



Effects of CTLA-4 Ig (Abatacept) On The Progression of Type 1 Diabetes In New Onset Subjects

(Protocol TN-09)

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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-09, Effects of CTLA-4 Ig (Abatacept) On The Progression of Type 1 Diabetes In New Onset Subjects 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.

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1. INTRODUCTION

1.1. Study Overview

Title	Effects of CTLA-4 Ig (Abatacept) On The Progression of Type 1 Diabetes In New Onset Subjects
IND Sponsor	Type 1 Diabetes Trial Network (TrialNet)
Conducted By	Type 1 Diabetes Trial Network (TrialNet)
Protocol Chair	Tihamer Orban, MD
Accrual Objective	108 subjects over two years
Study Design	The study is a two-group, multicenter, randomized, double-masked, placebo-controlled Phase II clinical trial. All groups will receive standard intensive diabetes treatment with insulin and dietary management. One-hundred eight (108) subjects will be randomly assigned in a 2:1 ratio to receive either two years of CTLA-4 Ig infusions (n=72) or placebo infusions (n=36).
Treatment Description	Abatacept (CTLA-4 Ig) is a fusion protein, which consists of CTLA linked to modified heavy-chain constant region of human IgG1. Abatacept binds to CD80 and CD86 receptors on the antigen-presenting cells and prevents them from binding to CD28 on the T cell for optimal T cell activation. Participants randomly assigned to CTLA-4 Ig treatments or placebo will receive a total of 27 IV infusions over two years.
Study Duration	All subjects will be treated and followed for two years, and will be followed for up to two additional years beyond the period of treatment. Enrollment is expected to occur over two years.
Objective	To assess the safety, efficacy, and mode of action of CTLA-4 Ig infusions for the treatment of individuals with new onset type 1 diabetes.
Primary Outcome	The primary statistical hypothesis to be assessed in this study is whether there is a difference in the mean C-peptide value at two years of follow-up for study subjects receiving CTLA-4 Ig versus those receiving placebo.
Secondary Goals	The study will also examine the effect of the proposed treatments on surrogate markers for immunologic effects, namely disease-specific outcomes and immunological outcomes.
Major Inclusion Criteria	Type 1 diabetes within past 3 months. Age 6-45 years. At least one diabetes associated autoantibody.

2. BACKGROUND AND SIGNIFICANCE

2.1. Rationale for intervention trials to preserve beta cell function in subjects with Type 1 diabetes

The onset of human Type 1 diabetes mellitus is the clinical manifestation of the β -cell failure caused by T cell mediated autoimmune destruction. This results in a life long dependence on daily insulin injections and exposure to both the acute and late complications of Type 1 diabetes mellitus (T1DM). T1DM is a particular burden to children and their families, representing one of the most common chronic childhood diseases. While T1DM can occur into adulthood, there is a bimodal peak age of onset, between ages 4-7 and ages 14-16 years. The world-wide incidence of T1DM is increasing, with the greatest increase in children under the age of 5 years. Despite the significant progress that has been made in its treatment, diabetes mellitus represents a severe burden on the individual and on society as well. Any intervention, which can stop or delay the complete loss of functional residual β -cell mass is significant as it may provide protection against hypoglycemia and provide improved metabolic control resulting in a delay in the micro and macro-vascular complications of diabetes.

Type 1 diabetes mellitus is an immune-mediated disease in which insulin-producing beta cells are completely destroyed resulting in life-long dependence on exogenous insulin. While this beta cell destruction process begins before clinical onset and continues after development of hyperglycemia and diagnosis, at the time of diagnosis subjects retain a significant amount of beta cell function as measured by C-peptide responses to a mixed meal tolerance test. However, this beta cell function deteriorates after diagnosis with the presumed eventuality of absent function over time. As shown in the Diabetes Control and Complications Trial, the persistence of residual beta cell function has been associated with important clinical outcomes, specifically reduction in severe hypoglycemia and complications. In this study, the rate of retinopathy and severe hypoglycemia were reduced by greater than 50% in those with sustained C-peptide when compared with those with undetectable C-peptide (1,2,3). Additionally, there is growing evidence that residual beta cell function shortly after the diagnosis of diabetes may be much greater than previously thought and may be as much as 50% of subjects without diabetes (4). Thus, an intervention that can enable continued endogenous insulin production would significantly improve the day-to-day management for subjects with diabetes and therefore reduce long-term complications.

2.2. Rationale for use of CTLA-4 Ig

Much of the knowledge gained in the field of T1DM autoimmunity comes from studies of animal models of diabetes autoimmunity. Especially well-studied models are the NOD mice (5,6) and BB rats (7, 8). It is now widely accepted that human Type 1 diabetes mellitus is mediated by β -cell specific T-cells (9, 10, 11, 12, 13). Co-stimulatory pathways are critical in T cell activation and play a central role in organ specific autoimmunity (14). Thus, emerging new agents, blocking these pathways hold great promise in human diabetes autoimmunity.

Several β -cell specific autoantigens have been described (ICA (15); insulin autoantibody IAA (16); GAD65-antibodies (17); IA2- antibodies (18)), but are considered as markers of the autoimmunity rather than aetiopathogenetic factors. A possible association between CTLA-4 DNA polymorphism and Type 1 diabetes mellitus in human has been described (19,20).

T lymphocytes are believed to play a major role in orchestrating the immune response. T cell activation is thought to involve a "two-signal" model (21). According to this "two-signal" model, "signal

1” is the interaction of the T cell receptor (TCR) with the antigen-MHC complex, meanwhile “signal 2” is the engagement of the co-stimulatory receptors (22,23). Both signals are required for optimal T cell activation; in the absence of signal 2, T cells fail to activate and become anergic. Signal 2 can be stimulatory and inhibitory as well. A large family of co-stimulatory molecules has been described and at the same time T-cell surface receptors providing inhibitory signals have also been reported (24). There is an apparent delicate balance between positive and negative regulatory signals, which drives the direction of the specific immune response. The B7-1/2 – CD28/CTLA-4 co-stimulatory pathway has been studied in depth and plays a crucial role in T-cell activation/tolerance. This is, arguably, the most important co-stimulatory pathway for T cell antigen activation.

