidity self-reactive T cell pool in the periphery represent a potential danger of pathogenic autoimmunity (1, 12), which could be accidentally activated by a sufficiently high level of self-antigens to initiate organ-specific autoimmune diseases during infections or injuries (1, 11, 13-15) and must be effectively controlled. We have thus postulated that self-nonself discrimination must continue in the periphery after the thymic negative selection to maintain self-tolerance (1, 12). In this regard, it has been shown that Qa-1/HLA-E restricted CD8+ T cell mediated regulatory pathway is important in maintaining peripheral selftolerance (16-24). We have further proposed and tested an "Avidity Model Of Peripheral T Cell Regulation", in which Qa-1/HLA-E restricted CD8+ T cells, by selectively down-regulating the intermediate avidity T cell pool, play a central role in control of organ specific autoimmune diseases in the conventional soluble protein antigen system in both mice (19, 20, 22) and humans (23). The peripheral self-reactive T cell repertoire is devoid of high avidity T cells due to thymic negative selection. Therefore, selective down-regulation of the intermediate avidity T cell pool enables the selective suppression on the overall immune responses to self-antigens, mediated by the intermediate avidity T cells, but not on effective immune responses to foreign antigens dominated by the high avidity T cells. This way, the Qa-1/HLA-E restricted CD8+ T cells are able to specifically control organ-specific autoimmune diseases or establish donor specific transplantation tolerance without damaging ongoing effective anti-infection and anti-tumor immunity (2, 19, 20, 22, 23, 25). The selective down-regulation of the intermediate avidity T cell pool by the Qa-1/HLA-E restricted CD8+ T cells is accomplished by a specific recognition of the unique common target structure, the signal peptide of heat shock protein 60 (Hsp60sp) associated with the non-polymorphic MHC Class 1b molecule Qa- 1/HLA-E, preferentially expressed on the intermediate avidity T cells (19, 20, 22, 23).

More relevant, we have shown a defect in the T cell regulatory pathway which normally controls autoreactive T cells that attack the body's own tissues and organs (23). A majority of people with Type 1 diabetes who were tested in the study were found to have this newly-identified cellular/molecular defect on HLA-E restricted CD8+ T cells, and we were able to successfully correct the defect in-vitro in most of the subjects tested by retriggering the CD8+ T cells with Avotres *in vitro* made of the autologous DC loaded with the synthetic oligopeptide from Hsp60sp (23).

Our preclinical human *in vitro* studies and murine *in vivo* studies formed the scientific basis for this IND application for trying AVT001 *in vivo.*

4.3 The Autoimmune Process of Type 1 diabetes

Development of autoimmune disorders is a result of breakdown in mechanisms that maintain unresponsiveness to self. Specifically, T1D is an automimmune disorder in which T cells are self-reactive or activated T cells that are activated against major antigenic components of pancreatic β cells are out of the control by the normal regulatory mechanisms. While these self-reactive T cells are under the control of peripheral regulatory mechanisms in healthy individuals, failure of control leads to the destruction of the β cells and consequent T1D. The autoimmune attack on pancreatic β cells is orchestrated by a variety of cells that produce cytokines and other toxic mediators. These self-reactive T cells work together with other lymphocytes and antigen-presenting cells to mediate this damage and have been shown in animal models to be important both in the early stages of diabetes development and in the final effector stages. They kill self-reactive T cells specific to self-antigens, which include β cell antigens, insulin and other key proteins associated with beta cell functions.

4.4 Autoreactive T cells in T1D

As reviewed in Wong 2008, many studies carried out in the NOD mouse model of T1D have indicated that both CD4+ and CD8+ T-cells inflict islet β-cell damage both at an early stage in diabetes development and at the final effector phase (26-28). There appears to be considerable similarity between the antigens recognized in humans and in the NOD animal models. In human studies, so far limited to testing peripheral blood lymphocytes, a variety of reactivities to putative autoantigens have been found, including proinsulin, GAD65, ppIAPP (pre-pro-islet amyloid polypeptide protein) and GFAP (glial fibrillary acidic protein). By the time diabetes occurs, it appears that there may be a broad spread of reactivity, which is different for each subject, and it will continue to be a major challenge to identify those antigenic epitopes that are important in the early phases of pathogenesis. Many of these antigenic epitopes represents attractive targets to go after for T1D therapeutic interventions.

Mechanisms by which T cells cause damage include production of cytotoxic cytokines such as IFNγ (interferon γ) and TNFα (tumour necrosis factor α), that have a direct cytotoxic action on the islets. In addition, these cytokines have an important role in up-regulation of MHC class I molecules, which could increase immune recognition of the cells. These T-cell-generated inflammatory cytokines can work together with cytokines produced by macrophages, such as IL (interleukin)-1, to have a synergistic damaging effect on islet β-cells. The CD8+ CTLs also exocytose granules from secretory lysosomes which contain cytotoxic proteins that include the pore-forming protein, perforin, and serine esterases, called granzymes. In summary, autoreactive T cells are considered as emerging targets for prospective antigen- specific therapeutic interventions.

4.5 Immunotherapeutic approaches to treat T1D

Many broad based immunosuppressive and antigen-specific immunoregulatory therapies have been and are currently being evaluated for their utility in the prevention and treatment of T1D. These may be administered as monotherapeis or in combination. Some of the below-mentioned have shown efficacy in initial clinical trials. Many tend to be small molecule inhibitors and monoclonal antibodies directed against cytokine and cytokine receptors, proteases, CD3 or CD20 receptors (i.e. Rituximab), GAD65, CTL14, heat shock proteins, or insulin-related molecules (29). Systemic non-antigen specific immunomodulator interventions include cycyclosporine A, azatiopirine, and anti-thymocyte globulin (ATG) plus prednisolone.

Other cell therapies using dendritic cells are also currently being tested as described briefly below. Additional examples of cell therapies include targeting regulatory T cells (Tregs). Approaches to

isolate and expand polyclonal and antigen-specific Tregs *in vitro* have been developed for immunotherapy and a clinical trial to test this hypothesis in the US (47)

Unlike those small molecule therapies and any currently tried cell-based therapies, AVT001 provides for a potential new type of cell-based therapeutic approach to treat and/or prevent T1D. AVT001, used as a vaccine, it may activate and correct the defective regulatory pathway mediated by HLA-E restricted CD8+ T cells in the identified subjects that normally function to selectively control the pathogenic self-reactive T cells that destroy β cells without damaging the normal on going anti-infection and anti-tumor immunity, a process called self-nonself discrimination (19, 20, 22, 23).

4.6 Dendritic cell based therapies for T1D

Dendritic cells (DCs) were first described by Ralph Steinman (30) nearly forty years ago. It is known that DCs existed in all lymphoid and most non-lymphoid tissues. DCs are antigen-presenting cells (APCs), which play a critical role in the regulation of the adaptive immune response. To perform this function, DCs are capable of capturing antigens, processing them, and presenting them on the cell surface along with appropriate costimulation molecules. The crucial function of DCs falls broadly into two categories, each of which involves antigen presentation. The first category of DCs function is antigen presentation and activation of T cells. The second category of DCs function has been suggested that a different class of DCs exist with the function of inducing and maintaining immune tolerance.

Cell therapy using dendritic cells use “immature” dendritic cells that are *in vitro* derived from monocyte precursors isolated from diabetic subjects and then either modified with and reinjected into the same individuals with the hope of resetting the immune system balance in the individual. There is a great deal of interest in how DCs might be exploited as a form of immunotherapy. DCs are being studied as adjuvants for vaccines or as a direct therapy to induce immunity against cancer. Human clinical trials are ongoing to use DCs to induce immunity to antigens against prostate cancer, lung cancer, melanoma and multiple myeloma etc. The first attempt to use DCs as cancer vaccines in humans was made by Edgar Engleman and Ronald Levy (31), who isolated DCs from subjects with non-Hodgkin's lymphoma who had failed conventional chemotherapy, loaded the cells with immunoglobulin idiotype obtained from the subject's tumor, and re-injected the antigen-loaded cells back into the subjects. Four subjects involved in this pilot clinical trial and all treatments were well tolerated, no significant side effects were associated with the infusion of antigen-loaded DCs. Since then, pilot clinical trials of antigen-pulsed DCs have been conducted in various types of cancer, including melanoma, prostate cancer, multiple myeloma, and non- small cell lung cancer. DC-based therapy as immunotherapy is also tried in human type 1 diabetes and a clinical trial to test this hypothesis has finished its phase I study in the US (Clinical Trials. gov identifier NCT00445913) (32). In general, it is widely accepted that DC-based therapy is consider a very safe therapy.

4.7 Dosing Rationale

Our preclinical human *in vitro* studies and murine *in vivo* studies formed the scientific basis for this clinical application to assess AVT001 *in vivo.* AVT001 is the study product name for autologous immature dendritic cells loaded with the synthetic oligopeptide from Hsp60sp. The study product represents an individualized preparation of autologous immature dendritic cells, derived by culture of the subject’s adherent primary monocytes with GM-CSF and IL-4 for 6 days, and loaded passively with the synthetic oligopeptide from Hsp60sp (QMRPVSRVL). In this study design

described in this protocol, three vaccines will be administered every 30 days (+/- 7 days but at least 21 days apart).

We feel strongly that the selection of the proposed route of administration, dose levels, dosing regimen, timing of vaccination relative to disease state, etc. in our clinical trial should rely on the information from similar human clinical trials rather than from animal studies. Particularly, it should not be heavily dependent on NOD/T1D model, which is a spontaneous disease animal model involving multiple mechanisms other than Qa-1 restricted CD8+ T cells and may not be exactly the same as human T1D subjects that we plan to test. For example, the subjects we propose to test are clearly defined to be the new onset T1D subjects who have the defect of the HLA-E restricted CD8+ T cells and the defect can be corrected *in vitro* by retriggering with AVT001. This design is based on the studies from human T1D subjects and its clinical regimen cannot be exactly mimicked and represented by the studies from NOD/T1D model. The best example in this case, which is almost identical to our circumstances is an iDC based therapy on T1D subjects (48) (32) versus its preclinical studies on NOD/T1D model (33), in that injections of 10 x 10^6 iDC every 2 weeks for total four times in humans versus a single injection of 2-3× 10^6 iDC in NOD mice.

In NOD mice we have used 0.5-2 x 10^6/mouse without any abnormality in protected mice, which dose range would be proportionally about 50-100 x 10^6/person in humans. However, in search for the information in other similar human clinical trials, the dosage range used has been reported to be between 2-50 x 10^6/dose every 2 weeks from two to 13 doses and showed no significant side effects (see Table below). Particularly, in one recently completed trial using iDC onT1D subjects, 10 x 10^6 iDC every 2 weeks for total four times were used (32), which is very close to our proposal, eg, 10 x 10^6 iDC every 4 weeks for total three times will be used. Finally, from our own studies, HLA-E restricted CD8+ T cells represent a very potent subset of CD8+ T cells, probably account for 1-2% of total CD8+ T cells. We calculate if the iDCs needed for activate such CD8+ T cells is at a ratio of 1:50-100, 10 x 10^6 of iDC would activate 500-10,000 x 10^6 of HLA-E restricted CD8+ T cells, which is in the range of 1-2% of total 10 x 10^{12} CD8+ T cells.

Table 1: **Summary of dosage and route of administration of dendritic cell-base vaccines in completed human clinical trails, which have been concluded as safe therapies**

Therapy to	Dose/schedule	Route	Number Subjects	Ref
NHL (Non-Hodgkin's lymphoma)	2-17×10^6 / 4 weeks × 4	i.v.	35	(34)
	2-17×10^6 / 4 weeks × 4	i.v.	10	(31)
Melanoma	5-50×10^6 / 2 weeks × 4	i.v./i.d./i.n.	28	(35)
	5-50×10^6 / 2 weeks × 4	i.v.	14	(36)
	3×10^6 +6/12×10^6 / 2 weeks × 5	i.d.+i.v.	13	(37)
Myeloma	0.5-11×10^6 / 4 weeks × 2	i.v.	12	(38)
	3.5-89×10^6 / 2 weeks × 3	i.v.	6	(39)
Prostate cancer	1-20×10^6 / 6-8 weeks × 4-5	i.v.	51	(40)

	10×10^6 / 2 weeks × 4	i.v.+i.d.	8	(41)
	$0.3\text{-}40\times10^6$ / 4 weeks × 2	i.v./i.d./i.l.	21	(42)
	10×10^6 / 2 weeks × 3	i.v.+i.d.	13	(43)
	$0.94\text{-}2.02\times10^8$ / 4 weeks × 3	i.v.	14	(44)
RCC (Renal cell carcinoma)	$5\text{-}10\times10^6$ / 4 weeks × 3-13	i.v./i.d.	35	(45)
	$10\text{-}50\times10^6$ +10×10^6 /2 weeks × 3	i.v.+i.d.	10	(46)
Type 1 diabetes	10×10^6 / 2 weeks × 4	i.d.	10	(32)

Based on the information from our own studies and the published results from clinical trials on DC-based therapies we have chosen a relatively low dose and regimen of AVT001 for safety consideration with a maximum potential for efficacy *in vivo* in our proposal.

We choose to vaccinate newly diagnosed T1D subjects that will be determined to have the defect of HLA-E restricted CD8+ T cells and the defect could be corrected by co-culture with AVT001 *in vitro*.

According to a 2017–2018 National Health Interview Survey (NHIS), National Center for Health Statistics, and Centers for Disease Control and Prevention report, as of 2018 an estimated 187,000 children and adolescents younger under 20 years of age had been diagnosed with with type 1 diabetes (49). Following the DSMB review of the safety data for the first 6 staggered adult subjects (18 years and older), no safety concerns were identified. Therefore, due to the safety established thus far and due to the high prevalence of disease in adolescents, this protocol is being amended (Amendment # 3) in a step-wise fashion to reduce the lower limit of age for eligibility to age 16.

5 Study Objectives and Design

Our working model for maintenance of T cell self-tolerance holds that a subset of HLA-E restricted regulatory CD8+ T cells that specifically recognize the target structure of Hsp60sp associated with HLA-E expressed on the pathogenic self-reactive T cells and selectively down-regulate these pathogenic T cells which are responsible for autoimmune diseases (19, 20, 22, 23). The failure of this HLA-E-restricted CD8+ T cell subset to recognize, or to suppress, its target population results in a permissive state in which organ-specific autoimmunity can emerge. We have identified defects in this CD8+ T cell system among nine of ten T1D subjects evaluated, and found the defect to be reversible in most of these *in vitro* by co-incubation with the synthetic oligopeptide from Hsp60sp-loaded autologous immature dendritic cells (AVT001).

5.1 Primary Outcomes

- To determine the safety and tolerability profile of three monthly doses of AVT001 (as compared to placebo) in subjects with T1D.

5.2 Secondary Outcomes

- To determine whether, among T1D subjects with *in vitro*-reversible defects in the CD8+ T cell regulatory system, infusion of AVT001 will lead to an *in vivo* reconstitution of this system.
- To determine whether, among T1D subjects with *in vitro*-reversible defects in the CD8+ T cell regulatory system, infusion of AVT001 will result in amelioration of β cell destruction as defined by preservation of stimulated C-peptide.

5.3 General Study Design

This is a double-blind, randomized, placebo-controlled study to evaluate the safety and tolerability of AVT001 cell-based therapy, and to assess its potential efficacy for the treatment of type 1 diabetes (T1D). A brief overview of the trial design is outlined in a schematic form in Figure 1.

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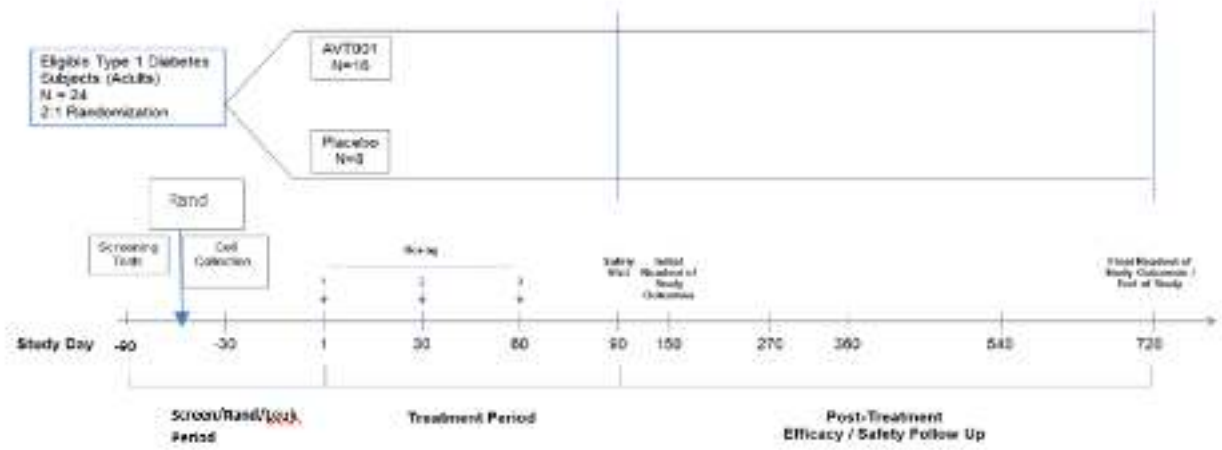
Figure 1: Study Design Schematic (Protocol AVT001-T1D-01)[1]



The trial will involve approximately 24 new-onset T1D subjects who have been identified as having: (a) a defect in HLA-E-restricted CD8+ T cell function putatively associated with pancreatic β cell destruction and (b) evidence that this defect of HLA-E-restricted CD8+ T cells can be corrected by *in vitro* exposure to AVT001.

Participants will be randomized, in a 2:1 ratio (AVT001:Placebo) to receive AVT001 or placebo by intravenous infusion.

- AVT001: 16 subjects to receive AVT001 through i.v. administration.
- Placebo: 8 subjects to receive saline through i.v. administration.

Subjects will be treated with 3 consecutive doses of AVT001, administered with one month (+/-7 days) intervals between doses. The primary time point for assessment will be 3 months post-last dose (Month 5), with longer-term follow-up through Month 24.

Administration of AVT001 will be performed in a double-blinded fashion as follows: the dosage regimen will be between 7 x 10^6 and 10 x 10^6 cells cells/infusion for each of the three infusions 30 (+/- 7) days apart. Each infusion will be administered by qualified clinical personnel in an established clinical research facility.

Subjects will be monitored for physical or reported symptoms as well as for vital signs (including temperature, heart rate, blood pressure, and arterial oxygen saturation) throughout the infusion procedure and for a minimum of 2 hours following the procedure. Although unlikely to be required, the clinical facility will include at the subject's side all medications and equipment necessary to treat cytokine release syndrome, acute anaphylactoid/infusion reactions, respiratory distress, and cardiac arrhythmia.

A placebo control arm using saline and DMSO is being used as the comparator.

[1] Amendment # 3 includes participants aged 16 and older.

5.4 Study Duration

There are three pre-defined periods in this study.

- Screening and cell collection period will last up to 3 months
- Treatment period will consist of 3 doses, ~30 days apart (Baseline, Month 1, Month 2) and a 1-month post-last dose assessment period (through Month 3). Thus, the treatment period is defined to be 3 months in duration.
- Post-Treatment Follow-up Period will extend 21 additional months (and thus through Month 24 of the study).

5.5 Subject Enrollment

The justification for the study design derives from the primary outcome of the proposed trial to assess the safety and tolerability of this therapy. This is a first time in man study and therefore exposure will be managed using a staggered enrollment that will allow for review of each subjects outcomes following the initial IV. This staggered enrollment and dosing for the first 6 subjects treated will be performed as follows:

- The second subject will be treated (dosed) only after the first subject has completed through the Month 1 safety visit and the Investigator and Medical Monitor have reviewed those data.
- Treatment (dosing) of subsequent subjects will be managed in a similar fashion, with subjects treated at a rate of one subject per month, with Investigator and Medical Monitor review of the Month 1 safety and tolerability outcomes for each subject before treatment of the subsequent subject is performed.

This will continue through the 6^{th} subject. Assuming an acceptable safety and tolerability profile is observed, enrollment of the remaining subjects will be determined by the sites accrual rate.

5.6 Data Safety Monitoring Board (DSMB)

A DSMB will be established that will monitor subject safety as well as data quality and adherence to safety rules. Additionally, the DSMB will review all safety/toxicity data for the trial and recommend trial modifications, suspension or termination, as needed.

The DSMB will meet after 3, 6 and 15 subjects have received at least one dose of double-blind study medication and have completed their in-clinic visit through at least one month post-dose. The DSMB will review *unblinded* data to assess the safety and tolerability of the treatments given. The DSMB will, following each of these meetings, provide the Sponsor with a summary of their findings and recommendations (if any) for protocol amendments.

A DSMB Charter will be written, to include details regarding meeting frequency, format, and membership.

5.6.1 Stopping Rules

Subjects will be clinically evaluated for evidence of toxicity from ≥ grade 3 toxicity as determined by CTCAE which is unmanageable, unexpected and (possibly, probably or definitely) related, and attributable to protocol therapy. Termination of enrollment would be considered with 2 dose limiting toxicities (DLTs) in the first 6 evaluable subjects and 3 in the first 9 evaluable subjects. In the event of a DLT for a given subject, doses will be discontinued, the subject clinically evaluated, and taken off study. The study will be stopped for DLT resulting in death of a subject.

5.7 Study Endpoints

Consistent with the study objectives, the primary endpoints will focus on the safety and tolerability of AVT001 treatment versus placebo.

The primary endpoints are:

(a) The incidence of treatment-emergent adverse events (TEAEs).
(b) Changes from baseline in clinical laboratory parameters.
(c) The incidence and severity of local IV-site reactions.
(d) Changes from baseline in vital signs and electrocardiograms.
(e) Incidence of severe hypoglycemic events.

Secondary objectives focus on the CD8+ T-cell regulatory system and therapeutic outcomes associated with T1D. These pharmacodynamic effects of AVT001 will be assessed in an exploratory manner.

Secondary endpoints will include:

(a) Assessment of the HLA-E-restricted CD8+ T cell regulatory activity ("potency assay")
(b) Changes from baseline in the area under the curve (AUC) of the stimulated C-peptide levels over the first 4 hours of a mixed meal tolerance test (MMTT)
(c) Changes from baseline in HbA1c
(d) Change from baseline in total daily insulin dose
(e) Changes from baseline in autoantibody levels

The primary time point for analysis is Month 5, at which point the primary assessment of the study objectives is performed.

6 Subject Selection and Withdrawal

6.1 Inclusion Criteria

Subjects who meet *all* of the following criteria are eligible for enrollment as study participants:

1. Diagnosis of type 1 diabetes, within 12 months of first dosing, confirmed by positive lab result for one or more of the following types of autoantibodies:
    a. Glutamic acid decarboxylase (GAD65)
    b. Insulinoma associated protein 2 (IA-2, also known as ICA-512)
    c. Zinc transporter 8 (ZnT8).
2. Age 16 or older and able to provide informed consent/assent.
3. Has a CD8+ T cell defect that is correctable *in vitro* by co-culture with immature dendritic cells loaded with the synthetic oligopeptide from Hsp60sp.
4. If a participant is female with reproductive potential, willing to avoid pregnancy through the duration of the trial.
5. Signed and dated written informed consent/assent.

