Antithymocyte Globulin (ATG) and pegylated granulocyte colony stimulating factor (GCSF) in New Onset Type 1 Diabetes

Protocol TN-19

Version: 3.0 March 30, 2016

IND # 107,185

Sponsored by the National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK), the National Institute of Allergy and Infectious Diseases (NIAID), the National Institute of Child Health and Human Development (NICHD), the National Center for Research Resources (NCRR), the Juvenile Diabetes Research Foundation International (JDRF), and the American Diabetes Association (ADA)

PREFACE

The Type 1 Diabetes TrialNet Protocol TN-19, ATG-GCSF in New Onset Type 1 Diabetes, describes the background, design, and organization of the study.

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

Glossary of Abbreviations

AE	Adverse event
APC	Antigen presenting cell
ATG	Anti-Thymocyte Globulin (Thymoglobulin®)
AUC	Area Under Curve
CBC	Complete blood count
CFR	Code of Federal Regulations
cGMP	Current Good Manufacturing Practice
CHO	Carbohydrates
CMV	Cytomegalovirus
CRF	Case report form
CRS	Cytokine Release Syndrome
DC	Dendritic Cell
DSMB	Data and Safety Monitoring Board
EBV	Epstein-Barr virus
FACS	Fluorescence activated cell sorting
FDA	US Food and Drug Administration
FOXP3	Forkhead box P3
FWA	Federal-wide Assurance
GAD	Glutamate decarboxylase
GCP	Good Clinical Practice
GCSF	Granulocyte colony-stimulating factor (Neulasta®)
HbA1c	Hemoglobin A1c
HBsAg	Hepatitis B surface antigen
HIV	Human immunodeficiency virus
ICA	Islet cytoplasmic antibodies
IEC	Independent Ethics Committee
IGRA	Interferon-γ release assays
IND	Investigational New Drug

IRB	Institutional Review Board
ITN	Immune Tolerance Network
JDRF	Juvenile Diabetes Research Foundation
LIFT	Long Term Investigative Follow-Up
MMTT	Mixed-meal tolerance test
NIDDK	National Institute for Diabetes and Digestive and Kidney Disease
NIH	National Institute of Health
NCI-CTCAE	National Cancer Institute *Common Terminology Criteria for Adverse Events*
NOD	Nonobese diabetic
OHRP	Office for Human Research Protections
PBMC	Peripheral Blood Mononuclear Cell
PCR	Polymerase chain reaction
PI	Principal Investigator
PO	Per Oral (by mouth)
QA	Quality Assurance
RMSE	Residual Mean Square Error
SAE	Serious adverse event
SOE	Schedule of events
SOP	Standard operating procedure
T1DM	Type 1 diabetes mellitus
Tregs	Regulatory T cells
TSDR	Treg Specific Demethylation region
ZnT8	Zinc Transporter 8

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1 INTRODUCTION

1.1 Study Overview

Title	ATG-GCSF in New Onset Type 1 Diabetes
IND Sponsor	National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK)
Conducted By	Type 1 Diabetes Trial Network (TrialNet)
Protocol Chair	Michael J. Haller, MD
Accrual Objective	84
Study Design	Three-arm, 1:1:1 randomized, placebo controlled, double-blinded trial in which at least 28 subjects will receive active ATG-GCSF, at least 28 subjects will receive ATG alone and at least 28 subjects will receive placebo alone within 100 days from diagnosis of T1D. An adaptive design will be used to determine the variance of C-peptide over time and may require additional subjects to be enrolled in each group to preserve power.
Treatment Description	ATG will be administered at a dose of 2.5mg/kg as two divided IV infusions of 0.5mg/kg and 2mg/kg. GCSF will be administered at a dose of 6mg SC or if <44.5 kg a dose of 100 mcg/kg every two weeks for a total of 6 doses.
Study Duration	Enrollment is expected to occur over two-three years. Subjects will be followed up to 24 months from randomization in this protocol.
Objective	To determine the safety and ability of low dose ATG plus GCSF and low dose ATG alone to retain/enhance C-peptide production in new onset T1D patients demonstrating residual beta cell function.
Primary Outcome	The primary statistical hypothesis to be assessed in the study is whether the 2 hour area under the curve (change in baseline to 12 months) in residual beta cell function (C-peptide) will differ between those treated with ATG and GCSF or ATG alone as compared with placebo.
Secondary Goals	The study will also examine the effect of the proposed treatments on surrogate markers for immunologic and metabolic outcomes
Major Inclusion Criteria	Type 1 diabetes diagnosed within the past 100 days Age $\geq$ 12 years < 46 At least one diabetes associated autoantibody

2 BACKGROUND AND SIGNIFICANCE

2.1 Introduction

Recent advances in our understanding of the pathogenesis of T1D have been somewhat overshadowed by our continued inability to develop a long lasting means to either reverse or prevent the disease (i.e., identify a cure), using a variety of interventions (e.g., cyclosporine, anti-CD3, anti-CD20, oral insulin) (1-3). The majority of attempts to reverse T1D in humans have, to date, involved the delivery of agents as monotherapy. As a result, we and others have recently questioned whether the delivery of therapeutics in combination might produce a synergistic response that would allow for the successful reversal of T1D (2, 3). The success of combination therapy for advancing the treatment of patients with HIV and cancer demonstrates a model for multi-agent therapy in diseases involving multiple pathways required for their successful treatment and cure.

As described below, based on strong preliminary data demonstrating efficacy and potential mechanisms leading to reversal of diabetes in NOD mice with a combination of ATG and GCSF, as well as preliminary safety and efficacy studies in humans with T1D utilizing ATG and GCSF as monotherapies and in combination, we will test the hypothesis that a short course of low dose ATG or a combination of low dose ATG plus GCSF will lead to preservation of C-peptide in patients with new onset T1D.

2.2 ATG Monotherapy

2.2.1 Rationale for ATG Therapy in T1D

Preclinical studies have shown that ALS treatment of the NOD mouse (18) and BB rat (19) with recent onset T1D can induce remission, supporting proof of principle for such an approach in humans. The case for combination of ATG with other agents received support from follow-up studies in new-onset NOD mice involving the addition of Exendin-4, a long-acting GLP-1 agonist shown to augment insulin secretion in rodents (22) as well as T1D (23) and T2D (24) subjects, where remissions were seen in 90% of combination treated mice. While our own NOD studies (Preliminary Data section) found ATG and GCSF to be a superior combination (in comparison to ALS and Exendin-4) both sets of data provide additional support for the concept of combination therapy and it's superiority to relatively ineffective monotherapy in terms of reversing T1D.

As further proof of the potential for translation from mouse to man, studies in human transplantation and various autoimmune conditions suggest that ATG may indeed induce tolerance. ATG has been successfully used as combination therapy in preventing rejection following organ transplantation. After induction with ATG, transplant recipients can be managed successfully with only limited maintenance monotherapy (25, 26), with weaning of some to as little as a single dose of tacrolimus per week. ATG, either alone or in combination with other agents, has also been used to treat a variety of autoimmune conditions, including Wegner's granulomatosis, lupus, rheumatoid arthritis, multiple sclerosis, scleroderma, aplastic anemia and myelodysplastic syndrome; with greater efficacy observed when used in combination (27-38). Extensive studies with ATG in a non-human primate model demonstrates its effectiveness as a single agent in T-cell depletion intravascularly and in peripheral lymphoid organs, as well as in prolongation of allograft transplantation of skin and heart (39).

Other studies also support ATG's possible efficacy in T1D. Specifically, early limited human studies with equine ATG and prednisone with new onset T1D suggested efficacy in prolonging

the honeymoon phase (11, 40). In a small randomized, placebo-controlled, single-blinded trial with ATG (ATG-Fresenius, Germany), T1D participants aged 18-35 years received a total dose of 18 mg/kg of ATG, administered in four infusions. Of the 17 study participants treated, 11 received drug while 6 received placebo. In terms of efficacy, increased glucagon-stimulated C-peptide levels, a lower insulin requirement, and lower glycosylated hemoglobin levels were reported in the ATG group (but not placebo group) at the 12 month study visit. Perhaps most promising, two ATG treated subjects achieved remission (i.e., off exogenous insulin for at least 1 month with fasting blood glucose below 126 mg/dl). (41).

Recently, a phase 2 study in humans with new-onset T1D and funded by the Immune Tolerance Network (ITN) was completed to determine if ATG (6.5mg/kg) could preserve C-peptide in new onset T1D. The study tested the hypothesis that selective deletion of lymphocytes would reset the immunologic rheostat, effect dynamic immune regulation and perhaps induce and maintain tolerance in T1D. While this study helped to establish the relative safety of ATG in humans with T1D, the study failed to demonstrate benefit (41b). Post-hoc analyses have suggested that an initial decline in beta cell function amongst those who were treated with active drug was followed by a relative preservation of beta cell function. We hypothesize that the initial decline in beta cell function was related to the severity of cytokine release syndrome and serum sickness. Furthermore, we hypothesize that the currently proposed protocol (utilizing ~1/3 of the ATG dose in combination with GCSF) will result in less severe cytokine release/serum sickness and, as is supported by our preliminary data, preservation/improvement in beta cell function 12 months after therapy.

2.2.2 Potential Mechanism of Action

ATG appears to induce both generalized immunosuppression and immunoregulation, with effects on APC and immunoregulatory cell function (42). It prevents B-cell proliferation and differentiation, as well as mediates T-cell suppressive effects via inhibition of proliferative responses to mitogens (43, 44). A single “typical” or “standard” dose of ATG reduces the total lymphocyte count by more than 85%. T-cell depletion may result from complement-dependent opsonization and cellular lysis, Fc-dependent opsonization, or Fas-mediated apoptosis; particularly at lower ATG concentrations, at which ATG exhibits preferential effects on pre-activated, as opposed to nonactivated, T-cells. T-cell depletion in peripheral blood persists for several days to several months following cessation of ATG administration. Recovery from treatment-induced lymphocyte depletion is gradual and total lymphocyte counts usually return to normal with 2 months after ATG administration.

Following ATG function, the CD4:CD8 T cell ratio remains significantly and persistently lower and the ratio may correlate with the outcome in transplantation studies (45-48). It has been hypothesized that ATG administration may induce a regulatory population of CD8+, CD57+ and CD28- cells that is critical for selective down regulation of pathogenic self-reactive CD4+ Th1 cells and the induction of tolerance.

Other studies have suggested alternative mechanisms to underlie the beneficial effects of ATG in vivo. As with anti-CD3, ATG may induce partial T-cell activation, leading to an anergic state (39, 53, 54). In cynomolgus monkeys, ATG appears to coat T cells, leading to a down regulation in surface expression of CD2, CD3, CD4, and CD8 molecules, along with impaired immune responses in mixed lymphocyte reactions (39). In addition, antibodies to adhesion molecules may interfere with cellular adhesion and endothelial interactions, as well as T-cell migration to sites of inflammation. ATG may also prevent co-stimulation of T cells by binding directly to APC, and may induce complement-mediated lysis of these cells; particularly in more mature APC (55). The survival of immature DC thus may be more tolerogenic. Antigens recognized by ATG

include CD86, CD32, CD4, CD11b, CD29, and CD51/61; some of which are shared by lymphocytes and DC. In addition, ATG contains antibodies that cross-react with B-cell surface antigens, allowing for induction of activated B-cell (another source of APC) apoptosis in vitro (56-58).