Both B cell molecules, B7-1 (25)(CD80) and B7-2 (26)(CD86) are ligands for CD28 and CTLA-4 (27) and are expressed on the surface of the antigen presenting cells (APC). The binding affinity of B7 molecules to CD28 and CTLA-4 on T cells differ substantially and this is important in T cell regulation (28). B7-2 is constitutively expressed on most APCs at a low level and is quickly upregulated, meanwhile B7-1 is only inducibly expressed after activation. CD28 is constitutively expressed on the T cell surface whereas CTLA-4 expression is upregulated 24- 48 hours after T cell activation (29). Engagement of CTLA-4 represents a crucial negative signal, inhibits TCR- and CD28 mediated signal transduction, also inhibits IL-2 production and terminates T cell response by inhibiting cell cycle progression (30,31). CTLA-4 (CD152) is currently viewed as major negative regulator of T cell activation and an important regulator of peripheral tolerance induction. The loss of CTLA-4 in CTLA-4 (-/-) mice leads to massive lymphoproliferation and fatal multiorgan tissue destruction (32). However, administration of CTLA-4 Ig prevents the lymphoproliferative disorder and the organ failures in these CTLA-4 (-/-) mice (33) indicating that the unopposed interaction of the B7 receptors with the intact CD28 is the cause of the fatal disorder. Further to this point, mice having no CTLA-4 and also missing both B7 receptors do not have lymphoproliferative disease (34). The B7-CD28 blockade by administration of CTLA-4 Ig inhibits Th1 (IL-2, INF- γ) but spares Th2 cytokines (IL-4 and IL-10) (35) and leads to Th2 type IgG isotype switching (IgG 1 upregulated) (36) in a rat renal allograft model. This divergent effect on Th1/Th2 balance makes it particularly attractive to use in T1DM, where there is a pathogenic Th1 bias.

The effect of CTLA-4 Ig has been studied in various animal models as well as in human trials. It has been used in a series of animal transplantation models, where it showed significant prolongation of transplanted organ survival (36,37,38) and in autoimmune models, where it slowed or prevented the autoimmune process (39, 40). It is also effective in animal pancreatic islet transplantation models (37) as well as in prevention in autoimmune models of diabetes (41). Lenschow and coworkers showed that co-stimulatory blockade with CTLA-4 Ig prevents diabetes in NOD mice model of T1DM, when administered after insulinitis developed but before frank diabetes ensues (41). Interestingly, in some transplantation models and in some animal models of autoimmunity CTLA-4 Ig effect persisted well beyond any detectable CTLA-4 Ig blood levels, leading to long-term graft survival (37) or long-term suppression of the autoimmunity (40).

There is now considerable clinical experience with abatacept (Bristol-Myers Squibb (BMS) CTLA-4 Ig). As discussed below, abatacept has been extensively tested in subjects with rheumatoid arthritis and is now FDA approved and in clinical use in that patient population. Individuals with psoriasis, multiple sclerosis, and juvenile idiopathic arthritis have also been studied. Clinical trials are currently underway in Crohn's disease, ulcerative colitis, and lupus nephritis.

CTLA-4 Ig is a novel immunosuppressive agent for Type 1 diabetes mellitus. It has shown strong beneficial effect in different animal models of transplantation and organ specific autoimmunity, including diabetes. The Ig Fc component of the abatacept molecule has been modified so that it neither fixes complement nor takes part in antibody-dependent cellular cytotoxicity (ADCC). Thus,

this agent inhibits/regulates T cell function, but does not deplete T cells in humans. The safety profile reported in human clinical trial appears to be better than any other immunosuppressive agent used to preserve beta cell function in T1DM (42,43,44,45,46). The combination of good safety profile and strong, therapeutically useful immuno-suppression/ modulation makes this drug particularly suitable for study in clinical trials for T1DM. At the time of clinical onset of type 1 diabetes mellitus, a significant number of insulin producing β cells are destroyed, but around 15% to 40% may still be capable of insulin production (47). Thus, by arresting or slowing down the autoimmune destruction of these β -cells, endogenous insulin production may be extended.

3. 3. CLINICAL AND PRE-CLINICAL DATA

There is a large body of data available on the CTLA-4 Ig costimulatory blockade in different animal models of transplantation and autoimmunity, including diabetes as well as recent data on clinical trials in patients with autoimmune diseases.

3.1. Animal Studies

3.1.1. Transplantation Models (other than Diabetes)

Blocking the B7/CD28 costimulatory pathway promotes long-term graft survival in many transplantation models, where CD4+ T cells are necessary for rejection. When given after initial graft injury, CTLA-4 was shown to prevent progression of chronic allograft rejection (48); in another renal allograft model, CTLA-4 prevented progressive proteinuria (49). CTLA-4 Ig alone or in conjunction with other therapy has been successfully used in cardiac transplantation (50), intestinal allograft, (51,52), and combined renal and cardiac transplantation studies (36). Grafts of the CTLA-4 Ig treated animals showed no tissue injury and also increased staining for IL-4 and IL-10 and also for IgG1 indicating that B7/CD28 blockade inhibits Th1 by sparing Th2 cytokines.

3.1.2. Transplantation Models (Diabetes)

CTLA-4 Ig has also been used with great success in pancreas or islet transplantation models. CTLA-4 Ig intervention successfully blocked human pancreatic islet rejection in mice and induced long term, donor specific tolerance (37). As a monotherapy, it did not protect pig islet xeno-transplantation in prediabetic NOD mice (53), however when the pig islets were microencapsulated, they were protected by CTLA-4 Ig (54). A muscle cell line (C2C12) was transfected with the cDNA for CTLA-4 Ig and was then co-transplanted with islet in the renal capsule of a diabetic mice by Chahine et al (55). The systemic CTLA-4 Ig levels were measurable up to 60 days and there was a significant prolongation of allogenic islets. O'Rourke et al have reported that a dendritic cell line, genetically modified to express CTLA-4 Ig prolonged islet allograft survival in streptozotocin-induced diabetic mice (56). Uchikoshi and coworkers have used the BB rat model, where the newly transplanted β -cells were threatened by both recurrent autoimmunity and alloimmunity. They used adenovirus-mediated CTLA-4 Ig gene transfer to the pancreaticoduodenal grafts and found that syngeneic DR-BB grafts were protected from recurrent autoimmunity and a fully histo-incompatible graft from LEW rats had a prolonged survival in diabetic BB rat recipients. The CTLA-4 Ig therapy prevented the alloimmune and also autoimmune destruction of the β -cells in type 1 diabetic host (57). In a non-human primate study CTLA-4 Ig monotherapy was administered to monkeys with allogenic pancreatic transplant. Humoral responses to the transplanted tissue were suppressed and animals had prolonged graft survival (58).

3.1.3. Autoimmunity Models (other than Diabetes)

Efficacy of B7/CD28 blockade has been demonstrated in several models of autoimmunity, including murine models of systemic lupus erythematosus (59), rheumatoid arthritis (60,61), T cell mediated autoimmune myocarditis (62), and experimental autoimmune encephalomyelitis (EAE; animal model of multiple sclerosis) (63, 64, 65). Immunohistological studies of the central nervous system in EAE animal model showed that CTLA-4 Ig therapy suppressed Th1 inflammatory response (IL-2 and IFN- γ), but spared Th2 cytokine expression (IL-4, IL-10 and IL-13) (66).