6.2 Exclusion Criteria

Subjects who meet *any* of the following criteria are *not* eligible for enrollment as study participants:

1. Poorly controlled diabetes despite insulin therapy, who in the opinion of the investigator would not be a good candidate for participation in a clinical trial
2. Screening hemoglobin <10.0 g/dL; leukocytes <3,000/uL; neutrophils <1,500/uL; lymphocytes <800/uL; platelets <100,000/uL
3. Screening Urine Albumin Excretion > 300mg/gmCr
4. Screening eGFR < 60 mL/min/1.73m^2
5. Screening ALT or AST > 1.5x upper limit of normal (ULN)
6. Screening bilirubin > 2.0 mg / dL, or > 3.0 mg / dL for participants with Gilbert's Syndrome
7. Current use of immunosuppressive or immunomodulatory therapies, including pharmacologic doses of systemic steroids (topical steroidal creams and inhaled steroids without large systemic absorption are allowed).
8. Coincident medical condition likely to require immunosuppressive or immunomodulatory therapies.
9. Coincident medical condition likely to limit short term (5 year) life expectancy (malignancy, symptomatic coronary artery disease, recent stroke)
10. Prior radiation therapy, immunotherapy (within 1 year of screening), or chemotherapy
11. Serologic evidence of current HIV-1 or HIV-2 infection
12. Serologic evidence of hepatitis C infection
13. Serologic evidence of acute or chronic active hepatitis B as measured by Core Ab positive and / or Surface Antibody antigen positive
14. Subjects with other autoimmune conditions (except compensated or treated autoimmune thyroid, celiac, alopecia, or vitiligo diseases)

15. Women who are pregnant (pregnancy testing during screening), breastfeeding, or planning pregnancy during the study period.
16. Inadequate venous access to support leukapheresis.
17. Any condition that in the opinion of the investigator would preclude the subject from participating in a clinical trial.
18. Abnormal screening ECG that in the opinion of the investigator or sponsor would pose a safety risk.

6.3 Early Withdrawal of Subjects

If a subject is discontinued from the study prematurely, the Investigator must select the primary reason for discontinuation on the End of Study eCRF. In addition, every effort should be made to complete the assessments listed under the "Early Term" column on the Schedule of Events.

Subjects withdrawn from the study will be considered evaluable for statistical assessment.

A subject may be removed from the study for the following medical or administrative reasons:

- Adverse Event: If a subject experiences an adverse event that the subject finds unacceptable or that, in the judgment of the Investigator or the Medical Monitor presents an unacceptable consequence or risk to the subject, the subject may be discontinued from further participation in the study.
- Administrative Discontinuation: After consultation with the Investigator or Medical Monitor, a subject may be discontinued from the study for failure to comply with protocol requirements. All instances of noncompliance must be documented in the eCRF.
- Refusal of Assessments: If for any reason, following dosing, the subject refuses further assessment during the study, the subject shall be discontinued from the study and the reasons for refusal documented. Reasonable efforts shall be made to monitor the subject for adverse events following such discontinuation. Such efforts shall be documented.

Standard supportive therapy will be maintained for subjects withdrawn from active treatment. _Every effort will be made to collect safety data from these subjects through the end of study.

7 Description of Study Drug

A study drug handling manual and study drug administration manual (Manual of Operating Procedures, ie, MOP) describes study drug management in detail. The MOP includes procedures for collection, transport, processing, storage, preparation (for dosing), and administration of study medication, as well as details regarding how the blinding is to be maintained during the infusion process. The following sections provide an overview of the key elements of those activities, but the MOP should be referred to for details.

Drug name and active ingredients: The study drug represents an individualized preparation of autologous immature dendritic cells, derived by culture from the subject's adherent primary monocytes with GM-CSF and IL-4 for 6 days, and loaded passively with the synthetic oligopeptide from Hsp60sp.

Formulation of dosage: AVT001 will be cryopreserved in infusible cryomedia in infusion bags. Each infusion bag will contain between 7 x 10^6 and 10 x 10^6 cells, and each subject randomized to AVT001 will have 3 such bags manufactured for infusion. The cryopreserved cells will be manufactured at the Dana Farber Cancer Institute Cell Manipulation Core Facility (DFCI-CMCF) and will be transported to the investigational site for infusion.

Route of administration: The infusions will be administered intravenously.

Frequency of administration: Three (3) infusions will be administered 30 (+/- 7) days apart. While there is a 7-day window for the infusions, they should occur at least 21 days apart.

Table 2 provides a summary description of the drug products, including the dosage form, the unit dose, and a physical description of the products.

Table 2: Investigational Product (Protocol AVT001-T1D-01)

	Investigational Product	
Product Name:	AVT001	Placebo
Dosage Form:	20 mL AVT001 suspension with 10% DMSO in Human Serum Albumin	20 mL saline with 10% DMSO in Human Serum Albumin
Packaging:	Cryopreservation bags	Cryopreservation bags
Route of Administration:	Intravenous (i.v.)	Intravenous (i.v.)
Physical Description:	Blinded (covered) bottle/tube	Blinded (covered) bottle/tube
Manufacturer:	DFCI-CMCF	DFCI-CMCF

7.1 Administration of Study Drug.

A study drug handling manual and study drug administration manual (Manual of Operating Procedures, ie, MOP) describes study drug management in detail.

Administration of the treatment regimen is as follows:

- On Study Day 1, the subject will receive study treatment (the AVT001 or placebo infusion).
- The interval between injections will be 30 (+/- 7) days. Subsequent injections will be at Study Day 30 and 60 (each ± 7 days and at least 21 days apart), for a total of 3 i.v. injections.

Subjects will be observed for at least 2 hours post-end of each infusion. Safety and tolerability assessments will be performed, including measurement of vital signs. Local tolerability at the infusion site (such as pain, swelling, tenderness, and erythema) will be assessed via adverse event reporting. Please refer to the study drug handling manual and study drug administration manual (Manual of Operating Procedures, ie, MOP) for details on monitoring.Receiving, Storage, Dispensing and Return

A study drug handling manual and study drug administration manual (Manual of Operating Procedures, ie, MOP) describes study drug management in detail.

7.2 Reconciliation of Drug Product

A study drug handling manual and study drug administration manual (Manual of Operating Procedures, ie, MOP) describes study drug management in detail.

8 Study Procedures

Study procedures are listed below and can also be found in the Schedule of Events (Table 3). No study procedures will be performed prior to obtaining informed consent.

8.1 Informed Consent

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

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

8.2 Screening/Randomization/Leukapheresis

8.2.1 Screening Study Day ≤ - 90

Screening assessments may be performed at more than one visit, if needed, and must be completed prior to randomization, to establish subject eligibility. Unless otherwise noted, all tests will be required within 90 days prior to treatment.

- Medical history including prior treatment, current medicines, and drug allergies
- Physical exam including blood pressure, pulse, temperature, weight
- Screening Laboratory studies including:
  - Complete Metabolic Panel (CMP) to include: glucose, BUN, creatinine, electrolytes, calcium, total protein, albumin, total bilirubin, AST, ALT, alkaline phosphatase
  - Complete Blood Count with differential (CBC w diff) [CBC may need to be repeated if not performed within acceptable safety timeframe prior to leukapheresis; see MOP]
  - HbA1c
  - eGFR (calculated)
  - Urine MicroAlbumin panel (urine microalbumin, urine creatinine, microalbumin/creatinine ratio)
  - Creatine Clearance (CrCl) (calculated)
- Measurement of auto-antibodies, including:
  - Insulin associated aAb (IAA)
  - Insulinoma associated protein 2 (IA-2, also known as ICA-512)
  - Glutamic acid decarboxylast (GAD65)
  - Zinc transporter 8 (ZnT8).
- Evaluation of HLA-E-restricted CD8+ T cells ("potency assay").
- A urine pregnancy test will be collected for females of child-bearing potential.
- ECG
- Viral studies comprising HIV-1, HIV-2, HBV Core Ab positive and / or Surface Antibody antigen positive, and HCV serology.

- Vein check

Screening Lab eligibility requirements

- Potency Assay results from Screening do not expire.
- Auto-antibody and Viral testing results from Screening expire 6 months from collection.
- All other safety labs results from Screening expire after 90 days from collection including urine pregnancy tests for women of child bearing potential.

8.2.2 Randomization

Upon successful completion of all eligibility requirements, study participants may be randomized to the trial. Each subject will be randomized to treatment in a 2:1 ratio of AVT001:Placebo. As this is a first-in-man study, block sizes of N=3 for the first six subjects will be used to allow for review of the initial six subjects with four AVT001 and two placebo-treated. Thereafter, the block sizes will be randomly assigned to ensure the integrity of the randomization schema while maintaining a 2:1 ratio of AVT001 to placebo.

8.2.3 Leukapheresis

Following randomization, all subjects (including those randomized to placebo arm as well as the AVT001 arm) will undergo leukapheresis according to standard clinical procedures.

The apheresis product will be delivered to Dana Farber Center Cancer Institute (DFCI), Cell Manipulation Core Facility (CMCF) for processing to produce AVT001. Detailed scheduling, coordination, communication, handling, and other procedures for all vein-to-vein operations are described in the MOP.

Leukapheresis is to be performed after randomization, with sufficient time for AVT001/placebo manufacture prior to the end of window between Screening Visit (Visit 1) and first infusion (Study Day 1, Visit 3). Initial manufacture of blinded IP is expected to take a minimum of 28 days.

8.3 Double-Blind Treatment Period

The double-blind treatment period includes 4 scheduled clinic visits. These visits will include study drug administration as well as assessment of safety endpoints.

8.3.1 Study Days 1, 30 ± 7 days, and 60 ± 7 days

On the first study day, a 4-hour Mixed Meal Tolerance Test (MMTT) will be performed as part of baseline evaluations. The meal test will be performed in the morning after an overnight fast, with no food or drink (with the exception of water). At the outset, a mixed meal, consisting of High Protein Boost will be administered. Blood samples for C-peptide and glucose will be collected at the following timepoints (in minutes, relative to administration of the mixed-meal): -10, 0, 15, 30, and then at 30 minute-intervals until the last time point of 240 minutes.

1-2 days prior to each study day, study personnel will contact the subject to confirm that they remain in generally good health and that there are no symptoms of acute illness.

Prior to dosing on Study Day 1, a review of eligibility should be performed and confirmed. If, prior to dosing, a randomized subject is not suitable (ineligible) for the trial, that subject will be considered a “randomization failure” and will be replaced.

The following procedures will be performed prior to study drug administration on each dosing day:

- Any changes to medical history including prior treatment, current medicines, and drug

allergies, should be recorded.

- Focused physical exam including blood pressure, pulse, temperature and weight.
- Laboratory studies including:
  - CMP
  - CBC w diff
  - HbA1c
  - Urine MicroAlbumin panel
  - CrCl (calculation)
-
- Measurement of auto-antibodies, including:
  - Insulin associated aAb (IAA)
  - Insulinoma associated protein 2 (IA-2, previously known as ICA-512)
  - Glutamic acid decarboxylase (GAD65)
  - Zinc transporter 8 (ZnT8).
- A urine pregnancy test will be collected for females of child-bearing potential.
- Any severe hypoglycemic events occurring since the prior study visit will be recorded. Note that non-severe events are not being collected in the database in this study, but will be monitored by the investigator, consistent with the site's standard of care.

Study drug will be administered during these visits. Laboratory data will be collected prior to initiation of the infusion of study drug.

Subjects will be observed for at least 2 hours post-end of each infusion. Safety and tolerability assessments will be performed during and following the infusion, including measurement of vital signs. Vital sign frequency peri-infusion is monitored per the site's standard of care and a recommendation for the minimal frequency is described in the MOP. Local tolerability at the infusion site (such as pain, swelling, tenderness, and erythema) will be assessed via adverse event reporting. Any adverse events or new concomitant medications will be recorded.

8.3.2 Study Day 90 ± 7 days

Subjects will return one month after the last infusion for safety and tolerability assessments. This will include a physical exam, insulin usage, vital signs, clinical laboratories CMP, CBC w diff, HbA1c, Urine MicroAlbumin panel, CrCl (calculated), adverse events, and concomitant medications. Local tolerability at the infusion site (such as pain, swelling, tenderness, and erythema) will be assessed via adverse event reporting. Samples for the potency assay and other glycemic measures will be collected.

- Measurement of auto-antibodies, including:
  - Insulin associated aAb (IAA)
  - Insulinoma associated protein 2 (IA-2, previously known as ICA-512)
  - Glutamic acid dexarboxylase (GAD65)
  - Zinc transporter 8 (ZnT8).

8.4 Long-Term Follow-Up Period

Continued data collection will be performed during the long-term follow-up period. There are 5 planned in-clinic visits during this period.

8.4.1 Study Day 150 ± 7 days

This visit will include a full assessment of safety, tolerability, and efficacy. This will include a physical exam, insulin usage, vital signs, clinical laboratories CMP, CBC w diff, HbA1c, Urine MicroAlbumin panel, CrCl (calculated) adverse events, and concomitant medications. A urine pregnancy test will be collected for females of child-bearing potential.

Samples for the potency assay and for pancreatic β-cell function (MMTT) and other glycemic measures will be collected.

- MMTT C-Peptide
- Measurement of auto-antibodies, including:
  - Insulin associated aAb (IAA)
  - Insulinoma associated protein 2 (IA-2, previously known as ICA-512)
  - Glutamic acid dexarboxylase (GAD65)
  - Zinc transporter 8 (ZnT8).
- Total daily insulin usage over the prior 3-7 days in the immediate period prior to the visit
- Any severe hypoglycemic events occurring since the prior study visit will be recorded.

8.4.2 Study Day 270 ± 14 days, 360 ± 14 days, 540 ± 14 days, and 720/Early Term ± 14 days

These visits will include a physical exam (can be symptom-driven except at Study Day 360, in which a full physical examination is required), insulin usage, vital signs, clinical laboratories CMP, CBC w diff, HbA1c, Urine MicroAlbumin panel, CrCl (calculated), adverse events, and concomitant medications. A urine pregnancy test will be collected for females of child-bearing potential at the Study Day 720/Early Term visit only.

Samples for the potency assay will be collected, and samples for pancreatic β-cell function will be collected.

- MMTT C-Peptide (MMTT will not be performed at the Day 270 visit, but will be collected at Day 360, 540, and 720/Early Term visits).
- Measurement of auto-antibodies, including:
  - Insulin associated aAb (IAA)
  - Insulinoma associated protein 2 (IA-2, previously known as ICA-512)
  - Glutamic acid decarboxylase (GAD65)
  - Zinc transporter 8 (ZnT8).
- Total daily insulin usage over the 3-7 days in the immediate period prior to the visit
- Any severe hypoglycemic events occurring since the prior study visit will be recorded.

8.5 ECGs

All scheduled ECGs will be performed after the subject has rested supine for approximately 5 minutes. ECGs will be collected during Screening and Day 90 ± 7 days

8.6 MMTT Description

The 4-hour Mixed Meal Tolerance Test (MMTT) will be performed as a measure of beta cell function at multiple visits during the study. The MMTT will be performed in the morning after an overnight fast, with no food or drink (with the exception of water). At the outset, a mixed meal, consisting of High Protein Boost will be administered. Blood samples for C-peptide and glucose will be collected at the following timepoints (in minutes, relative to administration of the mixed-meal): -10, 0, 15, 30, and then at 30 minute-intervals until the last time point of 240 minutes. An MMTT protocol is provided to the investigational centers that describes the procedures for performing the MMTT, including preparation and sampling times.

8.7 Prior and Concomitant Therapy and Insulin Usage

Study participants will continue all regularly prescribed medications, except for any diabetes medications other than insulin. For subjects who are already on diabetes medications and for whom it is clinically appropriate to discontinue, they will do a wash off period of at least 3 weeks prior to initiation of leukapharesis. The introduction of systemic immunosuppressive agents, however, will constitute grounds for withdrawal from the study. However, topical creams (e.g., steroidal) and inhaled steroids without large systemic absorption are allowed.

Insulin usage will be recorded, using the insulin log, 3-7 days, each day, prior to visit separately from prior/concomitant medication therapies. Insulin usage will include description of the daily total dose and regimen. Additional instructions for use are provided on the insulin log.

8.8 Immunoassessment "Potency" Assay Procedure

"Potency" Assay will be performed by the sponsor with the subject blood sample after the AVT treatment (or placebo) to test if the function of Qa-1/HLA-E restricted CD8+ T cell is restored. Please see "Schedule of Events" for the dates/frequency. The visit-specific blood volumes as well as specimen collection, handling, and transport procedures are described in the MOP.

"Potency" Assay will be also performed for "information only" with AVT001 made by DFCI-CMCF before applying to the subject. 2-3 vials of frozen ATVT001 ($1x10^6$ cells/vial) should be sent to the sponsor for such assessment.

9 Statistical Methodology

A separate document entitled "Statistical Analysis Plan" (SAP) will describe further details about the analyses to be conducted in this study. An overview of the general statistical methods is provided in the following sections.

9.1 Analysis Timing and Blinding Considerations

Consistent with the study design, the primary time point for analysis is Month 5. The primary analysis for this study will be performed after the last subject has completed the Month 5 study visit, at which time the study database will be locked and the treatment allocation codes unblinded for analysis. This analysis will form the primary basis for the assessment of the study objectives.

Continuing data collection through the long-term follow-up will be performed through Month 24. While Investigators and subjects will remain blinded to the treatment allocations, assessment and analysis of outcomes will be performed by the Sponsor in an on-going manner, to better understand the longer-term effects (on both safety and efficacy) of the therapy.

9.2 Sample Size Determination

The primary objective of this study is to assess the safety and tolerability of AVT001, as compared to placebo, in subjects with type 1 diabetes. A sample size of 14 subjects treated with AVT001 will provide at least 80% power to observe at least one occurrence of treatment-emergent adverse events with a true underlying incidence of 12%. The sample size provides the same power to detect at least one occurrence of local tolerability events relating to the i.v. administration of study drug.

Secondary objectives of this study include assessment of changes in pharmacodynamic markers, including (increases) in the CD8+ T-cell reg system, C-peptide levels, and HbA1c. The sample size of 14 subjects treated with AVT001 and 7 subjects treated with placebo will provide estimates of the mean and standard deviation of the treatment benefit of AVT001 as compared to placebo in these endpoints.

To account for an estimated 10% potential dropout prior to the completion of the Month 5 safety/efficacy assessment post-first dose, a total sample size of approximately 24 subjects will be randomized, in a 2:1 ratio of AVT001:Placebo.

9.3 Analysis Populations

There are two analysis populations defined for this study.

- The Safety population is defined as all subjects who receive at least one dose of study medication. Safety outcomes will be assessed using the Safety population.
- Pharmacodynamic outcomes will be assessed in subjects with at least one post-baseline assessment for the evaluation of the CD8+ T-cell reg system biomarker.

Other analysis populations may be defined in the SAP.

9.4 Baseline characteristics, Patient Disposition and Medication

Key baseline characteristics will be tabulated by treatment group. Patient disposition will be described, including patient completion status and reasons for premature discontinuation from the study. Prior and concomitant medications will be tabulated (with a focus on new-onset medications, ie, those that are started after study treatment has commenced).

9.5 Adverse Events

Adverse events will be reported using the MedDRA coding dictionary. Tabulations will include an overall incidence of at least one adverse event, incidence within body system, and incidence by preferred term. Each subject may only contribute once (ie, first occurrence) to each of the incidence rates, regardless of the number of occurrences. The incidence of adverse events will be presented as follows:

- The incidence of treatment-emergent adverse events (TEAEs), ie, those events that start on or after the first dose of study medication, will be tabulated by treatment for the Treatment Phase. This will include events starting within 30 days following the last dose.
- The incidence of post-treatment events (those events that start more than 30 days following the last dose) will be tabulated.

Subjects with serious adverse events (including deaths) and subjects who discontinue due to adverse events will be listed. Subjects who have other significant serious adverse events deemed to be of special interest because of clinical importance will also be listed.

9.6 Severe Hypoglycemic Events and Insulin Log

The number and subject incidence of severe hypoglycemic events (defined in Section 11.1.7) will be tabulated by subject incidence (number and percent of subjects) as well as frequency (number of events per subject).

The total daily dose of insulin will be tabulated by visit from the insulin logs. Daily insulin usage will be listed from these logs.

9.7 Clinical Laboratories, ECGs, and Vital Signs

Descriptive statistics at Baseline, on-treatment, and change from Baseline will be calculated for the subjects who have both Baseline and on-treatment evaluations. Shifts from Baseline to on-treatment will be examined. Potentially clinically significant laboratory values will be identified and listed.

9.8 Pharmacodynamic Outcomes

Changes over time in CD8+ T-cell reg system, C-peptide levels, HbA1c, and antibody values will be assessed. For purposes of data presentation, where appropriate, a mixed model for repeated measures (MMRM) will be used to present changes in these parameters over time. Details regarding this MMRM will be provided in the SAP.

9.8.1 CD8+ T-cell reg system

A “potency assay” has been established to measure the specific suppression (inhibition) of the CD8+ regulatory T-cells to the target cells as one parameter to evaluate the efficacy of the AVT001 treatment. The data of maximum suppression of CD8+ regulatory T-cells from treated T1D subjects before the treatment versus after the treatment, with healthy people as a system control, determines whether there is a regained suppressive function of the CD8+ regulatory T-cells following the AVT001 treatment. Such values might suggest the effectiveness of treatment. Analyses for these outcomes will be specified in the SAP.

9.8.2 C-Peptide AUC Calculation

The area under the stimulated C-peptide curve (AUC) over the first 4-hour period of a mixed meal glucose tolerance test will be conducted at baseline and Months 5, 12, 18, and 24. The AUC at each visit is computed using the trapezoidal rule that is a weighted sum of the C-peptide values over the 240 minutes. The weighted mean C-peptide is AUC/240 (pmol/mL). AUC values will then be log-

transformed using the function: log(C-peptide +1). This provides better normal distributional behavior of the random variable.

9.8.3 HbA1c, Antibodies

Changes from baseline in HbA1c and antibodies will be tabulated.

10 Risks and Discomforts

The Investigator's Brochure is to be reviewed by the Investigator prior to initiation of this study. A brief summary of potential risks and discomforts is provided in this section. The drug handling manual includes additional detail as well as rescue medications that may be required.

10.1 Venipuncture

Subjects may experience slight discomfort at the site and rarely develop an ecchymosis or superficial thrombosis.

10.2 Leukapheresis

The most common adverse reactions from leukapheresis are paraesthesias, dizziness, mild chest tightness, or cough related to the reinfusion of autologous red blood cells treated with citrate and ecchymoses at the catheter sites.

Potential adverse reactions related to leukapheresis include:

- Exacerbation of symptoms from an underlying medical condition including migraine headache, asthma/emphysema, or cardiovascular disease.
- Infection at catheter sites
- Hypotension, hypertension, or bradycardia from citrate toxicity
- Allergic reactions
- Anemia, if red blood cells cannot be reinfused

10.3 Response to AVT001

The response to AVT001 would be to activate HLA-E restricted CD8+ T cells *in vivo*, which would in turn selectively down-regulate any anti-self responses to control autoimmune diseases. In pre-clinical animal toxicology studies, the data showed no adverse effect/reaction to the treatment. In general, DC-based therapies are considered safe and tolerable therapies, with less toxicity comparable to checkpoint inhibitors in use or in trial.

There might be infusion site reactions such as swelling and irritation after the treatment of AVT001. In addition, there may be risks/discomforts involved in this study that are not known at this time.

11 Safety and Adverse Events

The primary objective of this study is to assess the safety and tolerability of AVT001, as compared to placebo, in subjects with type 1 diabetes.