2.3 Granulocyte colony-stimulating factor (GCSF) monotherapy

2.3.1 Rationale for GCSF Therapy in T1D

In a spontaneous diabetes model, GCSF treatment of NOD mice at 4 weeks of age for 5 consecutive days, repeated every 4 weeks thereafter until 16 weeks of age, prevented disease onset and insulitis when compared to injection of excipient (Figure 2) (59). Protection from diabetes onset correlated with the recruitment of both DC and CD4+CD25+ Treg cells. Indeed, the GCSF recipients showed peri-pancreatic lymph node accumulation of functional CD4+CD25+ Treg cells (60). Importantly, co-transfer of the functional Treg populations along with diabetogenic splenocytes into secondary NOD-SCID recipients actively suppressed diabetes. In addition, DC transferred from mice given a single 5-day-long GCSF treatment to secondary NOD recipients triggered enhanced accumulation of CD4+CD25+ Treg cells compared to recipients given non-GCSF treated DC. Thus, GCSF likely restores a balance between immunogenic and tolerogenic DC, resulting in the recruitment of functional CD4+CD25+ Treg cells.

Based on supportive data from animal models, several groups have moved on to initiate clinical trials of GCSF based therapies in human autoimmune disease. Perhaps the most extensive human data derives from studies of GCSF therapy in patients with Crohn disease. Several studies have now demonstrated improvement in clinical disease scores in patients with Crohn disease provided varying courses of GCSF (61-62). Specifically, a 12 week course of 300mcg daily GCSF, demonstrated marked reduction in the Crohn Disease Activity Index amongst 15 subjects with active disease (62). In a smaller study of Crohn disease patients with a history of severe endoscopic ileitis, a similar 12-week course of GCSF was associated with clinical remission of disease in all 5 subjects (61).

2.3.2 Potential Mechanism of Action

GCSF, an agent well known to help mobilize hematopoietic precursors from the bone marrow, has been used clinically for nearly 20 years to help repopulate peripheral cell counts in patients undergoing cancer therapy. However, a rapidly growing body of experimental and clinical evidence suggests that GCSF has the potential to be used in the treatment of autoimmune diseases. GCSF has been shown to favor the differentiation and mobilization of Treg cells, induce tolerogenic DC, and alter the balance between proinflammatory and anti-inflammatory soluble mediators both animals and in humans (Figure 3) (65-67). Specifically, GCSF can mobilize functional bone marrow $CD4^{+}CD25^{+}FoxP3^{+}$ Treg cells. Preclinical models of GCSF-induced inhibition of autoimmune and allogeneic T cell responses have demonstrated the potential efficacy of GCSF in treating Crohn disease, myasthenia gravis, and T1D.

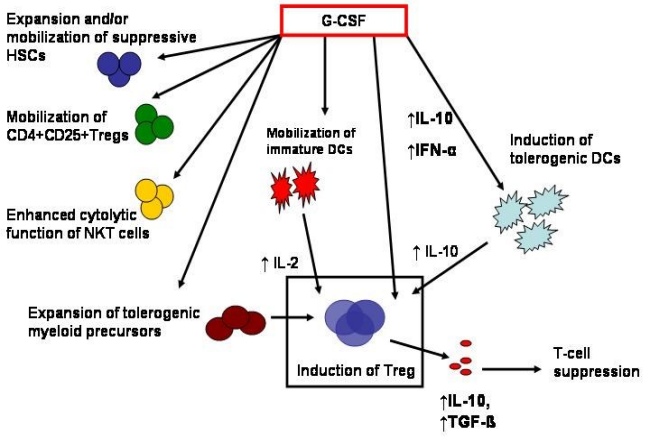

Figure 1. Effects of GCSF on immune function. The GCSF induced modulation of cytokine production, T cell polarization, and

DC functional profile is depicted schematically. GCSF can alter the balance between pro-inflammatory and anti-inflammatory soluble mediators, favor the differentiation and mobilization of Treg cells, and induce tolerogenic DC both in animals and in humans. *(Adapted from Rutella et al, J. Immunology 2005, 175: 7085–7091**, (65)***.

With this extensive experience of clinical use, GCSF therapy is known to be both safe and well tolerated. The side effect profile of short course GCSF therapy is limited; with frequency and severity of toxicities being dose dependent (greater with larger doses and longer duration) and minimal in comparison to many of the agents being used in current T1D intervention studies. Specifically, the most common side effect associated with GCSF therapy is bone pain (occurring in 10-20% of patients) which occurs 2-3 days after initiation of therapy. This discomfort is generally controllable with acetaminophen. Infrequent side effects include redness at injection site, fever, headache, chest pain and dizziness (occurring in less than 10% of patients).

2.4 Combination of ATG and GCSF

2.4.1 GCSF Enhances Reversal of Diabetes Afforded by murine ATG in the NOD

To determine the efficacy of a combination of ATG and GCSF in NOD mice with `new-onset' diabetes, female NOD mice were monitored 3 times per week for hyperglycemia (defined as a blood glucose > 240mg/dL) by tail bleed for up to 150 days as shown (Figure 2).

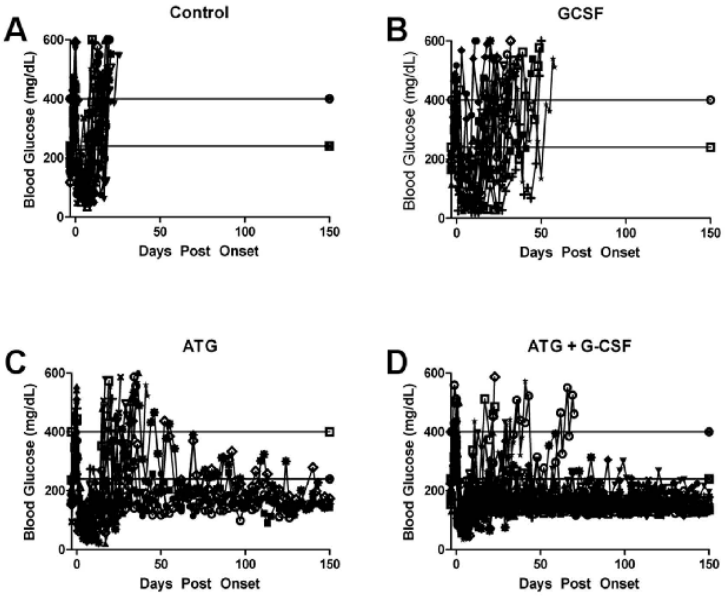

Figure 2. Blood glucose values were obtained for up to 150 days post-onset in NOD mice treated with (**a**) control (two IP injections of 500 ug rIgG), (**b**) GCSF (Neupogen (Amgen, Inc.) 6ug daily x 8weeks, (**c**) murine ATG (two IP injections of 500 ug murine ATG, or (**d**) murine ATG + GCSF. All received sustained release insulin for 3 weeks.

As shown (Figure 2), the administration of murine ATG alone to new-onset NOD mice resulted in durable (i.e., > 150 days post-onset) remissions from overt hyperglycemia in 33% (5/15) of treated animals, while neither control (0/16) nor GCSF monotherapy (0/14 mice) provided such reversions However, the combination of murine ATG and GCSF therapy resulted in a remission rate of 75%, a significantly greater rate of remission than was seen with murine ATG monotherapy (12/16; P = 0.0000006 versus control, P = 0.013 versus murine ATG).

Interestingly and very importantly, in terms of dosage in humans, <u>*GCSF improved the therapeutic capacity for diabetes reversal even when in combination with a suboptimal dose (290ug) of murine ATG*</u> Moreover, successful treatment with murine ATG was largely limited to

values of ≤380mg/dL (mean 317.2mg/dL; range 256-398mg/dL), whereas combination therapy of murine ATG and GCSF significantly increased the therapeutic ceiling to ~500 mg/dL (mean 401.8 mg/dL; range 264-500mg/dL). Additional studies demonstrated an improvement in IP glucose tolerance tests (IPGTT) at from the 60 to 120 day time points. This improvement in glucose control occurred in spite of the cessation of both murine ATG and GCSF therapies prior to the 60-day time point.

2.4.2 ATG + GCSF Combination Therapy Induces Immunomodulation

To address the question of whether GCSF-mediated enhancement of diabetes reversal was due to induction of immunoregulation, murine ATG and GCSF (as both mono- and combination-therapy) were administered to pre-diabetic 12-week-old female NOD mice for up to 8 weeks. Analysis of peripheral blood revealed marked leukocyte depletion in murine ATG-treated mice versus all other groups at 2 week, with movement back towards pre-treatment levels at 4 and 8 weeks post-induction. However, the addition of GCSF to murine ATG afforded a significant increase in leukocytes at 2 weeks versus murine ATG alone. In particular, GCSF increased the percentage of splenic macrophages and neutrophils. Both murine ATG as well as GCSF have been reported to induce a population of Treg *in vivo*. Predictably, all treatments utilized in these efforts herein demonstrated a reduced percentage of Treg at 2 weeks versus control animals due to either short-term depletion by murine ATG or mobilization of macrophages and neutrophils by GCSF. GCSF therapy led to an increase in Treg versus control as early as 4 weeks while combination therapy had the greatest increase in Treg versus all other treatments at 8 weeks. As indicated by the increase in Treg the immunomodulatory benefit afforded by GCSF continued through 8 weeks, despite the lack of mobilization of macrophages and neutrophils beyond 2 weeks. Thus the greatest percentage of Treg cells was observed in mice receiving combination therapy. The health of the islets at the endpoint of the pre-diabetic study was an important consideration. As such, insulitis scoring was performed to determine the degree of lymphocytic infiltration over the 8 weeks of therapy in pre-diabetic NOD mice. Combination therapy resulted in markedly lower insulitis intensity scores when compared with islets from control animals after 8 weeks In addition, insulin staining revealed improved beta cell area in animals receiving combination therapy versus murine ATG monotherapy, while control animals demonstrated a decline in beta cell area over the 8 week period

By combining these two monotherapies, however, the health of the islets was maintained relative to control as measured by insulitis scoring and beta cell area. These results indicate that combined treatment of murine ATG with GCSF offers a highly effective means for reversal of T1D in NOD mice.

2.5 Monotherapy and Combination Therapy Clinical Trial Updates

2.5.1 GCSF Monotherapy

In August of 2008, the University of Florida T1D research group embarked on a pilot (JDRF/NIH supported) study of GCSF monotherapy, in order to firmly establish the safety of GCSF and demonstrate the potential for efficacy in mobilizing Treg in the T1D population. The study used a 2:1 drug: placebo randomization scheme and AUC C-peptide as the primary efficacy outcome measure. Twenty-one study participants completed a 12 week course of the study drug (GCSF 6mg sq q 2 weeks versus placebo). Analysis of the 12 month follow-up data did not demonstrate any significant preservation of C-peptide in treated subjects. Drug was well tolerated with the most frequent complaints being bone pain or headache treatable with ibuprofen or acetaminophen. In addition, there were no issues with splenomegaly or concerning elevation of the absolute neutrophil count.

2.5.2 ATG Monotherapy

In late 2006, the ITN approved ATG monotherapy in new-onset T1D at a limited number of sites; the START trial (Study of Thymoglobulin to Arrest Type 1 Diabetes). After safety and tolerability of the drug was demonstrated, the age group was lowered to 12 years in the Fall of 2009 and the study was fully enrolled. The primary endpoint analysis was completed in 2012 and did not reveal any benefit for ATG monotherapy (at 6.5mg/kg) in preserving beta cell function in new onset subjects (41b).

2.5.3 ATG +GCSF

A phase IIa study of ATG (2.5mg/kg) and GCSF (6mg q 2 weeks x 6 doses) utilizing a 2:1 randomization scheme (Drug: placebo) is currently underway in “established” (diagnosed for 4 months to 2 years) T1D patients (Figure 3). The study is fully enrolled (n=25) and data from all 25 subjects at the 3, 6, and 9, and 12 month study visits demonstrate ***increased*** AUC C-peptide amongst the treated subjects in comparison to those who have received placebo.