3.1.4. Autoimmunity Models (Diabetes)

In BB rat model of diabetes, the recurrence of autoimmunity was completely prevented by adenovirus-mediated CTLA-4 Ig expression locally in the pancreas (57). Lenschow and colleagues (41) administered human CTLA-4 Ig to female nonobese (NOD) mice and prevented the development of diabetes when treated at the time of the onset of insulinitis (2-4 weeks of age). Treatment after 10 weeks of age did not protect the mice from diabetes. Histopathology of the pancreas showed that while the CTLA-4 Ig treatment blocked the development of diabetes, it did not reduce the severity of insulinitis. Their data indicate that B7/CD28 blockade acts at the time of insulinitis, but before the onset of disease. In a later paper, however, Lenschow reported use of murine CTLA-4 Ig at the same NOD mouse age as it was used in the original study using the human CTLA-4 Ig (2-4 weeks) and found acceleration of the disease (67). It is difficult to reconcile the seemingly contradictory results of different species of CTLA-4 Ig's effect (human CTLA-4 Ig protective versus murine CTLA-4 Ig accelerating effect on NOD diabetes); the answer may lie in the opposite effect of B7-1 versus B7-2 on diabetes in NOD mice (41) and may be due to differences in avidity of the murine versus human CTLA-4 Ig to B7-1 versus B7-2. However, it is also quite possible that CTLA-4 Ig acts via a novel pathway generating regulatory antigen presenting cells via indoleamine-2,3-dioxygenase (IDO) (68). Long-term survival of pancreatic islet allografts induced by the soluble fusion protein CTLA-4-immunoglobulin (CTLA-4 Ig) is dependent on an effective tryptophan catabolism in the host. In vitro, it is shown that CTLA-4 Ig regulates cytokine-dependent tryptophan catabolism in B7-expressing dendritic cells (DC). Data suggest that modulation of tryptophan catabolism is a means by which CTLA-4 functions in vivo and that it acts as a ligand for B7 receptor molecules that transduce intracellular signals important for CTLA-4's action (69). CTLA-4-Fc exposure induced increased IDO expression in PBMCs, as well as in monocyte-derived mature DC-s (70). Importantly, CTLA-4 regulates peripheral tolerance induction and promotes Th2 development (71). Most recently, use of human CTLA-4 Ig from Bristol-Myers Squibb (the drug we propose to use for the clinical trial), confirmed previous findings and showed marked prevention of diabetes in the NOD mice model (control NOD 80% diabetes at age 18 weeks versus 17% diabetes in the CTLA-4 Ig treated at 29 weeks; Sayegh et al. unpublished data).

3.2. Human Trials in other Autoimmune Diseases

Clinical trials with abatacept have been completed in individuals with rheumatoid arthritis, psoriasis, multiple sclerosis, and juvenile rheumatoid arthritis, with more than 8600 person-years of exposure. In addition, clinical trials are underway in Crohn's disease, ulcerative colitis and lupus nephritis and will shortly start in psoriatic arthritis.

3.2.1. Rheumatoid Arthritis

Results of a series of Phase I-III clinical trials involving 1955 subjects treated with abatacept and 989 placebo treated subjects, have led to FDA approval of the IV formulation of abatacept for treatment of Rheumatoid Arthritis (RA). Abatacept is indicated for reducing signs and symptoms, inducing major clinical response, slowing the progression of structural damage, and improving physical function in adult patients with moderately to severely active rheumatoid arthritis who have had an inadequate response to one or more Disease Modifying Anti-Rheumatoid Drugs (DMARDs), such as methotrexate or TNF antagonists. Abatacept may be used as monotherapy or concomitantly with DMARDs other than TNF antagonists.

Trials in RA included studies of monotherapy in individuals who had an inadequate response to DMARDs, in conjunction with MTX in those with inadequate response to MTX alone, and in conjunction with DMARDs in those with inadequate responses to TNF blocking therapy. These studies reported clinical efficacy within 3 months of therapy and through 1 year (72,73). Importantly open label extensions of these studies reported durable and sustained clinical responses through 18-48 months (unpublished data from BMS). For example, 50% of the 324 subjects initially randomized to abatacept/MTX who entered into the open label extension had no radiographic progression of structural damage at 2 years.

Adverse events possibly related to treatment were reported in 52.2% of abatacept treated and 46.1% of placebo treated adults, with the most frequent events in abatacept treated subjects being headache ($\geq 10\%$) and nausea. Treatment was discontinued due to these events in 3.4% of abatacept treated and 2.2% of placebo-treated subjects. Other common (1-10%) adverse effects include upper and lower respiratory tract infection, hypertension and flushing, cough, rash and fatigue.

3.2.2. Psoriasis

There have been 4 clinical studies of 177 subjects with psoriasis including a Phase I open label dose escalation study which suggested a 50% or greater sustained clinical improvement was achieved in 46% of all study subjects. The skin biopsies showed marked reduction in epidermal hyperplasia simultaneously with quantitative reduction of skin-infiltrating T cells. The T cell decline in the lesions was not due to intralesional apoptosis, but likely attributable to inhibition of T cell proliferation and recruitment (74). One of the other trials in psoriasis was stopped due to unexpected peri-infusion adverse events. Subsequently, these were found to be related to a constituent in the formulation (monocyte chemoattractant peptide -1), which was subsequently changed prior to further clinical trials.

3.2.3. Multiple Sclerosis

The dose-ranging study in subjects with relapsing-remitting multiple sclerosis (MS) was terminated by BMS when a data and safety monitoring board review detected an increase in enhancing T1 lesions and MS exacerbations in 1 of the randomized groups. A descriptive analysis of the primary efficacy endpoint, cumulative new or recurrent T1-enhancing lesions, reported that the 2 mg/kg group (median number of lesions = 8.0) was greater than that in the placebo group (median number of lesions = 5.5), however the 10 mg/kg group (median number of lesions = 1.5) median was less than that in the placebo group. The 10 mg/kg treatment group reported fewer protocol-defined exacerbations (20.6 versus 30.0%), fewer MS related AEs (52.9 versus 65.0%), and a lower mean annualized relapse rate (0.38 versus 0.73) compared with placebo. A similar pattern was observed for other efficacy endpoints (unpublished data).

3.3. Clinical Experience in Children: Juvenile Idiopathic Arthritis (JIA)

In a Phase 3, multi-center, multi-national, randomized, withdrawal study to evaluate the safety and efficacy of abatacept in children and adolescents with active polyarticular JIA, 190 children and adolescents, ages 6-16 (mean 12.4 + 3 years), were treated with open-label abatacept at 10 mg/kg IV for 4 months in a lead-in phase (Period A). One hundred twenty-two (122) subjects who responded according to the ACR 30 pediatric definition of improvement were randomized 1:1 into the double-blind withdrawal phase (Period B) either to continue abatacept (60 subjects), or to receive placebo (62 subjects) for 6 months or until the appearance of flare. Half (31/62) of those randomly assigned to continue abatacept completed Period B, while 82% (49/60) of those randomly assigned to placebo completed Period B. Those who did not respond in Period A, those who completed Period B, and those who flared in Period B were offered the option to receive abatacept in Period C, the open-label extension phase of the study. The difference in time to disease flare between the abatacept and placebo groups was statistically significant based on the log-rank test ($p = 0.0002$). The risk of disease flare among children and adolescent subjects continuing on abatacept was less than one-third that for the placebo subjects withdrawn from the abatacept treatment (hazard ratio = 0.31, 95% CI [0.16, 0.59]). When the proportions of subjects who flared were compared between the abatacept and placebo groups without accounting for time in the analysis, a result consistent with the primary efficacy analysis was obtained (20.0% versus 53.2%, chi square $p < 0.001$). The JIA Core Set Variables (ACR Pediatric components) continued to improve slightly or remained stable during Period B for abatacept-treated subjects, whereas most of these variables worsened in subjects randomized to placebo.

