NEW ONSET OF TYPE 1 DIABETES MYCOPHENOLATE MOFETIL – DACLIZUMAB CLINICAL TRIAL

(Protocol TN-02)

Preservation of Pancreatic Production of Insulin through Immunosuppression - 1 (POPPII-1) Study

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Preface

The protocol for the Type 1 Diabetes Protocol TN-02 – New Onset of Type 1 Diabetes Mycophenolate mofetil – Daclizumab Clinical Trial describes the background, design and organization of the study. The protocol will be maintained by the TrialNet Coordinating Center at The George Washington University over the course of the study through new releases of the entire 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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Introduction

1.1 Study Abstract

1.1.1 Rationale for the Study

The great body of evidence developed over the last 10 – 20 years suggests that type 1 diabetes in humans is a chronic, slowly progressive autoimmune disease [1] [2] [3] [4] [5]. The objective of this study is to identify immune intervention strategies that will prevent the progression of beta cell destruction from the time of onset of type 1 diabetes. The persistence of at least some beta cells should improve long-term diabetes care and prevent not only complications of the disease itself but also hypoglycemia, which is a consequence of its management. The aim is to arrest beta cell destruction in newly diabetic subjects because immune modulation may not work well alone once the autoimmune process has progressed to complete or near complete destruction of beta cells. The study's rationale is to demonstrate a meaningful preservation of islet function with minimal immune system side effects over the 4-year course of this study.

The data from this clinical trial could serve as the basis for a larger trial if the results are sufficiently positive, or they could suggest other combined intervention trials that might achieve either better efficacy or potentially preserve C-peptide without the need for continued immunosuppression.

The complications of long-term diabetes are well known, and the costs of caring for diabetes and its complications are currently greater than $100 billion a year. The Diabetes Control and Complications Trial (DCCT) and other studies have demonstrated that improved metabolic control can reduce the long-term complications of diabetes [6]. Thus, an intervention, which could restore normal islet function and maintain production of insulin would significantly improve the prognosis for metabolic control of diabetes and thus reduce long-term complications.

1.1.2 Design of Study

The study is a multi-center, three-arm, randomized, double-masked, placebo-controlled clinical trial. Comparisons will be made among the three groups, which are:

- Mycophenolate mofetil active drug with daclizumab (DZB) placebo IV,
- Mycophenolate mofetil active drug with daclizumab active IV,
- Mycophenolate mofetil placebo with daclizumab placebo IV.

Alteration in study design due to randomization error

After study enrollment was complete, an error in randomization was discovered so that 12 of 40 randomized subjects that were to have received active oral mycophenolate mofetil (MMF) and placebo daclizumab (DZB) infusion instead received placebo oral medication and active DZB infusion. This error has implications for the statistical analysis plan described in Section 8.0. However, study procedures for these subjects will not change. They will continue to be followed according to the protocol in a double-masked fashion.

1.1.3 Primary Hypothesis and Primary Outcome

The primary outcome of each participant is his or her area under the stimulated C-peptide curve over the first 2 hours of a 4-hour mixed meal glucose tolerance test (MMTT) conducted at the two-year visit. The primary null hypotheses for the study are:

- The mean C-peptide value for study subjects on mycophenolate mofetil (MMF) will not differ significantly from the mean value for placebo subjects.

- The mean C-peptide value for study subjects on MMF+ daclizumab (DZB) will not differ significantly from the mean value for placebo subjects.

1.1.4 Description of the Drugs

Mycophenolate mofetil

Mycophenolic acid (MPA) was originally discovered in 1896 and chemically characterized in 1952. Mycophenolate mofetil is rapidly absorbed following oral administration and hydrolyzed to form MPA, which is the active metabolite [7]. MPA is a potent, selective, uncompetitive, and reversible inhibitor of inosine monophosphate dehydrogenase (IMPDH), and, therefore, it inhibits the de novo pathway of guanosine nucleotide synthesis without incorporation into DNA. Because T- and B-lymphocytes are critically dependent for their proliferation on de novo synthesis of purines, while other cell types can utilize salvage pathways, MPA has potent cytostatic effects on lymphocytes. MPA inhibits proliferative responses of T- and B-lymphocytes to both mitogenic and allospecific stimulation. Addition of guanosine or deoxyguanosine reverses the cytostatic effects of MPA on lymphocytes. MPA also suppresses antibody formation by B-lymphocytes, and it prevents the glycosylation of lymphocyte and monocyte glycoproteins that are involved in intercellular adhesion to endothelial cells. Furthermore, MPA may inhibit recruitment of leukocytes into sites of inflammation and graft rejection. Mycophenolate mofetil does not inhibit early events in the activation of human peripheral blood mononuclear cells, such as the production of interleukin-1 (IL-1) and interleukin-2 (IL-2), but it does block the coupling of these events to DNA synthesis and proliferation.

Mycophenolic acid has been shown to be effective in a variety of treatment settings, such as therapy for autoimmune diseases like psoriasis and rheumatoid arthritis, anti-rejection therapy in patients who have received transplants, and in diabetic animal models.

Mycophenolic acid has been shown to be efficacious in severe cases of psoriasis that were refractory to conventional forms of therapy including methotrexate [8]. More than 70% of subjects gained control of previously refractory disease. Studies of 75 patients maintained on this agent for up to 13 years demonstrated that the frequent side effects of the gastrointestinal system, urinary tract in women, and viral infections reduced in frequency as patients remained on the drug and as the dose was lowered over the years of follow-up. It should be noted that this and other studies at this time used doses of up to 3600 mg a day or 65 mg/kg/day (by design, they dosed to toxicity), but long term follow-up studies have demonstrated that doses of 2400 - 3000 mg a day could maintain clinical remissions. Although cancer risks were originally cited as a concern in using this agent, long-term follow-up failed to demonstrate any statistically significant increase in cancer-risk for patients on Mycophenolic acid, compared to an age-matched cohort in the small group of 35 patients followed for more than 10 years [9].

Mycophenolate mofetil (MMF) has been used successfully to treat exacerbations of disease in a number of autoimmune disorders such as inflammatory eye disease [10], pemphigus vulgaris [11], inflammatory bowel disease [12], immune complex-mediated glomerulonephritis [13] and in rheumatoid arthritis (RA) [14]. In line with its known effect on B-lymphocytes, immunologic parameters such as RF titer were reduced after one year in the RA patients studied. All immunoglobulins were diminished with this therapy. Interestingly, the sera from patients treated with MMF inhibited lymphocyte stimulation to mitogens. No adverse side effects were reported in these studies.

Initially, MMF, in conjunction with Cyclosporine A and prednisone, was studied in two multi-center trials of cadaveric renal transplantation in Europe [15] and in the United States [16]. Both demonstrated benefits in nearly halving the number of either clinical or biopsy-proven rejection episodes when compared to placebo or azathioprine. More side effects such as gastrointestinal intolerance and viral infections were noted in the 3000mg group than in the 2000mg group. Mycophenolate mofetil has even been found to be useful in acute rejection episodes in renal transplantation; nearly 70% of subjects with acute rejection either improved or were stabilized with the drug. Similar efficacy data have emerged from transplantation trials in liver [17] and pancreas recipients [18]. The development of serious adverse side effects or complications of MMF has been noted in up to 15% of transplanted subjects on multi-drug immunosuppressive regimens. The level and type of toxicity was essentially equivalent to that seen on other triple therapy regimens. Toxicities included primarily neutropenia, CMV, other viral infections, and gastrointestinal disorders.

Mycophenolate prevents the development of diabetes in the BioBreeding (BB) rat model of diabetes. Efficacy was found at doses equivalent to 20 mg/kg/day or 1500 mg per day [19]. Similar to what was found using Cyclosporine alone in the BB rat, early and continued treatment with mycophenolate results in better protection. Short-term treatment with MMF was found to result in long term survival of islet allografts in chemically diabetic mice [20]. Specific tolerance induction was demonstrated in successful islet-transplanted mice; donor strain thyroid grafts were accepted while third party thyroid grafts were rejected [21]. Mycophenolate, like Cyclosporine, was not found to prevent islet allograft rejection in the spontaneously diabetic NOD mouse [21] nor in the BB rat when used as a single agent.

Daclizumab

ZENAPAX® (Daclizumab; DZB) is an immunosuppressive, humanized IgG1 recombinant monoclonal antibody produced by DNA technology that binds to CD25, the p55 alpha subunit of the interleukin-2 (IL-2) receptor expressed on the surface of activated lymphocytes. About 10% of DZB is the complementarity-determining regions of a murine anti-Tac antibody, and 90% is the constant domains of human IgG1 and the variable framework regions of the Eu myeloma antibody. DZB binding to CD25 inhibits IL-2 binding and the progression of T cells through the cell cycle. DZB, therefore, inhibits IL-2-mediated activation of lymphocytes, impairing the immune response to antigen. Whether the ability to respond to repeated or ongoing challenges of antigen returns to normal after DZB is cleared is unknown. All clinical experience with DZB to date comes from transplant populations, where the antibody is highly effective in ending rejection episodes and in halting graft versus host disease.

An important rationale in our proposed use of DZB is the blockade of IL2 signaling. Activated T cells deprived of cytokines, and particularly IL2, are known to respond by programmed cell death (seen as apoptosis). Activated T cells have been noted in the circulation at type 1 diabetes (T1D) onset [22] [23]. Although it is not known whether they have any relationship to the pathogenesis, it suggests that administration of this antibody could induce these potentially autoimmune cells to undergo apoptosis.

The efficacy of daclizumab in islet transplantation has been examined. Anti-IL2 receptor antibodies have been studied recently in several animal systems, and antibodies to the IL2 receptor appear to prevent allograft rejection in both mice and rats. In the diabetic BB rat, these antibodies can prolong isograft acceptance, but no significant prolongation is noted in diabetic NOD recipients [24]. Islet transplantation in autoimmune type 1 diabetic patients appears to be more successful when induction therapy with depleting T cell antibodies is incorporated into the

protocol [6]. Roep and colleagues demonstrated a reduction in peripheral T cell responses to autoimmune target antigens in those individuals given ATGAM at the time of islet cell transplantation but not in those who received conventional triple therapy. Their findings would suggest that the T cells responsible for autoimmune recurrence were significantly affected by T cell depletion given transiently during the engraftment of islets. The 'Edmonton protocol' incorporates the use of DZB induction therapy (5 doses) to minimize the toxicity associated with more broad-spectrum antibody therapies such as ATGAM or OKT3. The reported survival of all islet allografts performed to date utilizing this protocol suggests that DZB in combination with low dose Tacrolimus and Sirolimus may be achieving the same selective effect on the T cells responsible for autoimmune recurrence. Analysis of the differential effect on allograft rejection versus autoimmune recurrence will shed important information on how this result is obtained.

1.1.5 Secondary Goals

The study will examine the effect of the proposed treatment on surrogate markers for immunologic effects, namely disease-specific outcomes (autoantibody determinations, generation of alloantibodies, and T cell reactivity and frequency) and immunological outcomes (T cell reactivity to recall antigens and frequency of activated T cells).

Modulation of the immune response could lower autoantibody titers and either reduce or prevent the generation of autoantigenic T cell responses. Successful immunomodulation with this drug could be seen in the lack of generation of alloantibodies.