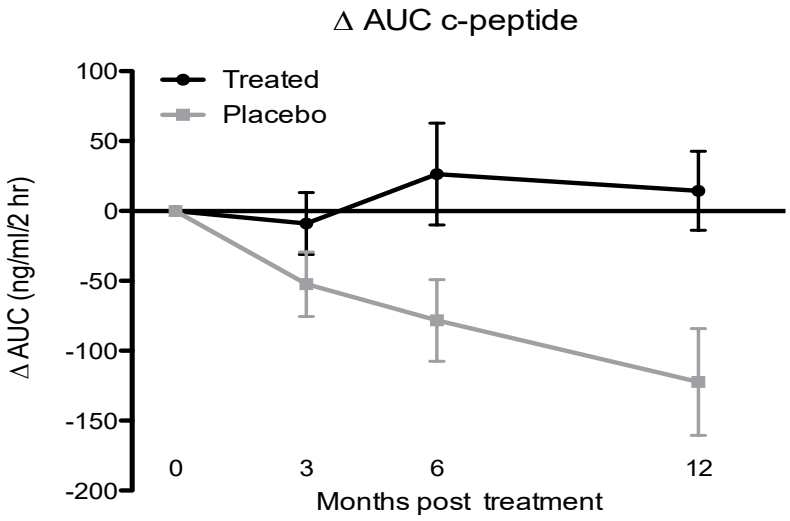

Figure 3. AUC C-peptide in Treated (ATG/GCSF) and Placebo Subjects with Established T1D. P=0.05 at 12 months

The dose of ATG for the pilot study and the current protocol was chosen based on a combination of the demonstrated safety and efficacy in treating human autoimmune and alloimmune diseases in adults and children, and the suggestion from our animal data indicating that lower dose ATG could be used successfully when utilized in combination with GCSF. Several autoimmune diseases (e.g., SLE, RA, Wegner's Granulomatosis, Systemic Sclerosis, and T1D) have been treated with some success with ATG based protocols utilizing doses from 2.5mg/kg to in excess of 10 mg/kg (68). In addition, induction therapy for kidney transplantation using low doses of ATG (2.5-5mg/kg) has demonstrated efficacy in protecting the graft and reducing acute rejection in both children and adults (69, 70).

In the ITN START study, subjects received ATG at a total dose of 6.5 mg/kg (0.5 mg/kg Day 1, then 2 mg/kg for next 3 doses). This dose has been effective and well tolerated in bone marrow and solid organ transplantation, and is well below the dose of 20 mg/kg that has been used for some transplantation protocols. Based on the observed efficacy in the mouse with combined low-dose ATG and GCSF therapy, the dose of ATG was reduced, albeit somewhat empirically, to 2.5 mg/kg with the hopes of minimizing side effects while still providing for efficacy in preserving beta cell function.

The dose and duration of GCSF was chosen on the basis of its safety as demonstrated to date in the T1D study completed at the University of Florida, and on the basis of other 12-week GCSF studies that have demonstrated potential for benefit in autoimmune disease (71).

Summary pre-clinical and preliminary clinical data:

Preclinical data demonstrate that combination therapy of ATG and GCSF has multiple beneficial mechanistic actions (e.g., increased Treg frequency, reduced islet inflammation, improved beta cell area, etc.) and dramatically extends the range of beta cell dysfunction allowable for effective and durable disease remission. Pilot clinical trial data have demonstrated efficacy in preserving C-peptide and have confirmed an acceptable safety profile. Collectively, these studies provide strong support for the performance of phase II human T1D trials with this combination of agents.

3 STUDY DESIGN

3.1 Overview

Summary of Inclusion and Exclusion Criteria

3.1.1 Inclusion Criteria

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

1. Must be $\geq$ 12 years < 46
2. Must have a diagnosis of T1D for less than 100 days at randomization
3. Willing to provide Informed Consent or have a parent or legal guardian provide informed consent if the subject is <18 years of age
4. Positive for at least one islet cell autoantibody; GAD65A, mIAA, if obtained within 10 days of the onset of insulin therapy, IA-2A, ICA, or ZnT8A

5. Must have stimulated C-peptide levels ≥ 0.2 pmol/ml measured during a mixed meal tolerance test (MMTT) conducted at least 21 days from diagnosis of diabetes.

   Randomization should occur within one month (37 days) of the MMTT. However, with prior approval by TrialNet, this window may be extended to allow randomization within 8 weeks (56 days).
6. Subjects who are EBV seronegative at screening must be EBV PCR negative within 30 days of randomization and may not have had signs or symptoms of an EBV compatible illness lasting longer than 7 days within 30 days of randomization
7. Be at least 6 weeks from last live immunization
8. Participants are required to receive killed influenza vaccination at least 2 weeks prior to randomization when vaccine for the current or upcoming flu season is available
9. Be willing to forgo vaccines during the treatment period and for 3 months following last dose of study drug
10. Be willing to comply with intensive diabetes management

3.1.2 Exclusion Criteria

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

1. Be immunodeficient or have clinically significant chronic lymphopenia: (Leukopenia (< 3,000 leukocytes /μL), neutropenia (<1,500 neutrophils/μL), lymphopenia (<800 lymphocytes/μL), or thrombocytopenia (<100,000 platelets/μL).
2. Have active signs or symptoms of acute infection at the time of randomization
3. Have evidence of prior or current tuberculosis infection as assessed by PPD, interferon gamma release assay or by history
4. Be currently pregnant or lactating, or anticipate getting pregnant within the two year study period
5. Require use of other immunosuppressive agents including chronic use of systemic steroids
6. Have evidence of current or past HIV, Hepatitis B or Hepatitis C infection
7. Have any complicating medical issues or abnormal clinical laboratory results that may interfere with study conduct, or cause increased risk to include pre-existing cardiac disease, COPD, sickle cell disease, neurological, or blood count abnormalities
8. Have a history of malignancies other than skin
9. Evidence of liver dysfunction with AST or ALT greater than 3 times the upper limits of normal
10. Evidence of renal dysfunction with creatinine greater than 1.5 times the upper limit of normal
11. Vaccination with a live virus within the last 6 weeks

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

13. Active participation in another T1D treatment study in the previous 30 days

14. Prior treatment with abatacept or anti-cd3

15. Known allergy to GCSF or ATG

16. Prior treatment with ATG or known allergy to rabbit derived products

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

3.2 Description of Treatment Groups

This protocol will enroll at least 84 participants who will be randomly assigned to the following groups:

- 28 participants will be assigned to receive ATG + GCSF
- 28 participants will be assigned to receive ATG + Placebo
- 28 participants will be assigned to receive Placebo + Placebo

3.3 Treatment Assignment and Double Masking

After participants sign the consent form they will be randomized to one of the three arms. The randomization method will be stratified by TrialNet study site. The participants will not be informed regarding the intervention assignment until the end of the study. The investigator and clinic personnel will also be masked as to study assignment. Laboratories performing assays for this protocol will be masked as to the identity of biological material to be studied.

3.4 Study Assessments

During the course of the study, participants will frequently undergo assessments of their insulin production, immunologic status, overall health and well-being and diabetes care (see schedule of assessments in Appendix A). Information about the subject's experience as a research participant will also be collected. The participants' insulin production will be measured by a series of mixed meal glucose tolerance tests (MMTT) conducted regularly during the study. The participants' diabetes control will be evaluated by measuring glycosylated hemoglobin (HbA1c) and clinical records including insulin types, doses, and timing and SMBG records. During the course of the study, samples will be drawn for storage in the National Institute for Diabetes and Digestive and Kidney Disease (NIDDK) Repository and at TrialNet Sites for future analysis.

3.5 Quality Assurance

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

3.6 Post-treatment Follow-up

At the conclusion of the study or when subjects reach 24 months, subjects will be encouraged to participate in further follow-up via participation in the TrialNet Long Term Investigative Follow-Up (LIFT).

4 PATIENT MANAGEMENT

4.1 Overview

Eligible subjects will receive either: (1) ATG (2.5mg/kg) IV followed by GCSF SQ (for those ≥ 44.5 kg, the dose is 6mg; for those <44.5 kg, the doses is 100 mcg/kg q 2 weeks x 6 doses), (2) ATG (2.5mg/kg) IV plus placebo, or (3) double placebo. Subjects will undergo screening visits to determine eligibility. Within 8 weeks of screening but preferably less than 37 days, eligible subjects will be admitted to an inpatient hospital or research facility for visit 0 and will be randomized to one of three treatment groups (ATG/GCSF, ATG/Placebo, and Placebo/Placebo). Individuals will receive oral and IV premedication and the first dose of ATG/placebo will be administered over a minimum of 12 hours (maximum of 20 hours) (Figure 4). CBC will be drawn 8 hours after the completion of dose 1 and will inform timing for infusion number 2. If labs are above threshold and at least 12 hours have passed since the completion of the first dose, the second dose of ATG/placebo will be administered over a minimum of 8 hours (maximum of 16 hours). Each ATG/placebo dose will be followed by steroid/saline infusion. The first dose of GCSF/Placebo will be given 6 hours after the end of the second ATG/placebo dose. Subsequent GCSF/placebo will then be given as outpatient every 2 weeks for a total of 6 doses over 5 visits. Subsequent visits will occur every 3-6 months through 24 months.

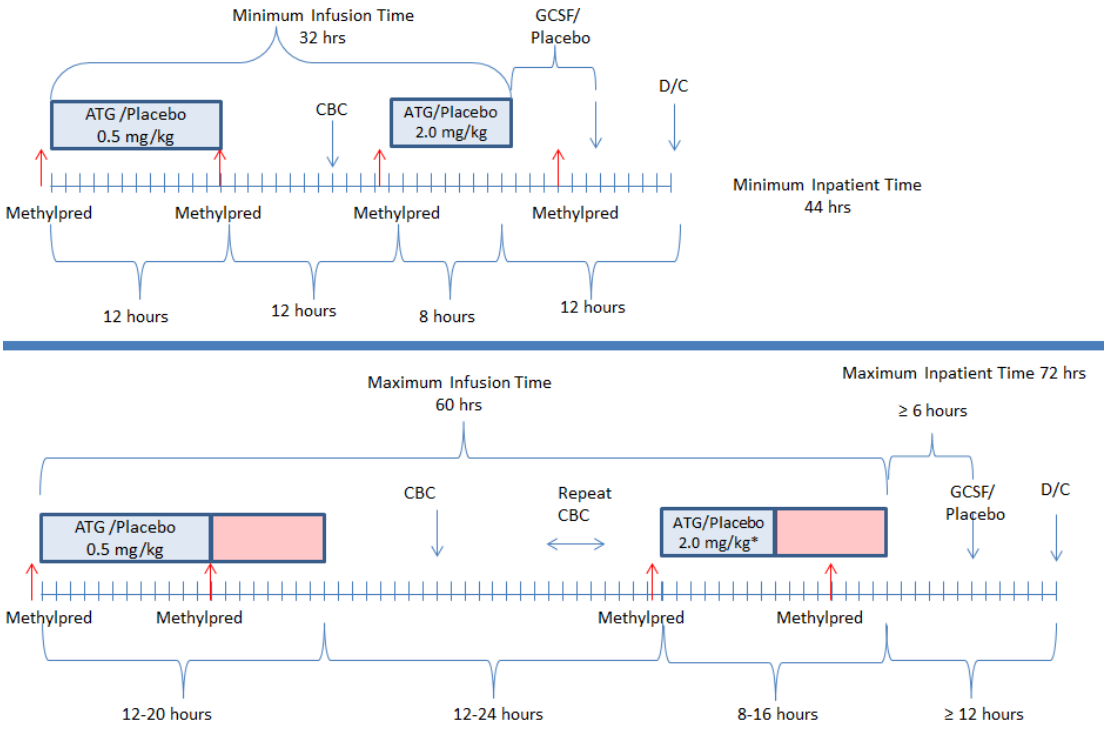

Figure 4. Minimum and Maximum Time Course for ATG Infusion and Hospitalization.
The top half of the figure demonstrates the ideal scenario for ATG infusion: First dose of ATG (0.5mg/kg) completed over 12 hours, CBC drawn 8 hours post infusion, second dose (2mg/kg) completed over 8 hours, first GCSF dose given 6 hours after completion of ATG, and D/C 12 hours after completion of ATG. The bottom half of the figure demonstrates the maximum allowable time for ATG infusion and first GCSF dosing if laboratory abnormalities or patient care issues require interrupting, postponing, or reducing the rate of ATG infusion

4.2 Screening

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

4.3 Randomization

Eligible study participants will be randomized by the TrialNet Coordinating Center at the baseline visit once eligibility has been confirmed. Subjects will be assigned a study randomization number corresponding to the treatment group assignment.