Treatment in children was well tolerated, although 13.2% reported headache, this did not result in discontinuation of treatment. Infections were reported with similar frequencies in abatacept (45%) and placebo (43.5%) treated groups with a slight increase in influenza 8.3% versus 6.5% in abatacept treated subjects. One subject had a varicella infection with normal clinical course. There were no safety issues from the evaluation of the laboratory data (Abatacept: Investigator's Brochure).

4. STUDY DESIGN

4.1. Overview

Type 1 diabetes results from immune mediated destruction of pancreatic islet beta cells. We hypothesize that CTLA-4 Ig (abatacept), by the virtue of blocking the co-stimulatory pathway of T-cell stimulation; will inhibit further destruction of the insulin producing beta cells in the pancreas by the auto-aggressive T-cells thereby preventing the progression of disease.

4.2. Summary of Inclusion and Exclusion Criteria

4.2.1. Inclusion Criteria

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

1. Be between the ages of 6 and 45 years
2. Be within 3-months (100 days) of diagnosis of type 1 diabetes based on American Diabetes Association (ADA) criteria
3. Must have at least one diabetes-related autoantibody present
4. 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 and within one month (37 days) of randomization
5. If a female participant with reproductive potential, she must be willing to avoid pregnancy and undergo pregnancy testing prior to each infusion
6. At least three months from last live immunization received
7. Be willing to forgo live vaccines for 3 months following last dose of abatacept/placebo
8. Be willing to comply with intensive diabetes management
9. Must weigh at least 20 kg (44 lbs) at study entry

4.2.2. Exclusion Criteria

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

1. Are immunodeficient or have clinically significant chronic lymphopenia
2. Have an active infection at time of randomization
3. Have a positive PPD test result
4. Be currently pregnant or lactating, or anticipate getting pregnant
5. On-going use of medications known to influence glucose tolerance
6. Require use of other immunosuppressive agents
7. Have serologic evidence of current or past HIV, Hepatitis B, or Hepatitis C infection
8. Have any complicating medical issues or abnormal clinical laboratory results that interfere with study conduct or cause increased risk to include pre-existing cardiac disease, COPD, neurological, or blood count abnormalities (such as lymphopenia, leukopenia, or thrombocytopenia)
9. Have a history of malignancies
10. Be currently using non-insulin pharmaceuticals that affect glycemic control
11. Be currently participating in another type 1 diabetes treatment study

4.3. 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 material will include a Patient Handbook and written consent forms. The consent form will be reviewed with participants (and their 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 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 signed consent/assent forms.

4.4. Description of Treatment Groups

This double-masked protocol will enroll a total of 108 participants who will be randomly assigned to the following groups:

- 72 participants will be assigned to receive intravenous infusion of 10mg/kg of CTLA-4 Ig at 0, 2 and 4 weeks following randomization, and then every month (28 days) thereafter for two years for a total of 27 doses.
- 36 participants will receive intravenous infusion of placebo at 0, 2, and 4 weeks following randomization, and then every month (28 days) thereafter for two years for a total of 27 doses

4.5. Treatment Assignment and Double Masking

Eligible participants who have provided written informed consent will be randomized to one of the treatment groups. The randomization method will be stratified by TrialNet study site. The participant, the clinical investigator and clinical personnel will be masked to the treatment assignment. Laboratories performing assays for this protocol will be masked as to the treatment assignment and the identity of each subject whose biological material is to be studied.

4.6. 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 1). The participant's insulin production will be measured by a series of mixed meal glucose tolerance tests (MMTT) conducted regularly during the study. The participant's diabetes control will be evaluated by measuring glycosylated hemoglobin (HbA1c) every three months.

Remaining samples will be stored in the National Institute for Diabetes and Digestive and Kidney Disease (NIDDK) Repository and at TrialNet Sites for future analysis. Samples will be stored only with the participant's permission. Participants who decline consent for storage of remaining samples will still be eligible to participate in this study.

4.7. 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 external quality surveillance of the performance of the central laboratories.

4.8. Post-treatment Follow-up

All subjects will be treated with 27 infusions of CTLA-4 Ig or placebo over a two year period. Subjects will also be asked to undergo additional follow-up post-treatment for up to two years with a visit every 6 months. Subjects with undetectable levels of C-peptide on the 30 month visit will not undergo any further MMTTs for assessment of C-peptide levels at subsequent visits.

TrialNet Approved 10/10/2008

5. PATIENT MANAGEMENT

5.1. Screening

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

5.2. Randomization

Eligible study participants will be randomized at the clinical sites at the baseline visit, and will be assigned a study randomization number to which a treatment group assignment has been made. The subject will receive the initial dose of the study drug via infusion of the assigned study treatment (either CTLA-4 Ig or placebo) at the baseline visit.

5.3. Intensive Diabetes Management

During the study period, all participants will receive “intensive” management of their diabetes. The goal of the treatment will be to keep the HbA1c levels within the currently recommended American Diabetes Association age-specific target range in the absence of 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 episodes of severe hypoglycemia will prompt review by the Safety Monitoring Committee with recommendations if any for changes in diabetes management conveyed to the clinical site in conjunction with the Clinic Monitoring Group.

5.4. Administration of CTLA-4 Ig/Placebo

The CTLA-4 Ig used in these human trials (abatacept) is a soluble fusion protein, which consists of the extracellular domain of the human CTLA-4 (CD152) and a fragment (hinge, CH2 and CH3 domains) of the Fc portion of human IgG1.

A urine pregnancy test will be given to females with reproductive potential. An interim medical history and exam with directed questioning regarding infectious symptoms will be performed before each study infusion is administered.

The study drug (CTLA-4 Ig/ placebo) is administered IV at 10 mg/kg over 30 minutes and not mixed or diluted with other medications. No routine pre-medication is needed; however medication such as acetaminophen and diphenhydramine may be administered according to investigator discretion as needed.

If mild hypersensitivity or an infusion related event develops, the study infusion should be temporarily interrupted. Bronchodilators and or saline infusions may be used if indicated for bronchospasm or mild hypotension. Subjects may be dosed with acetaminophen and diphenhydramine as needed. The study infusion can continue or be resumed upon improvement of patient symptoms.