11.1 Definitions

11.1.1 Unanticipated Problems Involving Risk to Subjects

Any incident, experience, or outcome that meets all of the following criteria:

- Unexpected in nature, severity, or frequency (i.e. not described in study-related documents such as the IRB-approved protocol or consent form, the investigators brochure, etc.)
- Related or possibly related to participation in the research (i.e. possibly related means there is a reasonable possibility that the incident experience, or outcome may have been caused by the procedures involved in the research)
- Suggests that the research places subjects or others at greater risk of harm (including physical, psychological, economic, or social harm).

11.1.2 Adverse Event

Adverse event means any untoward medical occurrence associated with the use of a drug in humans, whether or not considered drug related. Adverse events may include safety findings considered to be clinically significant by the Investigator. An adverse drug event can therefore be any unfavorable and unintended sign (including an abnormal laboratory finding), symptom, or disease temporally associated with the use of a medicinal product, whether or not it is related to the medicinal product. Reporting an adverse event does not necessarily reflect a conclusion by the Investigator that the event is causally related to the drug.

AE is any symptom, sign, illness or experience that develops or worsens in severity during the course of the study. Intercurrent illnesses or injuries should be regarded as adverse events. Abnormal results of diagnostic procedures are considered to be adverse events if the abnormality:

- results in study withdrawal
- is associated with a serious adverse event
- is associated with clinical signs or symptoms
- leads to additional treatment or to further diagnostic tests
- is considered by the investigator to be of clinical significance

A preexisting condition is one that is present at the start of the study. A preexisting condition should be recorded as an adverse event if the frequency, intensity, or the character of the condition worsens during the study period.

Each adverse event in this study will be assessed for Grade, where Grade of an AE refers to the severity of the AE. Grade will be assessed according to CTCAE Version 4.03 or higher. The CTCAE displays Grades 1 through 5 with unique clinical descriptions of severity for each AE that are based on this general guideline, with higher grades indicating worse severity.

The relationship of any AE to study treatment will be assessed using the following definitions:

Definite (must have all 4)

- Has a reasonable temporal relationship to the intervention
- Could not have readily been produced by the subject's clinical state or have been due to environmental or other interventions
- Follows a known pattern of response to intervention
- Disappears or decreases with reduction in dose or cessation of intervention and recurs with re-exposure

Probable (must have 3)

- Has a reasonable temporal relationship to the intervention
- Could not have readily been produced by the subject's clinical state or have been due to environmental or other interventions
- Follows a known pattern of response to intervention
- Disappears or decreases with reduction in dose or cessation of intervention

Possible (must have 2)

- Has a reasonable temporal relationship to the intervention
- Could not have readily been produced by the subject's clinical state
- Could not readily have been due to environmental or other interventions
- Follows a known pattern of response to intervention

Unlikely (must have 2)

- Does not have a temporal relationship to the intervention
- Could readily have been produced by the subject's clinical state
- Could have been due to environmental or other interventions
- Does not follow a known pattern of response to intervention
- Does not reappear or worsen with reintroduction of intervention

Unrelated

- The temporal relationship between treatment exposure and the adverse event is unreasonable or incompatible and/or adverse event is clearly due to extraneous causes (e.g., underlying disease, environment)

11.1.3 Serious Adverse Event (SAE)

Adverse events are classified as serious or non-serious. A *serious adverse event* is any AE that is:

- Fatal
- Life-threatening
- Requires or prolongs hospital stay
- Results in persistent or significant disability or incapacity
- A congenital anomaly or birth defect
- An important medical event

Any adverse event that results in hospitalization or prolonged hospitalization should be documented and reported as a serious adverse event unless specifically instructed otherwise in this protocol. Any

condition responsible for surgery should be documented as an adverse event if the condition meets the criteria for an adverse event.

Neither the condition, hospitalization, prolonged hospitalization, nor surgery are reported as an adverse event in the following circumstances:

- Hospitalization or prolonged hospitalization for diagnostic or elective surgical procedures for a preexisting condition. Surgery should *not* be reported as an outcome of an adverse event if the purpose of the surgery was elective or diagnostic and the outcome was uneventful.
- Hospitalization or prolonged hospitalization required to allow efficacy measurement for the study.
- Hospitalization or prolonged hospitalization for therapy of the target disease of the study, unless it is a worsening or increase in frequency of hospital admissions as judged by the clinical investigator.

All adverse events that do not meet any of the criteria for serious event should be regarded as *non-serious adverse events*.

11.1.4 General Physical Examination Findings

At screening, any clinically significant abnormality should be recorded as a preexisting condition. At the end of the study, any new clinically significant findings/abnormalities that meet the definition of an adverse event must also be recorded and documented as an adverse event.

Physical examinations can be symptom-driven except at Screening, Month 5, and Month 12, during which full examinations are required.

11.1.5 Post-study Adverse Event

All unresolved adverse events should be followed by the investigator until the events are resolved, the subject is lost to follow-up, or the adverse event is otherwise explained. At the last scheduled visit, the investigator should instruct each subject to report any subsequent event(s) that the subject, or the subject's personal physician, believes might reasonably be related to participation in this study. The investigator should notify the study sponsor of any death or adverse event occurring at any time after a subject has discontinued or terminated study participation that may reasonably be related to this study. The sponsor should also be notified if the investigator should become aware of the development of cancer or of a congenital anomaly in a subsequently conceived offspring of a subject that has participated in this study.

11.1.6 Abnormal Laboratory Values

A clinical laboratory abnormality should be documented as an adverse event if any one of the following conditions is met:

- The laboratory abnormality is not otherwise refuted by a repeat test to confirm the abnormality
- The abnormality suggests a disease and/or organ toxicity
- The abnormality is of a degree that requires active management; e.g. change of dose, discontinuation of the drug, more frequent follow-up assessments, further diagnostic investigation, etc.

11.1.7 Severe Hypoglycemia

Subject self-monitoring of blood glucose will be performed according to the standard of care as indicated by the investigator. This may include clinician-prescribed glucose monitoring devices, ie, glucose meters or continuous glucose monitoring devices (CGM). The number of severe hypoglycemic events will be collected as part of each study visit. Events are defined according to American Diabetes Association recommended criteria.

- Severe hypoglycemia denotes severe cognitive impairment requiring external assistance for recovery. Severe hypoglycemia is an event requiring assistance of another person to actively administer carbohydrates, glucagon, or take other corrective actions. Plasma glucose concentrations may not be available during an event, but neurological recovery following the return of plasma glucose to normal is considered sufficient evidence that the event was induced by a low plasma glucose concentration.
- In addition to the above criterion, for subjects using CGM, readings below the <54 mg/dL (3.0 mmol/L) for at least 15 minutes will be considered severe hypoglycemia

Note that non-severe hypoglycemic events will not be collected or recorded in the study database, but that the investigator will monitor those types of events according to the site's standard of care.

11.2 Recording of Adverse Events

At each contact with the subject, the investigator must seek information on adverse events by specific questioning and, as appropriate, by examination. Information on all adverse events should be recorded immediately in the source document, and also in the appropriate adverse event module of the case report form (CRF). All clearly related signs, symptoms, and abnormal diagnostic procedures results should recorded in the source document, though should be grouped under one diagnosis.

All adverse events occurring during the study period must be recorded. The clinical course of each event should be followed until resolution, stabilization, or until it has been determined that the study treatment or participation is not the cause. Serious adverse events that are still ongoing at the end of the study period must be followed up to determine the final outcome. Any serious adverse event that occurs after the study period and is considered to be possibly related to the study treatment or study participation should be recorded and reported immediately.

11.3 Reporting of Adverse Events

11.3.1 Adverse Event Reporting Period

The study period during which adverse events must be reported is defined as the period from the time of consent through the end of study or early termination, whichever is sooner. Reporting of Serious Adverse Events – Study Sponsor Notification by Investigator

A serious adverse event must be reported to the study sponsor by telephone within 24 hours of the event. A Serious Adverse Event (SAE) form must be completed by the investigator and faxed to the study sponsor within 24 hours. The investigator will keep a copy of this SAE form on file at the study site. Report serious adverse events by phone, facsimile or email to:

Name: Dr. Harvey Katzeff, MD

Address: Iqvia Biotech, 1700 Perimeter Park Drive, Morrisville, NC

Phone: 919.313.7111 (US Toll-free: 1-866-758-2798)

Fax: 919.313.1412 (US Toll-free: 1-866-761-1274)

Email: Safety-Inbox@novellaclinical.com

At the time of the initial report, provide as much of the following information as possible:

- Study identifier
- Study Center
- Subject number
- A description of the event
- Date of onset
- Current status
- Whether study treatment was discontinued
- The reason why the event is classified as serious
- Investigator assessment of the association and expectedness between the event and study treatment
- If the treatment blind was broken.

Within the following 48 hours, the investigator must provide further information on the serious adverse event in the form of a written narrative. This should include a copy of the completed Serious Adverse Event form, and any other diagnostic information that will assist the understanding of the event. Significant new information on ongoing serious adverse events should be provided promptly to the study sponsor.

11.3.2 IRB Notification

Each site's local policy for IRB reporting should be followed. All serious adverse events (SAEs) should be submitted to the Institutional Review Board/Independent Ethics Committee (IRB/IEC) within the timing of the local authority. The investigator should make an effort to obtain all hospital medical records including discharge summary confirming final diagnosis.

The death of a subject must be immediately (within 24 hours) reported to the IRB. Other Reportable Events

For clinical drug trials, the following events are also reportable to the IRB:

- Any adverse experience that, even without detailed analysis, represents a serious unexpected adverse event that is rare in the absence of drug exposure (such as agranulocytosis, hepatic necrosis, Stevens-Johnson syndrome).
- Any adverse event that would cause the sponsor to modify the investigators brochure, protocol or informed consent form, or would prompt other action by the IRB to assure protection of human subjects.
- Information that indicates a change to the risks or potential benefits of the research, in terms of severity or frequency. For example:
    - An interim analysis indicates that participants have a lower rate of response to treatment than initially expected.
    - Safety monitoring indicates that a particular side effect is more severe, or more frequent than initially expected.

- A paper is published from another study that shows that an arm of your research study is of no therapeutic value.

- Change in FDA safety labeling or withdrawal from marketing of a drug, device, or biologic used in a research protocol.
- Breach of confidentiality
- Change to the protocol taken without prior IRB review to eliminate apparent immediate hazard to a research participant.
- Incarceration of a participant when the research was not previously approved under Subpart C and the investigator believes it is in the best interest of the subject to remain on the study.
- Complaint of a participant when the complaint indicates unexpected risks or the complaint cannot be resolved by the research team.
- Protocol violation (meaning an accidental or unintentional deviation from the IRB approved protocol) that in the opinion of the investigator placed one or more participants at increased risk, or affects the rights or welfare of subjects.

11.3.3 FDA Notification by Sponsor

The study sponsor is required to report certain study events in an expedited fashion to the FDA. These written notifications of adverse events are referred to as IND safety reports. The following describes the safety reporting requirements by timeline for reporting and associated type of event:

- Within 7 calendar days

Any study event that is:

1. associated with the use of the study drug
2. unexpected,
3. fatal or life-threatening, and

- Within 15 calendar days

Any study event that is:

- associated with the use of the study drug,
- unexpected, and
- serious, but not fatal or life-threatening

-or-

- a previous adverse event that was not initially deemed reportable but is later found to fit the criteria for reporting (reporting within 15 calendar days from when event was deemed reportable).

Any finding from tests in laboratory animals that:

- suggest a significant risk for human subjects including reports of mutagenicity, teratogenicity, or carcinogenicity.

Sponsors are also required to identify in IND safety reports all previous reports concerning similar adverse events and to analyze the significance of the current event in light of the previous reports.

11.4 Protocol Deviations

The investigator will maintain a protocol deviation log, in accordance with the site's local policy for IRB reporting, that describes departures from the study protocol. If the impact on the protocol disrupts the study design, may affect the outcome (endpoints) or compromises the safety and welfare of the subjects, the deviation will be reported to the Sponsor (and the IRB in accordance with the site's local policy) within 10 business days.

Any departure from the protocol that meets the following criteria should be submitted to the Sponsor:

- May/can/have affects/affected subject safety
- Violate eligibility
- Dose adjustment
- Stopping criteria
- Affect sample size (adding more subjects, decreasing number of subjects, changing the number of subject in a specific arm/cohort)

11.5 Medical Monitoring

It is the responsibility of the Principal Investigators to oversee the safety of the study at their sites. This safety monitoring will include careful assessment and appropriate reporting of adverse events as noted above, as well as the construction and implementation of a site data and safety-monitoring plan. The Sponsor will be responsible for performing overall medical monitoring, including regular assessment of safety outcomes (e.g., review of laboratory data, review of adverse events and local tolerability outcomes across the sites).

12 Data Handling and Record Keeping

12.1 Confidentiality

Information about study subjects will be kept confidential and managed according to the requirements of the Health Insurance Portability and Accountability Act (HIPAA). Those regulations require a signed subject authorization informing the subject of the following:

- What protected health information (PHI) will be collected from subjects in this study
- Who will have access to that information and why
- Who will use or disclose that information
- The rights of a research subject to revoke their authorization for use of their PHI.

In the event that a subject revokes authorization to collect or use PHI, the investigator, by regulation, retains the ability to use all information collected prior to the revocation of subject authorization. For subjects that have revoked authorization to collect or use PHI, attempts should be made to obtain permission to collect at least vital status (i.e. that the subject is alive) at the end of their scheduled study period.

12.2 Source Documents

Source data is all information, original records of clinical findings, observations, or other activities in a clinical trial necessary for the reconstruction and evaluation of the trial. Source data are contained in source documents Examples of these original documents, and data records include: hospital records, clinical and office charts, laboratory notes, memoranda, subjects' diaries or evaluation checklists, pharmacy dispensing records, recorded data from automated instruments, copies or transcriptions certified after verification as being accurate and complete, microfiches, photographic negatives, microfilm or magnetic media, x-rays, subject files, and records kept at the pharmacy, at the laboratories, and at medico-technical departments involved in the clinical trial.

12.3 Case Report Forms

Clinical study data will be captured / collected in a clinical study database using electronic case report forms (eCRFs) in a CFR Title 21 part 11-compliant system. All data requested in the eCRF must be recorded, and reasons for missing data should be provided.

12.4 Records Retention

It is the investigator's responsibility to retain study essential documents according to regulatory requirements. These documents may be retained for a longer period if required by an agreement with the sponsor. In such an instance, it is the responsibility of the sponsor to inform the investigator/institution as to when these documents no longer need to be retained.

13 Study Monitoring, Auditing, and Inspecting

13.1 Study Monitoring Plan

This study will be monitored according to the study monitoring plan. The investigator will allocate adequate time for such monitoring activities. The Investigator will also ensure that the monitor or other compliance or quality assurance reviewer is given access to all the above noted study-related documents and study related facilities (e.g. pharmacy, diagnostic laboratory, etc.), and has adequate space to conduct the monitoring visit.

13.2 Auditing and Inspecting

The investigator will permit study-related monitoring, audits, and inspections by the EC/IRB, the sponsor, government regulatory bodies, and University compliance and quality assurance groups of all study related documents (e.g. source documents, regulatory documents, data collection instruments, study data etc.). The investigator will ensure the capability for inspections of applicable study-related facilities (e.g. pharmacy, diagnostic laboratory, etc.).

Participation as an investigator in this study implies acceptance of potential inspection by government regulatory authorities and applicable University compliance and quality assurance offices.

14 Ethical Considerations

This study is to be conducted according to US and international standards of Good Clinical Practice (FDA Title 21 part 312 and International Conference on Harmonization guidelines), applicable government regulations and Institutional research policies and procedures.

This protocol and any amendments will be submitted to a properly constituted independent Ethics Committee (EC) or Institutional Review Board (IRB), in agreement with local legal prescriptions, for formal approval of the study conduct. The decision of the EC/IRB concerning the conduct of the study will be made in writing to the investigator and a copy of this decision will be provided to the sponsor before commencement of this study. The investigator should provide a list of EC/IRB members and their affiliate to the sponsor.

All subjects for this study will be provided a consent form describing this study and providing sufficient information for subjects to make an informed decision about their participation in this study. This consent form will be submitted with the protocol for review and approval by the EC/IRB for the study. The formal consent of a subject, using the EC/IRB-approved consent form, must be obtained before that subject is submitted to any study procedure. This consent form must be signed by the subject or legally acceptable surrogate, and the investigator-designated research professional obtaining the consent.

Table 3 Schedule of Events (Protocol AVT001-T1D-01)

			Double-Blind Study Period (Visits 3-7)					Open-Label Study Period (Visits 8-11)		
Study Period	**Screen/Rand/Leuk**		**Double-Blind Treatment Period**			**Safety Assessment**	**Long-Term Follow-Up Period**			
Study Day	**≤ -90[2]**	*****	**1[3]**	**30±7**	**60±7**	**90±7**	**150±7**	**270±7**	**360±7**	**540±7, 720±7 / Early Term**
Study Month	**-3**	*****	**-**	**1**	**2**	**3**	**5**	**9**	**12**	**18, 24**
Visit Name	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10, 11**
Informed Consent	X									
Vein Check	X									
Randomization		X								
Medical History[4]	X		X							
Physical Exam[5]	X		X	X	X	X	X	X	X	X
Insulin Usage			X	X	X	X	X	X	X	X
Vital Signs[6]	X		X	X	X	X	X	X	X	X
MMTT for C-peptide			X				X		X	X
Severe Hypoglycemic Events Assessment			X	X	X	X	X	X	X	X
HbA1c and Antibody Samples	X		X	X	X	X	X	X	X	X
Laboratory Studies[7]	X		X	X	X	X	X	X	X	X
ECGs	X					X				
Viral Studies[8]	X									
Urine Pregnancy test	X		X	X	X		X			X (Day 720 /ET only)
Local tolerability of infusion site			X	X	X	X				
Leukapheresis		X[9]								
Potency Assay[10]	X (BD)	X (AVT)				X (BD)	X (BD)	X (BD)	X (BD)	X (BD)
Study Drug Administration[11]			X	X	X					
Adverse Events			On-going							
Prior/Conmeds	Prior Meds		Conmeds							

2 Screening assessments may be performed at more than 1 visit, if needed, and must be completed prior to randomization

3 At Study Day 1, a review of Medical history, symptom-driven PE, and review of eligibility should be performed prior to treatment. Details are included in note 4.

4 Medical history including prior treatment, current medicines, and drug allergies

5 Physical exam including blood pressure, pulse, temperature, weight. Can be symptom-driven physical examinations, except at Screening, Month 5, and Month 12.

6 Vital sign frequency peri-infusion is determined by site's standard of care; recommended (minimal) guidelines are provided in the MOP

7 Specified at each study visit: generally includes CMP, CBC w diff, Urine MicroAlbumin panel, CrCl (calculated); eGFR (calculated at screening only for eligibility)

8 HIV-1, HIV-2, HCV serology and HBV surface antigen

9 Leukapheresis to be performed after randomization, with sufficient time for IP/placebo manufacture prior to end of window between Screening Visit (Visit 1) and first infusion (Visit 3). Initial manufacture of blinded IP is expected to take a minimum of 28 days.

10 Blood draw (BD) or AVT001 (AVT) product (collection volumes and directions as per MOP)

11 Although visit windows allow a ±7 day window around visits, Study Drug administration must be at least 21 days between administration of double-blind study medication.

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16 Declarations of Sponsor and Investigator

Protocol Title: A Phase 1 / 2 Double-Blind, Randomized, Placebo Controlled Study of Safety, Tolerability and Potential Efficacy of AVOTRES Cell-Based Therapy (AVT001) in Patients with Type 1 diabetes

Protocol Number: AVT001-T1D-01

Protocol Version/ Date: Amendment 3 Dated 1Feb2021

Sponsor Name: Avotres Inc.

16. 1. Declaration of Sponsor

The above study protocol was reviewed and has been approved by the appropriate protocol review committee of the Sponsor. The information it contains is consistent with:

- The current risk-benefit evaluation of the IP;
- The moral, ethical, and scientific principles governing clinical research as set out in the Declaration of Helsinki and the principles of ICH guidelines for GCP and applicable local regulatory requirements.

The Investigator will be supplied with details of any significant or new findings, including adverse events (AE), related to the IP.

Sponsor
Signature: _____ Date:_____
Name (print): Hong Jiang, MD/Ph.D., Chief Scientific Officer, Avotres Inc.

16. 2. Declaration of Investigator

I confirm that I have read the above protocol. I understand it, and I will work according to the protocol and moral, ethical, and scientific principles governing clinical research as set out in the Declaration of Helsinki and the principles of ICH guidelines for GCP and according to applicable local regulatory requirements.

Investigator (Dr. Jason Gaglia)

Signature: _____ Date:_____
Name (print): Jason Gaglia, MD, MMSc
Institution name: Joslin Diabetes Center
Institution address: 1 Joslin Place, Boston, MA 02215

AMENDMENT 1: SUMMARY OF CHANGES FOR PROTOCOL AVT001-T1D-01

Investigational Product:

AVT001 Suspension for Intravenous Infusion
Hsp60sp-loaded Autologous Immature Dendritic Cells
($7x10^6$-$10x10^6$cells/dose)

Study Title:

A Phase 1 / 2 Double-Blind, Randomized, Placebo Controlled Study of Safety, Tolerability and Potential Efficacy of AVOTRES Cell-Based Therapy (AVT001) in Patients with Type 1 Diabetes

This document summarizes the changes from the original protocol (dated July 15, 2013) to Amendment 1 (dated January 28, 2019).

Amendment 1 maintains the same general study design as the original protocol. Modifications and clarifications have been made in Amendment 1, included both structural/formatting and content updates. The only key content updates are in reference to additions to the safety monitoring (eg, the addition of vital signs, ECGs), and further description of the DSMB reviews. There have been no other meaningful changes to the study design or procedures as described in the original protocol.

Because the structural changes to the format of the document resulted in renumbered the section headers, the first colum of the table in the following pages provides the Section Titles for the amended document only. This column also includes a brief description of the key change being described.

The second column includes the text/language from the original protocol.

The third column includes the revised/new language from the amended protocol.

The intent in this Summary of Changes document is not to provide documentation of each and every change (as the number of minor edits was substantial and would interfere with the clarity of this summary). Rather, the intent of this Summary of Changes is intended to communicate the key modifications in a clear, succinct and practical fashion.

Section Number / Section Title	Original Protocol	Amendment 1
All sections		The protocol document has had extensive formatting, structural and stylistic updates made throughout to better align with ICH and FDA guidances. The section numbers have also been updated and modified. Much of the detailed chemistry, manufacturing and control (CMC) and dosing procedures have been shifted to a manual of operating procedures (MoP) and allow for the removal of sections such as reconciliation and disposition processes.
Section 3: Synopsis	A "Study Summary" was provided.	An ICH-compliant synopsis has replaced the original 'Study Summary' table. Synopsis and protocol components have been aligned (e.g. objectives, endpoint, and inclusion / exclusion criteria.)
Section 3: (PI/Study Center) The first study center has been changed. Additional centers may be added as enrolment needs dictate.	Subjects will be recruited for this study from the clinical practices of the **Columbia University Medical Center** and its referral sources. Subjects will be required to give written informed consent to participate in the study before any screening tests or evaluations are conducted.	The first study center is now **the Joslin Diabetes Center, Boston,** with **Jason Gaglia**, MD, MMSc as the PI. However, an additional center may be added as enrollment and accrual needs dictate.
Section 4: Introduction		Minor updates to the background, disease description, and study treatment description have been made. AVT001 product name has been established.