4.4 Intensive Diabetes Management

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

Glucose levels should be checked frequently and records of the glucose levels communicated regularly to the study team. Records of communication with the participant will provide source documentation of this interaction.

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

4.5 Drug Administration

4.5.1 ATG

ATG is indicated for the treatment of renal transplant acute rejection in conjunction with concomitant immunosuppression. Each 10 mL vial contains 25 mg ATG (rabbit) as well as 50 mg glycine, 50 mg mannitol, and 10 mg sodium chloride.

4.5.2 ATG / Placebo Administration

All participants will be admitted to the hospital for the duration of the infusions and will be discharged no sooner than 12 hours after ATG infusion has been completed. Body weight at baseline (Time 0 – admission for the ATG/placebo infusion) will be used in calculating the doses for all infusions. Allowance in dose administration will be within +/- 5% of calculated dose.

To minimize the risk for thrombophlebitis associated with ATG infusion, 1000 units of heparin and 20 mg of hydrocortisone will be added to the ATG infusion bag for each dose given via peripheral intravenous administration. Since there is only a slight risk for thrombophlebitis for placebo infusion, the heparin and hydrocortisone will not be included in the infusion bag.

The first dose (0.5mg/kg) will be infused over a minimum of 12 hours, and the second dose (2mg/kg) over a minimum of 8 hours. The second dose should be given no less than 12 and no more than 24 hours after the previous dose. A maximum of 60 hours from the start of the first IV infusion will be allowed to complete all IV study drug (see parameters below). If a subject has not completed the two IV study drug infusions at the 60th hour, the infusion will be discontinued and they will not receive additional study drug. Vital signs will be checked every 30 minutes for the first 2 hours of the study drug infusion and thereafter at 60-minute intervals or as indicated for clinical signs or symptoms. Blood for laboratory testing should be drawn at least 8 hours after completion of each infusion in order to obtain reliable and comparable results.

Information about subject's clinical signs and symptoms related to ATG infusion will be captured on infusion CRFs. Those that are Grade 2 or more will be considered AEs and reported as such.

4.6 Premedication:

To reduce the risk of adverse reactions to ATG infusion, methylprednisolone 0.25 mg/kg IV will be given no less than 30 minutes before each infusion of active drug and 0.25 mg/kg IV will be given 12 hours (±15 min) after the start of each infusion of active drug.

Placebo group: A placebo (saline) infusion similar in appearance will be given no less than 30 minutes before and 12 hours (±15 min) after the start of each infusion.

Both groups: Participants in both groups will be pre-medicated with an antihistamine and acetaminophen PO at least 30 minutes before each infusion and every 4–6 hours as needed during the infusion, as follows:

- Diphenhydramine 1.25 mg/kg/dose to a maximum of 50 mg.
- Acetaminophen 10–15 mg/kg/dose to a maximum of 650 mg.

4.6.1 Cytokine Release Syndrome:

With ATG infusion, the subject may experience Cytokine Release Syndrome (CRS). The signs and symptoms can span a wide clinical spectrum.

Mild Reactions: For mild (grade 1) reactions per the NCI-CTCAE for CRS, the study medication will be continued. The investigator shall take one or more of the following actions, depending on the type of the reaction:

1. Administer additional doses of antihistamine and acetaminophen.
2. Reduce the rate of infusion by 50% or more.
3. For chills and rigors, meperidine may be considered.

Moderate Reactions: For moderate (grade 2) reactions per the NCI-CTCAE for CRS, the study medication may be interrupted. The investigator shall take the following actions, depending on the type of the reaction:

1. Interrupt infusion if any of the following occurs:
   a. Oral temperature of > 40.0 °C
   b. Symptomatic bronchospasm or pulmonary edema
   c. Allergy-related edema
   d. Hypotension
2. When the temperature is < 38.5 °C and signs and symptoms improve, restart ATG.
3. Closely monitor the subject with pulse oximetry and a blood pressure monitoring; provide ongoing nursing evaluation until at least 2 hours after the infusion is completed.
4. If necessary, glucocorticoids can be given every 6 hours at a dose of 0.5 mg/kg of methylprednisolone or equivalent.
5. If a subsequent dose of ATG further exacerbates the signs and symptoms of CRS despite following the above guidelines, the study treatment must be permanent discontinued.
6. Additional supportive or resuscitative measures (such as the use of epinephrine) may be needed if clinically indicated.

Severe Reactions: For severe (grade 3) reactions or greater per the NCI-CTCAE for CRS, the study medication will be permanently discontinued.

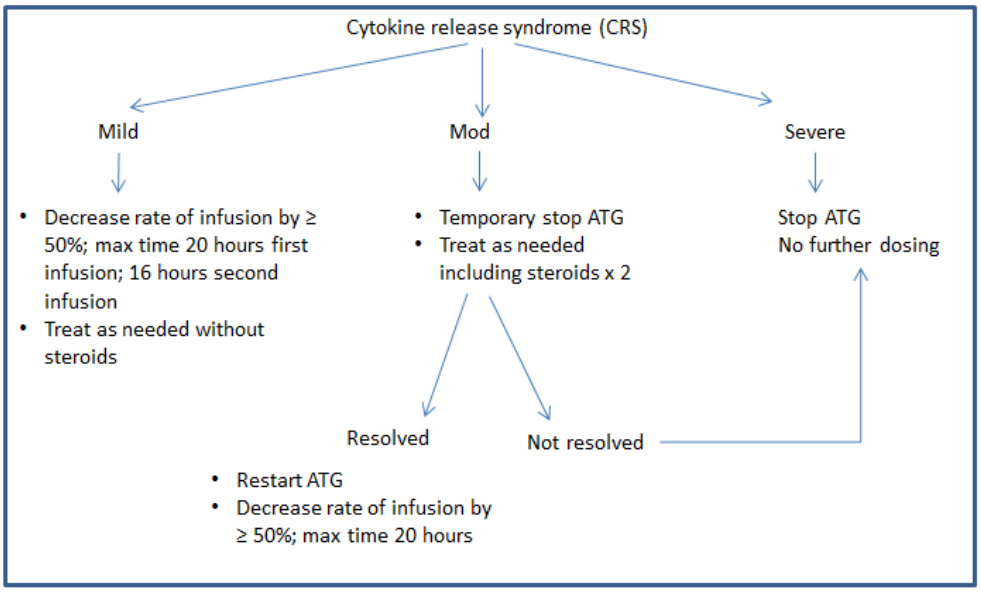

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Figure 5. Rate Adjustments in Response to Cytokine Release Syndrome

4.6.2 Allergic Reactions

Hypersensitivity: In rare cases, patients may experience hypersensitivity, which refers to immediate allergic, IgE mediated reactions to ATG. Such patients primarily develop skin rash and respiratory distress early in the course of the infusion (usually within the first hour). For such reactions, the investigator shall take one or more of the following actions:

1. Discontinue the infusion.
2. Apply appropriate resuscitation measures, including administration of 0.3–0.5 mL aqueous epinephrine (1:1000 dilution) subcutaneously.
3. Use other resuscitative measures, as clinically indicated, including oxygen, intravenous fluids, antihistamines, corticosteroids, pressor amines, and airway management.

Mild to Severe Reactions: For mild to severe (grade 3 or less) reactions per the NCI-CTCAE for allergic reactions, the study medication may be restarted at the discretion of the investigator. For those with severe (grade 3) reactions, any subsequent doses should be accompanied by pre-medication with additional corticosteroids.

Life-threatening Reactions: For life-threatening (grade 4) reactions per the NCI-CTCAE for allergic reactions, the study medication will be permanently discontinued.

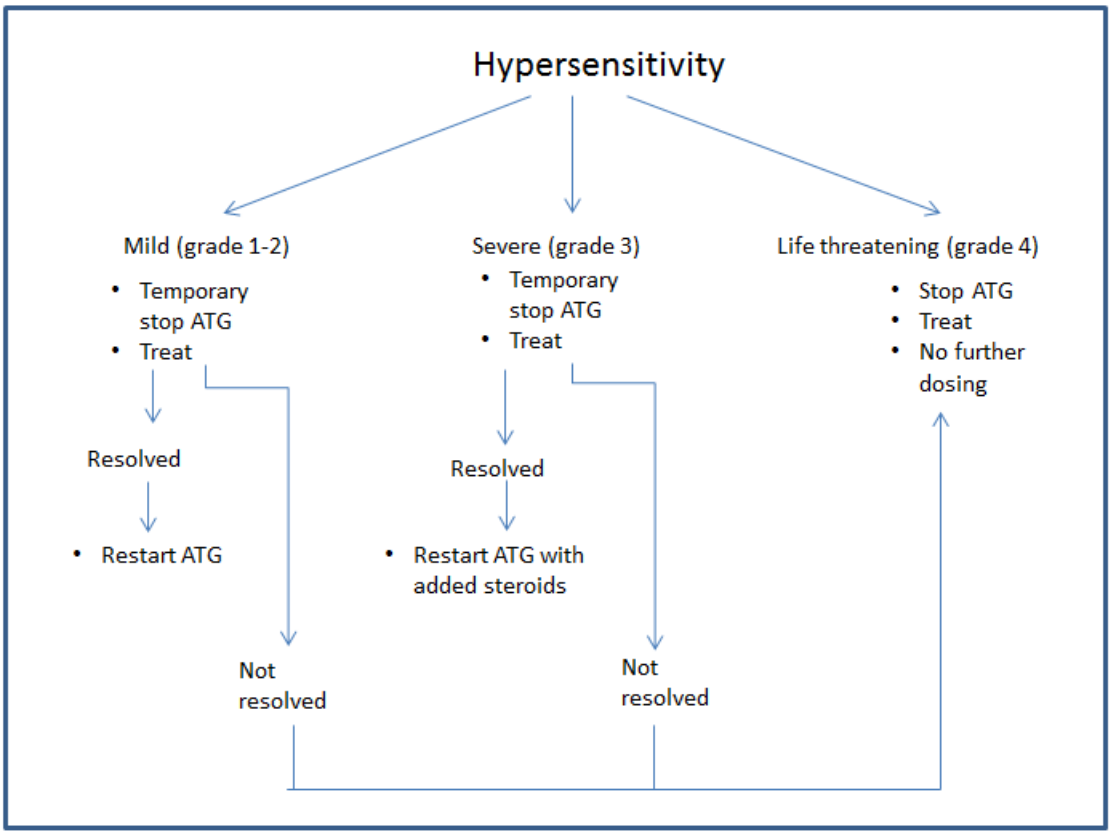


Figure 6. Rules for Temporary interruption or discontinuation of ATG due to Hypersensitivity Reaction

4.7 Modification or Discontinuation of ATG

4.7.1 Modification of second ATG dose to 1mg/kg

All ATG must be administered by the end of 60 hours. CBC with differential will be obtained 8 hours after the completion of infusion 1. If initial labs show acceptable values (WBC count >2,000 cells/mm3, neutrophil count >1,200 cells/mm3, and platelet count >75,000 cells/mm3), ATG will be continued at the full dose. If labs are outside of these parameters a second CBC can be obtained up to 24 hours after completion of the infusion.