Subjects will be monitored for 1 hour after the end of the infusion.

5.4.1. Dosing and Dose Withholding

The dose of 10 mg/kg was chosen based on demonstrated safety and efficacy in other human autoimmune diseases including children. Dosing will be according to the individual's weight during the previous visit unless the previous visit was more than three months previously. In that case, dosing will be according to the individual's weight on the day of the visit.

Subjects without previous exposure to EBV (EBV IgG and IgM negative during screening) will have EBV viral load determined at each study visit. Those that have evidence of active EBV infection before randomization will not be eligible for the study. Those that have evidence of active EBV infection after randomization will not receive additional study drug until resolution (determined by laboratory assessments and absence of signs and symptoms associated with active disease).

The study drug infusions will also not be given if any subject has had a symptomatic illness with fever, sore throat, or lymphadenopathy during the previous 10 days. The study drug infusions will also be withheld if there is grade 3 lymphopenia. Administration of subsequent infusions can occur upon clinical and laboratory resolution.

Subjects with grade 3 hypersensitivity or infusion reactions and/or those who require pressor support or epinephrine will not be restarted on therapy and will not receive subsequent doses.

5.4.2. 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 in 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 infusions, 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 all scheduled MMTT assessment visits. MMTT visits will not occur while an individual is pregnant.

In addition, if a participant on active treatment has undetectable C-peptide values on a follow-up MMTT, the participant will be brought back into the clinic in one month for a confirmatory MMTT. If this confirmatory MMTT again shows that all of the participant's stimulated C-peptide levels are undetectable, the participant will be switched from active treatment to placebo for the duration of their scheduled treatment period. The rationale is that the potential for benefit from the study treatment has been diminished once a participant's stimulated C-peptide has dropped below this level. The risk/benefit ratio is no longer sufficient to justify the risks associated with the study treatment. In order to maintain the masking of the study outcome, a random subset of placebo treated participants and/or those with a normal MMTT will also be brought back for an additional MMTT one month after undergoing a regularly scheduled MMTT.

5.4.3. Re-Entry into Study Treatment

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

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6. STUDY ASSESSMENTS

6.1. General Assessments

After screening to determine eligibility, study visits will occur for all subjects at baseline and 2, 4 weeks and every 28 days thereafter through 24 months and consist of study drug infusions and outcome assessments. At the end of two years, all subjects will continue with assessments up to an additional two years.

General assessments include:

- Medical history and routine or directed physical examination
- Concomitant medications
- Adverse events

6.2. Laboratory Assessments

The following laboratory assessments will be performed during the study:

- Chemistry (sodium, potassium, chloride, CO₂, glucose, BUN, creatinine, TSH)
- Liver function tests (ALT, AST, alkaline phosphatase, total protein, albumin, total and direct bilirubin)
- Hematology (complete blood count with differential and platelets)
- Purified protein derivative (PPD) test (screening)
- Urine pregnancy test as appropriate
- Serum IgG and IgM levels
- Autoantibodies (e.g. ICA, GAD65, ICA512, mIAA)
- Antibodies to HIV, hepatitis B (Anti-HBcAb, HbsAg), hepatitis C (HCV), Cytomegalovirus (CMV. IgG), Epstein-Barr Virus (EBV IgG and IgM)
- EBV viral load in individuals entering study as EBV serology negative
- Samples for thyroid antibodies, virology and other immunization titers

6.3. Mechanistic Outcome Assessments

TrialNet will perform immune and genetic assays to further understand mechanisms that may be underlying the Type 1 disease process and response to therapy. For this purpose, samples for PBMC, DNA, RNA, plasma, and serum will be obtained. HLA testing will be done. Subjects who are 18 months or more from their previous tetanus immunization will receive tetanus immunization at visit 26. At any time after the first month of study, subjects will receive their annual clinically indicated killed flu vaccine at the appropriate time of year. Responses to these immunizations will be determined through analysis of pre-and 4 week post-dose samples.

6.4. Metabolic Outcome Assessments

Metabolic assessments will consist of:

- Glucose records and reports of hypoglycemia
- Insulin dose

- HbA1c
- Mixed meal tolerance test (MMTT)

6.5. Laboratory Measures Related to CTLA-4 Ig Administration

Determination of immunogenicity to CTLA-4 Ig, receptor occupancy, and drug levels will be done to assess pharmacokinetic profiles according to the schedule of assessments (Appendix 1).

6.6. Visit Windows

The initial study infusion must begin within 100 days from the day of diagnosis and within 37 days from the screening MMTT. For the next two infusion visits (visit # 1, # 2), the window is +/- 3 days of the target date. The subsequent infusion visits should be within 7 days on either side of the targeted date to be permissible. Study doses outside of the window will not be made up (i.e. if treatment dose 5, scheduled for day 84 cannot be accomplished between days 77 and 91, this dose will not be given and the next dose will be on day 112 +/- 7 days). Further, the clock will not be reset (i.e. if treatment dose 5 is given on day 77 (day 84- 7 day window), treatment dose 6 target date remains day 112). In this way, no one will receive more than 27 study doses (infusions) over a two year period.

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7. PARTICIPANT SAFETY

As of December 22, 2006, 8,647 total person-years of exposure to abatacept have been documented in the abatacept clinical program with excellent safety profile and tolerability including in children.

7.1. Risks, Benefits, and Inclusion of Children

The risks of this study are presented below and in the informed consent form. This study will examine whether abatacept intervention 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. These additional resources include frequent in-person and other contact with study associate diabetes educator throughout the duration of the study. Further, the intervention has the prospect of direct benefit to a given subject and is likely to yield general knowledge about T1DM which is of importance for the understanding and amelioration of T1DM in children.

The study procedures, while greater than minimal risk, offers the possibility of benefit due to the close monitoring for all participants, including 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).

7.2. Expected Side Effects and Adverse Events

7.2.1. Infusion and Hypersensitivity Reactions

Between 5-9% of subjects in clinical trials of abatacept reported mild or moderate infusion reactions such as dizziness, hypertension, and headache. No severe infusion reactions have been reported.

Hypersensitivity reactions are rare, with mild urticaria, hypotension and dyspnea occurring in <0.9% of subjects. There have been two episodes of anaphylaxis reported (rate <1:1000).

7.2.2. Infectious Adverse Events

As with all immunomodulating agents, there is a risk of infectious adverse events. In clinical trials, the most frequent infections reported were nasopharyngitis, sinusitis, influenza, bronchitis and urinary tract infection. Infections were reported in 54% of abatacept and 48% of placebo treated subjects.

Serious infections such as cellulitis, bronchitis, acute pyelonephritis, pneumonia and diverticulitis were reported in 3% of treated subjects versus 1.9% of placebo group participants.