The T cells will be analyzed for antigen specificity, epitope recognition and cytokine production in order to determine if the therapies in this protocol have affected their function. With patient permission, frozen T-cells will be maintained as part of this process for comparison to untreated individuals and those with either prediabetes or chronic diabetes.

Mycophenolic acid and derivatives will be measured at one month of treatment to determine the pharmacokinetics of MMF in the adolescent/adult populations who will participate in this study. Understanding the factors that correlate with both efficacy as well as side effects of therapy are critically important in determining the risk benefit ratio for immunosuppressive therapies such as MMF and DZB. If we find that preservation of C-peptide can be achieved with lower levels of MMF, which would minimize the toxicity of the therapy, then dose reduction could be considered in this or subsequent protocols. If, however, we determine that the benefit of C-peptide preservation can only be achieved with high levels of MMF that are also associated with increased toxicity, then future long-term trials may not be indicated.

Other secondary goals include examining the effect of MMF and/or DZB on glycosylated hemoglobin (HbA1c), insulin dose, hypoglycemic episodes, rates of infection, and adverse events. The adverse events of interest include diarrhea as well as other gastrointestinal toxicities such as nausea, vomiting, gastritis and anorexia.

This study will also accrue additional information about immunologic, genetic and metabolic factors associated with type 1 diabetes by analyzing stored blood samples. New insights into immunological and genetic mechanisms controlling beta-cell loss in type 1 diabetes may lead to more effective strategies to more effectively treat (or prevent) the disease. Mechanistic studies will be conducted to compare mechanistic variables for subjects at baseline and over time between the treatment groups and other subgroups of interest. Stored samples could also be utilized to examine potential determinants of the complications of diabetes and of other conditions for which patients with T1D could be at increased risk.

1.2 Statement of Purpose

This protocol describes the background, design, and organization of the New Onset of Type 1 Diabetes Mycophenolate mofetil – Daclizumab Clinical Trial. The protocol was written by Dr. Peter Gottlieb and The George Washington University Biostatistics Center, and reviewed by the MMF/DZB Subcommittee. Any changes that occur to this protocol during the course of the trial require the formal approval of the TrialNet Steering Committee. The study protocol, along with the required informed consent forms, will be approved by each participating institution's Institutional Review Board (IRB).

1.3 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 (IRBs) at each of the participating clinical sites (or equivalent at international sites). HIPAA regulations will be followed by each participating institution in accordance with each institution's requirements.

2 Background

2.1 Clinical Problem

Type 1 diabetes in humans is a chronic, slowly progressive autoimmune disease. Therapies with immunosuppressive or modulatory agents for type 1 diabetes are being explored. Studies that detect autoantibodies to islet cell proteins have provided an important support to this assertion, and they have led to the identification and cloning of a number of target molecules, including insulin [25] [26], glutamic acid decarboxylase (GAD65 and GAD67) [27], islet cell antigen (ICA) 512/ islet antigen (IA)-2 [28] [29] phogrin or IA-2β [30], Imogen38 [31], heat shock protein [32], and others. Although autoantibodies may play a role in destruction of islet cells, evidence from animal models of type 1 diabetes suggests that T cells are primarily responsible for the development of disease [33] [34]

2.2 Biology/Physiology

Through histological examination of the pancreas, Gepts identified the T cell as a component of the mononuclear infiltration of the islets of Langerhans that leads to beta cell destruction [35]. Earlier research has shown that activated T cells (Ia+ or IL2 Receptor +) are present in the circulation at the time of diagnosis in humans [36] [37], which implies that the autoimmune destruction of islet cells is still ongoing. The “honeymoon” phase often follows the onset of clinical hyperglycemia, and it further suggests that some endogenous insulin production persists. The ability of the pancreas to secrete insulin in response to glucose or other secretagogues within the first year of diagnosis has been demonstrated in several studies. The ability of the islets to produce insulin, however, deteriorates over time, as demonstrated by C-peptide levels in previous immunotherapy trials [38] [22] [23] and other clinical studies [39].

Preservation of endogenous insulin is associated with better metabolic control than can be achieved in patients who must rely exclusively on exogenous insulin [39]. The Diabetes Control and Complications Trial (DCCT) and other studies have demonstrated that improved metabolic control can reduce the long-term complications of diabetes [24]. Thus, an intervention that could preserve islets and maintain production of insulin would significantly improve the prognosis for metabolic control of diabetes. One such intervention may be suppressing autoimmunity before insulin-producing beta cells are completely destroyed.

The autoimmune attack on beta cells observed in diabetics may be as aggressive as rejection episodes that occur in transplant recipients. In transplant recipients, rejection typically requires an anti-T cell antibody and steroids to terminate the episode. Less toxic interventions, particularly anti-CD3 and mycophenolate mofetil are now being explored. In transplant patients, these agents can restore organ function even when started after a rejection episode has begun. Similarly, immunosuppression in type 1 diabetes may preserve some beta cell function. A major question is whether we would have to maintain an immunosuppressive intervention in order to sustain any benefit, or whether there is any hope for restoring a state of tolerance to the islet cells. Recent evidence suggests that islet regeneration may occur even at the time of diagnosis of type 1 diabetes, which contributes more support for immunotherapies that can arrest the autoimmune attack on the beta cell.

Several new immunosuppressive agents show promise as therapy for diabetes. Some agents developed since Cyclosporine A include Tacrolimus (FK506), Sirolimus (Rapamycin), antibodies to CD52 (Campath) and CD25 (daclizumab), and mycophenolate mofetil (MMF).

With the exception of mycophenolate mofetil, all of these agents have undesirable and potentially unknown side effects.

Tacrolimus has much of the nephrotoxicity of Cyclosporine, and it has a mechanism of action related to that of Cyclosporine. Thus, maintaining any benefit would likely require continued treatment [7].

Sirolimus is a potentially attractive agent that blocks T cell proliferation, but it causes hypercholesterolemia, an undesirable side effect for diabetics who are predisposed to vascular disease [7]. As seen with many other agents, when Sirolimus is given early to prediabetic NOD mice, it prevents disease onset. However, it fails to reverse diabetes in hyperglycemic NOD mice [40] or to prevent adoptive transfer of disease when used as a single agent (Ron Gill, personal communication).

Rajotte and coworkers have preliminary data in islet transplantation suggesting that the 'Edmonton protocol' of daclizumab (Zenapax), low dose Tacrolimus and Sirolimus, may allow for successful islet engraftment in human type 1 diabetes patients. While this result is very exciting, it is not yet clear how this effect is being mediated. For example, the use of Sirolimus could be the key to this result, but it might be due to the particular combination of agents or the withholding of steroids from the regimen. We believe that tests of Sirolimus in new-onset type 1 diabetes are premature until more is known of its long-term safety and efficacy and until strategies that limit Sirolimus-induced hypercholesterolemia are developed.

The humanized Campath has very limited availability and still causes the cytokine syndrome associated with other anti-T cell antibodies. Further trials currently are not in progress despite some promising results in severe vasculitis.

2.3 Review of Previous Studies

The most extensive studies of immunosuppression in diabetes used Cyclosporine A, which blocks T lymphocyte IL-2 production and proliferation [6] [41]. Stiller and colleagues [6] demonstrated the effectiveness of Cyclosporine at maintaining C-peptide at 1 year from diagnosis in new onset patients. Adverse effects, especially nephrotoxicity, have limited the use of Cyclosporine in type 1 diabetes. Concern about adverse effects, similar to those of Cyclosporine, have generally discouraged further studies for type 1 diabetes.

The most comprehensive Cyclosporine trial, conducted at seven Canadian and five European centers, was a randomized, double-blind, placebo-controlled trial involving 188 patients aged 10-35 years who entered the study within six weeks of onset of symptoms (Canadian-European Randomized Trial Group) [41]. At six months, 39% of Cyclosporine A treated patients were off insulin compared with 19% of placebo group ($p<0.003$). At 12 months, 24% of Cyclosporine and 10% of placebo patients were off insulin. The difference between the Cyclosporine A and control groups had disappeared after 3 years. When Cyclosporine A was discontinued, all residual insulin secretion rapidly ended. As expected, adverse effects were increases in mean serum creatinine, hirsutism, and gum hypertrophy. The costs and morbidity of Cyclosporine A treatment led to its abandonment as a therapy for new-onset type 1 diabetes.

Interventions that could be evaluated in humans have generally been tested in non-obese diabetic mice first. Either spontaneous disease that arises between 3 and 9 months of age may be targeted, or newly diabetic animals can be transplanted with islets to study disease recurrence. Alterations in the radical scavenging potential of diet affects diabetes incidence in

the non-obese diabetic (NOD) mouse model of diabetes. These results led to formal trials of Nicotinamide as well as pilot studies of several radical scavengers in humans but with little success [22] [42]. A recent trial of Nicotinamide in high-risk prediabetic patients in Germany was unable to demonstrate the 80% beneficial effect that the study was powered to find [43]. Smaller scale trials of Nicotinamide in children did not preserve C-peptide relative to controls and may only have a small potential benefit in adults. These trials were undertaken despite concerns regarding the interference with poly-ADP ribose dependent DNA repair that can result in vitro from Nicotinamide and the potential for this drug to cause insulin resistance [44]. No Nicotinamide-treated subject seems to have had adverse consequences from a study.

The incidence of diabetes in (non-obese diabetic mouse) NOD mice is reduced by injections of complete Freund's adjuvant [45] and by infection with (Bacille Calmette-Guérin) BCG [46] attenuated mycobacteria. Because adverse effects of BCG immunization are limited to infants with severe combined immunodeficiency (who would not be at risk for diabetes), the Barbara Davis Center undertook a randomized placebo-controlled trial of BCG vaccine in 90 subjects [23]. There was no improvement in overall glycemic control, no preservation of C-peptide, no reduction in insulin dose, and no reduction in the level of autoantibodies among treated subjects.

Large-scale diabetes intervention studies are currently focusing on prediabetic subjects. The Diabetes Prevention Trial – 1 (DPT-1), for example, administered insulin to prediabetic subjects either subcutaneously or orally. Antigen-specific interventions when given alone are unlikely to have the power to arrest the autoimmune destruction that precedes clinical presentation of diabetes. Anti-T cell antibodies (anti-CD3), however, halt progression to diabetes in the NOD mouse even when started at the onset of hyperglycemia [47].

New immunosuppressive agents have become available that dramatically improve the survival of transplanted organs by preventing or reversing rejection. These agents interfere with immune responses and are effective in halting immune-mediated damage in life-threatening situations, such as severe graft versus host disease. These newer agents are increasingly being evaluated in less acute autoimmune situations, such as SLE, autoimmune anemias, and rheumatic syndromes. In the long term, it may be possible to develop antigen specific immunosuppression that might selectively induce tolerance to a transplant or re-induce tolerance to a self-antigen. Antigen specific therapies for type 1 diabetes are already under study in the Diabetes Prevention Trial to determine whether immune modulation, with minimal side effects, can prevent diabetes onset.

2.4 Known and Suspected Toxicity

Islet-cell toxicity is an important issue in the context of MMF in type 1 diabetes. In vitro studies have observed an increased rate of apoptosis in islet cell lines (HIT-1) cultured with Mycophenolic acid. In vivo, prolonged depletion of GTP induces beta-cell death compatible with apoptosis. This situation probably involves a direct impairment of GTP-dependent RNA-primed DNA synthesis, and it also appears to be modulated by small GTP-binding proteins.