The second ATG/placebo dose will be reduced from 2mg/kg to 1mg/kg if both the a 8 hour post infusion AND 24 hour post infusion platelet counts fall between 50,000 and 75,000 cells/mm3, or if the neutrophil count falls to a value >800 but <1,200 cells/mm3. Although the ATG package insert recommends reducing the dose by 50% if a patient has a total white blood cell (WBC) count of between 2000 and 3000 cells/mm3, it has been decided not to reduce the dose in this protocol for the following reasons:

- Because the primary focus of this study is initial T-cell depletion, premature or unnecessary reduction of the ATG dose may result in reduced efficacy.
- If the WBC count falls to a significantly lower level, i.e. <2000 cells/mm3, then ATG will be held

- Participants will be closely monitored for infectious disease risk and offered prophylaxis as warranted to minimize their risk
- GCSF is expected to minimize the time/severity of neutropenia

4.7.2 Discontinuation of ATG

The ATG/placebo will be discontinued if any one of the following is observed:

- A total WBC count <2,000 cells/mm3 that persists up to 24 hours after the time of the planned infusion.
- A neutrophil count <800 cells/mm3 that persists up to 24 hours after the time of the planned infusion.
- A platelet count <50,000 cells/mm3 that persists up to 24 hours after the time of the planned infusion.
- The participant experiences exacerbation of Cytokine Release Syndrome (CRS) twice consecutively despite the use of glucocorticoids.
- If the investigator believes that the study treatment is no longer in the best interest of the participant

Participants who prematurely discontinue study treatment will remain in the study and undergo all efficacy and safety assessments.

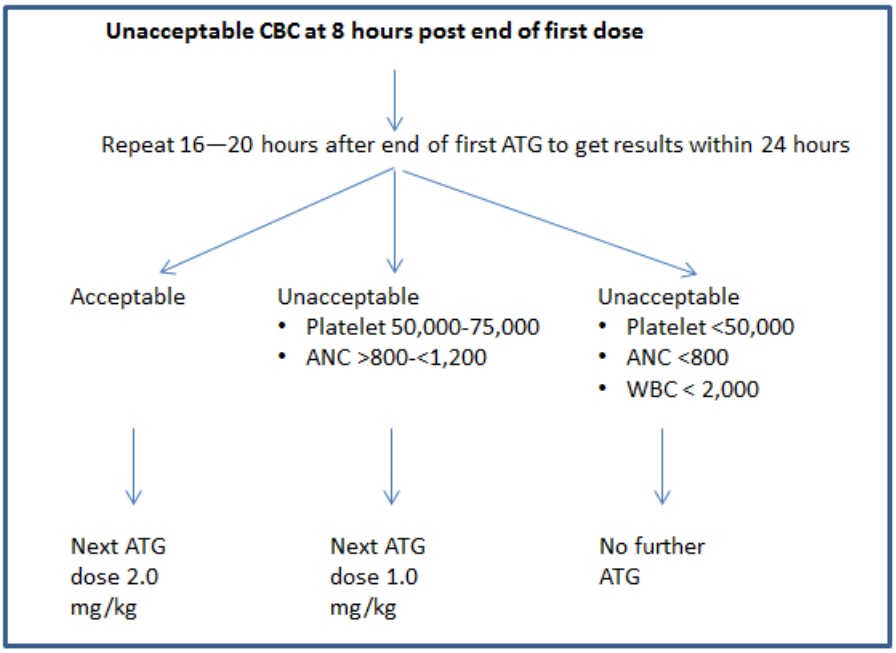


Figure 7. Rules for Reducing or Holding Second Dose of ATG

4.7.3 Serum Sickness

Serum sickness from host immunization against rabbit protein may occur 7–15 days after the first dose of ATG. The patient may require glucocorticoid treatment for supportive care. The dose will depend on the severity of signs and symptoms. Each subject who develops signs or symptoms concerning for serum sickness will undergo a centralized review to ensure consistent decision making on the use of steroids. For subjects greater than 50kg, we recommend Prednisone as follows: Days 1-3, 50 mg every 12 hours, Day 4, 40 mg every 12 hours, Day 5, 30 mg every 12 hours, Day 6, 20 mg every 12 hours, Day 7, 10 mg every 12 hours. For subjects less than 50kg we will provide Prednisone as follows: Days 1-3, 30mg every 12 hours, Day 4, 20mg every 12 hours, Day 5, 10 mg every 12 hours, Day 6, 5 mg every 12 hours, Day 7, 5 mg once. Clinical judgment should be used in augmenting additional steroid therapy to provide the least amount of steroid required to provide symptomatic relief of serum sickness.

4.8 GCSF

GCSF is a covalent conjugate of recombinant methionyl human granulocyte colony stimulating factor (filgrastim) and monomethoxypolyethylene glycol. GCSF is a Colony Stimulating Factor that acts on hematopoietic cells by binding to specific cell surface receptors, thereby stimulating proliferation, differentiation, commitment, and end cell functional activation. GCSF is supplied in 0.6 mL prefilled syringes for subcutaneous injection. Each syringe contains 6 mg GCSF (based on protein weight), in a sterile, clear, colorless, preservative-free solution (pH 4.0) containing acetate (0.35 mg), sorbitol (30.0 mg), polysorbate 20 (0.02 mg), and sodium (0.02 mg) in water for injection, USP. The standard 6mg dose will be given with the exception of subjects who weigh less than 44.5 kg. For subjects who weigh less than 44.5kg, the dose of GCSF will be given as 100mcg/kg/dose. Dosing calculations will be made based on the weight at the previous visit unless there has been more than a 10% change. Allowance in dose administered up to +/- 5% of calculated dose is permitted.

4.8.1 GCSF / Placebo Administration

GCSF/Placebo treatment will begin 6 hours after completion of the ATG / Placebo. GCSF/ Placebo will be given subcutaneously every 2 weeks for a total of 6 doses. Drug vehicle provided by the manufacturer will be used for the placebo injections.

4.9 Modification or Discontinuation of GCSF

Subjects will have a directed physical exam at each study visit and a CBC obtained every two weeks after the initiation of GCSF /placebo until they have completed study drug therapy. If at any time during the GCSF /placebo therapy the subject develops an ANC greater than 35 x 10^9/L, subsequent doses of GCSF /placebo will be given as placebo (regardless of study randomization) until the ANC is again lower than 35 x 10^9/L. A repeat CBC will be allowed once between scheduled doses to provide subjects an opportunity to normalize a previously elevated ANC. In addition, if splenomegaly is noted on physical exam, placebo will be given for the remainder of the drug course regardless of randomization.

4.10 Concomitant Mediations

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

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

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

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

4.11 Infectious Disease Screening

In general, no specific infectious diseases prophylaxis is warranted in this study. This decision is based on the limited duration and dose of exposure to ATG. In lieu of active serologic or virologic monitoring strategies, all subjects will be counseled on an ongoing basis on the importance of notifying their research centers about the presence of signs or symptoms suggestive of infection especially over the 1-2 months after the infusion. They will also be counseled on the importance of notifying the research center about potential exposures to varicella, influenza, or other infectious illnesses. In addition, during the first 2 weeks post ATG infusion, all research subjects will contacted on a daily basis and queried about the presence of signs or symptoms (e.g. fever, rhinorrhea, cough, sore throat, mouth sores, myalgia, arthralgia, etc) that might represent markers of active infection. Specific algorithm(s) for the evaluation and management of those patients identified as having signs and/or symptoms concerning for infection are provided in the manual of operations for this study and are directed based on the participant's specific symptomatology. This will provide general and in some case specific recommendations for the assessment of patients.

All subjects will have EBV serology determined prior to study enrollment. Subjects who are EBV seronegative at screening must be EBV PCR negative within 30 days of randomization and may not have had signs or symptoms of an EBV compatible illness lasting longer than 7 days within 30 days of randomization. Additional EBV or other infectious disease monitoring will be done only if clinically indicated.

5 STUDY ASSESSMENTS

See Appendix 1 for detailed schedule of assessments

5.1 General Assessments

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

• Medical history including lifestyle and participant experience assessment
• Physical exam
• Concomitant medications
• Adverse events

5.2 Laboratory Assessments

The following general laboratory assessments will be performed:

- Chemistry (sodium, potassium, chloride, CO2, glucose, BUN, creatinine)
- Liver function tests (ALT, AST, alkaline phosphatase, total protein, albumin, total and direct bilirubin)
- Hematology (complete blood count with differential and platelets)
- CD4/CD8 ratio
- Serum IgG levels

At screening, these additional laboratory assessments will be performed:

- Interferon-Gamma Release Assay Test (IGRA)
- Diabetes Autoantibodies
- Antibodies to HIV, hepatitis B (antiHBcAb, HBsAg), hepatitis C (HCV), Cytomegalovirus (CMV IgG, IgM), and Epstein-Barr Virus (EBV IgG, IgM, EBNACMV and EBV PCR

Additional assessments include:

- Pregnancy test before each injection for all sexually mature women

5.3 Mechanistic Outcome Assessments

TrialNet will perform immune and genetic assays to further understand mechanisms that may be underlying the type 1 diabetes disease process and response to therapy. For this purpose, samples for PMBC, DNA, RNA, plasma, and serum may be obtained.

5.4 Metabolic Outcome Assessments

Metabolic assessments will consist of:

- Glucose records and reports of hypoglycemia

- Insulin dose
- HbA1c
- Mixed meal tolerance test (MMTT): 2 hour MMTT at 3, 6, 9 months, 4 hour MMTT at screening, 12, 18, and 24 months

5.5 Visit Windows

Randomization must occur within 100 days from diagnosis of T1D. The initial treatment should begin within 100 days from the day of diagnosis and generally within 37 days from the screening MMTT. With prior approval, this window may be extended to 56 days. All subsequent treatment visits and follow up visits in Appendix 1 must occur within the time limits specified below:

	Visit Windows
Visit -1:	At least 2 weeks before visit 0 and at least 21 days from diagnosis.
Visit 0:	Randomization < 100 days from diagnosis of T1DM and within 37 days from Visit -1.
Visits 1 through 6:	±3 days
Visits 7 through 9:	±7 days
Visits 10 and 11:	±14 days

5.6 Withdrawal from treatment

The study will be conducted according to the intent-to-treat principle. This means that once randomized into the study, a participant will be expected to undergo all scheduled follow-up assessments and will remain within the assigned treatment group for purposes of statistical analysis regardless of the actual course of treatment administered. Withdrawal from treatment does not automatically entail withdrawal from the study. Withdrawal from the study will only occur if the participant dies or withdraws consent. Subjects who withdraw consent are classified as inactive but may again become active upon re-entry into the study, if they so choose.

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

A participant may elect to discontinue study medications, may be unable to continue them, or may be withdrawn (temporarily or permanently) at the discretion of the Principal Investigator if s/he determines that it is unsafe to continue or there is a significant change in the risk/benefit.