7.2.3. Immunizations

Although no data are available regarding the effects of vaccination in patients receiving abatacept therapy, vaccination with live vaccines is not recommended. The possibility exists for abatacept to affect host defenses against infections since the cellular immune response may be altered. Therefore concurrent use of live vaccines within 3 months of abatacept therapy may result in reduced efficacy of vaccine. All other vaccines are allowed. Tetanus and killed flu vaccine will be administered to subjects as part of the study (see Mechanistic Assessments).

7.2.4. Drug Interactions

Higher frequencies of adverse events were reported in subjects with rheumatoid arthritis also treated with leflunomide and TNF blockers.

7.2.5. Blood Glucose Testing

The maltose present in abatacept can interfere with the readings of blood glucose monitors that use test strips with glucose dehydrogenase pyrroloquinolinequinone (GDH-PQQ). GDH-PQQ based glucose monitoring systems may react with the maltose present in abatacept, resulting in falsely elevated blood glucose readings on the day of infusion. To circumvent this problem, the patients participating in the trial will be provided with glucometers and strips using non GDH-PQQ based glucose monitoring systems for the duration of the trial.

7.2.6. Other Reported Adverse Events

More than 10% of subjects reported nausea and headache. Among subjects with chronic obstructive pulmonary disease (COPD), more than 40% had acute exacerbations of COPD.

Antibodies directed against the abatacept molecule were assessed by enzyme-linked immunosorbent assays, in RA subjects treated for up to 3 years with abatacept. Sixty-two (62) of 2237 (2.8%) subjects developed binding antibodies. In subjects assessed for antibodies at least 56 days after discontinuation of abatacept, 15 of 203 (7.4%) subjects developed antibodies. Samples with binding activity to the CTLA-4 region of the molecule were assessed for the presence of neutralizing antibodies. Eight (8) of 13 evaluable subjects were shown to possess neutralizing antibodies. Overall, there was no apparent correlation of antibody development to clinical response or adverse events. However, the number of subjects that developed antibodies was too limited to make a definitive assessment. The clinical relevance of neutralizing antibody formation with abatacept is not known.

Similarly, antibodies directed against the abatacept molecule were assessed by enzyme-linked immunosorbent assays, in children and adolescents with JIA (age 6-16 years) treated with abatacept in a Phase 3, multi-center, multi-national, randomized, withdrawal study cited earlier. The presence of antibodies was transient and antibody titers were generally low. It did not correlate with any clinical findings, including an increase in incidence of SAEs, acute infusional AEs, or autoimmune disorders, diminution in clinical efficacy, or effect on serum concentrations of abatacept. Immunopositive subjects in the double-blind phase of the study were not at increased risk of experiencing SAEs, acute infusional AEs, or autoimmune disorders when re-initiating abatacept treatment in the open label follow up period.

Doses up to 50 mg/kg have been administered without apparent toxic effect.

There were no significant differences between abatacept and placebo subjects with respect to number of cancers, although more abatacept treated subjects developed lung cancer.

7.3. Pregnancy

Pregnant and lactating women will not be included in the study. Sexually active females must have a negative pregnancy test prior to enrolling in the study and will be required to use birth control during the study. At every study visit the sexual activity of female participants of reproductive age will be reassessed. If a subject who was previously sexually inactive becomes sexually active, she will be counseled about the need to use a reliable form of birth control. Female subjects will also be required to undergo pregnancy tests before each study infusion. Subjects will be requested to avoid pregnancy for 3 months following the last study infusion and instructed to use birth control.

7.4. 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 will take place in a facility that has resuscitation capabilities, and subjects will be closely monitored during and after the infusion.

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 infusion. No infusion will occur in those with signs or symptoms indicative of active infection. In addition, those subjects who were EBV serology negative at screening will undergo evaluation of EBV viral load at each visit. If laboratory evidence of active EBV infection is present, subjects will not receive additional study drug infusions until resolution of active infection indicated by laboratory evidence and absence of signs and symptoms of disease. In each instance, resumption of infusions will occur only after review of the data and consultation with the TrialNet infectious disease consultants.

Subjects will be counseled by study personnel and requested to avoid pregnancy for 3 months following the last study infusion as animal studies have indicated that CTLA-4 Ig crosses the placenta and is excreted in milk.

8. ADVERSE EVENT REPORTING AND SAFETY MONITORING

8.1. Adverse Event Definition

8.1.1. 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 to hypo or hyperglycemia that are Grade 2 or greater per the NCI CTCAE (see Section 8.1.4. Grading Event Severity below) must be reported to TNCC on the appropriate adverse event form. 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

In questioning the participant the questioning should be conducted in an objective manner.

8.1.2. Serious Adverse Event

For this trial, an adverse event associated with the treatment or study procedure that suggests a significant hazard, contraindication, side effect or precaution (as described below) is to be reported as a serious adverse event (SAE). A serious adverse event (experience) or reaction is any untoward medical occurrence that:

- results in death,
- is life-threatening,
- requires inpatient hospitalization or prolongation of existing hospitalization,
- results in persistent or significant disability/incapacity, or
- is a congenital anomaly/birth defect.

Important medical events that may not result in death, be life threatening, or require hospitalization may be considered serious adverse events when, based upon appropriate medical judgment, they may jeopardize the patient and may require medical or surgical intervention to prevent one of the outcomes listed above.

8.1.3. Unexpected Adverse Event

An adverse event is considered unexpected when the nature (specificity) or severity of the event is not consistent with the risks described in the protocol or informed consent document for a particular protocol.

8.1.4. Grading Event Severity

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 hyper and hypoglycemia. For this study, a reportable hypoglycemic event is defined as those resulting in loss of consciousness, seizure, or requiring assistance of others due to altered state of consciousness and hyperglycemic event is one resulting in DKA.

8.2. Adverse Event Reporting and Monitoring

Study personnel will assess adverse events and the use of concomitant medications throughout the study. Adverse events will be reported to the TrialNet Coordinating Center as described below. They will be graded as to severity according to common toxicity criteria or study-specific criteria and the investigator will make a determination as to the relation to therapy. Events will be assessed and reported in accordance with the ICH Guideline For Good Clinical Practice 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) of Grade 2 or greater severity regardless of relationship to therapy. For reporting serious adverse events (SAE), the TrialNet 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 Data and Safety Monitoring Board (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 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, as needed) of adverse events by treatment group assignment. Serious adverse events as well as adverse events leading to treatment discontinuation will be reviewed by the DSMB.

9. STATISTICAL CONSIDERATIONS AND ANALYSIS PLAN

Analyses of study data will be conducted to address the primary and secondary objectives of the trial, other stated objectives, and other interrelationships among elements of study data of interest to the investigators and of relevance to the objectives of the study. Such analyses may also entail the use of data from other studies in combination with data from this study. Likewise, data from this study may be used in combination with data from another study to address objectives of that study. Analyses by gender and race/ethnicity, as appropriate, are also planned.