Treatment of intact adult rat islets (the beta cells which replicate slowly) induced a modest, but definite, death by apoptosis over 1 to 3 day periods. Metz and colleagues speculate that more prolonged use of the new generation of immunosuppressive agents exemplified by MMF might have deleterious effects on the survival of islet or pancreas grafts. To date, this concern does not seem to be mirrored in impaired beta cell function in pancreatic or renal transplant patients treated with MMF [18] [48].

Psoriatic patients treated with mycophenolate have noted no increased incidence of diabetes with follow-up of up to 13 years [9]. In liver transplantation, where MMF was employed in regimens that were designed to withdraw steroids after 14 days, there was no increase in the incidence of hyperglycemia or graft rejection in patients maintained on Cyclosporine and MMF nor in patients on Tacrolimus and MMF [17].

Risks involved with immunosuppression generally pertain to malignancy and infection. Patients on immunosuppressive therapy are at increased risk for developing lymphoproliferative disorders and opportunistic infections. Use of DZB in combination with cyclosporine, MMF and prednisone showed no increase in incidence of lymphoproliferative disorders or opportunistic infections while significantly preventing rejection in cardiac allografts [39]. Low titers of anti-idiotype antibodies to daclizumab were detected in the DZB-treated patients with an overall incidence of 8.4%. We will store serum to be able to examine the amount of anti-idiotypic antibodies that are generated and determine whether their presence affects efficacy of therapy. No antibodies that affected efficacy, safety, serum DZB levels or any other clinically relevant parameter were detected in clinical studies to date. While in the circulation, DZB impairs the immune system's response to antigenic challenges. It is unknown whether the ability to respond to repeated or ongoing challenges with those antigens returns to normal after DZB is cleared. Subjects who may require immunizations during the conduct of the trial will be excluded from the protocol, and so by design we will try to minimize the risk for this potential problem as much as possible.

In previous studies, there were no differences in abnormal hematologic or chemical laboratory test results between placebo-treated and DZB-treated groups with the exception of fasting blood glucose. Fasting blood glucose was measured in a small number of placebo- and DZB-treated patients. A total of 16% (10 of 64 patients) of placebo-treated and 32% (28 of 88 patients) of DZB-treated patients had high fasting blood glucose values. Most of these high values occurred either on the first day post-transplant when patients received high doses of corticosteroids or in patients with diabetes.

The overall incidence of infectious episodes, including viral infections, fungal infections, bacteremia and septicemia, and pneumonia, was not higher in DZB-treated patients than in placebo-treated patients, with the exception of cellulitis and wound infections, which occurred in 4.1% of placebo-treated and 8.4% of DZB-treated patients. The types of infections reported were similar in both treatment groups. Cytomegalovirus infection was reported in 16% of the patients in the placebo group and 13% of the patients in the DZB group. It should be remembered that these complications took place in the setting of standard 3-drug therapy. With low-dose tacrolimus and sirolimus and gancyclovir prophylaxis, the Edmonton group has not noted significant infectious complications through this date.

3 Study Design

3.1 Overview

The study is a multi-center, three-arm, randomized, double-masked, placebo-controlled clinical trial. The total length of the study will be 4 years, with 1 year of recruitment. Participants will be followed for 2 years on treatment, and at least 1-year post treatment.

3.2 Enrollment

Study subjects will be recruited through the participating TrialNet clinical centers. Selection of subjects for enrollment in the study will be done through a screening process that will occur over a two to four week period. This screening process will occur between 3 weeks and 3 months of the date of diagnosis of type 1 diabetes in order to ensure that mixed meal test results will not be biased. The screening process will be divided into an initial screening visit and then a mixed meal tolerance test visit one week later. At remote study sites where subjects must travel a farther distance, steps will be taken to expedite the process so that the initial screening process and the mixed meal tolerance test can be conducted within one visit to the study clinic. Subjects will not be excluded if they develop type 1 diabetes in either arm of DPT-1, European Nicotinamide Diabetes Trial (ENDIT), or other such trials designed to prevent the onset of diabetes.

3.3 Inclusion Criteria

Potential participants must meet the following inclusion criteria:

1. Be within 3-months (100 days) of diagnosis of type 1 diabetes based on American Diabetes Association (ADA) criteria
2. Be between the ages of 8 and 45years
3. Must have stimulated C-peptide levels ≥ 0.2 pmol/ml (0.6 ng/ml). This test must be conducted at least 21 days from the diagnosis of diabetes and no more than one month (37 days) prior to the date of randomization.
4. Must have either detectable anti-GAD, anti-ICA512/IA-2, insulin autoantibodies (unless received insulin therapy for 7 days or more), or islet cell autoantibodies. The reason for inclusion of these enrollment criteria is to avoid inclusion of patients with “Type 1B diabetes mellitus”, which may not involve the immunologic criteria measured by the assays that will be utilized.
5. If participant has reproductive potential, he or she must be agreeable to an effective form of birth control (unless abstinence is the chosen method)
6. If participant is female with reproductive potential, she must be willing to undergo pregnancy testing and to report possible or confirmed pregnancies promptly during the course of the MMF/DZB study.
7. Must be willing to comply with intensive diabetes management. The goal of management will be an HbA1c of 7.0% for all participants, regardless of age. Participants will be expected to take a sufficient number of daily insulin shots to meet this goal. Alternatively, participants can use insulin pump therapy. Participants will also be expected to test their blood sugar at least 3-4 times per day. There will be a Certified Diabetes Educator working with study participants to achieve these goals.
8. Weigh at least 25 kg at the time of study entry

3.4 Exclusion Criteria

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

1. Have any complicating medical issues that would interfere with blood drawing or monitoring.
2. Have a Body Mass Index (BMI) that is greater than the 95th percentile for age and gender.
3. Have serologic evidence of HIV infection.
4. Have serologic evidence of Hepatitis B infection.
5. Have serologic evidence of Hepatitis C infection.
6. Have abnormal liver function tests.
7. Have a history of leukopenia and/or neutropenia.
8. Have a history of chronic peptic ulcer disease, erosive esophagitis, chronic inflammatory bowel disease and/or chronic colonic disease.
9. Have a positive PPD test result.
10. Have had any live vaccinations in the preceding 6 weeks (e.g. MMR-second dose, live flu vaccine, varicella vaccine, live polio vaccine, yellow fever vaccine).
11. Resides outside reasonable geographical proximity to the clinic (*i.e*., residence outside the state in which the Investigator and study reside, residence outside an immediately neighboring state, or residence outside an area that the Investigator considers reasonable). It is left to the Investigator's discretion to decide if a patient's geographical residence is prohibitive to complete study participation.
12. Require chronic use of steroids or other immunosuppressive agents for other conditions.
13. Require the use of any diabetes medications other than insulin.
14. Be currently pregnant or 3 months postpartum.
15. Be currently nursing or within 6 weeks of having completed nursing.
16. Anticipate getting pregnant, or fathering a child, during the study.

3.5 Informed Consent

At the beginning of the screening visit, participants will be given a written consent form by qualified study personnel (the Study Coordinator and/or Investigator or other designee). These research personnel will understand the research study, and will complete any necessary courses required by their Institutional Review Board prior to implementing the consent process. The consent process will occur in a quiet setting, and the participant will be given time to review the written consent form and ask questions prior to the initiation of study procedures. The consent form for this clinical study will be reviewed with participants (and their guardian in the case of adolescent participants) prior to performing any study-related assessments. Adolescent participants will be given the opportunity to discuss the study and consent form independently from their parent or guardian, which will allow these subjects to ask questions they might not have felt comfortable asking previously. In addition, the parent or guardian of adolescent participants will be given the opportunity to discuss the study independently from the participant. Asking the participant to explain the study in the participant's own words will assess the participant's understanding and autonomy. The participant will also be required to complete a short, written Volunteer Understanding Quiz that is designed to ensure that the subject understands the study, as well as what is being asked of him/her. If the subject is under the age of 18, the subject's parent or guardian will also be offered the opportunity to complete the Volunteer Understanding Quiz independently from the subject. TrialNet research personnel will review the completed quiz with the participant (and the participant's parent or guardian in the case of an adolescent participant), taking special care to review any questions the participant answered incorrectly and to answer any questions about the study. Then, qualified personnel as listed above will obtain written consent prior to the initiation of study procedures. The consent

form requests consent for each time the individual has blood drawn for these studies, and it informs the participant that samples will be drawn for storage, with the participant's permission, for future analyses. The participant will be given a copy of their signed consent forms (and assent forms where applicable).

Assent forms have also been developed for participants aged 8-17 years (unless local IRB requirements differ in procedure). Those within that age range will be given the consent and assent forms requested and will have the opportunity to discuss the study apart from their parent(s) or guardian(s). This will allow these individuals to ask questions they might not have felt comfortable asking previously. In addition, the parent(s) or guardian(s) will be given the opportunity to discuss the study apart from the child or adolescent.

A screening informed consent form has been developed that allows participants to be screened for this study without committing to full study participation. Participants will be provided with the full informed consent prior to screening, which will need to be reviewed and signed before participants proceed beyond the screening phase of this study. The screening informed consent form provides details about the procedures involved with being screened for participation and refers participants to the full informed consent for more details about the study. Participants will still have the option of signing the full informed consent prior to screening, if they desire.

Individuals in this age group will be re-consented at the first visit they attend after their eighteenth birthday.

Participants will also sign a consent form for HIV screening, which is a state-specific form. The participants will be informed that this information is a reportable condition to the respective state department of health. They will be told the results of their screening tests. If they are found to have evidence of HIV or Hepatitis B or C, they will be excluded from the study but referred for appropriate counseling by specialists in these areas.

Subjects enrolled at each of the 7 clinical sites affected will be informed of the randomization error. Subjects will be asked to continue follow-up as per protocol.

3.6 Assignment of the TrialNet Identification Number

Subjects who are screened for enrollment in the MMF/DZB study will receive a TrialNet identification number at the beginning of the screening visit. Randomized MMF/DZB subjects will receive a second identification number at the time of randomization. Because arbitrary identification numbers are susceptible to transcription errors, the participants will be further identified by the first three letters of their first name. No other identifiers will be used on the MMF/DZB study forms.

3.7 Treatment Groups

3.7.1 Description of Treatment Groups

The study groups were outlined in Section 1.1.2. Group assignment will be random and masked, and all study participants will follow the same schedule of study visits and procedures.

The sample of 120 participants will be randomly assigned to the following three groups:

- 40 participants will receive active MMF by mouth, at a twice-daily dose of 600 mg/m^2 (dependent on Body Surface Area, maximum 2000 mg/day) for the duration of the study **as well as** placebo daclizumab given as an intravenous

infusion of 1 mg/kg (maximum 100 mg) at the baseline visit (Week 0) and at the Week 2 follow-up visit.