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

5.7 Re-Entry into Study Treatment

In some circumstances, a participant may temporarily discontinue the study medication and/or not return to the study clinic for follow-up visits. If the participant decides to return for study injections and/or follow-up assessments at a later date, he or she will be allowed and encouraged to do so.

6 PARTICIPANT SAFETY

6.1 Risk, Benefits and Inclusion of Children

The risks of this study are presented in this protocol and in the informed consent form. This study will examine whether ATG-GCSF will preserve beta cell function, but there is no guarantee that this will occur.

There is the prospect of direct benefit to the individual subjects for their participation in the study. These potential benefits include the recognized benefits of being in a clinical study, including close monitoring and additional resources available to maintain tight glycemic control offered to all subjects, regardless of group assignment. Further, the intervention has the prospect of direct benefit to a given subject and is likely to yield general knowledge about T1DM that is of importance for the understanding and amelioration of T1DM in children.

The study procedures, while possibly slightly greater than minimal risk, offer the possibility of benefit in the close monitoring for all children. Assent of children along with consent of the parents will be obtained prior to any study procedures. This research proposal in children is therefore consistent with United States Department of Health and Human Services, Protection of Human Subjects, subpart D, section 46.405 (research involving greater than minimal risk but presenting the prospect of direct benefit to individual subjects) and with Subpart D. 50.52 (Clinical investigations involving greater than minimal risk but presenting the prospect of direct benefit to individual subjects).

6.2 Potential Risks

6.2.1 ATG

ATG has been widely used in transplantation and autoimmune disorders (42). With the low dose, the absence of other immunosuppressive medications, and the lack of severe medical conditions other than T1D we expect minimal side effects. In the ITN study, subjects receive ATG at a total

dose of 6.5 mg/kg, a dose which has been effective and well tolerated in bone marrow and solid organ transplantation, and well below the dose of 20 mg/kg that has been used for some transplantation protocols. For this study, the doses will be administered as 0.5 mg/kg during the first infusion and 2 mg/kg during the second infusion to reduce reactions. ATG will be given slowly by continuous IV infusion over at least a 12 hour period during the first dose and at least an 8 hour period during the second dose. Subjects will receive pre-medication with methylprednisolone, acetaminophen and diphenhydramine to minimize side effects from possible cytokine release syndrome (CRS), and will be closely observed in the hospital during the treatment period. Symptoms of CRS may include fever, chills, rigors, headache, tremor, nausea, vomiting, diarrhea, abdominal pain, muscle and joint pain, and malaise. Anaphylactic reactions have been rarely reported with ATG, but delayed allergic reactions with serum sickness are occasionally observed.

ATG contains a variety of antibodies that may cross-react with cell-surface markers. ATG causes lymphocyte depletion with marked depletion of lymphocytes occurring acutely. The circulating number of T cells increases with cessation of therapy, usually reaching pre-treatment levels by 2 months. Subjects are at low risk for opportunistic infection during this window of recovery and will receive close surveillance for primary viral infections, viral reactivations, and bacterial infections (76, 77).

ATG may also lead to leukopenia and thrombocytopenia. Effects are dose-dependent and are mainly encountered with over dosage. In up to 3% severe thrombocytopenia may occur but this is invariably seen in doses 4-5 times that which we are proposing. Thrombocytopenia in these subjects often occurred in a postoperative transplant setting and with other immunosuppressants. There was no concern for prolonged leukopenia or thrombocytopenia in our pilot ATG-GCSF study.

Transient abnormalities in liver function tests have been described in patients with aplastic anemia treated with ATG preparations. Such adverse events have not been noted in numerous other clinical settings in which ATG has been used, and it is not clear if this is related to the underlying disease or to associated medications used.

Conflicting data exists about the risk of EBV-related lymphoproliferative disease. Furthermore, in renal transplant patients, the overall risk in this transplant population is low (0.25%-0.85%), and patients received several immunosuppressive therapies and were on continuous immunosupression (42). There were no reported cases of EBV-associated lymphoproliferative disease in over 1,675 treated subjects (including children) treated for aplastic anemia.

6.2.2 GCSF

There are some potential risks associated with GCSF therapy. The side effect profile of GCSF injection will be monitored by close clinical observation of the subjects for 1 hour after the first GCSF injection as well as with daily contact to the subjects during the first 5 days of GCSF/placebo therapy and then weekly until the week 12 visit.

GCSF carries a risk (10-20% of patients) of bone pain and a risk (less than 10%) of headache, fever, pain or redness at the site of injection, and dizziness. According to the company USPI, there is also potential risk of Acute Respiratory Distress Syndrome, serious allergic reactions, and Sickle Cell crisis. There is also a risk that the subjects' WBC could become elevated while taking GCSF. A complete list of the potential risks associated with GCSF is further described in the package insert at http://pi.amgen.com/united_states/neulasta/neulasta_pi_hcp_english.pdf. This will be monitored by the study team at each center and the dose of study drug will be dispensed by the pharmacy as placebo regardless of randomization (to ensure blinding is

protected) if the ANC is > 35 x 10^9/L. There are also now a small number of reports in which recipients of GCSF have developed splenomegaly and/or splenic rupture. This risk will be explicitly discussed in the consent form and subjects will have close physical examination and follow-up to screen for any signs or symptoms. In addition, weekly contact for the month following therapy will be used to document any adverse reactions. The CBC and complete metabolic profile at each blood draw will be carefully reviewed for any clinically relevant abnormalities. There are no other known long-term risks from a 12 week course of GCSF.

6.3 Pregnancy

Female subjects with reproductive potential will be instructed to use effective means of birth control (which includes abstinence) from randomization until 3 months post last infusion of study drug to assure safety. They will also be asked to avoid pregnancy until last scheduled study visit at 24 months post randomization. This is to assure accurate endpoint measurements. They will undergo urine pregnancy testing at the start of every study visit. All pregnancies that are identified during the study must be followed to conclusion and the outcome of each must be reported. The investigator should be informed immediately of any pregnancy occurring in a female participant. Monitoring of the participant should continue until the conclusion of the pregnancy. Subjects that are found to be pregnant while on this study shall have treatment withheld, but will still be followed for safety and other study measures as appropriate. Treatment may only be resumed when subjects are no longer pregnant or nursing.

6.4 Protecting Against or Minimizing Potential Treatment Risks

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

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

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

7 ADVERSE EVENT REPORTING AND SAFETY MONITORING

7.1 Adverse Event Definition

7.1.1 Adverse Event

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

Throughout the study, the investigator must record all adverse events on source documents. Events not related hypoglycemia, or hyperglycemia which are Grade 2 or greater per the NCI CTCAE (see Section 7.1.5. Grading Event Severity below) must be reported to TNCC as AE. The investigator should treat participants with adverse events appropriately and observe them at suitable intervals until the events resolve or stabilize.

Adverse events may be discovered through:

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

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

7.1.2 Adverse Reaction

An adverse reaction means any adverse event caused by a drug. Adverse reactions are a subset of all suspected adverse events for which there is reason to conclude that the drug caused the event. Suspected adverse reaction means any adverse event for which there is a reasonable possibility that the drug caused the adverse event. For the purposes of safety reporting, "reasonable possibility" means there is evidence to suggest a causal relationship between the drug and the adverse event. A suspected adverse reaction implies a lesser degree of certainty about causality than an adverse reaction, which means any adverse event caused by a drug. Examples of evidence that suggest a causal relationship (reasonable possibility) between the drug and the adverse event include:

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

7.1.3 Serious Adverse Event/Reaction

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

1. Death. A death that occurs during the study or that comes to the attention of the investigator during the protocol-defined follow-up after the completion of therapy must be reported whether it is considered to be treatment related or not.
2. A life-threatening adverse event. A life-threatening event is any adverse therapy experience that, in the view of the investigator, places the participant at immediate risk of death from the reaction as it occurred.

3. Inpatient hospitalization or prolongation of existing hospitalization with the exception of hospitalization relating to initial diagnosis of type 1 diabetes.
4. Persistent or significant incapacity or substantial disruption of the ability to conduct normal life functions
5. Congenital anomaly or birth defect.
6. Important medical events that may not result in death, be life-threatening, or require hospitalization may be considered serious when, based upon appropriate medical judgment, they may jeopardize the patient or subject and may require medical or surgical intervention to prevent one of the outcomes listed above.

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

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

7.1.4 Unexpected Adverse Event

An adverse event/reaction is considered unexpected when the nature (specificity) or severity of the event is not consistent with the risks described in the Investigator's Brochure or the informed consent document. Unexpected refers to an experience that has not been previously observed. This includes events that occur more frequently than expected.

7.1.5 Grading Event Severity and Causality

TrialNet has adopted usage of the National Cancer Institute (NCI) Common Technology Criteria for Adverse Events (CTCAE) and/or study-specific criteria for classification to describe the severity of adverse events with the exception of hypoglycemia and hyperglycemia. Hypoglycemia and hyperglycemia will be reported as adverse events only in the case of requiring the assistance of others due to loss of consciousness or DKA. TrialNet Investigators will also provide an assessment of relationship of AE to study drug as not, unlikely, possibly, probably, or definitely related.

7.2 Adverse Event Reporting and Monitoring

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

The adverse event case report form for the protocol must be completed for all adverse events (AE). For reporting serious adverse events (SAE), the MedWatch Form should also be completed and faxed to the TNCC *within 24 hours of when the site was notified of the event.* This will be reviewed by the TrialNet Medical Monitor, the TrialNet Safety Monitoring Committee, and the DSMB as appropriate. Deaths must be reported immediately. Event outcome and other follow-up information regarding the treatment and resolution of the event will be obtained and

reported when available, if not known at the time the event is initially reported. The follow-up information should contain sufficient detail to allow for a complete medical assessment of the case and an independent determination of possible causality.

Adverse events will be assessed by the TrialNet Medical Monitor. The DSMB will conduct regular safety reviews approximately every three to six months (and otherwise as needed) of adverse events by treatment group assignment. Serious adverse events as well as adverse events leading to study discontinuation will be reviewed by the DSMB.

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

8 STATISTICAL CONSIDERATIONS AND ANALYSIS PLAN

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

Primary analysis of treatment effect will be conducted employing the intention-to-treat principle where all participants with measured one-year C-Peptide AUC mean are included regardless of treatment compliance (Complete Case Analysis).

8.1 Primary Outcome and Analyses

The primary outcome of each participant is the area under the stimulated C-peptide curve (Y_{AUC}) over the first 2 hours of a mixed meal glucose tolerance test conducted at the <u>one-year</u> visit. The Y_{AUC} is computed using the trapezoidal rule which translates into a weighted sum of the timed C-peptide values over the 120 minute MMTT. The more appealing quantity is the AUC mean which is $\frac{Y_{AUC}}{120}$ in nmol/L and will be denoted as Y_{Cp}.

Let $Y_{Cp}^{ATG/GCSF}, Y_{Cp}^{ATG}$, and $Y_{Cp}^{Control}$ represent the C-peptide AUC mean for study patients receiving both ATG and GCSF, receiving ATG alone, and those receiving placebo, respectively. Likewise, let $\mu_{Cp}^{ATG/GCSF}, \mu_{Cp}^{ATG}$, and $\mu_{Cp}^{Control}$ represent the population mean of C-Peptide for these groups at one year, respectively.

The primary statistical hypotheses to be assessed in the study are:

$$\mathrm{H}_0\colon \mu_{Cp}^{ATG/GCSF} = \mu_{Cp}^{Control} \text{ versus } \mathrm{H}_a\colon \mu_{Cp}^{ATG/GCSF} > \mu_{Cp}^{Control}$$

$$\mathrm{H}_0\colon \mu_{Cp}^{ATG} = \mu_{Cp}^{Control} \text{ versus } \mathrm{H}_a\colon \mu_{Cp}^{ATG} > \mu_{Cp}^{Control}$$

The primary analysis will be conducted on a transformed scale using the function $\log(Y_{Cp}+1)$. This provides better normal distributional behavior by the test statistic. The comparison between any two treatment arms will be based on a Wald test of treatment effect in an ANCOVA model adjusting for gender, baseline age, and baseline $\log(Y_{Cp}+1)$.