Section Number / Section Title	Original Protocol	Amendment 1
Section 5: Study Objectives and Design The study objectives have been better aligned to reflect the primary focus of the study on safety and tolerability. Pharmacodynamic outcomes have been defined as secondary. These changes are aligned with the synopsis as well.	**Primary Objectives** – To determine the safety and to Tolerability of the AVOTRES reagent. – To determine whether, among T1D subjects with in vitro-reversible defects in the CD8+ T cell regulatory system, **infusion of AVOTRES will lead to an *in vivo* reconstitution of this system.** **Secondary Objectives** – To determine whether, among T1D subjects with in vitro-reversible defects in the CD8+ T cell regulatory system, infusion of AVOTRES will result in amelioration of β cell destruction.	**Primary Outcomes** • To determine the safety and tolerability profile of three administrations of a single dose AVT001 (as compared to placebo) in T1D subjects with *in vitro*-reversible defects in the CD8+ T cell regulatory system **Secondary Outcomes** • To determine whether, among T1D subjects with in vitro-reversible defects in the CD8+ T cell regulatory system, **infusion of AVT001 will lead to an *in vivo* reconstitution of this system.** • To determine whether, among T1D subjects with *in vitro*-reversible defects in the CD8+ T cell regulatory system, infusion of AVT001 will result in β cell preservation assessed by stimulated C-peptide.
Section 5.3: Study Design and Section 5.4: Study Duration	The duration of the study was defined as 24 months, with an additional 2 years of 'follow-up' to assess survival/disease status.	The duration of the study has been revised *via* the definition of three 'periods' being defined for the study, ie, Screening, Treatment Period (through Month 3), and Post-Treatment Follow-up Period (through Month 12). Thus, the **primary time point for assessment in the study is now Month 5**, the duration of the 'follow-up' portion study has been shortened to extend out to 12 months post-first dose, and the longer-term followup may occur through up to Month 24.

Section Number / Section Title	Original Protocol	Amendment 1
Section 5.5: Subject Enrollment The number of patients subject to staggered enrollment prior to open enrollments is reduced from 9 to 6. Reducing the number of staggered patients: Given the latest evidence and track record of safety with dendritic cell therapies since the initial filing of this IND (**references 1-9**), and the fact that in this study there is no dose escalation and we are evaluating a single concentration range of AVT001 therapy for each subject, we are proposing to reduce the number of staggered subjects to 6 individuals, separated by 4 weeks each. We believe that this, in continued oversight of unblinded data by the DSMB, will ensure adequate safety monitoring during the course of the trial.	NOTE: No specific language in the protocol. Instead, the staggered enrollment applies as follows: We will monitor the first 9 patients to make sure that at least 6 subjects in the treatment group will be observed for 4 weeks for acute and subacute adverse events. The information obtained will allow the clinical PI and Sponsor to determine if the time interval of 2 weeks or 4 weeks is sufficient, we will consult with the FDA to lengthen or shorten the 4-week observation of patient staggering period in the clinical trial. See attached:	Subject exposure will be managed using a **staggered enrollment** that will allow for **review of each subjects outcomes** following the initial IV. This staggered enrollment will be in-place for the first 6 subjects treated. Subjects treated at a rate of one subject per month, with Investigator and Medical Monitor review of the Month 1 safety and tolerability outcomes for each subject before treatment of the subsequent subject is performed.

Section Number / Section Title	Original Protocol	Amendment 1
Section 5.6: DSMB	The Data Safety and Monitoring Board (DSMB) will monitor the data quality and adherence to safety rules. Additionally, the DSMB will review all safety/toxicity data for the trial and recommend trial suspension or termination as needed. Specific details of monitoring and audit frequency will be included in the Monitoring Plan (see Appendix A) but will be at least every 6 months Investigators will report all SAEs to the DSMB within 10 business days regardless of expectedness or attribution. SAE will be input into the CRF with follow up email to DSMB.	A DSMB will be established that will monitor subject safety as well as data quality and adherence to safety rules. Additionally, the DSMB will review all safety/toxicity data for the trial and recommend trial modifications, suspension or termination, as needed. The **DSMB will initially meet after 3 subjects** and then after 6 subjects have received at least one dose of double-blind study medication and have completed their in-clinic visit through at least one month post-dose. The DSMB **will review unblinded** data to assess the safety and tolerability of the treatments given. At the second meeting (in which 6 subjects are being evaluated), the DSMB will also review preliminary efficacy data (in an unblinded fashion). The DSMB will, following each of these meetings, provide the Sponsor with a summary of their findings and recommendations (if any) for protocol amendments. The interval of subsequent meetings of the DSMB will be determined by the DSMB Chair and Sponsor. A DSMB Charter will be written, to include details regarding meeting frequency, format, and membership.

Section Number / Section Title	Original Protocol	Amendment 1
5.7: Study Endpoints	Primary Study Endpoints (a) Safety and tolerability of the AVOTRES reagent. The primary analysis will describe the incidence of adverse events and laboratory abnormalities. (b) Tolerability of the AVOTRES reagent. (c) **Activity of the HLA-E-restricted CD8+ T cell regulatory function** over a 1-year period following treatment with AVOTRES. Secondary Study Endpoints (a) The change in the area under the curve (AUC) of the stimulated C-peptide levels over the first 2 hours of a mixed meal tolerance test (MMTT) 3 months, 6 months, 12 months, 18 months, and 24 months (b) Change in A1c (c) Change in total daily insulin dose (d) Number of major hypoglycemic events (defined as loss of consciousness, seizure, or requiring assistance from another person because of altered state of consciousness) (e) Reported hypoglycemic events confirmed with capillary blood glucose measurement less than 70 mg/dl. (f) Autoantibody levels	The study endpoints have been re-ordered to better reflect the primary and secondary objectives fo the study. Thus, the primary endpoints are related only to safety and tolability, while the secondary endpoints are related to the pharmacodynamic objectives. **The primary endpoints are:** (a) The incidence of treatment-emergent adverse events (TEAEs). (b) Clinically significant changes from baseline in clinical laboratory parameters. (c) The incidence and severity of local IV-site reactions. (d) Changes from baseline in vital signs and electrocardiograms. (e) Incidence of severe hypoglycemic events. **Secondary endpoints will include:** **(a) Assessment of the HLA-E-restricted CD8+ T cell regulatory activity ("potency assay")** (b) Changes from baseline in the area under the curve (AUC) of the stimulated C-peptide levels over the first 4 hours of a mixed meal tolerance test (MMTT) (c) Changes from baseline in HbA1c (d) Change from baseline in insulin usage (e) Changes from baseline in autoantibody levels

Section 6: Subject Selection (Inclusion / Exclusion Criteria) The same underlying study population has been retained. Minor changes to individual inclusion and exclusion criteria have been made to better-define the eligibility requirements. Removed insulin prerequisite from original version. Removing the insulin therapy requirement: The criterion for requirement of insulin therapy per trial was revised to defer to the primary treating physician to provide standard of care insulin therapy, in order to provide maximal flexibility for the treating healthcare providers.	Subjects who meet all of the following criteria are eligible for enrollment as study participants: Diagnosis of Type 1 Diabetes by one or more of the following types of antibodies: anti-GAD65 (glutamic acid decarboxylase), anti–islet-cell antibody 512 (ICA512), anti-IA2 and anti-insulin antibody.subjects 1. Age 18 or older and able to provide informed consent for participation. 2. Must have stimulated C-peptide levels □0.2 pmol/ml measured during a mixed meal tolerance test (MMTT) 3. subjectsSubjectsRecent onset (diagnosis within the past 12 months) T1D subjects who screen positive for a CD8+ T cell defect that is correctable in vitro by co-culture with immature dendritic cells loaded with self-peptide Hsp60sp. 4. All subjects will be using insulin at the time of enrollment. 5. If a participant is female with reproductive potential, she must be willing to avoid pregnancy and have a negative pregnancy test during the 12 months of treatment and for an additional 3 months. 6. Signed and dated written informed consent Subjects who meet any of the following criteria are not eligible for enrollment as study participants: 1. SubjectsSubjectsSubjectsHemoglobin <10.0 g/dL; leukocytes <3,000/uL; neutrophils <1,500/uL; lymphocytes <800/uL; platelets <100,000/uL 2. Urine Albumin Excretion < 300mg/gmCr 3. eGFR > 60 mL/min/1.73m2 4. ALT/AST < 3x normal 5. Current use of immunosuppressive or immunomodulatory therapies, including pharmacologic doses of systemic steroids 6. Coincident medical condition likely to require immunosuppressive or immunomodulatory therapies	**6.1 Inclusion Criteria** Subjects who meet all of the following criteria are eligible for enrollment as study participants: 1. **Diagnosis of type 1 diabetes, within the past 12 months but at least 1 month prior to screening**, confirmed by positive lab result for one or more of the following types of autoantibodies: a. Glutamic acid decarboxylase (GAD65) b. Insulinoma associated protein 2 (IA-2, also known as ICA-512) c. Zinc transporter 8 (ZnT8). 2. Age 18 or older and able to provide informed consent. 3. Subjects who **screen positive for a CD8+ T cell defect that is correctable *in vitro*** by co-culture with immature dendritic cells loaded with the synthetic oligopeptide from Hsp60sp. 4. If a participant is female with reproductive potential, she must be willing to avoid pregnancy and have a negative pregnancy test through the duration of the trial. 5. Signed and dated written informed consent. **6.2 Exclusion Criteria** Subjects who meet any of the following criteria are not eligible for enrollment as study participants: 1. Subjects on existing treatment with poorly controlled glycemic control despite insulin therapy, who in the

	7. Coincident medical condition likely to limit short term (5 year) life expectancy (malignancy, symptomatic coronary artery disease, recent stroke) 8. Prior radiation therapy, immunotherapy, or chemotherapy 9. Serologic evidence of current HIV-1 or HIV-2 infection 10. Evidence of current hepatitis B as demonstrated by HBSAg or circulating hepatitis B genomes 11. Serologic evidence of hepatitis C infection 12. SubjectsAutoimmune disease aside from T1D 13. Pregnant or breastfeeding 14. Any chronic illness which, in the opinion of the principal investigator, should preclude participation in the trial 15. Adequate venous access to support leukapheresis	opinion of the investigator would not be a good candidate for participation in a clinical trial 2. Screening hemoglobin <10.0 g/dL; leukocytes <3,000/uL; neutrophils <1,500/uL; lymphocytes <800/uL; platelets <100,000/uL 3. Screening Urine Albumin Excretion > 300 mg/gmCr 4. Screening eGFR < 60 mL/min/1.73m^2 5. Screening ALT or AST > 1.5x upper limit of normal (ULN) 6. Screening bilirubin > 2.0 mg / dL 7. Current use of immunosuppressive or immunomodulatory therapies, including pharmacologic doses of systemic steroids. However, topical steroidal creams and inhaled steroids without large systemic absorption are allowed. 8. Coincident medical condition likely to require immunosuppressive or immunomodulatory therapies. 9. Coincident medical condition likely to limit short term (5 year) life expectancy (malignancy, symptomatic coronary artery disease, recent stroke) 10. Prior radiation therapy, immunotherapy, or chemotherapy 11. Serologic evidence of current HIV-1 or HIV-2 infection 12. Serologic evidence of hepatitis C infection 13. Serologic evidence of acute or chronic active hepatitis B as measured by Core

Section Number / Section Title	Original Protocol	Amendment 1
		Ab positive and / or Surface Antibody antigen positive 14. Subjects with non-T1D autoimmune conditions (except autoimmune thyroid and/or celiac diseases) 15. Women who are pregnant, breastfeeding, or planning pregnancy during the study period. 16. Inadequate venous access to support leukapheresis. 17. Any condition that in the opinion of the investigator(s) would preclude the subject from participating in a clinical trial. 18. Abnormal baseline ECG that in the opinion of the investigator or sponsor would pose a safety risk.

Section 6.3 Early Withdrawal of Sujects	Criteria for Premature Termination from the Study Subjects may withdraw from the study prior to the expected completion date for the following reasons: • Unacceptable toxicity and other safety reasons • Progression or disease relapse • Subject consent withdrawal • Decision by the investigators that withdrawing is in the patient's best interest • Death Standard supportive therapy will be maintained for subjects withdrawn from active treatment. Data Collection and Follow-up of Withdrawn ubjects Every effort will be made to collect toxicity information on withdrawn subjects. Subjects withdrawn prematurely from the trial will be followed-up for survival and disease status by the principal investigator or clinical investigators via clinic visit, telephone contact, or medical records review every 6 months for 2 years. Other methods that will be used for follow-up will include contacting next of kin, contacting referring or primary physicians, use of certified letters to the patient, and social security index.	If a patient is discontinued from the study prematurely, the Investigator must select the primary reason for discontinuation on the End of Study eCRF. In addition, every effort should be made to complete the assessments listed under the End of Treatment column on the Schedule of Events. Patients withdrawn from the study will be considered evaluable for statistical assessment. A patient may be removed from the study for the following medical or administrative reasons: • Adverse Event: If a patient experiences an adverse event that the patient finds unacceptable or that, in the judgment of the Investigator or the Medical Monitor presents an unacceptable consequence or risk to the patient, the patient may be discontinued from further participation in the study. • Administrative Discontinuation: After consultation with the Investigator or Medical Monitor, a patient may be discontinued from the study for failure to comply with protocol requirements. All instances of noncompliance must be documented in the eCRF. • Refusal of Assessments: If for any reason, following dosing, the patient refuses further assessment during the study, the patient shall be discontinued from the study and the reasons for refusal documented. Reasonable efforts shall be made to monitor the patient for adverse events following such discontinuation.

Section Number / Section Title	Original Protocol	Amendment 1
		Such efforts shall be documented. Standard supportive therapy will be maintained for subjects withdrawn from active treatment. Every effort will be made to collect safety data from withdrawn subjects.
Section 7: Description of Study Drug		Updated section in alignment with CMC procedures of drug supply including selection of **Dana Farber Cancer Institute Cell Manufacturing Core Facility** (DFCI-CMCF)
Section 8: Study Procedures The entire Study Procedures section has been updated to better-define the evaluations to be performed at each visit. The visit timing and assessments at each visit have been modified to allow for better and more meaningful data collection that is consistent with the study objectives and design.	See the original **Schedule of Events** at the end of this document	See the amended **Schedule of Events** at the end of this document. More details have been provoed regarding the procedures to be performed at each study visit. Additional safety assessments have been added, including vital signs and ECGs Visits have been added (and removed) to match the study design. MMTT Description has been added Many supply related details have been taken out and be placed into a manual of operating procedures (MoP). Study visit ranges have been modified to ensure timely yet convenient windows for a site visit from patients to maximize adherence to the schedule. Assay procedure clarifications have been added.

Section Number / Section Title	Original Protocol	Amendment 1
Section 9: Statistical Methodology The statistical methods sections have been rewritten to better reflect the timing of the analysis, to describe the analysis populations, and details as to how safety and tolerability data will be analyzed.		The entire Statistical Methods section has been rewritten and now better matches the study design and objectives.
Section 9.1: Sample Size Determination The sample size methodology has been updated to reflect the primary objective of the study (safety and tolerability).	Analysis assumes that the proportion of subjects given placebo exhibiting reversal at 12 months will be 0 and that at least 60% of the subjects receiving AVOTRES will exhibit in vivo evidence of reversal of the defect. With a randomization of 2:1 subjects to Avotres or Placebo. **N = 14 subjects randomized to Avotres and N = 7 subjects to placebo**. There would be 94% power to detect this difference in proportions of 0.6 with a one sided α <0.05 using the Fisher's exact test. Allowing for up to **10%** of subjects being unavailable for evaluation the total sample size is increased to **N = 24**.	The primary objective of this study is to assess the safety and tolerability of AVT001, as compared to placebo, in subjects with type 1 diabetes. A sample size of **14 subjects treated with AVT001** will provide at least 80% power to observe at least one occurrence of treatment-emergent adverse events with a true underlying incidence of 12%. The sample size provides the same power to detect at least one occurrence of local tolerability events relating to the i.v. administration of study drug. Secondary objectives of this study include assessment of changes in pharmacodynamic markers, including (increases) in the CD8+ T-cell reg system, C-peptide levels, and HbA1c. The sample size of 14 subjects treated with AVT001 and **7 subjects treated with placebo** will provide estimates of the mean and standard deviation of the treatment benefit of AVT001 as compared to placebo in these endpoints. To account for an estimated **10% potential dropout** prior to the completion of the Month 5 safety/efficacy assessment post-first dose, a total sample size of approximately **24 subjects** will be randomized, in a 2:1 ratio of AVT001:Placebo.

Section Number / Section Title	Original Protocol	Amendment 1
Section 11: Safety and Adverse Events		Clarification of safety endpoints has been added, as well as more robust description of safety data collection.
Section 11.3.2: Reporting of Serious Adverse Events – Study Sponsor Notification by Investigator The information of medical monitor for SAE has been updated	Name: Hong Jiang, MD, PhD Address: Columbia University Medical Center PH - 8 East, Ste. 101, 622 West 168th St New York, NY 10032 Phone: 212-305-9984/201-944-3352 Fax: 212-305-6070 Email: hj4@columbia.edu	Name: Dr. Kristy Freeman Woods, MD, MPH, FACP Address: Novella Clinical, 1700 Perimeter Park Drive, Morrisville, NC Phone: 919.313.7111 (US Toll-free:1-866-758-2798) Fax: 919.313.1412 (US Toll-free:1-866-761-1274) Email: Safety-Inbox@novellaclinical.com
Table 3: Schedule of Events	The original schedule of events can be found at the end of this document.	The amended schedule of events can be found at the end of this document. Changes included the addition of new visits, removal of select visits, addition of new safety procedures (ECG, vital signs), and more details on safety data collection.

Original Schedule of Events

VISIT	SCREEN	APH	VISIT	VISIT	VISIT	VISIT	VISIT	VISIT	VISIT	VISIT	VISIT
PROCEDURES	**SCREEN**	**APH**	**VAC#1**	**VAC#2**	**VAC#3**	**F/U**	**F/U**	**F/U**	**F/U**	**F/U**	**F/U**
STUDY DAY	***-60***	***-30***	***0***	***30***	***60***	***90***	***120***	***180***	***365***	***---***	***---***
STUDY MONTH	***-1***	***0***	***---***	***1***	***2***	***3***	***4***	***6***	***12***	***18***	***24***
STUDY YEAR	***---***	***---***	***---***	***---***	***---***	***---***	***---***	***---***	***1***	***---***	***2***
History[1]	X					X					
Physical Exam[2]	X					X			X		X
Pancreatic □ cell Function[3]	X		X	X	X	X		X	X	X	X
Laboratory	X		X	X	X	X			X		X
Adverse Event	X		X	X	X	X					
Viral Studies[5]	X										
Survival and Disease											
Serum Pregnancy	X										
Leukapheresis		X									
Potency Assay[7]	X	X				X		X	X	X	X

VISIT NAME	VISIT	VISIT	VISIT	VISIT
PROCEDURES	**LTFU**	**LTFU**	**LTFU**	**LTFU**
STUDY DAY	***---***	***---***	***---***	***---***
STUDY MONTH	***30***	***36***	***42***	***48***
STUDY YEAR	***---***	***3***	***---***	***4***
History[1]				
Physical Exam[2]		X		X

Pancreatic β cell Function[3]		X		X
Laboratory Studies[4]		X		X
Adverse Event Assessment				
Viral Studies[5]				
Survival and Disease	X	X	X	X
Serum Pregnancy[6]				
Leukapheresis				
Potency Assay[7]				

Amended Schedule of Events

Study Period	Screening		Double-Blind Treatment Period			Safety Assessment	Long-Term Follow-Up Period			
Study Day	**-60±7**	**-30±14**	**1[1]**	**30±7**	**60±7**	**90±7**	**150±7**	**270±7**	**360±7**	**560±7, 720±7 / Early Term**
Study Month	**-2**	**-1**	**-**	**1**	**2**	**3**	**5**	**9**	**12**	**18, 24**
Visit Name	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10, 11**
Informed Consent	X									
Vein Check	X									
Randomization[2]		X								
Medical History[3]	X		X							
Physical Exam[4]	X		X			X	X	X	X	X
Insulin Usage			X	X	X	X	X	X	X	X
Vital Signs	X		X	X	X	X	X	X	X	X
MMTT for C-peptide			X				X		X	X
Severe Hypoglycemic Events Assessment			X	X	X	X	X	X	X	X
HbA1c and Antibody Samples	X		X	X	X	X	X	X	X	X
Laboratory Studies[5]	X		X	X	X	X	X	X	X	X
ECGs	X									
Viral Studies[6]	X									
Urine Pregnancy test	X		X	X	X		X			X (Day 720 /ET only)
Local tolerability of infusion site			X	X	X	X				
Leukapheresis		X								
Potency Assay[7]	X (BD) [7]	X (AVT) [7]				X (BD) [7]	X (BD) [7]	X (BD) [7]	X (BD) [7]	X (BD) [7]
Study Drug Administration[8]			X	X	X					
Adverse Events			On-going							
Prior/Conmeds	Prior Meds		Conmeds							

[1] At Study Day 1, a review of Medical history, symptom-driven PE, and review of eligibility should be performed prior to treatment. Details are included in note 4.
[2] Randomization should occur after successful completion of leukapheresis.
[3] Medical history including prior treatment, current medicines, and drug allergies
[4] Physical exam including blood pressure, pulse, temperature, weight. Can be symptom-driven physical examinations, except at Screening, Month 5, and Month 12.
[5] CBC/differential, creatinine, AST, ALT, and total bilirubin; including creatinine clearance and albuminuria
[6] HIV-1, HIV-2, HTLV-1/2, and HCV serology and HBV surface antigen
[7] Blood draw (BD) ~80 mL, collected into Sodium Heparin blood collection tubes or AVT001 (AVT) product
[8] Although visit windows allow a ±7 day window around visits, Study Drug administration must be at least 21 days between administration of double-blind study medication.

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Summary of Protocol AVT001-T1D-01 From Amendment No. 1 to No. 2 Changes

Below is a by-section summary of changes associated with this protocol amendment. Minor schedule clarifications, updated versioning, as well as insignificant grammatical or format changes may not be listed. Specific language changes are either summarized in underlined/~~strikethrough~~ text or included in the tracked changes version of the protocol amendment and are not all entirely duplicated verbatim in this summary.

Section	Summary of Change
DSMB	Updated to include a third DSMB safety meeting and to remove the primary efficacy evaluation.
Eligibility	Select inclusion and exclusion criteria have been updated in the protocol to ensure consistency and clarity. In addition, inclusion criterion # 1 has been revised to reflect a previously approved administrative protocol change.
Investigational product, dosage and mode of administration	Revised to clarify that subjects randomized to AVT001 will have 3 such bags manufactured for infusion
Duration of treatment and Figure 1: Study Design Schematic	Screening and cell collection period revised to up to 3 months.
Efficacy Assessment	The following additional language has been inserted: The SAP will also detail the HLA-E restricted CD8+ regulatory T cell function analysis.
General Study Design	Removed text as indicated: Subjects will be monitored for physical or reported symptoms as well as for vital signs (including temperature, heart rate, blood pressure, and arterial oxygen saturation) ~~and changes in physical exam~~ throughout the infusion procedure and for a minimum of 2 hours following the procedure.
Stopping Rules	Added clarification of protocol attribution to include possibly, probably or definitely.
Early Withdrawal of Subjects	Last sentence in section revised as indicated: Every effort will be made to collect safety data from ~~withdrawn~~ these subjects through the end of study.
Table 2: Investigational Product	Dosage Form for both IP and Placebo clarified to include 10% DMSO in Human Serum Albumin
Screening/ Randomization/ Leukapheresis	Entire section revised and reordered to better describe sequencing of screening, randomization and leukapheresis, to reflect a previously approved administrative protocol change, to ensure consistency with the Schedule of Events, to refer the reader to the Manual of Operations for more detailed product handling and procedures, to add specificity of required lab and other assessments, and to add granularity around the randomization schema.