- 40 participants will receive active MMF by mouth, at a twice-daily dose of 600 mg/m^2 (dependent on Body Surface Area, maximum 2000 mg/day) for the duration of the study **as well as** active daclizumab given as an intravenous infusion of 1 mg/kg (maximum 100 mg) at the baseline visit (Week 0) and at the Week 2 follow-up visit.
- 40 participants will receive MMF placebo by mouth, at a twice-daily dose of 600 mg/m^2 (dependent on Body Surface Area, maximum 2000 mg/day) for the duration of the study **as well as** placebo daclizumab given as an intravenous infusion of 1 mg/kg (maximum 100 mg) at the baseline visit (Week 0) and at the Week 2 follow-up visit.

The MMF/placebo dose for each participant will be calculated according to a study specific Body Surface Area Dosing Chart. Dosing will be recalculated at every follow-up visit in order to consider a participant's weight change between visits, which would influence their study drug dosage. The MMF/placebo will be dispensed at Week 0 and every 3 months during the remainder of the study.

3.8 Assignment to Groups

After participants sign the consent form, complete the screening visit(s) including the mixed meal tolerance test, meet all of the inclusion criteria and none of the exclusion criteria, and complete the baseline procedures, they will be randomized to one of the three study groups: (i) active MMF and placebo DZB; (ii) active MMF and active DZB; (iii) placebo MMF and placebo DZB.

Due to an error in randomization, 12 participants intended to be randomized to the MMF only treatment group with active MMF and placebo DZB instead received active DZB and placebo MMF. This, "DZB only" group then constitutes a fourth study group, the statistical implications of which are included in the revised analysis plan.

3.9 Randomization Method

Participants will be randomized, in equal numbers, to the three arms of the study. The randomization method will be stratified by TrialNet study site. This approach ensures that study site will not be a potential confounder.

3.9.1 Level of Masking

The study will be double-masked, in that the participant and those involved in patient care at the clinics will be masked to the participant's treatment group assignment. The TrialNet Central Pharmacy and the staff at the Coordinating Center will know to which treatment group each participant is assigned.

3.9.2 Randomization Number Assignment

The Coordinating Center will generate a randomization schedule for the study sites. The randomization number will be a four digit code, where each study participant's code is unique. The randomization numbers will be obtained from an automated telephone system at the Coordinating Center. The randomization number will in no way reflect the treatment group the participant has been assigned to. The pharmacist at each study site will receive study

medication identifiable only by randomization number. After a subject is randomized, the Study Coordinator will contact the site pharmacist to obtain bottle(s) of study medication labeled with the subject's randomization number. The randomization number will also determine if a participant gets an active or placebo DZB IV infusion.

3.10 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. The participant's insulin production will be measured by a series of mixed meal glucose tolerance tests that will occur every six months during the two-year treatment period and for up to two-years after treatment has stopped. The participant's average blood glucose level will be evaluated by measuring glycosylated hemoglobin (HbA1c) every three months during treatment and every six months after treatment has stopped. The subjects will also be monitored for the presence of autoantibodies that are involved in the destruction of insulin producing beta cells in the pancreas. These autoantibodies will be measured every 3 months for the first year of treatment, followed by every 6 months during the second year of therapy.

The immunologic status of each study subject will be evaluated by measuring a number of different parameters. Participants will have blood drawn for a complete blood count (CBC) with differential every week for the first month of treatment, twice a month for the second and third months, monthly for the remainder of the first year of treatment and then every three-months for the remainder of the treatment period to ensure that there are no abnormalities in cell counts or distribution. Antibody titers to rubella and viral flu will be measured every 6 months during the course of treatment. Flow cytometry will be utilized every week for the first month of therapy, followed by every 3 months for the first year, then on an annual basis to monitor the proportion of depleted versus coated CD4 and CD25 T-cells.

During the two-year treatment period, participants will be closely monitored for a new or re-activated infection with Cytomegalovirus (CMV) or Epstein-Barr Virus (EBV) (see Appendix 2). At every visit to the clinic (except Week 1 and 3, Month 15 and 21) participants will have blood drawn to check for any abnormalities in blood chemistries.

At every visit to the clinic, subjects will undergo a physical examination (except Week 1 and 3). Participants will also be asked about any adverse events of treatment that might have occurred in the interval between visits. At each visit to the clinic subjects will be asked about their insulin regimen/requirements and blood glucose monitoring as a means to assess their diabetes care. At two years, additional glucose monitoring will be done to assess glycemic variation.

3.11 Samples for Storage

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 Sites for future analysis. These samples will be collected only with the subject's permission. **Table 1** lists the samples and volumes for collection and storage for future analysis. Subjects who decline consent for these sample collections will still be eligible to participate in this study.

Table 1. Mechanistic Samples

Specimen	Assay	Blood Volume
Serum	Autoantibody isotypes and proteomics	5 ml
RNA	Gene Expression – Gene Chip/RT PCR	6 ml
Frozen PBMC	ELISPOT/Tetramers/Archive	10 – 30 ml†
Plasma†	Proteomics	
DNA	Genotype analysis	5 ml

Footnotes:

† Plasma will be collected following extraction of T-cells. Approximately 6-7 ml of plasma will be obtained per 10 ml whole blood collected.

If ≤ 10 ml of blood is available – Collect 6 ml for RNA and the remaining available blood for storage of serum.
If 10 – 20 ml of blood is available – Collect 6 ml for RNA, ≥ 10 ml for frozen PBMC and remaining available blood for storage of serum.
If 20 – 30 ml of blood is available – Collect 6 ml for RNA, ≥ 10 ml for frozen PBMC, 5 ml for storage of serum and remaining available blood for DNA.
If > 30 ml of blood is available – Collect 6 ml for RNA, 5 ml for storage of serum, 20-30 ml for frozen PBMC and remaining available blood for DNA.

The mechanistic samples for collection and storage in the NIDDK Repository are:

a) 6 ml of whole blood for RNA extraction for gene expression studies.

b) 30 ml whole blood for PBMC extraction and freezing for ELISPOT and tetramer assays.

c) 5 ml whole blood for DNA extraction.

d) 5 ml whole blood for serum for autoantibody isotypes.

e) All leftover plasma following PBMC extraction (estimated volume is 6 -7 ml per 10 ml whole blood) for proteomic studies.

f) Any additional material that remains after collection and preparation of whole blood samples for items a) to e) will also be sent to the NIDDK Repository sites.

The total volume of whole blood for collection for the stored samples will not exceed maximal allowable volumes according to current NIH guidelines (specifically, no more than 3.0 ml/kg body weight at any single visit and no more than 7.0 ml/kg body weight over 6 weeks). Adjustments in sample collection procedures will be made for smaller subjects, if needed.

Samples will be drawn for RNA extraction and storage twice in the first month of the study, at the 3-month study visit, then approximately every 6 months for the rest of the study. T-cells will be collected from a blood sample and frozen for storage at the baseline and 3-month study visit, then approximately every 6 months for the rest of the study. DNA will be stored once during the study, from a blood sample taken at the baseline visit. Serum will also be stored for future proteomic and autoantibody isotype analysis twice in the first month of the study, every 3 months for the first year of the study, followed by every 6 months for the second year of the study.

3.12 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. These additional collections will only be taken from subjects aged 18 years and older who weigh at least 110 lbs (50 kg).

4 Patient Enrollment

4.1 Screening Visit

New onset T1D patients will be identified at the participating TrialNet study centers. The family will be asked if they would be interested in participating in a research project. Those indicating interest are referred to one of the Investigators or Study Coordinators for a description of the study. TrialNet research personnel authorized to present the study to families have attended an approved IRB course and are registered by the IRB. Essential components of the presentation are that the family is being invited to participate in research, that participation is voluntary, and that participation may be ended at any time at the subject's request. TrialNet research personnel will provide the participant with a full description of the study, the inclusion and exclusion criteria, the procedures involved, the study groups and the randomization process, time commitments, and the schedule of follow-up visits. The participant (and their parent or guardian in the case of adolescent participants) will then be required to complete a short, written Volunteer Understanding Quiz that is designed to ensure that the subject understands the study, as well as what is being asked of him/her. This quiz must be completed prior to obtaining informed consent.

After a participant signs the written consent form, the participant will receive a TrialNet identification number described in Section 3.6, and the screening data collection form will be completed. Blood will be taken to examine whether a subject is eligible for the MMF/DZB study. Labs, the mixed meal tolerance test, and other screening procedures described in Section 5.2.1 also will be conducted.

4.2 Baseline Visit

At the baseline study visit, the lab results from the screening visit, the results from the MMTT, and the information from the screening and baseline data collection forms will be reviewed to assess whether a subject is eligible to be randomized and enrolled in the MMF/DZB study. Subjects who do not meet all of the inclusion criteria or have one of the exclusion criteria will be referred back to the attending diabetologist for standard education, treatment, and care. Subjects not eligible for the MMF/DZB study, however, will be offered the opportunity to participate in another TrialNet study, if one is available.

Those meeting the eligibility criteria will be randomized to one of the study groups, and they will be assigned a randomization number. The subject **must** be randomized within one-month of having undergone the screening MMTT. The TrialNet Central Pharmacy will dispense and ship a three-month supply of MMF/placebo to the clinical sites before participants are randomized so that all participants will have their study medication at the baseline visit. The TrialNet Central Pharmacy will also dispense and ship the initial DZB/placebo IV infusion kit to the clinic sites so that participants will have their initial infusion at the end of the baseline visit. Since the amount of medication will vary from subject to subject, the Study Coordinator will dispense a participant-specific, three-month supply of MMF/placebo at the baseline visit. As a participant begins month 2 of the study with the original three-month supply of dispensed medication, the Study Coordinator will fax an order to the TrialNet Central Pharmacy to send another three-month supply of MMF/placebo for that participant. Therefore, the next three-month supply can be dispensed to the participant at their Month 3 follow-up visit.

The randomized participants will be asked to take their first dose of MMF/placebo in the study clinic. Randomized participants will also receive their first IV infusion of DZB/placebo at the end of the baseline visit.

5 Patient Management

5.1 Randomization

Study participants will be randomized at the clinical sites at the end of the baseline visit, and they will receive a randomization number. When it is time to randomize a subject at the baseline visit, the Investigator or Study Coordinator will call a designated phone line at the Coordinating Center. The phone line will verify the TrialNet research personnel and the study site, and a computer system will give the randomization number. This number will correspond to a randomization number on one of the bottles of medication the TrialNet Central Pharmacy pre-shipped to the study site, and that study medication will be given to the randomized participant. The randomization number will also determine if a participant gets an active or placebo DZB IV infusion. The randomization number will correspond to an infusion kit pre-shipped to the study site by the TrialNet Central Pharmacy. This infusion kit will be either DZB or DZB placebo.

5.2 Patient Management after Randomization

5.2.1 Visit Schedules, Tests, and Procedures at Each Visit

At each follow-up visit after baseline, all participants will receive enough medication to maintain a three-month supply. The TrialNet Central Pharmacy will maintain a six-month supply of the study medication for each study participant, and will contact Roche for additional supplies as needed.

Patients with type 1 diabetes usually are seen every 3 months and generally have blood drawn by fingerstick or vein at each of these visits. The major change for participants in this study is that they will have more frequent visits to the clinical sites for a mixed meal tolerance test to evaluate endogenous islet cell function. It is anticipated that a participant would have approximately 14 outpatient visits to any of these facilities over their 2-year treatment period.