8.2 Secondary Outcome and Analyses

Additional analyses of the primary outcome to determine the effect of ATG with and without GCSF include:

- C-peptide AUC mean at 3, 6, 9, 12, 18, and 24 months using the ANCOVA mentioned above.

- The hazard rate of C-peptide failure (C-peptide failure defined as the first occurrence at which the 2 hour peak C-peptide < 0.2 nmol/L during a MMTT) using the proportional hazards model[82] while adjusting for baseline level of C-peptide, gender and baseline age.

- Longitudinal analyses of C-Peptide AUC means 3, 6, 9, 12, 18, and 24 months using a mixed effects model[72] with a random intercept and slope by subject, adjusted for the baseline level of C-peptide, gender and baseline age. The mean intercept and slope will be compared between treatment groups.
- Treatment interactions with the covariates baseline C-peptide, gender and baseline age will be analyzed with a homogeneity test categorizing the continuous variables into 3 approximately equal groups; ladder plots will be constructed. Other variables to be tested for treatment interactions are HbA1c levels, HLA , other genotype and immune phenotypes, and race/ethnicity, as appropriate.

Additional secondary objectives to determine the effect ATG with and without GCSF has on the following:

- HbA1c, Insulin dose (units/kg) and Blood glucose over time by treatment group using ANCOVA.
- Adverse events
    - Number and severity.
    - The rates of severe adverse events will be computed (total number of events divided by total subject years of follow-up).
- Hypoglycemia
    - Number of major hypoglycemic events (defined as loss of consciousness, seizure, or requiring assistance from another person because of altered state of consciousness).
    - Reported hypoglycemic events confirmed with capillary blood glucose measurement less than 70 mg/dl.
    - The rates of severe hypoglycemic will be computed (total number of events divided by total subject years of follow-up).
- For individuals with continuous glucose monitoring data available

- Area under the curve and number of events less than 70 mg/dl on the continuous glucose monitoring record.
- Hyperglycemia measured as the area under the curve and number of events greater than 180 mg/dl on the CGMS record prior to each study visit.
- Glycemia and glycemic variability prior to each MMTT visit
- The daily mean level of glucose, as well as the levels before and after meals will be computed.
- Measures of diurnal variability in glucose will be measured by the J-value, standard deviation of glucose values, and the mean amplitude of glycemic excursion (MAGE).

8.3 Additional Outcomes and Analyses

Additional outcomes of interest include the effects of ATG with and without GCSF treatment with regard to the Mechanistic Studies assessed from blood draws as outlined in the Schedule of Assessments. These measures include, but are not limited to, exploration of pharmaco-genetic signatures that may differentiate response to treatment, and the relationship between hsCRP and IL-1β levels and beta cell function and/or other metabolic measures.

Additional analyses will compare the results in this trial to other trials using ATG and GCSF and other TrialNet studies. Data in this trial will be used in conjunction with other TrialNet data for exploratory analysis.

8.4 Sample Size and Power Calculations

The primary analysis will compare the difference in C-Peptide between experimental and placebo treatment groups at 12 months using $\log(Y_{Cp}+1)$ transformation and ANCOVA model adjusting for gender, baseline age and the baseline value of $\log(Y_{Cp}+1)$. Estimates of the mean and standard deviation of $\log(Y_{Cp}+1)$ (expressed algebraically as: $\hat{\mu}_{\log(Y_{Cp}+1)}$ and $\hat{\sigma}_{\log(Y_{Cp}+1)}$) in the placebo group were derived from the last four TrialNet studies of early onset disease. The 90% confidence bound estimates were used to provide good confidence that this trial's study population characteristics fall within these limits to assure the advertised statistical power; the estimates are $\hat{\mu}_{\log(Y_{Cp}+1)}=0.360$ and $\hat{\sigma}_{\log(Y_{Cp}+1)|X}=0.167$ (i.e., the residual mean squared error regressing on gender, baseline age and C-peptide). The geometric-like mean of Y_{Cp} for the placebo group is $\exp(0.36)-1=0.433$ nmol/L.

Using standard equations for the comparison of two means and a 1:1:1 allocation, a sample size of 78 participants (26 per group) with complete data would provide power of 85% to detect a 50% increase in the geometric-like mean in either experimental treatment group (compared to the control) using the Wald test (from the adjusted linear model) at the 0.025 level (one-sided). Thus the overall Type I error for both tests is approximately 0.05.

Assuming that 10% of the participants will have missing data (one-year MMTT was not done or subject withdrew prior to the one-year assessment), the sample size goal for this study will be set at 84 participants (28 per group).

The study will be closed to additional participants when the total number then randomized plus a fraction of those in screening (i.e., screening for eligibility) is expected to provide the proper number of eligible participants. Participants who had already conducted the initial screening visit at that time will be allowed to complete screening and be randomized if both consenting and eligible.

In the situation where both hypotheses are rejected in favor of the experimental regimen, then it is appropriate to decide which experimental regimen should be considered first for additional clinical trials. If ATG and ATG+GCSF truly differ in efficacy, it is not expected that the difference would be as large as the effect size used in this design. Thus requiring the difference to reach statistical significance would be too stringent due to the lack of statistical power. The plan is to employ the method of Simon[81] to select the treatment with the largest geometric-like mean in C-Peptide regardless of how small the difference is over the other experimental regimen. Formally, the decision rule is to select the experimental regimen with highest predicted mean based on the fitted linear regression model of C-Peptide used in the formal hypothesis tests. Given the one experimental treatment is associated with 25% higher mean (half of the design effect size) compared to the other experimental treatment, the probability is 96% that the most efficacious regimen will be selected with this decision rule. Even if the increase is only 12.5% (i.e., a fourth of the design effect size), the probability is 82% in making the right choice. Since under the null ($\mu_{Cp}^{ATG} = \mu_{Cp}^{ATG/GCSF}$) we consider selecting either treatment a non-error there is no contribution to the type I error, and therefore, no further adjustment is required to the α-level of the initial two pair-wise tests.

8.5 Sample Size Re-estimation

The Residual Mean Square Error (RMSE) and the mean of the control group are two parameters used in determining the sample size goal (N_{SSG}=78) which are directly tied to the study cohort enrolled. The former value is directly proportional to the sample size and the latter applies its influence in defining the effect size. Thus the advertised statistical power of this trial may differ from the actual power if either differs from the initial values assumed. Consequently, the plan is to estimate both values using the accumulated data (internal interim estimate, IIE) when approximately half the subjects (N_{SSG}/2) have had their 1 year C-Peptide assessment. Since the initial values used are based on real data from four previous TrialNet studies, the re-estimation of each parameter will be a two-term weighted average using the number of subjects on which the IIE is based as the one weight (N_{IIE}) and the remaining number of subjects participants to be enrolled as the other weight (N_{SSG}-N_{IIE}). Algebraically, the re-estimate will be computed:

$$\hat{\sigma}^2_{\mathrm{Re}-estimate} = \frac{N_{IIE}\hat{\sigma}^2_{IIE} + (N_{SSG} - N_{IIE})\hat{\sigma}^2_{Initial}}{N_{SSG}}$$

Similarly, re-estimation of the mean of the control group will be calculated. These new estimates will be used to calculate a new sample size goal for the trial.

It is important to note that this adaptive procedure is a non-comparative interim analysis (i.e., the observed treatment effect has no influence on the sample size re-estimation). Therefore, this analysis has no effect on the type I error. Note the re-estimation can be conducted by the analyst in a blinded fashion. The goal is to assure adequate statistical power at the completion

of the trial by assuring the two design parameters reflect accurately the study population being enrolled.

8.6 Interim Monitoring Plan

Interim analyses will be conducted periodically during the study and will be reviewed by the TrialNet DSMB for assessment of effectiveness and safety. The Lan-DeMets[74] spending function with an O'Brien-Fleming boundary will be used to protect the type I error probability from early and multiple testing and to assess the significance of the interim results that emerge during the trial[82]. The spending function that approximates the O'Brien-Fleming boundaries is:

$$\alpha_1(t^*) = 2 - 2\Phi\left[\frac{Z_{\alpha/2}}{\sqrt{t^*}}\right]$$

where t^* is the information fraction $(0 < t^* \leq 1), \alpha_1$ is the $\alpha\text{-}level$ of the interim (one-sided) test and α is the type I error for one of the pair-wise tests (i.e., 0.025). The monitoring plan will allow for early termination based on the treatment effect on C-peptide values at 1 year of follow-up using the ANCOVA model described above.

The DSMB will also be informed if there is a serious lack of evidence of a treatment effect (i.e. futility analysis). The boundaries are based on the paper by Lachin[83]. The study arm should be "closed" based on the futility of rejecting the null hypothesis at the completion of the trial if: $z_{ATG}(t^*) \leq 0.1$ when $0.5 \leq t^* < 0.75$ or if $z_{ATG}(t^*) \leq 1.0$ when $t^* \geq 0.75$ (z is the Wald test of the treatment effect coefficient). The same rule would be applied to the ATG/GCSF treatment group. These $t^* = 0.5 \text{ and } 0.75$ are equivalent to when there are 39 and 59 participants with one-year C-peptide results, respectively. Lachin showed that a onetime use of either boundary for the design parameters above $(\theta \equiv Z_{1-\alpha} + Z_{1-\beta} = 3.00)$ raises the type II error to approximately 0.15414 and 0.15611, respectively. For larger values of t^* the increase to the error probability is even less. Furthermore, by the laws of probability a single use of each rule will increase the type II error no more than the sum of the increase (i.e., 0.00414+0.00611 = 0.0103).