9.1. Primary Outcome and Analyses

The primary outcome of each participant is the area under the stimulated C-peptide curve (AUC) over the first 2 hours of a 4-hour mixed meal glucose tolerance test conducted at the two-year visit. The AUC is computed using the trapezoidal rule that is a weighted sum of the C-peptide values over the 120 minutes. By the mean value theorem of integral calculus, the weighted mean C-peptide in pmol/mL is simply $AUC/120$.

The primary statistical hypothesis to be assessed in the study is whether:

- The mean C-peptide value for study subjects receiving CTLA-4 Ig differs significantly from the mean value for placebo subjects.

The primary analysis will employ the weighted mean derived from the 2 hour AUC for each participant transformed as $\log(\text{mean C-peptide}+1)$. The comparison between the two treatment arms will be based on a t-test of treatment effect in an ANCOVA model adjusting for gender, baseline age and baseline $\log(\text{C-peptide}+1)$ (75).

9.2. Secondary Outcomes and Analyses

Additional analyses of the primary outcome will include:

- A log rank test of the difference in the hazard function between groups in the incidence of the loss of the 2 hour peak C-peptide < 0.2 pmol/mL on a semi-annual MMTT (76), and
- Longitudinal analyses (75) using mixed effects models with a random intercept and slope of the C-peptide values over the post-treatment period, adjusted for the baseline level of C-peptide. The average intercept and slope will be compared between groups adjusting for age, gender and the baseline $\log(\text{C-peptide}+1)$.

Additional secondary objectives are to examine how CTLA-4 Ig affects the following:

- Mean area under the stimulated C-peptide curve (AUC) curve at 12 months
- Mean area under the stimulated C-peptide curve (AUC) over 4 hours at 24 months
- HbA1c levels over time
- Insulin dose (units/kg) over time
- Number of severe hypoglycemic events
- Number and severity of adverse events

The mean levels of quantitative variables (e.g. HbA1c and insulin dose) over all follow-up values will be compared between groups using a normal errors longitudinal analysis (75).

The prevalence of a binary characteristic (e.g. yes/no or positive/negative) at a single visit (e.g. a history of hypoglycemia during follow-up) will be assessed using a logistic regression model (76). The prevalence of a binary characteristic over time will be assessed using generalized estimating equations (75).

The rates of severe hypoglycemic events and severe adverse events will be computed (total number of events divided by total patient years of follow-up) and the rates compared using a Poisson regression model, allowing for over-dispersion using a quasi-likelihood model as appropriate. Tests of significance will employ a robust estimate of the variance (76).

The above analyses will also be conducted to adjust for age, gender, baseline $\log(C\text{-peptide}+1)$ and baseline HbA1c; and by race/ethnicity, as appropriate. Analyses will also be conducted to examine the effect of HLA or other genotype.

Analyses will also be conducted to assess heterogeneity of the effect of treatment group (the group difference) as a function of age, gender, baseline $\log(C\text{-peptide}+1)$ and baseline HbA1c; and by race/ethnicity and HLA or other genotype. Heterogeneity will be assessed using a test of treatment group by covariate interaction in an appropriate regression model.

9.3. Additional Outcomes and Analyses

Additional outcomes of interest include

- Change in autoantibody levels and/or B cell function
- Antigen specific and non-specific T and B cell subset enumeration and function
- Responses to vaccination (tetanus and killed flu)

The effects of CTLA-4 Ig on immune function will be assessed from regular blood draws during and after the course of the treatment period. The specimens will allow an assessment of whether immune cell reactivity can be found for autoantigen-specific peptides. Cell proliferation, cytokine production, and other methodologies including microarrays will also be used to measure changes (if any) in immune responses.

Treatment specific data such as PK results and titers of CTLA-4 Ig specific antibodies (if any) will also be analyzed and correlated to the drug effect (if any) on beta cell preservation.

The analyses of each outcome will be conducted using the methods for a quantitative outcome, binary outcome, or rate as described in Section 9.2 above..

9.4. Sample Size and Power Calculations

The primary analysis will compare the difference between the treated group versus the placebo group in the levels of the 2 hour AUC-mean using the $\log(\text{mean } C\text{-peptide}+1)$ in an ANCOVA model adjusting for gender, baseline age, and baseline $\log(C\text{-peptide}+1)$. Estimates of $\log(\text{mean } C\text{-peptide}+1)$ and root mean square error (RMSE) in the placebo group were obtained from prior studies (3). Among subjects with baseline C-peptide > 0.2 pmol/ml and age ≥ 12 years, the mean $\log(C\text{-peptide} + 1)$ values is 0.248 with RMSE = 0.179.

The corresponding geometric-like mean C-peptide value is 0.282 pmol/mL obtained using the inverse transformation $\exp(0.248) - 1$. Using standard equations for the comparison of two means, it was determined that a sample size of 108 subjects randomized to active treatment versus placebo in a 2:1 ratio would provide power of 85% to detect a 50% increase in the geometric-like mean C-peptide with active treatment relative to the placebo group using a test at the 0.05 level (one-sided), with 10% loss to follow-up.

Thus, this study will employ 72 subjects in the active treatment group and 36 subjects in the placebo group (108 total) who are scheduled to be followed for a total of four years on double-masked treatment.

The exact final sample size cannot be fixed to be exactly 108 subjects because of staggered patient entry. The study will be closed to new subjects entering the screening phase when the total number then randomized plus a fraction of those in screening is expected to provide at least 108 randomized subjects. Subjects who had already conducted the initial screening visit at that time will be allowed to complete screening and be randomized if still both consenting and eligible.

9.5. 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 spending function with an O'Brien-Fleming boundary will be used to protect the type I error probability (77) and to assess the significance of the interim results that emerge during the trial. The monitoring plan will allow for early termination based on the treatment effects on C-peptide values at 1 year and also at 2 years of follow-up. DSMB reports will also include conditional power analyses conducted both under the study hypotheses and under the current trend of the data (78) to allow early termination due to futility – i.e. lack of beneficial treatment effect. Additional analysis will assess potential adverse outcomes of treatment and will assess the incidence of all severe adverse events.

10. ETHICAL CONSIDERATIONS AND COMPLIANCE WITH GOOD CLINICAL PRACTICE

10.1. 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.

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

10.2. Participating Centers

Participating TrialNet clinical sites must have a Federal-wide Assurance (FWA) 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 at each of the participating clinical sites. HIPAA 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 in this study. Medical and research records should be maintained at each site in the strictest confidence. However, as a part of the quality assurance and legal responsibilities of an investigation, the investigational 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.

10.3. Informed Consent

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

The informed consent form must be updated or revised whenever 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 patient's participation in the study.

10.4. Study Subject Confidentiality

Study records with the study subject's information for internal use at the clinical sites will be secured at the study site during the study. At the end of the study, all records will continue to be kept in a secure location. There are no plans to destroy the records.

Study subject data, which is for reporting purposes, will be stored at The TrialNet Coordinating Center. Case report forms 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, and the data collection forms will be electronically scanned and saved in electronic format for long-term storage. All paper copies of the forms will ultimately be destroyed after the data is transferred.