Section	Summary of Change
Double-Blind Treatment Period	The following additional language has been inserted: If, prior to dosing, a randomized subject is not suitable (ineligible) for the trial, that subject will be considered a "randomization failure" and will be replaced. Vital sign frequency peri-infusion is monitored per the site's standard of care and a recommendation for the minimal frequency is described in the MOP. Specificity of required laboratory assessments have been added/clarified.
Long-Term Follow-Up Period	Specificity of required laboratory assessments have been added/clarified.
Prior and Concomitant Therapy and Insulin Usage	The following additional language has been inserted: Additional instructions for use are provided on the insulin log.
Immunoassessment "Potency" Assay Procedure	Entire section revised to read: "Potency" Assay will be performed by the sponsor with the subject blood sample after AVT treatment (or placebo) to test if the function of Qa-1/HLA-E restricted CD8+ T cell is restored. Please see "Schedule of Events" for the dates/frequency. The visit-specific blood volumes as well as specimen collection, handling, and transport procedures are described in the MOP. "Potency" Assay will be also performed for "information only" with AVT001 made by DFCI-CMCF before applying to the subject. 2-3 vials of frozen ATVT001 (1x106 cells/vial) should be sent to the sponsor for such assessment.
Baseline characteristics, Patient Disposition and Medication	Entire section added as follows: Key baseline characteristics will be tabulated by treatment group. Patient disposition will be described, including patient completion status and reasons for premature discontinuation from the study. Prior and concomitant medications will be tabulated (with a focus on new-onset medications, ie, those that are started after study treatment has commenced).
Severe Hypoglycemic Events and Insulin Log	The following additional language has been inserted: The total daily dose of insulin will be tabulated by visit from the insulin logs. Daily insulin usage will be listed from these logs.
CD8+ T-cell reg system	Entire section added as follows: A "potency assay" has been established to measure the specific suppression (inhibition) of the CD8+ regulatory T-cells to the target cells as one parameter to evaluate the efficacy of the AVT001 treatment. The data of maximum suppression of CD8+ regulatory T-cells from treated T1D subjects before the treatment versus after the treatment, with healthy people as a system control, determines whether there is a regained suppressive function of the CD8+ regulatory T-cells following the AVT001 treatment. Such values might suggest the effectiveness of treatment. Analyses for these outcomes will be specified in the SAP.

Section	Summary of Change
Adverse Event Reporting Period	First paragraph revised as follows: The study period during which adverse events must be reported is defined as the period from the time of consent through the end of study or early termination, whichever is sooner. ~~as the period from the initiation of dosing (Day 1) to the end of the study treatment follow-up. For this study, the study treatment follow-up is defined as 30 days following the last administration of study treatment. SAEs that occur prior to Study Day 1 should also be reported.~~ In addition, the medical monitor is changed from Dr. Kristy Freeman Woods to Dr. Harvey Katzeff and Novella Clinical is changed to Iqvia Biotech.
Case Report Forms	Added that eCRFs are maintained in a CFR Title 21 part 11-compliant system.
Ethical Considerations	Removed reference to Appendix 4 for a copy of the Subject Informed Consent Form as this is a stand-alone document.
Table 3: Schedule of Events	Clarified which visits are blinded and which are not. Double-Blind Study Period (Visits 3-7) Open-Label Study Period (Visits 8-11) Clarified several footnotes to conform to protocol procedures, to clarify laboratory assessments, to correct Potency Assay collection requirements, and to reflect a previously approved administrative protocol change

Summary of Protocol AVT001-T1D-01 From Amendment No. 2 to No. 3 Changes

Below is a by-section summary of changes associated with this protocol amendment. Minor schedule clarifications, updated versioning, as well as insignificant grammatical or format changes may not be listed. Specific language changes are either summarized in underlined/~~strikethrough~~ text or included in the tracked changes version of the protocol amendment and are not all entirely duplicated verbatim in this summary.

Section	Summary of Change
Inclusion	Inclusion now permits participants aged 16 and older. Assent required, where applicable.
Exclusion	Exclusion criterion for bilirubin cut-off modified to include those with Gilbert's Syndrome as follows: Screening bilirubin > 2.0 mg / dL, or > 3.0 mg / dL for participants with Gilbert's Syndrome
Dosing Rationale	The following additional language has been added for clarification around rationale for including older adolescents aged 16-17: According to a 2017–2018 National Health Interview Survey (NHIS), National Center for Health Statistics, and Centers for Disease Control and Prevention report, as of 2018 an estimated 187,000 children and adolescents younger under 20 years of age had been diagnosed with with type 1 diabetes (49). Following the DSMB review of the safety data for the first 6 staggered adult subjects (18 years and older), no safety concerns were identified. Therefore, due to the safety established thus far and due to the high prevalence of disease in adolescents, this protocol is being amended (Amendment # 3) in a step-wise fashion to reduce the lower limit of age for eligibility to age 16. [With new reference]
Screening	Clarified that urine pregnancy testing expires after 90 days from collection (instead of 30 days)

STATISTICAL ANALYSIS PLAN

AVT001-T1D-01

A Phase 1 / 2 Double-Blind, Randomized, Placebo Controlled Study of Safety, Tolerability and Potential Efficacy of AVOTRES Cell-Based Therapy (AVT001) in Patients with Type 1 Diabetes

AUTHOR: MARIANA HILDESHEIM

VERSION NUMBER AND DATE: V1.0, 28MAR2022

STATISTICAL ANALYSIS PLAN SIGNATURE PAGE

Statistical Analysis Plan v1.0 (dated 28Mar2022) for protocol AVT001-T1D-01.

	Name	Signature	Date (DDMmmYYYY)
Author:	Mariana Hildesheim	DocuSigned by Mariana Hildesheim Mariana Hildesheim I approve this document 28-Mar-2022 | 21:26:19 JST 63E0BBDB276D464FA64BAB373E9E96F3	28-Mar-2022 | 21:26:26 JST
Position:	Sr Biostatistician		
Company:	IQVIA Biotech		

Upon review of this document, the undersigned approves this version of the Statistical Analysis Plan, authorizing that the content is acceptable for the reporting of this study.

	Name	Signature	Date (DDMmmYYYY)
Approved by:	Tuochuan Dong	DocuSigned by Tuochuan Dong Tuochuan Dong I approve this document 28-Mar-2022 | 07:20:27 PDT 890E07643E004E7AA3DC06C7F6163497	28-Mar-2022 | 07:20:51 PDT
Position:	Biostatistician		
Company:	Avotres, Inc.		

MODIFICATION HISTORY

Unique Identifier for this Version	Date of the Document Version	Author	Significant Changes from Previous Version
0.1	22Dec2021	Mariana Hildesheim	Not Applicable – First Version
0.2	20Jan2022	Mariana Hildesheim	Planned analyses revised to condense TLF counts
0.3	04Mar2022	Mariana Hildesheim	Sponsor review comments addressed
0.4	16Mar2022	Mariana Hildesheim	Sponsor review comments addressed
1.0	28Mar2022	Mariana Hildesheim	Final version

TABLE OF CONTENTS

1. INTRODUCTION

This statistical analysis plan (SAP) describes the rules and conventions to be used in the presentation and analysis of efficacy and safety data for protocol AVT001-T1D-01. It describes the data to be summarized and analyzed, including specifics of the statistical analyses to be performed.

This SAP is based on protocol amendment 3, dated 01Feb2021, inclusive of 2 administrative change letters to amendment 3, dated 16Jun2021 and 03Nov2021.

2. STUDY OBJECTIVES AND ENDPOINTS

2.1. PRIMARY OBJECTIVE AND ENDPOINTS

Objectives	Endpoints
To determine the safety and tolerability profile of three doses of AVT001 (as compared to placebo) in T1D subjects with*in-vitro*-reversible defects in the CD8+ T cell regulatory system.	The incidence of treatment-emergent adverse events (TEAEs). Clinically significant changes from baseline in clinical laboratory parameters. The incidence and severity of local IV-site reactions. Changes from baseline in vital signs and electrocardiograms. Incidence of severe hypoglycaemic events.

2.2. SECONDARY OBJECTIVES AND ENDPOINTS

Objectives	Endpoints
To determine whether, among T1D subjects with *in vitro*-reversible defects in the CD8+ T cell regulatory system, infusion of AVT001 will lead to an *in vivo* reconstitution of this system. To determine whether, among T1D subjects with *in vitro*-reversible defects in the CD8+ T cell regulatory system, infusion of AVT001 will result in amelioration of β cell destruction as assessed by stimulated C-peptide.	Assessment of the HLA-E-restricted CD8+ T cell regulatory activity ("potency assay"). Changes from baseline in the area under the curve (AUC) of the stimulated C-peptide levels over the first 4 hours of a mixed meal tolerance test (MMTT). Changes from baseline in HbA1c. Change from baseline in insulin usage. Changes from baseline in autoantibody levels.

2.3. EXPLORATORY OBJECTIVES AND ENDPOINTS

Although not specifically defined in the protocol, the following endpoints will be examined as exploratory.

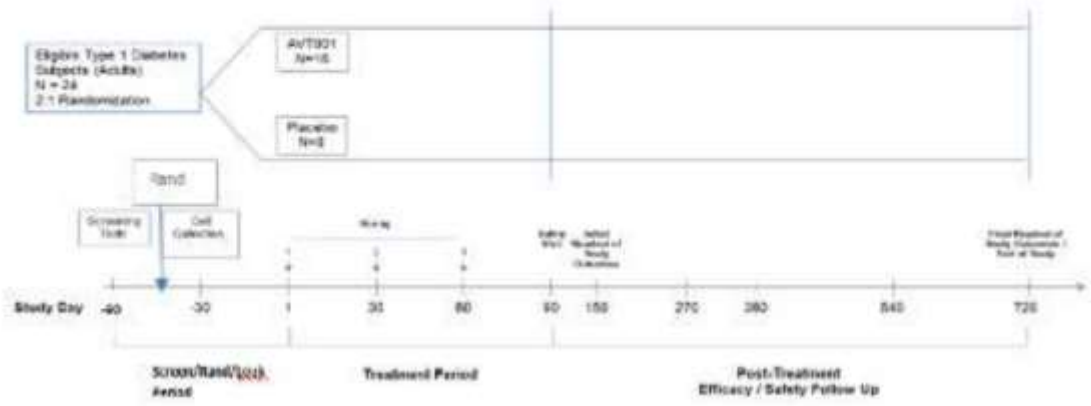
Objectives	Endpoints
To explore the effects of infusion of AVT001 on stimulated and unstimulated C-peptide and glucose measurements made over the first 4 hours of a mixed meal tolerance test (MMTT).	Changes from baseline in C-peptide and Glucose measured over the first 4 hours of a MMTT: • Unstimulated C-peptide • Delta AUC C-peptide • Cmax C-peptide • Delta Cmax C-peptide • Total AUC glucose • Unstimulated glucose • Delta AUC glucose • Cmax glucose • Delta Cmax glucose

3. STUDY DESIGN

3.1. GENERAL DESCRIPTION

This study is a phase 1 / 2 double-blind, randomized, placebo-controlled study to evaluate the safety and tolerability of AVT001 cell-based therapy, and to assess its potential efficacy for the treatment of type 1 diabetes (T1D). A brief overview of the trial design is outlined in Table A.

Table A: Study Design



3.2. SCHEDULE OF EVENTS

Schedule of events can be found in Table 3 of the protocol.

3.3. CHANGES TO ANALYSES FROM PROTOCOL

Potency assay data will be listed in the final Clinical Study Report. In addition, as an exploratory endpoint, it will be evaluated internally by Avotres and will be reported separately.

4. PLANNED ANALYSES

The following analyses will be performed for this study:

- Primary analysis after the last subject completes the Month 5 study visit (after study unblinding). All available data will be analyzed.
- Final analysis

4.1. DATA SAFETY MONITORING BOARD (DSMB)

A DSMB was established to monitor subject safety as well as data quality and adherence. Data reports for review by the members were generated from the electronic data capture (EDC) system by the IQVIA Biotech Data Management team. The IQVIA Biotech unblinded randomization statistician had a copy of the randomization schedule on hand at the closed sessions to provide the DSMB members with unblinded treatment assignments if needed.

Refer to the DSMB Charter for more details about the DSMB composition, responsibilities, and meeting frequency.

4.2. PRIMARY ANALYSIS

The primary analysis will take place for this study once the last subject completes the Month 5 study visit. The study database will be locked at this time and the treatment allocation codes unblinded for analysis. All analyses described in this plan will be produced. This analysis will form the primary basis for the assessment of the study objectives.

All planned analyses identified in the SAP will be performed by IQVIA Biotech Biostatistics following Sponsor Authorization of this SAP, Sponsor authorization of the analysis sets, database lock (DBL), and general study unblinding.

4.3. FINAL ANALYSIS

The final analysis will take place once the last subject completes the Month 24 long-term follow-up visit. All analyses described in this plan will be produced.

5. ANALYSIS POPULATIONS

5.1. ALL SCREENED SUBJECTS ANALYSIS POPULATION

The all screened subjects (SCRN) analysis population will contain all subjects who provide informed consent for this study.

5.2. SAFETY POPULATION

The safety (SAF) population is defined as all subjects who receive at least one dose of study medication. If there is any doubt whether a subject was treated or not, he/she will be assumed treated for the purposes of analysis.

For analyses and displays based on SAF, subjects will be classified according to the actual treatment received.

5.3. PHARMACODYNAMIC POPULATION

The pharmacodynamic (PD) population is defined as all subjects in the safety population with at least one post-baseline assessment for the evaluation of the CD8+ T-cell reg system biomarker. If there is any doubt whether a subject was treated or not, he/she will be assumed treated for the purposes of analysis.

For analyses and displays based on PD, subjects will be classified according to the actual treatment received.

5.4. PROCESS FOR ANALYSIS POPULATION ASSIGNMENT

For the PD population, the identification and agreement of protocol deviations or events which affect PD results will be performed between the biostatistician, and the sponsor, prior to DBL, with sponsor authorization of any excluded subjects or their data.

6. GENERAL CONSIDERATIONS

6.1. REFERENCE START DATE AND STUDY DAY

Study Day will be calculated from the reference start date and will be used to show start/stop day of assessments and events. Reference start date is defined as the day of the first injection of study drug i.e., Day 1.

Study Day will be computed as follows:

- Study Day = (Date of event –Date of first injection of study drug) + 1 if the date of the event is on or after the date of the first injection of study drug
- Study Day = (Date of event – Date of first injection of study drug) if the date of the event is prior to the date of the first injection of study drug

In the situation where the event date is partial or missing, Study Day and any corresponding durations will appear partial or missing in the listings.

6.2. BASELINE

Unless otherwise specified, baseline is defined as the last non-missing measurement taken prior to the first injection of study drug (including unscheduled assessments). In the case where the last non-missing measurement and the date and time of the first injection of study drug coincide, that measurement will be considered pre-baseline, but adverse events (AEs) and medications commencing on the date and time of the first injection of study drug will be considered post-baseline.

6.3. UNSCHEDULED VISITS, RETESTS, AND EARLY TERMINATION DATA

For by-visit summaries, data recorded at the nominal visit will be presented. That is, unscheduled, retest (same visit number assigned), and early termination measurements will not be included in by-visit summaries but might contribute to the baseline timepoint and/or maximum value, where required (e.g. shift table).

Listings will include scheduled, unscheduled, retest and early discontinuation data.

6.4. WINDOWING CONVENTIONS

No visit windowing for analysis purposes will be performed for this study.

6.5. COMMON CALCULATIONS

Change from baseline will be calculated as:

- Change from baseline = Test value at post-baseline visit – Baseline value

Percent change from baseline will be calculated as:

- Percent change from baseline (%) = (Change from baseline at post-baseline visit / Baseline value) * 100%

For select parameters where data is available, change and percent change will also be calculated from diagnosis.

7. STATISTICAL CONSIDERATIONS

For continuous data, descriptive statistics (i.e., n [number of subjects with available data], mean, standard deviation [SD], median, 25th and 75th percentiles, and minimum, and maximum values) will be presented by treatment group and visit, when applicable.

For categorical data, the number and percentages of subjects in each category will be presented by treatment group and visit, when applicable

7.1. SAMPLE SIZE CALCULATION

The sample size of 14 subjects treated with AVT001 will provide at least 80% power to observe at least one occurrence of treatment-emergent adverse events with a true underlying incidence of 12%. The sample size provides the same power to detect at least one occurrence of local tolerability events relating to the i.v. administration of study drug.

To account for an estimated 10% potential dropout prior to the completion of the Month 5 safety/efficacy assessment post-first dose, a total sample size of approximately 24 subjects will be randomized, in a 2:1 ratio of AVT001:Placebo.

7.2. MISSING DATA

Missing efficacy data will not be imputed.

Missing safety data will not be imputed, except for missing AE severity and relationship data (refer to Section 17.1.1). Partial or completely missing AE and medication dates will be handled as described in APPENDIX 1.

7.3. STATISTICAL TESTS

All statistical tests will be conducted at the two-sided 5% significant level, unless otherwise specified in the description of the analyses. Confidence Intervals (CIs) will be two-sided with 95% coverage.

7.4. MULTIPLE COMPARISONS/ MULTIPLICITY

No adjustment for multiple comparisons and multiplicity will be performed. Only nominal p-values will be

provided for the efficacy endpoints.

No statistical testing will be performed for the safety endpoints.

7.5. MULTICENTER STUDIES

This study will be conducted by one investigator at one center. Pooling of data for analysis is not applicable.

7.6. ADJUSTMENTS FOR COVARIATES AND FACTORS TO BE INCLUDED IN ANALYSES

Mixed-effect Model for Repeated Measurements (MMRM) analyses C-peptide total AUC will be adjusted for treatment, HbA1c, total insulin, BMI, potency assay, study visit, and treatment by study visit interaction. Section 16.2 for further details.

7.7. EXAMINATION OF SUBGROUPS

No subgroup analyses are planned.

7.8. SOFTWARE VERSION

All analyses will be conducted using SAS version 9.4.

8. OUTPUT PRESENTATIONS

The presentation of data in outputs will follow IQVIA Biotech Data Display Standards. Refer to the TFL shells document for additional information.

9. DISPOSITION AND WITHDRAWALS

All subjects who provide informed consent will be accounted for in this study.

9.1. DISPOSITION

Number of subjects screened will be presented overall for the SCRN analysis population. Number and percentage of subjects with screen failure and reason for screen failure will also be presented overall based on the SCRN analysis population. Number of subjects randomized will be presented overall and by treatment group for the SCRN analysis population.

Number and percentages of subjects treated, who completed/discontinued early from treatment (including reason for withdrawal), and who completed/discontinued early from the study (including reason for withdrawal) will be provided overall and by treatment group based on the SAF analysis population.

A listing showing inclusion and exclusion of each subject from each analysis population, including reason for exclusion, will be provided.

9.2. PROTOCOL DEVIATIONS

A listing of protocol deviations identified by the study team (important or not) will be provided.

10. DEMOGRAPHIC AND OTHER BASELINE CHARACTERISTICS

The following demographic and other baseline characteristics collected during the screening visit will be reported for this study:

- Age (years) – calculated relative to date of consent
- Age groups
- Sex
- Childbearing potential (for female subjects only)
- Race
- Ethnicity
- Weight (kg)
- Height (cm)

- Body Mass Index (BMI) (kg/m^2)

Continuous demographic and other baseline characteristics will be summarized using descriptive statistics overall and by treatment group based on the SAF analysis population. For categorical demographic and other baseline characteristics, number and percentage of subjects in each category will be provided overall and by treatment group based on the SAF analysis population. No statistical testing will be carried out for demographic or other baseline characteristics.

10.1. DERIVATIONS

BMI, in kg/m^2, will be calculated as follows:

- BMI (kg/ m^2) = weight (kg)/ [height (m)^2]

11. SURGICAL AND MEDICAL HISTORY

Surgical and medical history are defined as any surgeries that happened before the first injection of study drug and any medical conditions/diseases that started and stopped before the first injection of study drug.

Surgical and medical history will be coded using the Medical Dictionary for Regulatory Activities (MedDRA), version 22.0, and will be summarized by System Organ Class (SOC) and Preferred Term (PT) overall and by treatment group based on the SAF analysis population. A subject having more than one surgery/medical condition/disease within the same SOC/PT will be counted only once for that SOC or PT.

All surgical and medical history will be listed.

12. DISEASE HISTORY

The following disease history characteristics will be summarized overall and by treatment group based on the SAF analysis population:

- Time since diagnosis (days) – calculated relative to date of first infusion of study drug
- Use of insulin pump (yes/no)
- HbA1c at diagnosis, screening, and baseline (%)
- Change and percent change in HbA1c from diagnosis to baseline (%)

- Weight at diagnosis, screening, and baseline (kg)
- Change and percent change in weight from diagnosis to baseline (kg)
- BMI at diagnosis, screening, and baseline (kg/m^2)
- Change and percent change in BMI from diagnosis to baseline (kg/m^2)

All disease history characteristics will be listed.

12.1. DERIVATIONS

'Time since' disease history characteristics, in days, will be calculated as follows:

- Time since diagnosis (days) = (Date of first infusion of study drug - Date of diagnosis)

Change from diagnosis will be calculated as follows:

- Change from diagnosis = Test value at screening or first dosing visit – Diagnosis value

Percent change from baseline will be calculated as:

- Percent change from diagnosis (%) = (Change from diagnosis at screening or fist dosing visit / diagnosis value) * 100%

BMI, in kg/m^2, will be calculated as follows:

- BMI (kg/ m^2) = weight (kg)/ [height $(m)^2$]

13. MEDICATIONS

All medications will be coded using the World Health Organization (WHO) Drug Global dictionary, version B3 March 2020. Prior medications are defined as any medication that stopped before the date of first dose of study drug. Concomitant medications are defined as any medication that were ongoing or ended at the date of first dose of study drug, or any medication that started on or after the date of first dose of study drug.

Partially or completely missing medication start and stop dates will be handled as described in APPENDIX 1.

Prior and concomitant medications will be summarized separately by Anatomical Therapeutic Class (ATC) level 2 and preferred drug name by treatment arm and overall based on the safety analysis population. A subject having more than one medication within the same ATC Level 2 or preferred drug name will be counted only once for that ATC Level 2 or preferred drug name.

All medications (prior and concomitant) will be listed.

14. EXPOSURE TO STUDY DRUG

Exposure to the study drug will be summarized by treatment group based on the SAF analysis population for the following measures:

- Treatment duration, weeks
- Cumulative drug exposure, mL
- Total number of doses administered, n
- Dose intensity, %
- Any dose modification (dose held, interrupted, reduced, or permanently withdrawn)

14.1. DERIVATIONS

Measures of drug exposure will be calculated as follows:

- Treatment duration (weeks) = (Date of last injection of study drug – Date of first injection of study drug + 30.4167) /7
- Cumulative drug exposure (mL) = the sum of "Volume Administered (mL)" recorded on the *Dose* eCRF page, across all study dosing days
- Total number of doses administered = count of records on the *Dose* eCRF page where the response to the question "Was IP/Placebo administered at this visit" is Yes.
- Dose intensity (%) = (Cumulative drug exposure (mL) / Total planned dose (mL))*100, where total planned dose = (20 mL * 3)

The dates of first and last AVT001 administration will be taken from the eCRF *Dose* page.

15. COMPLIANCE WITH STUDY DRUG

Because IP will be administered intravenously to the subjects enrolled in the study in accordance with the protocol, compliance will not be assessed.