All participants will be required to have blood draws for a CBC with differential on a weekly basis for the first month of treatment, twice a month for the second and third months of treatment, monthly for the remainder of the first year of treatment and then every three-months for the remainder of the treatment period. Ideally, all blood draws for a CBC would be done at the study sites. However, if it is inconvenient for a participant to have the blood drawn at the study site, the blood draw can be done at the participant's primary care physician's office or qualified independent laboratory. The study site will provide instructions to the physician or laboratory for how to process the blood and to transmit the results.

Monthly blood draws for EBV and CMV monitoring will be obtained at the study sites. If it is inconvenient for the participant to attend the study site for this blood draw, the blood draw will be done at their primary care physician's office, and the study site will give instructions to the physician about how to process and ship the blood. Ideally, the monthly visits would be done at the study sites.

At each clinic visit, participants will see their diabetologists to review blood sugar control and hypoglycemic episodes. Each clinical site will have a Certified Diabetes Educator to assist subjects in their diabetes care. In addition to diabetes care, they will review issues related to immunosuppression (weight loss, diarrhea, infections, warts). Routine clinical laboratory tests will be performed (CBC with differential, LFT's, C-peptide, Glucose, HbA1c, CMV serology, EBV serology, and blood draws for examining the T cells). A central biochemistry laboratory has

been established for TrialNet. At the central laboratory, serum for mycophenolate and daclizumab levels will be stored, batched, and forwarded to the appropriate laboratories to measure levels. One tube will be drawn for MMF and DZB levels. Since DZB levels are only drawn at weeks 2 and 4, a 10 ml red top tube will be drawn at those visits to supply enough serum for batching both DZB and MMF levels. For all other blood draws, where only mycophenolate levels are analyzed, a 5 ml red top will be drawn. Draws will always be taken prior to administering any medication. At Week 4 of the study, blood samples for an MMF pharmacokinetic (PK) analysis will be drawn from all participants. This analysis will take 2-hours and will require three 4 ml blood samples from each subject. These samples will be analyzed by a TrialNet central laboratory. Autoantibody analysis will be performed at TrialNet central laboratories for all study centers. C-peptide measurements will be examined by a TrialNet central laboratory.

Laboratory specimens collected at the local clinical sites will be forwarded to TrialNet central laboratories for analysis. These specimens will be labeled by a study assigned specimen number, the date of collection, and the first three letters of the participant's first name.

With the participant's permission, samples will also be collected and stored at the NIDDK Repository for later testing. Blood for DNA testing will be stored, with the participant's permission, when blood is drawn for HLA determination. T-cells will be stored, with the participant's permission, at the baseline and 3-month study visit, then approximately every 6 months for the rest of the study. Blood for RNA testing will be stored, with the participant's permission, twice in the first month of the study, at the 3-month study visit, then approximately every 6 months for the rest of the study. Serum will also be stored for autoantibody isotype and proteomic analysis, with the participant's permission, when blood is drawn for autoantibody testing. Samples will be available for use by investigators within or outside of TrialNet for research related to the development and treatment of type 1 diabetes. The utilization of these samples will be subject to NIDDK policies and procedures.

At every study visit the sexual activity of participants of reproductive age will be re-assessed. If a subject who was previously sexually inactive becomes sexually active, he or she will be counseled about the need to use a reliable form of birth control. Female subjects will also be required to undergo urine pregnancy tests at every visit to the clinic.

All subjects will have additional blood draws for a CBC with differential at Week 6, 10, Month 4, 5, 7, 8, 10 and 11. In addition to the schedule detailed below, all subjects will have blood drawn for monitoring of EBV viral load (by PCR) and serology on a monthly basis for the first 12 months of the treatment period. All subjects will also have blood drawn on a monthly basis for monitoring of CMV viral load (by PCR) and serology for the first three months of the study. After this, viral monitoring will occur every 3-months for the remainder of the treatment period and every six months until study end.

Week –1 (a and b) Screening Visit Schedule

Week –1a:

- Consent process
- Screening data collection form completed
- Urine pregnancy test, if female
- ECG acquisition
- Labs drawn: CBC with differential, chemistries, serum for autoantibodies and extra serum stored for isotype and proteomic analysis, HbA1c, HIV, Hep B and C, Varicella, EBV serology and PCR, CMV serology and PCR

- PPD test with a 48-72 hour follow-up
- MMTT scheduled for Week –1b screening and preparatory guidelines given
- Blood glucose diary given to patient

Week –1b:

- 4-hour mixed meal tolerance test

Week 0 Baseline Visit Schedule

- Evaluate lab results (from Week –1a and –1b) and eligibility for randomization
- Urine pregnancy test, if female
- Labs drawn: CBC with differential, chemistries, HLA determination and extra blood stored for genetic testing , baseline C-peptide, immune testing (CD4/CD25/apoptosis), rubella titers, viral flu titers, T-cells (ELISPOT), RNA (stored), T-cells and extra plasma (stored)
- Baseline data collection form completed (includes medical history, physical examination, insulin requirements and regimen assessments, and hypoglycemia assessment)
- Randomization number obtained
- Study medication dispensed
- Participant takes first dose of study medication
- DZB IV (active or placebo) administered

Week 1 Visit Schedule

- Follow-up data collection form completed (includes insulin requirements and regimen assessments, and hypoglycemia assessment)
- Labs drawn: CBC with differential, immune testing (CD4/CD25/apoptosis)
- Adverse events form completed, if necessary

Week 2 Visit Schedule

- Follow-up data collection form completed (includes physical examination, insulin requirements and regimen assessments, and hypoglycemia assessment)
- Urine pregnancy test, if female
- Labs drawn: CBC with differential, chemistries, immune testing (CD4/CD25/apoptosis), mycophenolate mofetil and daclizumab levels, EBV PCR/serology
- DZB IV (active or placebo) administered
- Adverse events form completed, if necessary

Week 3 Visit Schedule

- Follow-up data collection form completed (includes insulin requirements and regimen assessments, and hypoglycemia assessment)
- Labs drawn: CBC with differential, RNA (stored)
- Adverse events form completed, if necessary

Week 4 Visit Schedule

- Follow-up data collection form completed (includes physical examination, insulin requirements and regimen assessments, and hypoglycemia assessment)
- Urine pregnancy test, if female
- Labs drawn: CBC with differential, chemistries, serum for autoantibodies and extra serum stored for autoantibody isotype and proteomic analysis, immune testing (CD4/CD25/apoptosis), daclizumab levels, MMF pharmacokinetic (PK) analysis, rubella titers and viral flu titers, EBV PCR/serology, CMV PCR/serology
- Adverse events form completed, if necessary

Month 2 Visit Schedule

- Follow-up data collection form completed (includes physical examination, insulin requirements and regimen assessments, and hypoglycemia assessment)
- Urine pregnancy test, if female
- Labs drawn: CBC with differential, chemistries, immune testing (CD4/CD25/apoptosis), EBV PCR/serology, CMV PCR/serology
- Adverse events form completed, if necessary

Month 3 Visit Schedule

- Follow-up data collection form completed (includes physical examination, insulin requirements and regimen assessments, and hypoglycemia assessment)
- Urine pregnancy test, if female
- 2- hour mixed meal tolerance test
- Labs drawn: CBC with differential, chemistries, serum for autoantibodies and extra serum stored for autoantibody isotype and proteomic analysis, mycophenolate mofetil levels, HbA1c, C-peptides and glucoses (from MMTT), EBV PCR/serology, CMV PCR/serology, immune testing (CD4/CD25/apoptosis), T-cells (ELISPOT), RNA (stored), T-cells and extra plasma (stored)
- Study medication (MMF/Placebo) collected/dispensed
- Adverse events form completed, if necessary

Month 6 Visit Schedule

- 2-hour mixed meal tolerance test
- Follow-up data collection form completed (includes physical examination, insulin requirements and regimen assessments, and hypoglycemia assessment)
- Urine pregnancy test, if female
- Labs drawn: CBC with differential, chemistries, serum for autoantibodies and extra serum stored for autoantibody isotype and proteomic analysis, mycophenolate mofetil levels, HbA1c, EBV PCR/serology, CMV PCR/serology, immune testing (CD4/CD25/apoptosis), C-peptides and glucoses (from MMTT), rubella titers, viral flu titers, T-cells (ELISPOT), RNA (stored), T-cells and extra plasma (stored)
- Study medication (MMF/Placebo) collected/dispensed
- Adverse events form completed, if necessary

Month 9 Visit Schedule

- Follow-up data collection form completed (includes physical examination, insulin requirements and regimen assessments, and hypoglycemia assessment)
- Urine pregnancy test, if female
- Labs drawn: CBC with differential, chemistries, serum for autoantibodies and extra serum stored for autoantibody isotype and proteomic analysis, HbA1c, EBV PCR/serology, CMV PCR/serology
- Study medication (MMF/Placebo) collected/dispensed
- Adverse events form completed, if necessary

Month 12 Visit Schedule

- 2- hour mixed meal tolerance test
- Follow-up data collection form completed (includes physical examination, insulin requirements and regimen assessments, and hypoglycemia assessment)
- Urine pregnancy test, if female
- ECG

- Labs drawn: CBC with differential, chemistries, serum for autoantibodies and extra serum stored for autoantibody isotype and proteomic analysis, mycophenolate levels, HbA1c, immune testing (CD4/CD25/apoptosis), EBV PCR/serology, CMV PCR/serology, C-peptides and glucoses (from MMTT), rubella titers, viral flu titers, T-cells (ELISPOT), RNA (stored), T-cells and extra plasma (stored)
- Study medication (MMF/Placebo) collected/dispensed
- Adverse events form completed, if necessary

Month 15 Visit Schedule

- Follow-up data collection form completed (includes physical examination, insulin requirements and regimen assessments, and hypoglycemia assessment)
- Urine pregnancy test, if female
- Labs drawn: CBC with differential, HbA1c, EBV PCR/serology, CMV PCR/serology
- Study medication (MMF/Placebo) collected/dispensed
- Adverse events form completed, if necessary

Month 18 Visit Schedule

- 2-hour mixed meal tolerance test
- Follow-up data collection form completed (includes physical examination, insulin requirements and regimen assessments, and hypoglycemia assessment)
- Urine pregnancy test, if female
- Labs drawn: CBC with differential, chemistries, serum for autoantibodies and extra serum stored for autoantibody isotype and proteomic analysis, mycophenolate mofetil levels, HbA1c, EBV PCR/serology, CMV PCR/serology, C-peptides and glucoses (from MMTT), rubella titers, viral flu titers, T-cells (ELISPOT), RNA (stored), T-cells and extra plasma (stored)
- Study medication (MMF/Placebo) collected/dispensed
- Adverse events form completed, if necessary

Month 21 Visit Schedule

- Follow-up data collection form completed (includes physical examination, insulin requirements and regimen assessments, and hypoglycemia assessment)
- Urine pregnancy test, if female
- Labs drawn: CBC with differential, HbA1c, EBV PCR/serology, CMV PCR/serology
- Study medication (MMF/Placebo) collected/dispensed
- Adverse events form completed, if necessary

Month 24 Visit Schedule

- 4-hour mixed meal tolerance test
- Follow-up data collection form completed (includes physical examination, insulin requirements and regimen assessments, and hypoglycemia assessment)
- Urine pregnancy test, if female
- Medication Withdrawal Form completed
- ECG
- Labs drawn: CBC with differential, chemistries, serum for autoantibodies and extra serum stored for autoantibody isotype and proteomic analysis, mycophenolate mofetil levels, HbA1c, C-peptides and glucoses (from MMTT), EBV PCR/serology, CMV PCR/serology, immune testing (CD4/CD25/apoptosis), rubella titers, viral flu titers, T-cells (ELISPOT), RNA (stored), T-cells and extra plasma (stored)
- Study medication (MMF/Placebo) collected
- Adverse events form completed, if necessary

- Glycemic excursion profile, assessed by two day recording of home blood glucose monitor values 8 times during the day and/or five day records from continuous glucose monitoring.