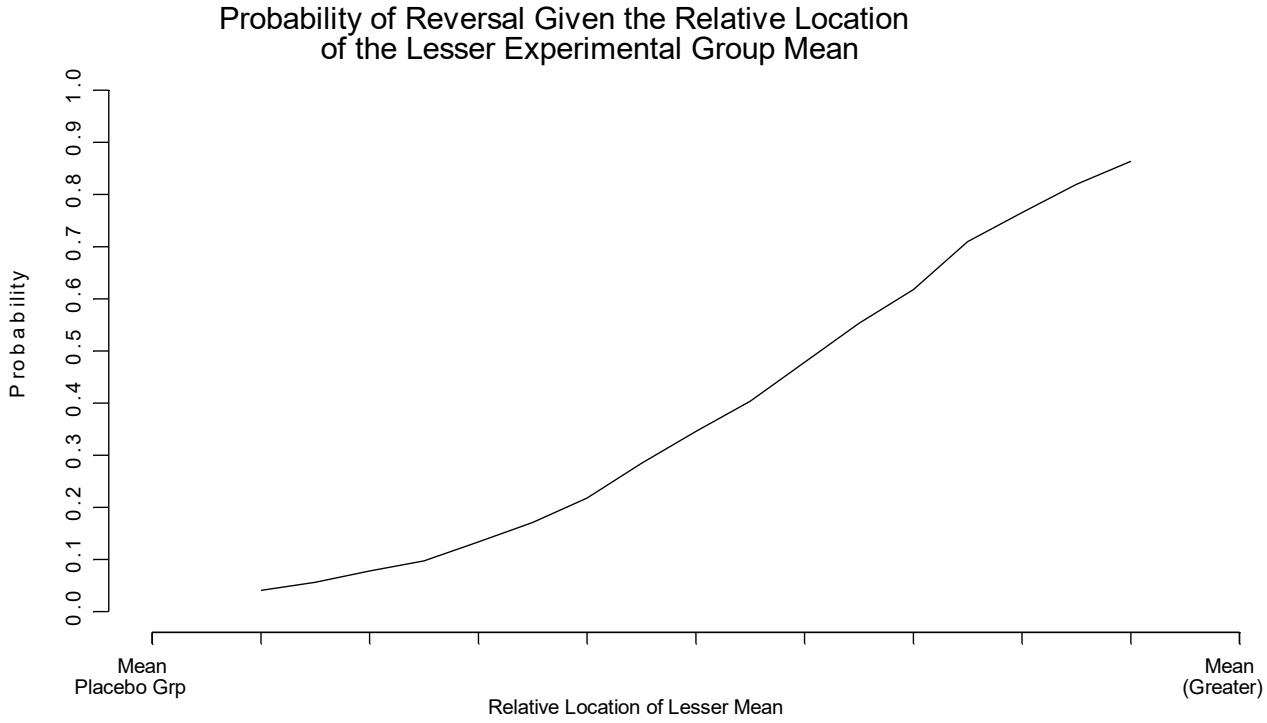
8.7 Interim Analysis Study Modification

Whether Lan-DeMets boundary is crossed or a futility decision rule indicates closure of an experimental arm, the course of action for the study is as follows. In case the futility decision rule indicates closure of one of the experimental arms (and the governing bodies concur), the trial should continue randomizing between the other remaining experimental treatment and placebo (1:1 ratio) since the corresponding hypothesis remains unaddressed. However, in the circumstance where the Lan-DeMets boundary is crossed the normal course of action would be to close the placebo arm. This would incapacitate the second formal hypothesis test thus leaving only the issue of which experimental treatment is "best" as the remaining question. However, it may or may not be reasonable to make this decision as well. To evaluate the evidence at the interim the plan is to use stochastic curtailment and compute the probability that the currently 'best' treatment (as defined by the decision rule above) is not the 'best' when the full sample size is reached. In the normal employment of stochastic curtailment in futility analysis the parameters used for the simulation are based on the alternative hypothesis. The

analogous parameters for this situation is to set the mean of the currently 'best' treatment to be lower than the other experimental treatment mean and determine the probability of a reversal. As with the normal application of stochastic curtailment, we will use the probability of 0.20 or less to stop accrual completely and invoke the decision rule for selecting the 'best' treatment immediately. Alternatively, the study would continue to its planned completion, randomizing between the two active treatment arms, until the projected probability of a re-ordering of the outcome (change in the selection of the superior treatment arm) is less than 0.2.

Our choice for setting the parameters for simulation is to set the 'best' treatment group at 25% lower than the other experimental treatment mean; these two parameters being centered at the midpoint between the two estimated experimental group means. This is consistent with the difference between the two treatment arms specified in the Simon selection at the completion of the trial (described above), should both arms be superior to the placebo.

The graph below provides an estimated probability of a 'best' selection reversal conditioned on the interim data (simulated under the condition of crossing the Lan-DeMets boundary and t* = 0.5). The probability is primarily a function of the relative position of the 'lesser' mean to the positions of the placebo mean and the 'greater' mean (all means being estimates from the interim data). That is, the closer the 'lesser' mean is to the placebo, the less likely there could be a reversal in the selection of the 'best' treatment.



Additional analysis will assess potential adverse outcomes of treatment and will assess the incidence of all severe adverse events.

9 ETHICAL CONSIDERATIONS AND COMPLIANCE WITH GOOD CLINICAL PRACTICE

9.1 Statement of Compliance

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

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

9.2 Participating Centers

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

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

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

9.3 Informed Consent

The process of assuring that individuals (and parent/guardian if less than 18 years of age) are making an informed decision about participating in this study includes both verbal and written communication. Written materials include a Volunteer Handbook, Volunteer Understanding Assessment, and written consent forms. There are several consent forms for this study. One is a Screening consent form that describes the procedures, risks, and benefits, and determines eligibility for the study. The second is the Intervention consent form, which describes the procedures, risks, and benefits for the remainder of the study. A third consent form is for use at clinical sites that will be performing the post-treatment visits, but not the treatment visits. The consent forms will be reviewed with participants (and their parent/guardian in the case of participants under 18 years of age) and the participant will be given time to review the written consent form and ask questions. An assent form has also been developed for participants less than 18 years of age (unless local IRB/REB requirements differ in procedure).

As part of the informed consent process, the participant and/or parent or guardian (if the participant is less than 18 years of age) will also be required to complete a short, written Volunteer Understanding Assessment that is designed to ensure that the subject understands the study, as well as what is being asked of him/her. The participant will be given a copy of their consent/assent forms.

The consent process will be conducted by qualified study personnel (the Trial Investigator or Study Coordinator and/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 there is new, clinically significant information applicable to the safety of the participants, when indicated for a protocol amendment, and/or whenever any new information becomes available that may affect a subject's participation in the study.

Subjects will be re-consented if they reach the age of 18 years while enrolled in the study.

9.4 Study Subject Confidentiality

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

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

9.5 Risks and Benefits

The risks of this study are presented in this protocol, and informed consent form. There is no guaranteed benefit to subjects for their participation in the study.

Special consideration regarding risks and benefits for children is described in section 6.2.

10 STUDY ADMINISTRATION

10.1 Organizational Structure

This study is part of Type 1 Diabetes TrialNet, which is funded by the National Institutes of Health. Funding will also be provided by the Helmsley Charitable Trust. Funding will cover the costs of administration and laboratory tests associated with this study.

10.2 Role of Industry

Sanofi will provide the ATG for the study, and will also be providing funding to support the conduct of the study. Amgen will be providing GCSF/Placebo.

10.3 Groups and Committees

10.3.1 ATG-GCSF Study Chair Committee

The Study Chair and TrialNet Executive Committee will receive periodic reports from the TNCC on the progress of the study. These will include accrual rates and baseline demographic characteristics. Interim data summaries provided to others (except those that could lead to unmasking of study outcome) will first be supplied to the Study Chair for review. Criteria and results of ongoing monitoring of the TrialNet labs in terms of reproducibility will also be provided on a routine basis and reported on during TN19 ATG-GCSF Study Chair Committee meetings, as scheduled. As appropriate, abstracts and manuscripts dealing with the progress of the trial shall be directed by the TN19 ATG-GCSF Study Chair Committee.

10.3.2 TrialNet Chairman's Office and TNCC

The TrialNet Chairman's Office and TNCC will work together in providing leadership to the TrialNet study group to include protocol and manual preparation, training for clinical sites, development of statistical design for each study, and analysis of study results. The TNCC will also coordinate interactions among the participating TrialNet Clinical Centers, test laboratories including TrialNet Core Laboratories and other subcontract laboratories, NIDDK, and other sponsoring agencies.

10.3.3 Clinical Sites

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

10.3.4 Safety Monitoring Subcommittee

The Type 1 Diabetes TrialNet Safety Monitoring Subcommittee (SMS) is responsible for establishing policies and procedures for assurance of safety monitoring of TrialNet protocols and of TrialNet subjects. The Safety committee will review all serious AEs and receive summary reports of all AEs. This committee will be masked as to treatment assignment.

10.3.5 Clinical Site Monitoring

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

10.3.6 Medical Monitor and Data Safety and Monitoring Board (DSMB)

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

An independent physician will be designated to serve as the medical monitor for this study who will maintain regular contact with the study and the Study Chair. (S)he will review all adverse event reports, masked to treatment assignment, and will file event reports with regulatory authorities as appropriate.

The DSMB will meet approximately every 3 months and as needed to review indicators of safety. In addition, they will meet every 6 months to review the interim effectiveness and potential toxicity of the study treatments based on interim analyses of indicators of effectiveness and safety prepared by the TNCC separately by treatment group. The DSMB will independently evaluate whether there are grounds to modify or discontinue the study.

10.4 Sample and Data Storage

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

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

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

10.5 Preservation of the Integrity of the Study

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

10.6 Participant Reimbursement and Compensation

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

APPENDIX 1 - Schedule of Assessments

Week of Trial					2	4	6	8	10						
Month of Trial										3	6	9	12	18	24
Day of Trial		0	1	2											
Visit number	-1	0[A]	0[B]	0[C]	1	2	3	4	5	6	7	8	9	10	11
ATG/Placebo: Infusion 1 and 2			X	X											
GCSF/Placebo q 2 wk[6]				X	X	X	X	X	X						
History	X	X													
Physical exam [8]	X	X			X	X	X	X	X	X	X	X	X	X	X
TB Test – IGRA	X														
CBC with differential[2] (local)	X		X		X	X	X	X	X	X	X	X	X	X	X
CD4/CD8 Ratio[3]		X				X		X		X	X	X	X		
Chemistries	X	X			X	X	X	X	X	X	X	X	X	X	X
Pregnancy test (if female of reproductive potential)	X	X			X	X	X	X	X	X	X	X	X	X	X
Urinalysis	X	X			X	X	X	X							
Flu Vaccination[7]	X														
HIV, Hep B, Hep C serology	X														
IgG		X													
EBV and CMV serology and PCR[4]	X												X		
Serum for autoantibodies	X														
Mechanistic Assessments[5]		X	X	X	X	X	X	X	X	X	X	X	X	X	X
Adverse Events Assessments[6]			X	X	X	X	X	X	X	X	X	X	X	X	X
Hemoglobin A1c	X									X	X	X	X	X	X
Mixed Meal Tolerance Test (2 hr)										X	X	X			
Mixed Meal Tolerance Test (4 hr)	X												X	X	X
Diabetes Assessment		X			X	X				X	X	X	X	X	X

[2] CBC obtained 8 hours after completion of infusion 1. CBC can be repeated up to 24 hours after completion of infusion 1 if initial CBC is abnormal. The repeat CBC will inform dosing for infusion 2.
[3] CD4/CD8 ratio will continue to be monitored until the CD4 count is above 500. CD4/CD8 ratio can be collected at screening visit or baseline Visit 0[A] and may also be done at local laboratory if necessary to obtain results.
[4] Viral PCR/Serology: All subjects will have serology and PCR for EBV and CMV at screening and at 1 year. Subjects who are EBV serology negative at screening must be PCR negative within 30 days of receiving ATG/Placebo. Additional EBV PCR testing may be obtained locally if necessary to obtain results within study windows. Subsequent EBV PCR/Serology will only be performed for symptomatic subjects who were EBV seronegative at screening. Subsequent CMV PCR will be performed for symptomatic subjects regardless of initial CMV serology status.
[5] May include samples for RNA, plasma, serum, DNA, measures of B and T cell number and function to understand the effect of therapy on the immune system and infectious disease. The schedule for these assessments may vary as appropriate. At no time will the blood draw volume exceed what is allowable according to the subject's age and body weight (for subjects <18 years, 5 mL/kg per visit, 9.5 mL/kg in an 8 week period).
[6] Weekly contact with participant will occur.
*Visit 0 [A-C] will occur over a 3 day period. During the Baseline Visit, the participant is admitted to the hospital to undergo ATG/placebo infusions.
[7] Participants are required to receive killed influenza vaccination at least 2 weeks prior to randomization when vaccine for the current or upcoming flu season is available.

[8] Full physical exam including Tanner staging at screening. Full exam also at randomization, week 12, month 12 and month 24. Tanner staging required at month 12 and 24 if Tanner stage 1 or 2 on previous exam. Directed exam as indicated by symptoms in addition to HEENT and spleen exam at weeks 2-10, month 6,9, and 18.

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