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11. STUDY ADMINISTRATION

This study is part of Type 1 Diabetes TrialNet, which is funded by the National Institutes of Health. Funding will cover the costs of administration and laboratory tests associated with this study during the participant's period of follow-up. Bristol-Myers Squibb will provide CTLA-4 Ig free of charge for the participant's entire length of treatment and will support some laboratory tests related to drug administration.

11.1. Groups and Committees

11.1.1. CTLA-4 Ig (Abatacept) Study Committee

The CTLA-4 Ig (Abatacept) Study Committee, the TrialNet Clinic Monitoring Group, Laboratory Monitoring Group, Steering Committee and Data and Safety Monitoring Board will receive periodic reports from the TrialNet Coordinating Center on the progress of the study. These will include accrual rates and baseline demographic characteristics.

As appropriate, abstracts and manuscripts dealing with the progress of the CTLA4-Ig (Abatacept) Study shall be prepared by the Study Committee under the guidance of the TrialNet Publications and Presentations Committee under the policies established by TrialNet.

11.1.2. TrialNet Chairman's Office and TrialNet Coordinating Center

The TrialNet Chairman's Office and TrialNet Coordinating Center (TNCC) will collaboratively provide leadership to the TrialNet study group to include protocol and manual preparation, training for clinical sites, development of statistical design for each study, analysis of study results and the preparation of publications and presentations. The TNCC will also coordinate interactions among the participating TrialNet Clinical sites, laboratories including TrialNet core laboratories and other subcontract laboratories, NIDDK, and other sponsoring agencies.

11.1.3. Clinical Sites

Each Principal Investigator at the participating TrialNet clinical site will oversee all operations. The clinical sites will forward all laboratory and data collection form information to the TrialNet Coordinating Center for analysis. Conference calls and site visits, as needed, will facilitate evaluation of the trial management.

11.1.4. Clinical Site Monitoring

In order to conduct this study consistent with established research principles and ICH-GCP guidelines, there may be site visits conducted during the study to evaluate study conduct. All sites will be monitored by the Coordinating Center and appropriate TrialNet committees for patient enrollment, compliance with protocol procedures, completeness and accuracy of data entered on the case report forms (CRFs), and the occurrence and reporting of adverse events (AEs) and serious adverse events (SAEs).

11.1.5. Data and Safety Monitoring Board (DSMB)

The DSMB will meet approximately every 6 months to review interim analyses of efficacy and adverse events prepared by the Coordinating Center. All adverse events will be recorded on the adverse event forms, which will be provided to the local IRBs, per their reporting requirements, and to the Coordinating Center. The DSMB will independently evaluate whether the adverse events constitute grounds to discontinue the study.

11.2. Sample and Data Storage

Data collected for this study will be sent to the TrialNet Coordinating Center. After the study is completed, 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 the subject's approval, when TrialNet is completed, remaining samples will be stored at the NIDDK Repository Sites. Samples could be used to learn more about causes of type 1 diabetes, its complications and other conditions for which individuals with diabetes are at increased risk and how to improve treatment. The results of these future analyses will not be made known to the participant.

Since the stored samples will be fully de-identified upon the completion of TrialNet, it will no longer be possible to identify samples. Thus, whereas participants can choose to change their mind about having their remaining blood stored during the existence of TrialNet, this is not possible when 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 proposals approved by the NIDDK. The NIDDK will convene an external panel of experts to review requests for access to samples.

11.3. 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 site will not report the data collected from its site alone. All presentations and publications using TrialNet study data must protect the main objectives of the trial. Data that could be perceived as threatening the masking 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 given by the TrialNet Publications and Presentations Committee. Study results should be discussed with the news media only upon authorization of the Publications and Presentations Committee and the Executive Committee, and never before the results are presented. Any written statements about this study that are shared with national media should be approved by the Publications and Presentations Committee and/or Executive Committee before release.

11.4. Participant Reimbursement and Compensation

Participants will be compensated for each visit attended in the study.

12. STUDY TIMELINE

It is anticipated that patient enrollment will occur during the first two years of the trial. All subjects will be followed until two years after initial treatment, with up to two years further follow-up after the treatment period has ended.

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APPENDIX 1 - Schedule of Assessments

	Visit	-1	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28-31	
	year																															
	~day of Trial:	-1 ¹	0	14	28	56	84	112	140	168	196	224	252	280	308	336	364	392	420	448	476	504	532	560	588	616	644	672	700	728	⁸	
History		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	
Physical exam ²		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	
Adverse Events Assessments			X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	
CBC with Differential		X	X	X		X	X			X				X			X			X				X			X			X	X	
Chemistries		X	X		X			X			X				X		X			X				X			X			X	X	
PPD Test		X																														
Viral serology (EBV, CMV, HIV, Hep B and C)		X																														
EBV viral load ³		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X			
Urine Pregnancy Test		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Serum for Autoantibodies		X					X				X				X		X			X				X			X			X	X	
Study drug Administration			X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X		
PK, receptor occupancy, immunogenicity						X	X ⁴	X ⁴			X			X			X			X							X			X		
Immunizations;tetanus/flu/ response ⁵																													X			
Hemoglobin A1c			X				X				X			X			X			X				X			X			X	X	
MMTT (4-hour)		X																												X		
MMTT (2-hour)							X				X						X							X							X	
Serology/viral monitoring ⁶			X								X			X			X							X						X	X	
Mechanistic Assessments ⁷		X	X			X	X				X			X			X							X						X	X	

1. **Screening Visit:** Screening MMTT must be at least 3 weeks after diagnosis and within one month (37 days) of randomization.

2. **Physical Exam:** Routine exam at screening and quarterly, directed exam prior to administration of study drug infusion.

3. **EBV viral load:** To be determined in individuals who were previously unexposed (EBV IgG and IgM negative at screening). Those with evidence of active EBV infection will not receive further study drug infusions until resolution of infection.

4. **Study drug level and receptor occupancy and immunogenicity analysis:** Collect pre and post-dose.

5. **Immunizations and response:** At study visit 26, subjects will have a pre-immunization blood draw and then receive a tetanus immunization with follow up blood draw assessing immune response at study visit 27. Subjects will have a pre-immunization blood draw and then receive killed flu vaccine annually during a study visit at least one month from study entry occurring at a clinically appropriate month with follow up blood draw assessing immune response at next visit.

6. **Serology/viral monitoring:** Anti-thyroid antibodies as well as titers to childhood immunizations and illnesses will be measured on these samples as well as tested for viral load if clinically indicated.

7. **Mechanistic Assessments:** includes samples for RNA, plasma, serum, DNA, measures of B and T cell number and function. The schedule for these assessments may vary as appropriate. At no time will the blood draw volume exceed what is allowable according to the subject's body weight (3ml/kg for subjects <18).

8. **Follow-Up After 24 Months:** Visits will be conducted approximately every 6 months.

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