Month 27 Visit Schedule (3 months post-treatment)

- Labs drawn: CBC with differential, chemistries,
- Adverse events assessed

Month 30 – 48 (every 6 months)

- Post-treatment follow-up form completed (includes physical examination)
- 2-hour mixed meal tolerance test
- Labs drawn: CBC with differential, chemistries, immune testing (CD4/CD25/apoptosis), HbA1c, EBV PCR/serology, RNA (stored), T-cells and extra plasma (stored)
- Adverse events assessed

5.2.2 Doses and Dose Changing Rules and Schedules

The dose of MMF (CellCept) or placebo (up to 250 mg 4 tabs BID PO) is participant specific based on the participant's body surface area (BSA), which is defined as

$$BSA = \sqrt{\{[height\ (cm) \times weight\ (kg)] / 3600\}}.$$

The BSA chart (in the study Manual of Operations) provides the dose for a given BSA. Each participant's BSA will be calculated at baseline and every three months thereafter for the duration of the study in case changes in dose need to be made. The data collection forms at baseline and at each follow-up visit recalculate the participant's body surface area and the participant's appropriate dose.

A participant's dose also can change because of an adverse event. This type of dosing change is described in Section 6.9. If a participant's dose is changed because of an adverse event, the date of the change, total dose per day, frequency, and the reason for the change will be documented.

5.2.3 How Masking is Maintained

The clinical sites and anyone involved in patient care will be masked to the participant's study group assignment. The TrialNet Central Pharmacy will be unmasked and will maintain a list of the randomization numbers and their respective treatment group assignments. The Coordinating Center will also be unmasked because it will prepare reports to the Data and Safety Monitoring Board to describe the study's progress. To maintain the masked study design at the clinical sites, only the four-digit randomization number will be used to identify a participant's study medication. The TrialNet Central Pharmacy and the Coordinating Center will keep a list of the treatment group assignments for the randomization numbers.

At the end of the study, a designated person at each clinical site will receive from the Coordinating Center a list of treatment assignments, allowing them to unmask their study subjects. TrialNet research personnel who see or treat the study subjects cannot be unmasked until the end of the entire study, except under the circumstances described in Section 6.10.

5.2.4 Compliance and Adherence

Pill counts will be used to assess participant compliance with daily doses of the study medication. The baseline and follow-up data collection forms capture the number of pills dispensed and collected. Because a participant's dose can change over the course of the study, the data management system will include algorithms to calculate the expected number of pills taken, which will be based on the number of pills dispensed, the number of pills collected, and the changes in dose. TrialNet research personnel will record the medication dispensation, collection, and changes in dose information.

If a participant neglects to bring back to the clinic the participant's medication bottles from the last clinic visit, TrialNet research personnel will schedule a time for the participant to bring the bottles back to the clinic. If an additional trip to the clinic is not feasible, the participant will be asked to bring the bottles at the next scheduled study visit.

Measurements of blood MMF levels will be used as a second level of compliance monitoring.

5.2.5 Withdrawal From Treatment, Continued Follow-up

The study will follow the intent-to-treat principle. This means that once randomized into the study, a participant will undergo all scheduled follow-up assessments and will remain in the assigned treatment group until he or she either dies or withdraws consent for further participation, *regardless of whether he or she is continuing to receive study medication*. Thus withdrawal from treatment does not automatically entail withdrawal from the study. Withdrawal from treatment can occur for a number of reasons, some of which are outlined below. Withdrawal from the study and its scheduled follow-up assessments should only occur if the participant dies or withdraws consent.

A participant may elect to discontinue study medication, may be unable to continue taking them, or may be withdrawn at the discretion of the Principal Investigator under the following circumstances:

- Any need for live vaccination.
- Adverse effect of immunosuppression, such as leukopenia, opportunistic infections, and weight loss.
- Pregnancy.
- A need to start on another immunosuppressive medication – such as systemic steroids.
- Missed 2 or more consecutive follow-up visits.
- Evidence of EBV or CMV reactivation or infection.

If a participant withdraws, or is withdrawn from medication for any reason, the Medication Withdrawal form will be completed and the participant will be encouraged to continue with all scheduled follow-up visits. TrialNet research personnel will make every effort to keep participants in the study even if the participant is no longer taking the study medication, or is not fully compliant with the medication schedule. If a person fails to attend a visit, TrialNet research personnel will contact the participant to reschedule and encourage the participant to come back for their follow-up evaluations.

5.2.6 Withdrawal from Active Treatment

If a participant on active MMF has all stimulated C-peptide values less than 0.05 pmol/ml, 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 below 0.05 pmol/ml, the participant will be switched from active treatment to placebo. If this confirmatory MMTT does not show that all of the participant's stimulated C-peptide levels are below 0.05 pmol/ml, the participant will continue with the study medication as before. The Coordinating Center will track each participant's stimulated C-peptide levels as the study progresses. If the Coordinating Center recognizes that a participant's stimulated C-peptide levels are all below 0.05 pmol/ml, the Coordinating Center will notify the clinical site that the subject needs to be brought back in for a confirmatory MMTT. If the Coordinating Center recognizes that the results of a confirmatory MMTT are all below 0.05 pmol/ml, the Coordinating Center will notify the TrialNet Central Pharmacy to switch the participant to placebo MMF in the next shipment to the clinical center. The participant will then be given this 3-month supply of placebo MMF at the end of the next scheduled clinic visit and will continue with placebo MMF for the remainder of the study. The rationale for this change is that once a participant's stimulated C-peptide has dropped below this level, the potential for benefit from this protocol has been diminished. The risk/benefit ratio is no longer sufficient to justify the risks associated with the study medication. In order to maintain the masking of the study outcome, a random subset of participants with a normal MMTT will also be brought back in for an additional MMTT one month after undergoing a regularly scheduled MMTT.

5.2.7 Re-Entry into the Study

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

5.2.8 Post-Treatment Follow-up

Although the experiences of the trials with Cyclosporine suggest that therapy should be life-long, it is also apparent that MMF and/or DZB may help engender a tolerant state, which could be long lasting. Additionally, retrospective data from islet transplant patients suggest that use of an agent that depletes T cells initially appears to substantially reduce subsequent recurrence of autoimmunity. These findings suggest that by using MMF alone or in conjunction with DZB, autoreactive T cells may be eliminated, and tolerance to islets may be re-established, which may allow for successful discontinuation of therapy.

Treatment will be stopped at the Month 24 visit for all participants, regardless of assigned treatment group. Participants will be asked to return to the clinical site 3 months after stopping treatment to evaluate whether there are any issues or concerns that need to be addressed. At this time routine clinical laboratory tests will be performed (CBC with differential, chemistries). After this 3 month post-treatment visit, participants will be asked to return in 3 months and then every 6 months until the end of the entire study. The purpose of the post-treatment follow-up period is to evaluate the long-term benefits and side effects of the treatment. The minimum length of post-treatment follow-up will be 1-year (for participants recruited at end of the 1-year recruitment period), and the maximum will be 2-years (for participants recruited at the very beginning of the recruitment period). The average post-treatment follow-up will be 1.5 years. At the visits starting 6 months post-treatment, subjects will be given a 2-hour mixed meal glucose tolerance test, and a physical examination will be completed. The following tests will also be conducted at each of these 6-month visits: viral monitoring, immune testing

(CD4/CD25/apoptosis), HbA1c, T cell testing, CBC with differential and chemistries. These tests are to ensure that hematocrit and white blood cell counts are normal and that the liver is functioning normally in subjects after therapy is discontinued.

6 Participant Safety

6.1 Expected Side Effects and Adverse Events

Immune suppression may increase the risk of infection or lymphoproliferative disease. On a practical basis, however, this risk is minimal. The long term safety of mycophenolate in psoriasis patients taking the drug for over 13 years suggests that there may be an increased rate of viral infections such as Herpes simplex, but no evidence of increased frequency of lymphoproliferative disorders or other malignancies have been seen.

Mycophenolate mofetil may cause gastrointestinal toxicity, including nausea, vomiting, diarrhea, gastritis, and anorexia. Mycophenolate mofetil also is known to cause leukopenia.

Although we do not anticipate significant hyperglycemia to develop because the proposed regimen does not include prednisone, cyclosporine or high doses of tacrolimus, signs of hyperglycemia, such as elevated HbA1c with preserved C-peptide, will be monitored and considered an adverse effect of treatment.

6.2 Management of Side Effects and Adverse Events

All adverse events will be documented on the adverse events data collection forms, will be managed by the TrialNet research personnel at the clinical sites, and will be monitored by the TrialNet Medical Monitor and the Data and Safety Monitoring Board (DSMB). If a participant dies at any point of the study, regardless of adherence to the study medication and compliance with the schedule of follow-up assessments, the death will be reported to the DSMB and investigated. The TrialNet Medical Monitor will evaluate all serious adverse events to determine if they need to be reported to the Food and Drug Administration (FDA).

To determine the effects of the drug on general immune responses, the T cell proliferative studies will include responses to conventional antigens, and changes in these responses with treatment will be monitored closely to determine whether recall responses are altered. The TrialNet Medical Monitor will evaluate the results of these studies, on an individual basis, to check for any changes in the general immune response. In the event that changes are apparent, titers of antibodies to previous vaccinations (such as Rubella) will be measured to determine whether the repeated treatments have resulted in a loss of protective immunity. If titers have been diminished, re-immunization or withdrawal from the study treatment will be considered on an individual basis initially. These data will also be regularly reviewed and evaluated by the DSMB.

Diarrhea, nausea, abdominal pain and other GI complications will be treated as follows: infectious causes for diarrhea (enteropathogens) should be ruled out and treated if detected. MMF/placebo dose reduction may take place as outlined below. If diarrhea persists, the MMF/placebo dose should be changed from BID to TID dosing or reduced by 25% initially, if it persists, 50% or if severe, discontinued. If diarrhea continues and infectious causes have been excluded, then agents such as Lomotil or tincture of opium can be used judiciously. When diarrhea persists for longer than 48-72 hours, with or without fever, a CMV PCR test will be run. If a participant is positive, study medication will be discontinued. If diarrhea persists in a CMV positive participant despite discontinuation of study drug, then treatment with gancyclovir should be considered. Persistent diarrhea in a participant who tested negative for CMV may result in the need for consultation with a gastroenterologist to undergo endoscopy to determine the cause of condition.