16. EFFICACY ENDPOINTS

Unless otherwise indicated, all efficacy summaries and figures will be presented by treatment group and visit, when appropriate, based on the SAF analysis population.

16.1. PRIMARY EFFICACY

There are no primary efficacy endpoints in this study.

16.2. SECONDARY EFFICACY

The secondary efficacy endpoints analyzed in this SAP are:

- Change from baseline in the area under the curve (AUC) of the stimulated C-peptide levels over the first 4 hours of a mixed meal tolerance test (MMTT).
- Change from baseline in HbA1c.
- Change from baseline in total, basal, and bolus daily insulin dose.
- Changes from baseline in autoantibody levels.

16.2.1. SECONDARY EFFICACY ENDPOINT VARIABLES AND DERIVATIONS

16.2.1.1. Change from Baseline in the Area under the Curve (AUC) of the stimulated C-peptide levels over the first 4 hours of a mixed meal tolerance test (MMTT)

The area under the stimulated C-peptide curve (AUC) over the first 4-hour period of a mixed meal glucose tolerance test will be calculated using the trapezoidal rule that is a weighted sum of the C-peptide values over the 240 minutes. Missing C-peptide levels at any given timepoint will not be imputed. In the calculation of the AUC when C-peptide levels are missing, a line will be drawn from the last timepoint with a non-missing C-peptide to the next timepoint

with non-missing C-peptide.

Change from baseline in C-peptide AUC will be computed for each post-baseline visit where MMTT was a scheduled assessment.

16.2.1.2. Change from Baseline in HbA1c

HbA1c will be measured at the first screening visit and at each dosing and long-term follow-up visit as per the schedule of events (refer to protocol Table 3).

Change from baseline in HbA1c will be computed for each post-baseline visit.

16.2.1.3. Change from Baseline in Total Daily Insulin Dose, Total Daily Basal Insulin Dose, and Total Daily Bolus Insulin Dose

Total daily insulin will be entered by subjects on an insulin log for 3-7 days prior to each study visit starting with Study Day 1.

Change from baseline in total daily insulin dose, total daily basal insulin dose, and total daily bolus insulin dose will be computed for each post-baseline visit.

16.2.1.4. Change from Baseline in Autoantibody Levels

Antibody levels to Glutamic acid decarboxylase (GAD65), Insulinoma associated protein (IA-2), Zinc transporter 8 (ZnT8), and Insulin associated aAb (IAA) will be measured at the first screening visit and at each dosing and long-term follow-up visit as per the schedule of event (refer to Protocol Table 3).

Change from baseline for each antibody type will be computed for each post-baseline visit.

16.2.2. Analysis of Secondary Efficacy Endpoints

16.2.2.1. Continuous Secondary Efficacy Endpoints

Observed and change from baseline for C-peptide AUC, HbA1c, total daily insulin dose, total daily basal insulin dose, total daily bolus insulin dose, and each of 4 antibodies will be summarized by treatment group and visit.

For the analysis of C-peptide AUC, a Mixed-effect Model for Repeated Measurements (MMRM) will be performed based on the PD analysis population using the observed-case data. This model will include the change from baseline

variable as the dependent variable, its baseline observed value as covariate, treatment group (active and placebo), visit, HbA1c, BMI, total insulin, potency assay, and treatment group-by-visit interaction as fixed effects. A backward stepwise variable selection process will be used to remove any non-relevant or highly correlated variables before fitting the final model. Restricted Maximum Likelihood (ReML) estimation will be used. An unstructured (UN) covariance structure will be used to model the within-patient error and an adjustment to the degrees of freedom will be made using the Kenward-Roger's approximation. In the event this model does not converge, the heterogeneous Toeplitz, heterogeneous first-order autoregressive (AR(1)), Toeplitz, and AR(1) covariance structures will be tested one after the other in the order listed here i.e., the heterogeneous Toeplitz covariance structure will be tested first and if this model converges, the other covariance structures will not be tested. If this model does not converge, then the heterogeneous AR(1) will then be tested. The same process will be repeated for each covariance structure one after the other as listed above until the model converges.

The Least Square (LS) mean estimates will be provided for each treatment group for each visit along with their standard error (SE). The difference of LS means between the treatment groups (active vs. placebo) and associated SE, two-sided 95% CI, and p-value will also be provided for each visit.

All remaining secondary endpoints (HbA1c, insulin, and auto-antibody levels) will be summarized univariately by generating summaries of the observed values and changes from baseline (both observed and percent changes) by study visit.

16.3. EXPLORATORY EFFICACY

The exploratory efficacy endpoints analyzed in this SAP are:

- Change from baseline in unstimulated C-peptide of a MMTT.
- Change from baseline in the change in the AUC of C-peptide (Delta AUC) over the first 4 hours of a MMTT.
- Change from baseline in the maximum concentration of C-peptide (Cmax) during a MMTT.
- Change from baseline in the maximum C-peptide levels (Delta Cmax) over the first 4 hours of a mixed meal tolerance test (MMTT).
- Change from baseline in the total area under the curve (AUC) of the stimulated glucose levels over the first 4 hours of a mixed meal tolerance test (MMTT).

- Change from baseline in unstimulated glucose of a MMTT.
- Change from baseline in the change in the AUC of glucose (Delta AUC) over the first 4 hours of a MMTT minus the unstimulated glucose AUC prior to MMTT.
- Change from baseline in the maximum concentration of glucose (Cmax) during a MMTT.
- Change from baseline in the maximum change from baseline concentration (Delta Cmax) of the stimulated glucose levels over the first 4 hours of a mixed meal tolerance test (MMTT)

16.3.1. EXPLORATORY EFFICACY ENDPOINT VARIABLES AND DERIVATIONS

16.3.1.1. Change from Baseline in unstimluated C-peptide of a MMTT

Unstimulated C-peptide will be defined as the average of C-peptide at time -10 and 0 minutes of a MMTT.

16.3.1.2. Change from Baseline in the change in AUC of C-peptide (Delta AUC) of a MMTT

The change in AUC of C-peptide (Delta AUC) will be defined as total AUC C-peptide over the first 4 hours of a MMTT minus the unstimulated C-peptide AUC at time -10 and 0 minutes of the same MMTT.

16.3.1.3. Change from Baseline in the maximum concentration of C-peptide (Cmax) of a MMTT

The maximum concentration of C-peptide will be defined as the maximum C-peptide value over the first 4 hours of a MMTT.

16.3.1.4. Change from Baseline in the maximum change from baseline concentration (Delta Cmax) of the stimulated C-peptide levels of a mixed meal tolerance test (MMTT)

The maximum change from baseline in C-peptide concentration (Delta Cmax) over the first 4-hour period of a mixed meal glucose tolerance test is defined as the maximum concentration of C-Peptide during the MMTT test minus the baseline concentration of C-Peptide, which is calculated as the average of C-Peptide at time -10 min and 0 min of the same MMTT test. Missing C-peptide levels at any given timepoint will not be imputed.

The maximum delta C-peptide Cmax will be computed for each post-baseline visit where MMTT was a scheduled assessment.

16.3.1.5. Change from Baseline in the AUC of the stimulated glucose of a MMTT

The area under the stimulated glucose curve (AUC) over the first 4-hour period of a mixed meal glucose tolerance test will be calculated the trapezoidal rule as described in Section 16.2.1.

16.3.1.6. Change from Baseline in unstimluated glucse of a MMTT

Unstimulated glucose will be defined using the approach described in Section 16.3.1.1.

16.3.1.7. Change from Baseline in the change in AUC of glucose (Delta AUC) of a MMTT

The change in AUC of glucose (Delta AUC) over the first 4-hour period of a MMTT will be defined using the approach described in Section 16.3.1.2.

16.3.1.8. Change from Baseline in the maximum concentration of glucose (Cmax) of a MMTT

The maximum glucose concentration (Cmax) over the first 4-hour period of a MMTT will be derived using the approach described in Section 16.3.1.3.

16.3.1.9. Change from Baseline in the maximum change from baseline concentration (Delta Cmax) of the stimulated glucose levels of a MMTT

The maximum change from baseline in glucose concentration (Delta Cmax) over the first 4-hour period of a MMTT will be derived using the approach described in Section 16.3.1.4.

16.3.2. ANALYSIS OF EXPLORATORY EFFICACY ENDPOINTS

16.3.2.1. Continuous Exploratory Efficacy Endpoints

Observed and change from baseline for all exploratory endpoints will be summarized by treatment group and visit. Univariate summaries of the observed values and changes from baseline (both observed and percent change) will be generated by study visit.

17. SAFETY ENDPOINTS

All safety summaries will be presented by treatment group based on the SAF population. There will be no statistical comparisons between the treatment groups for safety data.

17.1. ADVERSE EVENTS

- Prior adverse events (AEs) are defined as any AE that started or worsened in severity on or after the date of signed informed consent but before the first dose of study drug.
- Treatment-emergent AEs (TEAEs) are defined as any AE that starts on or after the first dose of study medication. This includes events that start within 30 days following the last dose.
- Post-treatment AEs are defined as adverse events that start more than 30 days following the last dose.

See APPENDIX 1 for handling of partial dates for AEs. In the case where it is not possible to define an AE as treatment emergent or not, the AE will be classified by the worst case; i.e. treatment emergent.

AEs will be coded using the MedDRA dictionary, version 22.0.

An overall summary of number and percentage of subjects within each of the categories described in the sub-sections below will be provided by treatment group based on the SAF analysis population. Should a subject experience multiple events within a category, the subject will be counted only once for that category. Each adverse event summary table will include a sub-table summarizing TEAEs and a sub-table summarizing post-treatment AEs.

All AEs (prior, TEAE, and post-treatment) will be listed. The listing will include a column to identify potential dose-limiting toxicities as collected on the AE CRF page.

17.1.1. ALL TEAES AND POST-TREATMENT AES

Number and percentage of subjects with at least one TEAE will be presented by SOC and PT. Should a subject experience multiple events within a SOC or PT, the subject will be counted only once for that SOC or PT. Subjects with at least one post-treatment AE will be summarized similarly.

Number and percentage of subjects with at least one TEAE will be broken down further by maximum severity. Subjects with at least one post-treatment AE will be summarized similarly.

17.1.1.1. CTCAE Grading for AEs

Severity will be classified according to the Common Terminology Criteria for AEs (CTCAE), version 4.03. TEAEs or post-treatment AEs starting after the first injection of study drug with a missing CTCAE grade will be classified as grade 3. Should a subject experience multiple events within a SOC or PT, only the subject's worst CTCAE grade will be counted for that SOC or PT.

17.1.1.2. Relationship to Study Drug

Relationship to study drug, as indicated by the Investigator, will be classified as unrelated, unlikely related, possibly related, probably related, or definitely related (increasing severity of relationship). A summary of TEAEs related to study drug by SOC and PT will be prepared. A sub-table of post-treatment AEs related to study by SOC and PT will also be prepared.

A "Related" AE is defined as a TEAE or a post-treatment AE with a relationship to study drug of possibly related, probably related, or definitely related, while a "Non-related" AE is defined as a TEAE or post-treatment AE with a relationship to study drug of unrelated or unlikely related. TEAEs or post-treatment AEs with a missing relationship to study drug will be regarded as related to study drug. Should a subject experience multiple events within a SOC or PT, only the subject's worst relationship will be counted for that SOC or PT.

17.1.2. THE LISTING OF ALL AES WILL INCLUDE A COLUMN FOR RELATEDNESS.ADVERSE EVENTS WITH AN OUTCOME OF DEATH

AEs with an outcome of death are those events which are recorded as "Fatal" on the AE page of the eCRF.

17.1.3. THE LISTING OF ALL AES WILL INCLUDE A COLUMN FOR OUTCOME.SERIOUS ADVERSE EVENTS

Serious adverse events (SAEs) are those events recorded as "Serious" on the AE page of the eCRF. A summary of serious TEAEs by SOC and PT will be prepared. A sub-table of post-treatment serious AEs by SOC and PT will also be prepared. Should a subject experience multiple events within a SOC or PT, the subject will be counted only once for that SOC or PT.

A listing of all SAEs will be provided

17.1.4. AEs Leading to Discontinuation of Study Drug

TEAEs leading to permanent discontinuation of study drug are those events recorded as "Drug Permanently Discontinued" on the AE pages of the eCRF. A summary of TEAEs leading to permanent discontinuation of study drug by SOC and PT will be prepared. A sub-table of post-treatment AEs leading to discontinuation of study drug by SOC and PT will also be prepared.

A listing of all AEs leading to permanent discontinuation of study drug will be provided.

17.1.5. Severe Hypoglycaemic Adverse Events

Severe hypoglycaemic adverse events will be the AEs coded to the "Hypoglycaemia" Preferred Term (PT). A listing of all severe hypoglycaemic events will be provided. A summary table of severe hypoglycaemic adverse events may be provided if needed.

17.1.6. Local IV-Site Reactions

Local IV-site reactions will be the AEs coded to the "Injection site reaction" MedDRA High Level Term (HLT). A listing of all IV-site reactions will be provided. A summary table of local IV-site reaction adverse events may be provided if needed.

17.2. Deaths

A listing of all deaths will be provided.

17.3. Laboratory Evaluations

A urine pregnancy test will be performed at screening. Chemistry, hematology, and urinalysis (microalbumin panel) will be performed as per the schedule of events (refer to protocol, Table 3). A list of laboratory parameters to be included in the outputs is included in APPENDIX 2.

Quantitative laboratory parameters reported as "< X", i.e. below the lower limit of quantification (BLQ) or "> X", i.e. above the upper limit of quantification (ULQ), will be converted to X for the purpose of quantitative summaries, but will be presented as recorded, i.e. as "< X" or "> X" in the listings.

The following summaries will be provided by treatment group based on the SAF analysis population for each

chemistry, hematology, and urine microalbumin laboratory parameter:

- Observed and change from baseline in Standard International (SI) units by visit (for quantitative parameters)
- Shift from baseline to the worst post-baseline observed value according to the Common Terminology Criteria for Adverse Events (CTCAE) toxicity grades (for quantitative parameters with available CTCAE toxicity grades; refer to Section 17.3.11)
- Shifts from baseline to the maximum/minimum post-baseline observed value according to normal range criteria (for quantitative parameters without CTCAE toxicity grades; refer to Section 17.3.12)

All laboratory data will be listed.

17.3.1. CTCAE Toxicity Grades

Quantitative laboratory parameters with available CTCAE toxicity grades will be categorized as follows where higher grades representing a more severe toxicity.

- Grade 1 (i.e., mild)
- Grade 2 (i.e., moderate)
- Grade 3 (i.e., severe)
- Grade 4 (i.e., life-threatening)
- Grade 5 (i.e., death)

Although not defined in the CTCAE toxicity grading system, version 4.03, non-missing laboratory parameter results not meeting any of the 5 grades defined in the CTCAE toxicity grading system will be categorized as 'No Event' for the purpose of the shift from baseline summaries.

17.3.2. Laboratory Normal Ranges

Quantitative laboratory parameters will be compared with the relevant laboratory normal ranges in SI units and categorized as:

- Low: Below the lower limit of the laboratory normal range.
- Normal: Within the laboratory normal range (upper and lower limit included).

- High: Above the upper limit of the laboratory normal range.

17.4. VITAL SIGNS

The following vital sign parameters will be collected for this study as per the schedule of events (refer to protocol, Table 3):

- Systolic blood pressure (SBP) (mmHg)
- Diastolic blood pressure (DBP) (mmHg)
- Pulse rate (beats per minute [bpm])
- Oxygen saturation (%)
- Body temperature (ºC)
- Weight (kg)

The following summaries will be provided by treatment group based on the SAF analysis population for each vital sign parameter:

- Observed and change from baseline by visit and timepoint
- Number and percentages of subjects with at least one markedly abnormal post-baseline observed value/change from baseline (refer to Section 17.4.1)

All vital sign data will be listed. Vitals signs meeting one of the markedly abnormal criteria in Section 17.4.1 will be identified.

17.4.1. VITAL SIGNS MARKEDLY ABNORMAL CRITERIA

Markedly abnormal vital sign observed values and/or change from baseline will be identified in accordance with the following predefined markedly abnormal criteria:

Variable	Unit	Low	High
SBP	mmHg	≤ 90 mmHg AND change from baseline ≤ -20 mmHg	≥ 180 mmHg AND change from baseline ≥ 20 mmHg

Variable	Unit	Low	High
DBP	mmHg	≤ 50 mmHg AND change from ≤ -15 mmHg	≥ 105 mmHg AND change from baseline ≥ 15 mmHg
Pulse rate	bpm	≤ 50 bpm AND change from baseline ≤ -15 bpm	≥ 120 bpm AND change from baseline ≥ 15 bpm
Oxygen saturation	%	< 94 %	Not applicable
Body temperature	°C	Not applicable	≥ 38.3 °C AND change from baseline ≥ 1.1 °C
Weight	kg	Percent change from baseline ≤ -7.0 %	Percent change from baseline > 7.0 %

17.5. ECG EVALUATIONS

The following electrocardiogram (ECG) parameters will be measured for this study as per the schedule of events (refer to protocol, Table 3):

- PR interval (msec)
- RR interval (msec)
- QRS interval (msec)
- QT (uncorrected) interval (msec)
- Overall ECG evaluation (Investigator's judgment)
    - Normal
    - Abnormal, not clinically significant (NCS)
    - Abnormal, clinically significant (CS)

The following summaries will be provided by treatment group based on the SAF analysis population for each ECG

parameter:

- Observed and change from baseline by visit (for quantitative parameters)
- Number and percentages of subjects with at least one markedly abnormal post-baseline observed value/change from baseline (for quantitative parameters; refer to Section 17.5.11)
- Shift from baseline in overall ECG interpretation to the worst post-baseline assessment

All ECG data will be listed. ECG results meeting one of the markedly abnormal criteria in Section 17.5.1 will be identified.

17.5.1. ECG MARKEDLY ABNORMAL CRITERIA

Markedly abnormal quantitative ECG parameters will be identified in accordance with the following predefined markedly abnormal criteria:

- Observed values for QT (uncorrected) interval will be classified as:
    - > 450 msec
    - > 480 msec
    - > 500 msec
- Change from baseline for QT (uncorrected) interval will be classified as:
    - >30 msec increase from baseline
    - >60 msec increase from baseline

It is to be noted that the previous categories are not mutually exclusive, but cumulative. For example, if a subject worst post-baseline QT post-baseline observed value is 490 mmHg, then this subject will be reported once under QT > 450 msec and once under QT > 480 msec.

18. DATA NOT SUMMARIZED OR PRESENTED

Data that will not be summarized or listed are:

- Results of serology tests performed (i.e., HIV, Hepatitis A, B, and C) at screening for the purpose of

determining eligibility.

APPENDIX 1. PARTIAL DATE CONVENTIONS

ALGORITHM FOR PRIOR ADVERSE EVENTS, TREATMENT-EMERGENT ADVERSE EVENTS, AND POST-TREATMENT ADVERSE EVENTS

START DATE	END DATE	ACTION
Known	Known/Partial/ Missing	If (AE start date < study drug start date) or AE is a pre-initial dose event, then PRAE. If study drug start date ≤ AE start date and AE start date <= last drug date_30 and AE is not a pre-initial dose event, then TEAE. If study drug start date <= AE start date and AE start date > last drug date + 30 then PTAE
Partial, but known components show that AE started before study drug start date or AE is a pre-initial dose event	Known/Partial/ Missing	PRAE
Partial and known components show that AE started on or after study drug start date	Known/Partial/ Missing	Impute missing AE start day as the first of the month or the first study drug dose date, whichever is later. If (AE start date < study drug start date) or AE is a pre-initial dose event, then PRAE. If study drug start date ≤ AE start date and AE start date <= last drug date+30 and AE is not a pre-initial dose event, then TEAE. If study drug start date <= AE start date and AE start date > last drug date+30 then PTAE

Note: If an AE started prior to first dose of study drug but worsened after treatment started, it will be reported as a separate adverse event with a new AE start date. A pre-initial dose event is defined as an AE that is entered in the EDC as occurring prior to infusion (Month 0, Day 1).

ALGORITHM FOR PRIOR / CONCOMITANT MEDICATIONS

START DATE	STOP DATE	ACTION
Known	Known or ongoing	If medication stop date < study drug start date, assign as prior; Otherwise, assign as concomitant.
	Partial	If known components of medication stop date show that medication stopped before study drug start date, assign as prior; Otherwise, assign as concomitant.
	Missing, not ongoing	Can never be assigned as prior, therefore assign as concomitant.
Partial	Known or ongoing	If medication stop date < study drug start date, assign as prior; Otherwise, assign as concomitant.
	Partial	If known components of medication stop date show that medication stopped before study drug start date, assign as prior; Otherwise, assign as concomitant.
	Missing, not ongoing	Cannot be assigned as prior, therefore assign as concomitant.
Missing	Known or ongoing	If medication stop date < study drug start date, assign as prior; Otherwise, assign as concomitant.
	Partial	If known components of medication stop date show that medication stopped before study drug start date, assign as prior; Otherwise, assign as concomitant.
	Missing, not ongoing	Assign as concomitant.

APPENDIX 2. LABORATORY ASSESSMENTS

Chemistry (SI unit)

- Blood urea nitrogen (BUN) (mmol/L)
- Creatinine (µmol/L)
- Calcium (mmol/L)
- Chloride (mmol/L)
- Potassium (mmol/L)
- Sodium (mmol/L)
- Bicarbonate (mmol/L)
- Glucose (mmol/L)
- Alkaline phosphatase (ALP) (U/L)
- Alanine transaminase (ALT) (U/L)
- Aspartate transaminase (AST) (U/L)
- Albumin (g/L)
- Total protein (g/L)
- Total bilirubin (µmol/L)
- HbA1c, L/L
- Creatinine Clearance, mL/s

Hematology (SI unit)

- Hemoglobin (g/L)
- Hematocrit
- Red blood cells (RBC) (x10E12/L)
- White blood cell (WBC) count total (x10E9/L)
- Platelets (x10E9/L)
- Absolute neutrophils count (x10E9/L)
- Absolute lymphocyte count (x10E9/L)
- Absolute monocyte count (x10E9/L)
- Absolute eosinophils count (x10E9/L)
- Absolute basophils count (x10E9/L)

Urine Microalbumin Panel
• Urine Microalbumin (mg/dL) • Urine Creatinine (mg/dL) • Microalbumin/Creatinine Ratio (ratio)

STATISTICAL ANALYSIS PLAN

AVT001-T1D-01

A Phase 1 / 2 Double-Blind, Randomized, Placebo Controlled Study of Safety, Tolerability and Potential Efficacy of AVOTRES Cell-Based Therapy (AVT001) in Patients with Type 1 Diabetes

AUTHOR: CARLOS ALEJANDRO DIAZ

VERSION NUMBER AND DATE: V2.0, 06DEC2022

STATISTICAL ANALYSIS PLAN SIGNATURE PAGE

Statistical Analysis Plan v2.0 (dated 23Nov2022) for protocol AVT001-T1D-01.

	Name	Signature	Date (DDMmmYYYY)
Author:	Carlos Alejandro Diaz		06Dec2022
Position:	Sr. Biostatistician		
Company:	IQVIA Biotech		

Upon review of this document, the undersigned approves this version of the Statistical Analysis Plan, authorizing that the content is acceptable for the reporting of this study.

	Name	Signature	Date (DDMmmYYYY)
Approved by:	Tuochuan Dong		06Dec2022
Position:	Biostatistician		
Company:	Avotres, Inc.		