As indicated in the schedule of evaluations, a CBC with differential will be drawn weekly for the first month of treatment, bi-weekly in the second and third months, monthly for the remainder of the first year of treatment and then every three-months for the remainder of the treatment period in order to monitor participants for leukopenia. If the CBC (WBC count) is < 3500/mm^3, a 25% decrease in MMF/placebo dose will be implemented. If CBC is < 3000/mm^3, a 50% decrease in MMF/placebo dose will be implemented. If the WBC count decreases even lower (< 2500/mm^3), MMF/placebo will be stopped completely. If a study participant develops leukopenia, the MMF/placebo doses will be withheld until leukopenia has been resolved. After a dose interruption, every effort should be made within 14 days after resolution of leukopenia, unless medically warranted, to reinstate mycophenolate mofetil/placebo in stepwise increments of 500 mg until the protocol recommended MMF/placebo dose is achieved. The participant's WBC count will be rechecked by repeating a CBC blood draw every 2 to 3 days until the WBC count is > 3000/mm^3. Then, the participant's WBC count will be checked on a weekly basis. After the WBC count is normalized at > 3000/mm^3, the study medication will be restarted in the stepwise increments listed above while continuing weekly CBC monitoring of WBC count. If this approach is not possible, mycophenolate mofetil/placebo dose should be increased gradually until an optimal tolerable dose is achieved.

The CBC drawn at every study visit will also be utilized to monitor participants for neutropenia. If the absolute neutrophil count (ANC) is < 1500/mm^3, a 25% reduction in MMF/placebo dose will be implemented. If the absolute neutrophil count (ANC) is < 1250/mm^3, a 50% reduction in MMF/placebo dose will be implemented. If the absolute neutrophil count (ANC) is < 1000/mm^3, MMF/placebo will be stopped completely. If a study participant develops neutropenia, the MMF/placebo dose will be withheld until neutropenia has been resolved. After a dose interruption, every effort should be made within 14 days after resolution of neutropenia, unless medically warranted, to reinstate MMF/placebo in stepwise increments of 500 mg until the protocol recommended MMF/placebo dose is achieved. The participant's ANC will be rechecked by repeating a CBC blood draw every 2 to 3 days until the ANC is > 1500/mm^3. Thereafter, the participant's ANC will be checked on a weekly basis. After the ANC is normalized at > 1500/mm^3, the study medication will be restarted in the stepwise increments listed above while continuing weekly CBC monitoring of the ANC. If this approach is not possible, mycophenolate mofetil/placebo should be increased gradually until an optimal tolerable dose is found.

Because DZB/placebo will be administered only two times during the study (Week 0 and Week 2), it is unlikely that the DZB/placebo dose will need to be adjusted because of adverse events.

6.3 EBV Monitoring

To maximize safety, participants will be tested for antibody to EBV at the screening visit. Throughout the study, participants will be monitored by genome and serology. All subjects will be monitored at Week 2 and 4, then monthly for the first year of treatment, followed by every 3 months for the rest of the 2-year treatment period and every six months until study end. All subjects will have blood drawn for EBV PCR (viral load) and serology at all of these visits. Only the PCR sample will be analyzed immediately for all subjects. For EBV seronegative subjects the serology sample will also be analyzed immediately, but for EBV seropositive subjects the sample will only be run if there is clinical indication, the PCR is elevated or for purposes of retrospective analyses.

Seropositive study subjects (at study entry) - If the viral load is ≤ 2000 DNA copies/ml, then the indicated monitoring scheme will be maintained. If the viral load > 2000 DNA copies/ml,

weekly monitoring by EBV PCR will be implemented. In either case, if a 5-fold increase in viral load is detected, study medication will be discontinued permanently. The participant will be followed as indicated until the viral load is normalized. If the participant is mildly symptomatic, the participant will continue to be closely followed. If the participant develops more severe signs or symptoms, appropriate treatment will be considered at the discretion of the local principal investigator or primary care physician. The local principal investigator may consult with the TrialNet Infectious Disease Consultants to determine the best treatment plan. Once the viral load has normalized the study subject will return to the indicated monitoring scheme, but study medication will not be continued.

Seronegative study subjects (at study entry) - If EBV PCR is positive (viral load > 2000 DNA copies/ml) and EBV serology (IgG or IgM) is positive, study medication will be discontinued permanently. The study subject will be monitored on a weekly basis until normalized. If either EBV PCR or EBV serology is positive and the other is negative, a repeat sample will be drawn within one-week. If the participant is mildly symptomatic, the participant will continue to be closely followed. If the participant develops more severe signs or symptoms, appropriate treatment will be considered at the discretion of the local principal investigator or primary care physician. The local principal investigator may consult with the TrialNet Infectious Disease Consultants to determine the best treatment plan.

Participants who develop an infection typical of EBV (characterized by 3 days of fever, sore throat, and lymphadenopathy) will have additional blood drawn for EBV PCR and serology independent of the screening proposed above.

6.4 CMV Monitoring

To maximize safety, participants will be tested for antibody to CMV at the screening visit. All study subjects will be monitored on a monthly basis for the first three months of the study, followed by monitoring every 3-months for the rest of the 2-year treatment period. Subjects will have blood drawn for CMV PCR (viral load) and serology at all of these visits. Only the PCR sample will be analyzed immediately for all subjects. The serology sample will only be run if there is clinical indication, the PCR level is elevated or for purposes of retrospective analyses.

Seropositive study subjects (at study entry) - If the viral load increases 5-fold or is > 10,000 DNA copies/ml, study medication will be discontinued. An eye exam will be given to rule out CMV retinitis. An examination will be performed to rule out pneumonia and gastrointestinal involvement as indicated by history and physical examination. Those with an active infection will be treated with gancyclovir. The study subject will be monitored by CMV PCR until the viral load has normalized. Once active infection has resolved, continuation of study medication will be decided on a case-by-case basis.

Seronegative study subjects (at study entry) - If the viral load becomes positive (> 2000 DNA copies/ml), study medication will be discontinued. Active infections will be treated as above. Once resolved, the decision to continue study medication will be made on a case-by case basis.

If a study subject develops a **symptomatic** infection of the eye, lungs or intestines, study medication will be discontinued and blood samples will be drawn for laboratory analyses. Study medication will be withheld until the infection has been determined not to be associated with CMV, or if the infection is related to CMV, until the viral load is less than 2000 DNA copies/ml or

stable. Continuing study medication will only occur when more than 1 month of stable data is obtained. The dose of study medication will then be titrated up on a weekly basis. Continued weekly screening of CMV PCR will be performed until the viral load has normalized.

6.5 Papillomavirus Monitoring

Female participants who are sexually active are at an increased risk for becoming infected with Human Papillomavirus. By also being on immunosuppressive therapies, such as MMF and DZB, the infection may be more likely to progress to cervical cancer. Female study subjects who are sexually active are recommended to have annual cervical assessments to detect cancerous and pre-cancerous conditions as part of normal clinical care. These records will be requested from the participant's primary care physician. If the study subject is found to have an abnormal cervical assessment, study medication may be withheld until an evaluation of the abnormal result has been undertaken.

6.6 Varicella Virus (Chickenpox)

To maximize safety, study subjects will be tested for previous exposure to varicella virus at the screening visit. If the study subject does not have antibodies to this virus, the varicella vaccine will be recommended. Participants who do not have antibodies and are exposed to patients with either chickenpox or shingles, will be offered VZIG IV within 96 hours of exposure to prevent infection, or will be offered treatment with Acyclovir beginning one week following exposure.

6.7 Herpes Simplex Virus

All subjects will be monitored closely for signs and symptoms of infection or re-activation of herpes simplex virus (HSV). If a subject is already on prophylaxis at the time of study entry, the subject will be encouraged to continue it during the entire study. At the first episode of documented herpetic infection, the subject will be treated with antiviral agents to resolve the episode after which prophylaxis will be considered, but not required. Study medication will be maintained during this time. If there is a second episode of a documented herpetic infection the subject will be treated with an antiviral agent to resolve the episode. The subject will remain on study medication. Prophylaxis will be provided, with adjustment as needed, for the remainder of the study and for two additional weeks after its completion. If a third episode should occur, study medication will be permanently withdrawn and treatment will again be provided. After the study medication is withdrawn, prophylaxis will be continued and adjusted as needed for an additional two weeks.

6.8 Immunosuppression and Pregnancy

There is little known about the relationship between immunosuppression and pregnancy. In kidney transplantation, fertility generally returns after transplantation with a pregnancy success rate exceeding 90 percent after the first trimester. There is, however, a slight increase in spontaneous abortion, and intrauterine growth retardation and/or premature delivery can occur in one-half to two-thirds of cases. Women are usually advised to wait at least one year after living-related-donor transplantation and 2 years after cadaver transplantation to avoid complications arising from rejection. The rationale for the delay is that with kidney transplantation, the dose of steroids is relatively low, and the Azathioprine dose is stable. Neither of these drugs appears to have an adverse effect on the fetus. Although not recommended, women who become pregnant in the first 6-12 months post-transplantation are likely to have a successful pregnancy.

Mycophenolate mofetil is a Category D drug that causes fetal resorptions and malformations in rats and rabbits. Mycophenolate mofetil has been associated with an increased risk of first trimester pregnancy loss and an increased risk of congenital malformations, specifically external ear, other facial abnormalities (including cleft lip and palate), and anomalies of the distal limbs, heart esophagus, and kidney. Female participants with reproductive potential must have a negative pregnancy test prior to entry. A communication from Roche Laboratories, Inc. indicates that mycophenolate mofetil reduces blood levels of the hormones in oral contraceptive agents and could theoretically reduce its effectiveness [49]. It is required that all female participants use one form of birth control, and it is recommended that two forms be used. Female patients should begin using their chosen contraceptive method 4 weeks prior to starting mycophenolate mofetil therapy and should continue contraceptive use during therapy and for 6 weeks after stopping therapy if planning for a pregnancy. Male participants, if sexually active, will also be required to use one form of effective birth control, unless abstinence is the chosen method. TrialNet research personnel will counsel participants about the increased risks to the fetus of spontaneous abortion, malformations, and intrauterine growth retardation. If pregnancy occurs, the study medication will be stopped immediately, and the participants will continue with the scheduled follow-up visits. If a pregnancy occurs in the partner of a male participant, the participant will be strongly encouraged to inform his partner of the potential risks to the fetus.

Daclizumab is considered a Category C drug. Since this drug will only be used at the initiation of treatment, its effects on a pregnancy 2 years later should be negligible.

6.9 MMF/Placebo Adjustment for Adverse Events

Listed below are suggested guidelines for dose reduction and interruptions of MMF/placebo for adverse events.

Recommended Dose Reduction Guidelines for Adolescents and Adults				
CellCept (MMF)/Placebo*	Daily dose reduction (step 1)	Daily dose reduction (step 2)	Daily dose reduction (step 3)	Daily dose interruption
	25% reduction	50% reduction	0 tablets	0 tablets

*TID dosing should be attempted prior to reducing the dose in the case of an adverse event that might be related to mycophenolate mofetil/placebo.

The Investigator should attempt at his/her discretion to return the study subject to the standard maintenance dose upon resolution of the adverse event. Participants will continue to be followed for the remainder of the study even if they discontinue therapy.