MODIFICATION HISTORY

Unique Identifier for this Version	Date of the Document Version	Author	Significant Changes from Previous Version
0.1	22Dec2021	Mariana Hildesheim	Not Applicable – First Version
0.2	20Jan2022	Mariana Hildesheim	Planned analyses revised to condense TLF counts
0.3	04Mar2022	Mariana Hildesheim	Sponsor review comments addressed
0.4	16Mar2022	Mariana Hildesheim	Sponsor review comments addressed
1.0	28Mar2022	Mariana Hildesheim	Final version
1.1	26Ago2022	Carlos Alejandro Díaz	Text updates to include the changes described in the memo-to-file (22Apr2022) to amend the SAP v1.0, as well as to include the 'Supplemental Analysis' described in section 4.3, and additional MMRM in section 16.2.2.1.
1.2	10Nov2022	Carlos Alejandro Díaz	Draft version including Day 360 Analysis
2.0	06Dec2022	Carlos Alejandro Díaz	Final version sign-off, including Day 360 Analysis

TABLE OF CONTENTS

1. INTRODUCTION

This statistical analysis plan (SAP) describes the rules and conventions to be used in the presentation and analysis of efficacy and safety data for protocol AVT001-T1D-01. It describes the data to be summarized and analyzed, including specifics of the statistical analyses to be performed.

This SAP is based on protocol amendment 3, dated 01Feb2021, inclusive of 2 administrative change letters to amendment 3, dated 16Jun2021 and 03Nov2021. The SAP V2.0 amendment was done to include the supplemental analysis at Day 360.

2. STUDY OBJECTIVES AND ENDPOINTS

2.1. PRIMARY OBJECTIVE AND ENDPOINTS

Objectives	Endpoints
To determine the safety and tolerability profile of three doses of AVT001 (as compared to placebo) in T1D subjects with*in-vitro*-reversible defects in the CD8+ T cell regulatory system.	Incidence of treatment-emergent adverse events (TEAEs). Clinically significant changes from baseline in clinical laboratory parameters. Incidence and severity of local IV-site reactions. Changes from baseline in vital signs and electrocardiograms. Incidence of severe hypoglycaemic events.

2.2. SECONDARY OBJECTIVES AND ENDPOINTS

Objectives	Endpoints
To determine whether, among T1D subjects with *in vitro*-reversible defects in the CD8+ T cell regulatory system, infusion of AVT001 will lead to an *in vivo* reconstitution of this system. To determine whether, among T1D subjects with *in vitro*-reversible defects in the CD8+ T cell regulatory system, infusion of AVT001 will result in amelioration of β cell destruction as assessed by stimulated C-peptide.	Assessment of the HLA-E-restricted CD8+ T cell regulatory activity ("potency assay"). Changes from baseline in the area under the curve (AUC) of the stimulated C-peptide levels over the first 4 hours of a mixed meal tolerance test (MMTT). Changes from baseline in HbA1c. Change from baseline in insulin usage. Changes from baseline in autoantibody levels.

2.3. EXPLORATORY OBJECTIVES AND ENDPOINTS

Although not specifically defined in the protocol, the following endpoints will be examined as exploratory.

Objectives	Endpoints
To explore the effects of infusion of AVT001 on stimulated and unstimulated C-peptide and glucose measurements made over the first 4 hours of a mixed meal tolerance test (MMTT).	Changes from baseline in C-peptide and Glucose measured over the first 4 hours of a MMTT: • Unstimulated C-peptide • Delta AUC C-peptide • Cmax C-peptide • Delta Cmax C-peptide • Total AUC glucose • Unstimulated glucose • Delta AUC glucose • Cmax glucose • Delta Cmax glucose

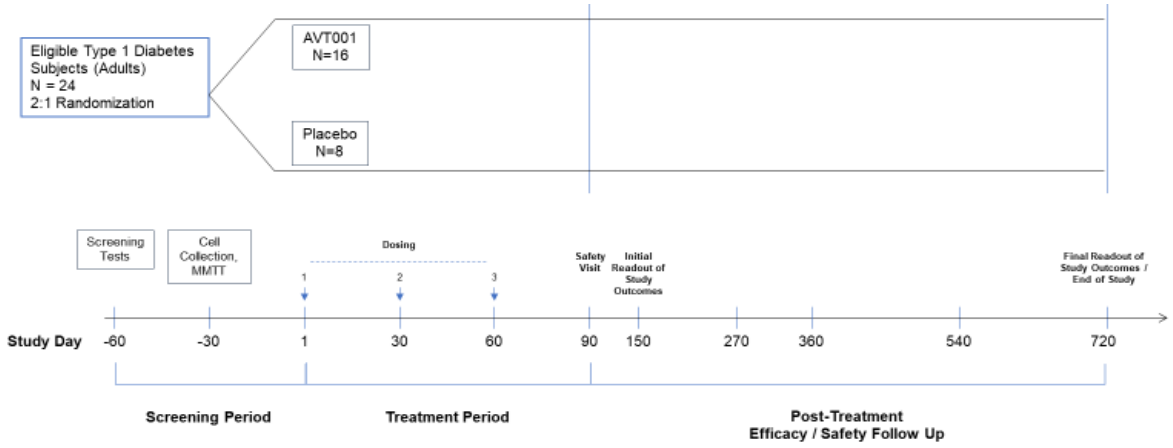
3. STUDY DESIGN

3.1. GENERAL DESCRIPTION

This study is a phase 1 / 2 double-blind, randomized, placebo-controlled study to evaluate the safety and tolerability of AVT001 cell-based therapy, and to assess its potential efficacy for the treatment of type 1 diabetes (T1D). A brief overview of the trial design is outlined in Table A.

Table A: Study Design

3.2. SCHEDULE OF EVENTS



Schedule of events can be found in Table 3 of the protocol.

3.3. CHANGES TO ANALYSES FROM PROTOCOL

Potency assay data will be listed in the final Clinical Study Report (CSR).

In addition, as an exploratory endpoint, the potency assay data will be evaluated internally by Avotres and will be reported separately.

A supplemental analysis that will occur after the last subject completes the Month 12 study visit (Day 360) has been added to SAP v2.0.

4. PLANNED ANALYSES

The following analyses will be performed for this study:

- Primary analysis after the last subject completes the Month 5 study visit (after study unblinding). All available data will be analyzed.
- Supplemental analysis after the last subject completes the Month 12 study visit (Day 360).

4.1. FINAL ANALYSIS AFTER THE LAST SUBJECT COMPLETES THE MONTH 24 LONG-TERM FOLLOW-UP VISIT. DATA SAFETY MONITORING BOARD (DSMB)

A DSMB was established to monitor subject safety as well as data quality and adherence. Data reports for review by the members were generated from the electronic data capture (EDC) system by the IQVIA Biotech Data Management team. The IQVIA Biotech unblinded randomization statistician had a copy of the randomization schedule on hand at the closed sessions to provide the DSMB members with unblinded treatment assignments if needed.

Refer to the DSMB Charter for more details about the DSMB composition, responsibilities, and meeting frequency.

4.2. PRIMARY ANALYSIS

The primary analysis had taken place for this study after the last subject completes the Month 5 study visit (SAP v1.0 was finalized on 28MAR2022 and amended via memo-to-file on 22APR2022). Data used of the primary analysis was extracted and locked 29APR2022 04:04, except the potency assay. Unblinding was performed on 17MAY2022, after the transfer of potency assay data from Avotres had been completed. All analyses described in SAP v1.0 and the binding amendment plan were produced and delivered to the sponsor on 10JUN2022. This analysis formed the primary basis for the assessment of the study objectives.

All planned analyses identified in the SAP had been performed by IQVIA Biotech Biostatistics following Sponsor Authorization of this SAP, Sponsor authorization of the analysis sets, database lock (DBL), and general study unblinding.

4.3. SUPPLEMENTAL ANALYSIS

A supplemental analysis will take place for this study after the last subject completes the Month 12 study visit (Day 360). The study database will undergo an interim lock at this time. All analyses described in the current document will be produced for the supplemental analysis. This analysis will add information to the assessment of the study objectives, since data at Day 360 is generally accepted by the T1D medical community as sufficient long-term data to evaluate the efficacy and safety of a clinical candidate (AVT001). Moreover, data at Day 360 will facilitate the discussion of the development milestones for AVT001.

All planned analyses identified in the SAP will be performed by IQVIA Biotech Biostatistics following Sponsor authorization of this SAP, and database lock (DBL). The study unblinding has already occurred at the time of the primary analysis.

4.4. FINAL ANALYSIS

The final analysis will take place once the last subject completes the Month 24 long-term follow-up visit. The SAP amendment for the final analysis will be updated before the corresponding DBL.

5. ANALYSIS POPULATIONS

5.1. ALL SCREENED SUBJECTS ANALYSIS POPULATION

The all screened subjects (SCRN) analysis population will contain all subjects who provide informed consent for this study.

5.2. SAFETY POPULATION

The safety (SAF) population is defined as all subjects who receive at least one dose of study medication. If there is any doubt whether a subject was treated or not, he/she will be assumed treated for the purposes of analysis.

For analyses and displays based on SAF, subjects will be classified according to the actual treatment received.

5.3. PHARMACODYNAMIC POPULATION

The pharmacodynamic (PD) population is defined as all subjects in the safety population with at least one post-baseline assessment for the evaluation of the CD8+ T-cell reg system biomarker. If there is any doubt whether a subject was treated or not, he/she will be assumed treated for the purposes of analysis.

For analyses and displays based on PD, subjects will be classified according to the actual treatment received.

5.4. PROCESS FOR ANALYSIS POPULATION ASSIGNMENT

For the PD population, the identification and agreement of protocol deviations or events which affect PD results will be performed between the biostatistician, and the sponsor, prior to DBL, with sponsor authorization of any excluded subjects or their data.

6. GENERAL CONSIDERATIONS

6.1. REFERENCE START DATE AND STUDY DAY

Study Day will be calculated from the reference start date and will be used to show start/stop day of assessments and events. Reference start date is defined as the day of the first injection of study drug i.e., Day 1.

Study Day will be computed as follows:

- Study Day = (Date of event –Date of first injection of study drug) + 1 if the date of the event is on or after the date of the first injection of study drug
- Study Day = (Date of event – Date of first injection of study drug) if the date of the event is prior to the date of the first injection of study drug

In the situation where the event date is partial or missing, Study Day and any corresponding durations will appear partial or missing in the listings.

6.2. BASELINE

Unless otherwise specified, baseline is defined as the last non-missing measurement taken prior to the first injection of study drug (including unscheduled assessments). In the case where the last non-missing measurement and the date

and time of the first injection of study drug coincide, that measurement will be considered pre-baseline, but adverse events (AEs) and medications commencing on the date and time of the first injection of study drug will be considered post-baseline.

6.3. UNSCHEDULED VISITS, RETESTS, AND EARLY TERMINATION DATA

For by-visit summaries, data recorded at the nominal visit will be presented. That is, unscheduled, retest (same visit number assigned), and early termination measurements will not be included in by-visit summaries but might contribute to the baseline timepoint and/or maximum value, where required (e.g. shift table).

Listings will include scheduled, unscheduled, retest and early discontinuation data.

6.4. WINDOWING CONVENTIONS

No visit windowing for analysis purposes will be performed for this study.

6.5. COMMON CALCULATIONS

Change from baseline will be calculated as:

- Change from baseline = Test value at baseline or post-baseline visit – Baseline value

Percent change from baseline will be calculated as:

- Percent change from baseline (%) = (Change from baseline at baseline or post-baseline visit / Baseline value) * 100%

For select parameters where data is available, change and percent change will also be calculated from diagnosis.

7. STATISTICAL CONSIDERATIONS

For continuous data, descriptive statistics (i.e., n [number of subjects with available data], mean, standard deviation [SD], median, 25th and 75th percentiles, and minimum, and maximum values) will be presented by treatment group and visit, when applicable.

For categorical data, the number and percentages of subjects in each category will be presented by treatment group and visit, when applicable

7.1. SAMPLE SIZE CALCULATION

The sample size of 14 subjects treated with AVT001 will provide at least 80% power to observe at least one occurrence of treatment-emergent adverse events with a true underlying incidence of 12%. The sample size provides the same power to detect at least one occurrence of local tolerability events relating to the i.v. administration of study drug.

To account for an estimated 10% potential dropout prior to the completion of the Month 5 safety/efficacy assessment post-first dose, a total sample size of approximately 24 subjects will be randomized, in a 2:1 ratio of AVT001:Placebo.

7.2. MISSING DATA

Missing efficacy data will not be imputed, except for missing potency assay on Visit 3/Day 1, as described in APPENDIX 1.

Missing safety data will not be imputed, except for missing AE severity and relationship data (refer to Section 17.1.1). Partial or completely missing AE and medication dates will be handled as described in APPENDIX 1.

7.3. STATISTICAL TESTS

All statistical tests will be conducted at the two-sided 5% significant level, unless otherwise specified in the description of the analyses. Confidence Intervals (CIs) will be two-sided with 95% coverage.

7.4. MULTIPLE COMPARISONS/ MULTIPLICITY

No adjustment for multiple comparisons and multiplicity will be performed. Only nominal *p-values* will be provided for the efficacy endpoints.

No statistical testing will be performed for the safety endpoints.

7.5. MULTICENTER STUDIES

This study will be conducted by one investigator at one center. Pooling of data for analysis is not applicable.

7.6. ADJUSTMENTS FOR COVARIATES AND FACTORS TO BE INCLUDED IN ANALYSES

Mixed-effect Model for Repeated Measurements (MMRM) analyses C-peptide total AUC will be adjusted for treatment group, visit, HbA1c, BMI, total daily insulin divided by weight, potency assay, and treatment group-by-visit interaction as fixed effects. See section 16.2 for further details.

7.7. EXAMINATION OF SUBGROUPS

No subgroup analyses are planned.

7.8. SOFTWARE VERSION

All analyses will be conducted using SAS version 9.4.

8. OUTPUT PRESENTATIONS

The presentation of data in outputs will follow IQVIA Biotech Data Display Standards. Refer to the TFL shells document for additional information.

9. DISPOSITION AND WITHDRAWALS

All subjects who provide informed consent will be accounted for in this study.

9.1. DISPOSITION

Number of subjects screened will be presented overall for the SCRN analysis population. Number and percentage of subjects with screen failure and reason for screen failure will also be presented overall based on the SCRN analysis population. Number of subjects randomized will be presented overall and by treatment group for the SCRN

analysis population.

Number and percentages of subjects treated, who completed/discontinued early from treatment (including reason for withdrawal), and who completed/discontinued early from the study (including reason for withdrawal) will be provided overall and by treatment group based on the SAF analysis population.

A listing showing inclusion and exclusion of each subject from each analysis population, including reason for exclusion, will be provided.

9.2. PROTOCOL DEVIATIONS

A listing of protocol deviations identified by the study team (important or not) will be provided.

10. DEMOGRAPHIC AND OTHER BASELINE CHARACTERISTICS

The following demographic and other baseline characteristics collected during the screening visit will be reported for this study:

- Age (years) – calculated relative to date of consent
- Age groups
- Sex
- Childbearing potential (for female subjects only)
- Race
- Ethnicity
- Weight (kg)
- Height (cm)
- Body Mass Index (BMI) (kg/m^2)

Continuous demographic and other baseline characteristics will be summarized using descriptive statistics overall and by treatment group based on the SAF analysis population. For categorical demographic and other baseline characteristics, number and percentage of subjects in each category will be provided overall and by treatment group based on the SAF analysis population. No statistical testing will be carried out for demographic or other baseline

characteristics.

10.1. DERIVATIONS

BMI, in kg/m^2, will be calculated as follows:

- BMI (kg/ m^2) = weight (kg)/ [height (m)^2]

11. SURGICAL AND MEDICAL HISTORY

Surgical and medical history are defined as any surgeries that happened before the first injection of study drug and any medical conditions/diseases that started and stopped before the first injection of study drug.

Surgical and medical history will be coded using the Medical Dictionary for Regulatory Activities (MedDRA), version 22.0, and will be summarized by System Organ Class (SOC) and Preferred Term (PT) overall and by treatment group based on the SAF analysis population. A subject having more than one surgery/medical condition/disease within the same SOC/PT will be counted only once for that SOC or PT.

All surgical and medical history will be listed.

12. DISEASE HISTORY

The following disease history characteristics will be summarized overall and by treatment group based on the SAF analysis population:

- Time since diagnosis (days) – calculated relative to date of first infusion of study drug
- Use of insulin pump (yes/no)
- HbA1c at diagnosis, screening, and baseline (%)
- Change and percent change in HbA1c from diagnosis to baseline (%)
- Weight at diagnosis, screening, and baseline (kg)
- Change and percent change in weight from diagnosis to baseline (kg)
- BMI at diagnosis, screening, and baseline (kg/m^2)

- Change and percent change in BMI from diagnosis to baseline (kg/m^2)

All disease history characteristics will be listed.

12.1. DERIVATIONS

'Time since' disease history characteristics, in days, will be calculated as follows:

- Time since diagnosis (days) = (Date of first infusion of study drug - Date of diagnosis)

Change from diagnosis will be calculated as follows:

- Change from diagnosis = Test value at screening or first dosing visit – Diagnosis value

Percent change from baseline will be calculated as:

- Percent change from diagnosis (%) = (Change from diagnosis at screening or fist dosing visit / diagnosis value) * 100%

BMI, in kg/m^2, will be calculated as follows:

- BMI (kg/ m^2) = weight (kg)/ [height $(m)^2$]

13. MEDICATIONS

All medications will be coded using the World Health Organization (WHO) Drug Global dictionary, version B3 March 2020. Prior medications are defined as any medication that stopped before the date of first dose of study drug. Concomitant medications are defined as any medication that were ongoing or ended at the date of first dose of study drug, or any medication that started on or after the date of first dose of study drug.

Partially or completely missing medication start and stop dates will be handled as described in APPENDIX 1.

Prior and concomitant medications will be summarized separately by Anatomical Therapeutic Class (ATC) level 2 and preferred drug name by treatment arm and overall based on the safety analysis population. A subject having more than one medication within the same ATC Level 2 or preferred drug name will be counted only once for that ATC Level 2 or preferred drug name.

All medications (prior and concomitant) will be listed.

14. EXPOSURE TO STUDY DRUG

Exposure to the study drug will be summarized by treatment group based on the SAF analysis population for the following measures:

- Treatment duration, weeks
- Cumulative drug exposure, mL
- Total number of doses administered, n
- Dose intensity, %
- Any dose modification (dose held, interrupted, reduced, or permanently withdrawn)

14.1. DERIVATIONS

Measures of drug exposure will be calculated as follows:

- Treatment duration (weeks) = (Date of last injection of study drug – Date of first injection of study drug + 30.4167) /7
- Cumulative drug exposure (mL) = the sum of "Volume Administered (mL)" recorded on the *Dose* eCRF page, across all study dosing days
- Total number of doses administered = count of records on the *Dose* eCRF page where the response to the question "Was IP/Placebo administered at this visit" is Yes.
- Dose intensity (%) = (Cumulative drug exposure (mL) / Total planned dose (mL))*100, where total planned dose = (20 mL * 3)

The dates of first and last AVT001 administration will be taken from the eCRF *Dose* page.

15. COMPLIANCE WITH STUDY DRUG

Because IP will be administered intravenously to the subjects enrolled in the study in accordance with the protocol, compliance will not be assessed.

16. EFFICACY ENDPOINTS

Unless otherwise indicated, all efficacy summaries and figures will be presented by treatment group and visit, when appropriate, based on the SAF analysis population.

16.1. PRIMARY EFFICACY

There are no primary efficacy endpoints in this study.

16.2. SECONDARY EFFICACY

The secondary efficacy endpoints analyzed in this SAP are:

- Change from baseline in the area under the curve (AUC) of the stimulated C-peptide levels over the first 4 hours of a mixed meal tolerance test (MMTT).
- Change from baseline in HbA1c.
- Change from baseline in total, basal, and bolus daily insulin dose.
- Changes from baseline in autoantibody levels.

16.2.1. SECONDARY EFFICACY ENDPOINT VARIABLES AND DERIVATIONS

16.2.1.1. Change from Baseline in the Area under the Curve (AUC) of the stimulated C-peptide levels over the first 4 hours of a mixed meal tolerance test (MMTT)

The area under the stimulated C-peptide curve (AUC) over the first 4-hour period of a mixed meal glucose tolerance test will be calculated using the trapezoidal rule that is a weighted sum of the C-peptide values over the 240 minutes. Missing C-peptide levels at any given timepoint will not be imputed. In the calculation of the AUC when C-peptide levels are missing, a line will be drawn from the last timepoint with a non-missing C-peptide to the next timepoint with non-missing C-peptide.

Change from baseline in C-peptide AUC will be computed for baseline and for each post-baseline visit where MMTT was a scheduled assessment.

16.2.1.2. Change from Baseline in HbA1c

HbA1c will be measured at the first screening visit and at each dosing and long-term follow-up visit as per the schedule of events (refer to protocol Table 3).

Change from baseline in HbA1c will be computed for baseline and for each post-baseline visit.

16.2.1.3. Change from Baseline in Total Daily Insulin Dose, Total Daily Basal Insulin Dose, and Total Daily Bolus Insulin Dose

Total daily insulin will be entered by subjects on an insulin log for 3-7 days prior to each study visit starting with Study Day 1. Change from baseline in total daily insulin dose, total daily basal insulin dose, and total daily bolus insulin dose will be computed for baseline and for each post-baseline visit.

Because insulin is heavily dependent on weight at the same visit, the following measures will also be computed for baseline and for each post-baseline visit: change from baseline in total daily insulin dose divided by weight, total daily basal insulin dose divided by weight, and total daily bolus insulin dose divided by weight. This additional summary is included per the 22Apr2022 memo-to-file to amend the SAP v1.0.

16.2.1.4. Change from Baseline in Autoantibody Levels

Antibody levels to glutamic acid decarboxylase (GAD65), insulinoma associated protein (IA-2), zinc transporter 8 (ZnT8), and insulin associated aAb (IAA) will be measured at the first screening visit and at each dosing and long-term follow-up visit as per the schedule of event (refer to Protocol Table 3).

Change from baseline for each antibody type will be computed for baseline and for each post-baseline visit.

16.2.2. Analysis of Secondary Efficacy Endpoints

Observed value and change from baseline for C-peptide AUC, HbA1c, total daily insulin dose, total daily basal insulin dose, total daily bolus insulin dose, and each of 4 antibodies will be summarized by treatment group and visit.

16.2.2.1. Continuous Secondary Efficacy Endpoints – C peptide AUC

For the primary analysis of C-peptide AUC, a Mixed-effect Model for Repeated Measurements (MMRM) will be performed based on the PD analysis population using the observed-case data. This model will include the change

from baseline variable as the dependent variable, its baseline observed value, age, and time from diagnosis to the first dose as covariates, treatment group (active and placebo), visit, HbA1c, BMI, total daily insulin divided by weight, potency assay, and treatment group-by-visit interaction as fixed effects. The fixed effects and covariates specified in SAP v1.0 were revised per the 22APR2022 memo-to-file to amend the SAP v1.0.

A backward stepwise variable selection process will be used to remove any non-relevant or highly correlated variables before fitting the final model. Restricted Maximum Likelihood (ReML) estimation will be used. An unstructured (UN) covariance structure will be used to model the within-patient error and an adjustment to the degrees of freedom will be made using the Kenward-Roger's approximation. In the event this model does not converge, the heterogeneous Toeplitz, heterogeneous first-order autoregressive (AR(1)), Toeplitz, and AR(1) covariance structures will be tested one after the other in the order listed here i.e., the heterogeneous Toeplitz covariance structure will be tested first and if this model converges, the other covariance structures will not be tested. If this model does not converge, then the heterogeneous AR(1) will then be tested. The same process will be repeated for each covariance structure one after the other as listed above until the model converges.

The Least Square (LS) mean estimates will be provided for each treatment group for each visit along with their standard error (SE). The difference of LS means between the treatment groups (active vs. placebo) and associated SE, two-sided 95% CI for each visit, and *p-value* will also be provided only for Visits at Day 150 and Day 360.

In addition, the following MMRMs for C-peptide AUC will be analyzed as exploratory analyses:

- MMRM, based on the data locked for the primary analysis when the last subject completes the Month 5 study visit (Day 150), with the data at Visit 3/Day 1 included. A separate backward stepwise variable selection process will be performed.
- MMRM, based on the data locked for the primary analysis when the last subject completes the Month 5 study visit (Day 150), with the data at Visit 3/Day 1 excluded. A separate backward stepwise variable selection process will be performed.
- MMRM, based on the data locked for the supplemental analysis when the last subject completes the Month 12 study visit (Day 360), with the data at Visit 3/Day 1 excluded. A separate backward stepwise variable selection process will be performed.
- MMRM, based on the data locked for the supplemental analysis when the last subject completes the Month 12 study visit (Day 360), with the data at Visit 3/Day 1 excluded. The full model will be fitted directly without any variable selection process. The primary analysis performed in June 2022 for Day 150 reported results from the full model without any variable selection process, and with the data at Visit 3/Day 1 excluded.

16.2.2.2. Continuous Secondary Efficacy Endpoints – HbA1C

For the analysis of HbA1C, a Mixed-effect Model for Repeated Measurements (MMRM) will be performed based on the PD analysis population using the observed-case data. This model will include the change from baseline variable as the dependent variable, its baseline observed value, age, and time from diagnosis to the first dose as covariates, treatment group (active and placebo), visit, C-peptide AUC, BMI, total daily insulin divided by weight, potency assay, and treatment group-by-visit interaction as fixed effects.

The MMRM modeling and selection procedures described in section 6.2.2.1 will be used to select an appropriate model for this endpoint.

The Least Square (LS) mean estimates will be provided for each treatment group for each visit along with their standard error (SE). The difference of LS means between the treatment groups (active vs. placebo) and associated SE, two-sided 95% CI for each visit, and *p-value* will also be provided only for Visits at Day 150 and Day 360.

16.2.2.3. Continuous Secondary Efficacy Endpoints – Total Daily Insulin Divided by Weight

For the analysis of total daily insulin divided by weight, a Mixed-effect Model for Repeated Measurements (MMRM) will be performed based on the PD analysis population using the observed-case data. This model will include the change from baseline variable as the dependent variable, its baseline observed value, age, and time from diagnosis to the first dose as covariates, treatment group (active and placebo), visit, C-peptide AUC, BMI, HbA1C, potency assay, and treatment group-by-visit interaction as fixed effects.

The MMRM modeling and selection procedures described in section 6.2.2.1 will be used to select an appropriate model for this endpoint.

The Least Square (LS) mean estimates will be provided for each treatment group for each visit along with their standard error (SE). The difference of LS means between the treatment groups (active vs. placebo) and associated SE, two-sided 95% CI for each visit, and *p-value* will also be provided only for Visits at Day 150 and Day 360.

16.2.2.4. Continuous Secondary Efficacy Endpoints – Antibody Levels to Glutamic Acid Decarboxylase (GAD65), Insulinoma Associated Protein (IA-2), Zinc Transporter 8 (ZnT8), and Insulin Associated aAb (IAA)

For the analysis of each of the antibody levels to glutamic acid decarboxylase (GAD65), insulinoma associated protein (IA-2), zinc transporter 8 (ZnT8), and insulin associated aAb (IAA), a Mixed-effect Model for Repeated Measurements (MMRM) will be performed based on the PD analysis population using the observed-case data. This

model will include the change from baseline variable as the dependent variable, its baseline observed value, age, and time from diagnosis to the first dose as covariates, treatment group (active and placebo), visit, C-peptide AUC, HbA1C, BMI, total daily insulin divided by weight, potency assay, and treatment group-by-visit interaction as fixed effects.

The MMRM modeling and selection procedures described in section 6.2.2.1 will be used to select an appropriate model for these endpoints.

The Least Square (LS) mean estimates will be provided for each treatment group for each visit along with their standard error (SE). The difference of LS means between the treatment groups (active vs. placebo) and associated SE, two-sided 95% CI for each visit, and *p-value* will also be provided only for Visits at Day 150 and Day 360.

16.3. EXPLORATORY EFFICACY

The exploratory efficacy endpoints analyzed in this SAP are:

- Change from baseline in unstimulated C-peptide of a MMTT.
- Change from baseline in the change in the AUC of C-peptide (Delta AUC) over the first 4 hours of a MMTT.
- Change from baseline in the maximum concentration of C-peptide (Cmax) during a MMTT.
- Change from baseline in the maximum C-peptide levels (Delta Cmax) over the first 4 hours of a mixed meal tolerance test (MMTT).
- Change from baseline in the total area under the curve (AUC) of the stimulated glucose levels over the first 4 hours of a mixed meal tolerance test (MMTT).
- Change from baseline in unstimulated glucose of a MMTT.
- Change from baseline in the change in the AUC of glucose (Delta AUC) over the first 4 hours of a MMTT minus the unstimulated glucose AUC prior to MMTT.
- Change from baseline in the maximum concentration of glucose (Cmax) during a MMTT.
- Change from baseline in the maximum change from baseline concentration (Delta Cmax) of the stimulated glucose levels over the first 4 hours of a mixed meal tolerance test (MMTT)

16.3.1. EXPLORATORY EFFICACY ENDPOINT VARIABLES AND DERIVATIONS

16.3.1.1. Change from Baseline in unstimluated C-peptide of a MMTT

Unstimulated C-peptide will be defined as the average of C-peptide at time -10 and 0 minutes of a MMTT.

16.3.1.2. Change from Baseline in the change in AUC of C-peptide (Delta AUC) of a MMTT

The change in AUC of C-peptide (Delta AUC) will be defined as total AUC C-peptide over the first 4 hours of a MMTT minus the unstimulated C-peptide AUC at time -10 and 0 minutes of the same MMTT.

16.3.1.3. Change from Baseline in the maximum concentration of C-peptide (Cmax) of a MMTT

The maximum concentration of C-peptide will be defined as the maximum C-peptide value over the first 4 hours of a MMTT.

16.3.1.4. Change from Baseline in the maximum change from baseline concentration (Delta Cmax) of the stimulated C-peptide levels of a mixed meal tolerance test (MMTT)

The maximum change from baseline in C-peptide concentration (Delta Cmax) over the first 4-hour period of a mixed meal glucose tolerance test is defined as the maximum concentration of C-Peptide during the MMTT test minus the baseline concentration of C-Peptide, which is calculated as the average of C-Peptide at time -10 min and 0 min of the same MMTT test. Missing C-peptide levels at any given timepoint will not be imputed.

The maximum delta C-peptide Cmax will be computed for baseline and for each post-baseline visit where MMTT was a scheduled assessment.

16.3.1.5. Change from Baseline in the AUC of the stimulated glucose of a MMTT

The area under the stimulated glucose curve (AUC) over the first 4-hour period of a mixed meal glucose tolerance test will be calculated the trapezoidal rule as described in Section 16.2.1.

16.3.1.6. Change from Baseline in unstimluated glucose of a MMTT

Unstimulated glucose will be defined using the approach described in Section 16.3.1.1.

16.3.1.7. Change from Baseline in the change in AUC of glucose (Delta AUC) of a MMTT

The change in AUC of glucose (Delta AUC) over the first 4-hour period of a MMTT will be defined using the approach described in Section 16.3.1.2.

16.3.1.8. Change from Baseline in the maximum concentration of glucose (Cmax) of a MMTT

The maximum glucose concentration (Cmax) over the first 4-hour period of a MMTT will be derived using the approach described in Section 16.3.1.3.

16.3.1.9. Change from Baseline in the maximum change from baseline concentration (Delta Cmax) of the stimulated glucose levels of a MMTT

The maximum change from baseline in glucose concentration (Delta Cmax) over the first 4-hour period of a MMTT will be derived using the approach described in Section 16.3.1.4.

16.3.2. Analysis of Exploratory Efficacy Endpoints

16.3.2.1. Continuous Exploratory Efficacy Endpoints

Observed and change from baseline for all exploratory endpoints will be summarized by treatment group and visit. Univariate summaries of the observed values and changes from baseline (both observed and percent change) will be generated by study visit.

17. Safety Endpoints

All safety summaries will be presented by treatment group based on the SAF population. There will be no statistical comparisons between the treatment groups for safety data.

17.1. Adverse Events

- Prior adverse events (AEs) are defined as any AE that started or worsened in severity on or after the date of signed informed consent but before the first dose of study drug.
- Treatment-emergent AEs (TEAEs) are defined as any AE that starts on or after the first dose of study

medication. This includes events that start within 30 days following the last dose.

- Post-treatment AEs are defined as adverse events that start more than 30 days following the last dose.

See APPENDIX 1 for handling of partial dates for AEs. In the case where it is not possible to define an AE as treatment emergent or not, the AE will be classified by the worst case; i.e. treatment emergent.

AEs will be coded using the MedDRA dictionary, version 22.0.

An overall summary of number and percentage of subjects within each of the categories described in the sub-sections below will be provided by treatment group based on the SAF analysis population. Should a subject experience multiple events within a category, the subject will be counted only once for that category. Each adverse event summary table will include a sub-table summarizing TEAEs and a sub-table summarizing post-treatment AEs.

All AEs (prior, TEAE, and post-treatment) will be listed. The listing will include a column for relatedness (see section 17.1.1.2), a column for outcome, and a column to identify potential dose-limiting toxicities as collected on the AE CRF page. Listings of subsets of AEs will include these columns as well.

17.1.1. ALL TEAES AND POST-TREATMENT AES

Number and percentage of subjects with at least one TEAE will be presented by SOC and PT. Should a subject experience multiple events within a SOC or PT, the subject will be counted only once for that SOC or PT. Subjects with at least one post-treatment AE will be summarized similarly.

Number and percentage of subjects with at least one TEAE will be broken down further by maximum severity. Subjects with at least one post-treatment AE will be summarized similarly.

17.1.1.1. CTCAE Grading for AEs

Severity will be classified according to the Common Terminology Criteria for AEs (CTCAE), version 4.03. TEAEs or post-treatment AEs starting after the first injection of study drug with a missing CTCAE grade will be classified as grade 3. Should a subject experience multiple events within a SOC or PT, only the subject's worst CTCAE grade will be counted for that SOC or PT.

17.1.1.2. Relationship to Study Drug

Relationship to study drug, as indicated by the Investigator, will be classified as unrelated, unlikely related, possibly related, probably related, or definitely related (increasing severity of relationship). A summary of TEAEs related to

study drug by SOC and PT will be prepared. A sub-table of post-treatment AEs related to study by SOC and PT will also be prepared.

A "Related" AE is defined as a TEAE or a post-treatment AE with a relationship to study drug of possibly related, probably related, or definitely related, while a "Non-related" AE is defined as a TEAE or post-treatment AE with a relationship to study drug of unrelated or unlikely related. TEAEs or post-treatment AEs with a missing relationship to study drug will be regarded as related to study drug. Should a subject experience multiple events within a SOC or PT, only the subject's worst relationship will be counted for that SOC or PT.

17.1.2. AEs with an Outcome of Death

AEs with an outcome of death are those events which are recorded as "Fatal" on the AE page of the eCRF.

17.1.3. Serious Adverse Events

Serious Adverse Events (SAEs) are those events recorded as "Serious" on the AE page of the eCRF. A summary of serious TEAEs by SOC and PT will be prepared. A sub-table of post-treatment serious AEs by SOC and PT will also be prepared. Should a subject experience multiple events within a SOC or PT, the subject will be counted only once for that SOC or PT.

A listing of all SAEs will be provided.

17.1.4. AEs Leading to Discontinuation of Study Drug

TEAEs leading to permanent discontinuation of study drug are those events recorded as "Drug Permanently Discontinued" on the AE pages of the eCRF. A summary of TEAEs leading to permanent discontinuation of study drug by SOC and PT will be prepared. A sub-table of post-treatment AEs leading to discontinuation of study drug by SOC and PT will also be prepared.

A listing of all AEs leading to permanent discontinuation of study drug will be provided.

17.1.5. Severe Hypoglycaemic Adverse Events

Severe hypoglycaemic adverse events will be the AEs coded to the "Hypoglycaemia" Preferred Term (PT). A listing of all severe hypoglycaemic events will be provided. A summary table of severe hypoglycaemic adverse events may be provided if needed.

17.1.6. LOCAL IV-SITE REACTIONS

Local IV-site reactions will be the AEs coded to the "Injection site reaction" MedDRA High Level Term (HLT). A listing of all IV-site reactions will be provided. A summary table of local IV-site reaction adverse events may be provided if needed.

17.2. DEATHS

A listing of all deaths will be provided.

17.3. LABORATORY EVALUATIONS

A urine pregnancy test will be performed at screening. Chemistry, hematology, and urinalysis (microalbumin panel) will be performed as per the schedule of events (refer to protocol, Table 3). A list of laboratory parameters to be included in the outputs is included in APPENDIX 2.

Quantitative laboratory parameters reported as "< X", i.e. below the lower limit of quantification (BLQ) or "> X", i.e. above the upper limit of quantification (ULQ), will be converted to X for the purpose of quantitative summaries, but will be presented as recorded, i.e. as "< X" or "> X" in the listings.

The following summaries will be provided by treatment group based on the SAF analysis population for each chemistry, hematology, and urine microalbumin laboratory parameter:

- Observed and change from baseline in Standard International (SI) units by visit (for quantitative parameters)
- Shift from baseline to the worst post-baseline observed value according to the Common Terminology Criteria for Adverse Events (CTCAE) toxicity grades (for quantitative parameters with available CTCAE toxicity grades; refer to Section 17.3.11)
- Shifts from baseline to the maximum/minimum post-baseline observed value according to normal range criteria (for quantitative parameters without CTCAE toxicity grades; refer to Section 17.3.12)

All laboratory data will be listed.

17.3.1. CTCAE TOXICITY GRADES

Quantitative laboratory parameters with available CTCAE toxicity grades will be categorized as follows where

higher grades representing a more severe toxicity.

- Grade 1 (i.e., mild)
- Grade 2 (i.e., moderate)
- Grade 3 (i.e., severe)
- Grade 4 (i.e., life-threatening)
- Grade 5 (i.e., death)

Although not defined in the CTCAE toxicity grading system, version 4.03, non-missing laboratory parameter results not meeting any of the 5 grades defined in the CTCAE toxicity grading system will be categorized as 'No Event' for the purpose of the shift from baseline summaries.

17.3.2. LABORATORY NORMAL RANGES

Quantitative laboratory parameters will be compared with the relevant laboratory normal ranges in SI units and categorized as:

- Low: Below the lower limit of the laboratory normal range.
- Normal: Within the laboratory normal range (upper and lower limit included).
- High: Above the upper limit of the laboratory normal range.

17.4. VITAL SIGNS

The following vital sign parameters will be collected for this study as per the schedule of events (refer to protocol, Table 3):

- Systolic blood pressure (SBP) (mmHg)
- Diastolic blood pressure (DBP) (mmHg)
- Pulse rate (beats per minute [bpm])
- Oxygen saturation (%)
- Body temperature (ºC)

- Weight (kg)

The following summaries will be provided by treatment group based on the SAF analysis population for each vital sign parameter:

- Observed and change from baseline by visit and timepoint
- Number and percentages of subjects with at least one markedly abnormal post-baseline observed value/change from baseline (refer to Section 17.4.1)

All vital sign data will be listed. Vitals signs meeting one of the markedly abnormal criteria in Section 17.4.1 will be identified.

17.4.1. VITAL SIGNS MARKEDLY ABNORMAL CRITERIA

Markedly abnormal vital sign observed values and/or change from baseline will be identified in accordance with the following predefined markedly abnormal criteria:

Variable	Unit	Low	High
SBP	mmHg	≤ 90 mmHg AND change from baseline ≤ -20 mmHg	≥ 180 mmHg AND change from baseline ≥ 20 mmHg
DBP	mmHg	≤ 50 mmHg AND change from ≤ -15 mmHg	≥ 105 mmHg AND change from baseline ≥ 15 mmHg
Pulse rate	bpm	≤ 50 bpm AND change from baseline ≤ -15 bpm	≥ 120 bpm AND change from baseline ≥ 15 bpm
Oxygen saturation	%	< 94 %	Not applicable
Body temperature	°C	Not applicable	≥ 38.3 °C AND change from baseline ≥ 1.1 °C
Weight	kg	Percent change from baseline ≤ -7.0 %	Percent change from baseline > 7.0 %

17.5. ECG EVALUATIONS

The following electrocardiogram (ECG) parameters will be measured for this study as per the schedule of events (refer to protocol, Table 3):

- PR interval (msec)
- RR interval (msec)
- QRS interval (msec)
- QT (uncorrected) interval (msec)
- Overall ECG evaluation (Investigator's judgment)
    - Normal
    - Abnormal, not clinically significant (NCS)
    - Abnormal, clinically significant (CS)

The following summaries will be provided by treatment group based on the SAF analysis population for each ECG parameter:

- Observed and change from baseline by visit (for quantitative parameters)
- Number and percentages of subjects with at least one markedly abnormal post-baseline observed value/change from baseline (for quantitative parameters; refer to Section 17.5.11)
- Shift from baseline in overall ECG interpretation to the worst post-baseline assessment

All ECG data will be listed. ECG results meeting one of the markedly abnormal criteria in Section 17.5.1 will be identified.

17.5.1. ECG MARKEDLY ABNORMAL CRITERIA

Markedly abnormal quantitative ECG parameters will be identified in accordance with the following predefined markedly abnormal criteria:

- Observed values for QT (uncorrected) interval will be classified as:

- > 450 msec
- > 480 msec
- > 500 msec

- Change from baseline for QT (uncorrected) interval will be classified as:
  - >30 msec increase from baseline
  - >60 msec increase from baseline

It is to be noted that the previous categories are not mutually exclusive, but cumulative. For example, if a subject worst post-baseline QT post-baseline observed value is 490 mmHg, then this subject will be reported once under QT > 450 msec and once under QT > 480 msec.

18. DATA NOT SUMMARIZED OR PRESENTED

Data that will not be summarized or listed are:

- Results of serology tests performed (i.e., HIV, Hepatitis A, B, and C) at screening for the purpose of determining eligibility.

APPENDIX 1. PARTIAL DATE CONVENTIONS AND MISSING DATA IMPUTATION

ALGORITHM FOR PRIOR ADVERSE EVENTS, TREATMENT-EMERGENT ADVERSE EVENTS, AND POST-TREATMENT ADVERSE EVENTS

START DATE	END DATE	ACTION
Known	Known/Partial/ Missing	If (AE start date < study drug start date) or AE is a pre-initial dose event, then PRAE. If study drug start date ≤ AE start date and AE start date <= last drug date_30 and AE is not a pre-initial dose event, then TEAE. If study drug start date <= AE start date and AE start date > last drug date + 30 then PTAE
Partial, but known components show that AE started before study drug start date or AE is a pre-initial dose event	Known/Partial/ Missing	PRAE
Partial and known components show that AE started on or after study drug start date	Known/Partial/ Missing	Impute missing AE start day as the first of the month or the first study drug dose date, whichever is later. If (AE start date < study drug start date) or AE is a pre-initial dose event, then PRAE. If study drug start date ≤ AE start date and AE start date <= last drug date+30 and AE is not a pre-initial dose event, then TEAE. If study drug start date <= AE start date and AE start date > last drug date+30 then PTAE

Note: If an AE started prior to first dose of study drug but worsened after treatment started, it will be reported as a separate adverse event with a new AE start date. A pre-initial dose event is defined as an AE that is entered in the

EDC as occurring prior to infusion (Month 0, Day 1).

ALGORITHM FOR PRIOR / CONCOMITANT MEDICATIONS

START DATE	STOP DATE	ACTION
Known	Known or ongoing	If medication stop date < study drug start date, assign as prior; Otherwise, assign as concomitant.
	Partial	If known components of medication stop date show that medication stopped before study drug start date, assign as prior; Otherwise, assign as concomitant.
	Missing, not ongoing	Can never be assigned as prior, therefore assign as concomitant.
Partial	Known or ongoing	If medication stop date < study drug start date, assign as prior; Otherwise, assign as concomitant.
	Partial	If known components of medication stop date show that medication stopped before study drug start date, assign as prior; Otherwise, assign as concomitant.
	Missing, not ongoing	Cannot be assigned as prior, therefore assign as concomitant.
Missing	Known or ongoing	If medication stop date < study drug start date, assign as prior; Otherwise, assign as concomitant.
	Partial	If known components of medication stop date show that medication stopped before study drug start date, assign as prior; Otherwise, assign as concomitant.
	Missing, not ongoing	Assign as concomitant.

ALGORITHM FOR POTENCY ASSAY

As specified in the inclusion criteria 3 in the protocol, a subject is eligible for enrollment as the study participant, only if the subject "has a CD8+ T cell defect that is correctable in vitro by co-culture with immature dendritic cells loaded with the synthetic oligopeptide from Hsp60sp".

Therefore, for the potency assay result of each patient on Visit 3/Day 1 (before the study treatment), should be imputed as the same as the corresponding potency assay result on Visit 1/Screening.

For potency assay results that are missing on any visits other than Visit 3/Day 1, no imputation will be performed.

APPENDIX 2. LABORATORY ASSESSMENTS

Chemistry (SI unit)

- Blood urea nitrogen (BUN) (mmol/L)
- Creatinine (µmol/L)
- Calcium (mmol/L)
- Chloride (mmol/L)
- Potassium (mmol/L)
- Sodium (mmol/L)
- Bicarbonate (mmol/L)
- Glucose (mmol/L)
- Alkaline phosphatase (ALP) (U/L)
- Alanine transaminase (ALT) (U/L)
- Aspartate transaminase (AST) (U/L)
- Albumin (g/L)
- Total protein (g/L)
- Total bilirubin (µmol/L)
- HbA1c, L/L
- Creatinine Clearance, mL/s

Hematology (SI unit)

- Hemoglobin (g/L)
- Hematocrit
- Red blood cells (RBC) (x10E12/L)
- White blood cell (WBC) count total (x10E9/L)
- Platelets (x10E9/L)
- Absolute neutrophils count (x10E9/L)
- Absolute lymphocyte count (x10E9/L)
- Absolute monocyte count (x10E9/L)
- Absolute eosinophils count (x10E9/L)
- Absolute basophils count (x10E9/L)

Urine Microalbumin Panel

- Urine Microalbumin (mg/dL)
- Urine Creatinine (mg/dL)
- Microalbumin/Creatinine Ratio (ratio)

Summary of Change, SAP v2.0 vs SAP v1.0

- **Added supplementary analysis for Day 360**
- **Defined Change from Baseline at baseline, so that the baseline data can be included in MMRM**
- **Defined the imputation rule for Potency Assay results on Day 1 (which was not planned to be collected on Day 1 per protocol), based on the Potency Assay results from Screening**
- **In addition to Total Daily Insulin usage, also added Total Daily Insulin usage divided by weight as a secondary endpoint**
- **Added additional MMRM analysis for C-peptide AUC, HbA1c, Total Daily Insulin usage, and 4 auto-antibodies.**