6.10 Rules and Procedures for Unmasking Randomized Participants

In most cases the management of minor symptoms will be the same regardless of a participant's treatment group assignment, and TrialNet research personnel generally will not need to be unmasked. If a situation arises in which it is essential for TrialNet research personnel to be unmasked to treat a study subject, the MMF/DZB Principal Investigator at the clinical site will contact the Coordinating Center within 24 hours of the event. The TrialNet Central Pharmacy will maintain a list of each participant’s group assignment, and it will be able to unmask a participant’s treatment group assignment. In the case of a serious adverse event or any adverse event for which the study medication is discontinued permanently, TrialNet

research personnel will not be unmasked unless it is essential to know the study group in order to treat the participant. In addition, if pregnancy occurs, the diabetologist and the participant will be unmasked.

If TrialNet research personnel are unmasked about a given study subject's treatment group assignment, the subject will still be encouraged to continue with the schedule of follow-up assessments.

In general, except for the case of a pregnancy, the study subject should not be unmasked until the end of the study.

7 Adverse Event Reporting and Safety Monitoring

7.1 Adverse Event Definitions

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 specifically associated with the treatment and study procedures.

Throughout the study, the investigator must record adverse events on the appropriate adverse event form, regardless of the severity. 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.

7.1.2 Serious Adverse Event

For this trial, an adverse event associated with the treatment or study procedures 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.

7.1.3 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 required intervention.

7.1.4 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.

7.2 Adverse Event Reporting and Monitoring

Adverse events will be reported to the TrialNet Coordinating Center. 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). 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 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 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 study discontinuation will be reviewed by the DSMB.

7.3 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.

8 Statistical Considerations

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.

The randomization error has affected the number of treatment groups in the trial and the number of subjects that could be entered into a concurrently randomized analysis comparing the treatment groups. Prior to discovery of the error, 126 subjects had been randomly assigned to one of the three original treatment groups in 13 clinical sites. In seven of these, the randomization was flawed with the assignment of 12 subjects to the DZB only group among 35 entered. Technically, this leaves 91 subjects who were properly concurrently randomized to the original three groups that could be employed in analyses in comparison to the MMF only treatment group, approximately 30 per group. In addition, among all 13 study centers, and the 126 subjects entered, a total of 83 were correctly allocated to either the MMF+DZB group or to placebo, approximately 40 per group.

8.1 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.

Owing to the randomization error, the primary analysis was modified to remove the comparison of the two active therapies versus each other, as this comparison would have low power, particularly if both of the treatments were effective.

The primary statistical hypothesis to be assessed in the study is whether either of the MMF-only treatment, or the combination MMF+DZB treatment, retards the rate of decline in beta cell function. This implies the hypotheses

- The mean C-peptide value for study subjects on mycophenolate mofetil (MMF) differs significantly from the mean value for placebo subjects.
- The mean C-peptide value for study subjects on MMF+ daclizumab (DZB) differs significantly from the mean value for placebo subjects.

The primary analyses will employ the weighted mean derived from the 2 hour AUC for each participant transformed as *log(mean C-peptide+1)*. The comparison between any 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(C-peptide+1)* [50]. The adequacy of the model will be evaluated using the Shapiro-Wilk [51] test for normality of the residuals and the White [52] test for homoscedasticity.

If either test of model adequacy fails at the 0.05 level, the primary outcome will be tested using a Wilcoxon rank sum test of the residuals from the ANCOVA model above with the treatment

term dropped. The residuals can also be added to the overall mean *log(mean C-peptide+1)* to yield covariate adjusted C-peptide values for each patient. The distribution of these adjusted values will also be summarized using percentiles, such as the quartiles of the distribution, in each group.

The Holm closed-sequential procedure for multiple tests [53] will be used to control the type I error probability to not exceed 0.05, one-sided, for the set of two primary pairwise group comparisons MMF versus placebo, and MMF/DZB versus placebo.

8.2 Secondary Outcomes and Analyses

The comparison of the MMF only therapy versus the MMF+DZB combination therapy will be a secondary comparison with no adjustment for multiple tests. All analyses specified herein for the comparison of the two active therapies versus placebo will also be conducted comparing the two active therapies.

Additional analyses will include the comparisons among groups using:

- 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 semiannual MMTT [54], and
- longitudinal analyses 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 [55] adjusting for age, gender and the baseline *log(C-peptide+1)*.

Additional secondary objectives are to examine how MMF alone or in conjunction with DZB affects the following:

- HbA1c
- Total number of hypoglycemic events
- Number of major cases of hypoglycemic events

Hypoglycemia is defined as any blood glucose level < 50 mg/dl or hypoglycemic symptoms. Major hypoglycemia is defined as loss of consciousness, seizure, or requiring assistance from another person. Minor hypoglycemia is defined as the presence of hypoglycemic symptoms but without requiring assistance from another person.

The mean HbA1c over all follow-up values will be compared between groups using a normal errors longitudinal analysis [55]. The rate of hypoglycemic 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 [54]. Both sets of analyses will be adjusted for age, gender, baseline *log(C-peptide+1)* and baseline HbA1c.

For each of these analyses the Holm procedure will be used to control the type I error probability at the 0.05 level for the set of two primary pair-wise group comparisons of MMF versus placebo, and MMF/DZB versus placebo.

8.3 Additional Analyses

Additional outcomes of interest include

- Insulin dose (units/kg)
- Change in autoantibody levels
- T cell reactivity
- T cell frequency
- T cell reactivity to recall antigens
- Frequency of activated T cells
- Number of infections
- Number of allergies

The effects of MMF alone and MMF in conjunction with DZB on T-cell reactivity to autoantigens will be assessed from blood draws every 3 months during the course of the treatment period. The specimens will allow an assessment of whether reactivity can be found for autoantigen-specific peptides. Proliferation, cytokine production, and other methodologies will measure T cell responses. T cell lines and clones will be made from antigen-reactive individuals who may be asked to return for repeat blood sampling.

The study will examine immunologic effects of MMF alone and MMF in conjunction with DZB (defined by CD4/CD25/apoptosis).

The analyses of each quantitative outcome will be conducted using a normal errors longitudinal regression model and of each event using a Poisson regression model, with the Holm adjustment for multiple comparisons.

8.4 Sample Size and Power

The primary analysis will compare the difference between each treated group separately versus the placebo group (two pair-wise comparisons) in the levels of the 2 hour AUC-mean using the *log(mean C-peptide+1)* in an ANCOVA model adjusting for gender, baseline age, and baseline *log(C-peptide+1)*. Estimates of *log(mean C-peptide+1)* and root mean square error (RMSE) in the placebo group were obtained from prior studies (Palmer et al. 2004).

Among subjects that met the eligibility constraints of baseline C-peptide > 0.2 pmol/ml and age $\geq$ 12 years, the mean log(C-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. Power was then computed using standard equations for the comparison of two means. The original sample size of 120 subjects total (40 per group) provides 85% power to detect a 65% difference in the geometric-like mean C-peptide for any one of the three possible pairwise comparisons among the three treatment groups using a test at the 0.025 level (one-sided, adjusted for 3 comparisons), with 10% loss to follow-up.

Thus, the primary analysis will only compare each active therapy versus placebo. The comparison of the MMF/DZB group versus placebo will employ all subjects entered into those two groups, with a sample size of about 40 subjects per group. This will provide power of 85% to detect a 61% increase in the geometric-like mean C-peptide with the combination treatment group relative to the placebo group using a test at the 0.025 level (one-sided, adjusted for 2 comparisons), with 10% loss to follow-up.

Owing to the randomization error, the comparison of the MMF only versus placebo group will only include those subjects concurrently randomized to these groups among the 6 sites in which the randomization was implemented correctly, about 30 per group. This will provide 80% power to detect a 67% increase, or 85% power to detect a 72% increase, in the geometric-like mean

C-peptide with the MMF only treatment group relative to the placebo group using a test at the 0.025 level (one-sided, adjusted for 2 comparisons), with 10% loss to follow-up.

The secondary comparison of MMF only versus the combination will also be restricted to the subjects concurrently randomized with about 30 subjects per group. This comparison will provide 80% power to detect a 59% increase, or 85% power to detect a 64% increase, in the geometric-like mean C-peptide with the MMF/DZB combination treatment group relative to the MMF only group using a test at the 0.05 level (one-sided), with 10% loss to follow-up.

Finally, secondary analyses will also compare the DZB only treatment group versus the concurrently randomized placebo group, and also the MMF/DZB group, using those subjects concurrently randomized into the 7 sites with the flawed randomization, with about 12 subjects per group. This sample size will provide 80% power to detect a 96% difference between groups.

8.5 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 [56] for the primary outcome analyses, 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 initial study design and under the current trend of the data [57].

9 Study Administration

9.1 Organizational Structure

This study is part of TrialNet, which is funded by the National Institutes of Health through R01 grant awards. Funding will cover the costs of administration and laboratory tests associated with this study during the participant's period of follow-up. Roche Laboratories will provide mycophenolate mofetil free of charge for the participant's entire length of treatment. Study subjects will be compensated for each study visit attended. In addition to this, minor travel expenses and parking will be reimbursed.

9.2 Groups and Committees

9.2.1 Clinical Sites

Each Principal Investigator at the participating TrialNet clinical site will oversee all operations and will be the study participant's primary diabetologist. The clinical sites will forward all laboratory and data collection form information to The George Washington University Coordinating Center for analysis. Quarterly conference calls and site visits, as needed, will facilitate evaluation of the trial management.

9.2.2 Clinical Site Monitoring

In order to conduct this study 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 of adverse events (AE) and serious adverse events (SAEs).

9.2.3 Data and Safety Monitoring Board (DSMB)

The DSMB will meet approximately every 6 months to review efficacy issues and adverse events prepared by the Coordinating Center. All adverse events will be recorded on the adverse event forms, which will be sent 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. Events such as increased rates of neutropenia, infection, hospitalization, or other potentially untoward effects of mycophenolate mofetil and daclizumab will be assessed.

9.3 Study Subject Confidentiality

Confidential binders with the study subject's information for internal use at the clinical sites will be kept in locked file cabinets in the locked Study Coordinator's office at each study site. At the end of the study, all records will be kept in a locked storeroom at each study site. There are no plans to destroy the records.

Study subject data from all the data collection forms, which is for reporting purposes, will be stored at The George Washington University Coordinating Center. Case report forms sent to the Coordinating Center will identify participants by the unique TrialNet Identification Number, study randomization number, and the first 3 letters of their first name. No other identifiers will be used. 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 stored at a secured storeroom. No data will be destroyed.

HLA testing for type 1 diabetes is for research purposes only and will give a relative indication of the likelihood of the diagnosis of type 1 diabetes. The HLA typing will not be made available to the participant and his or her physician. It is unlikely that this information will be beneficial to the participant's health. Only the study subject with diabetes will be assessed and since no other family members will be assessed for this study, there should be little direct possibility of discovery of non-parentage.

DNA will be stored for future use with the permission of the study subject, so that as new markers of type 1 diabetes are developed, subjects can be tested for them. This information will be important because type 1 diabetes may be a family of disorders, and different genes within different families or populations may contribute to disease development. The results of these future analyses will not be made known to the participant as it is extremely unlikely that this information will be beneficial to the patient's health.

9.3.1 Sample and Data Storage

Blood samples to be stored for research purposes will be located at the NIDDK Repository 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. The 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.

As long as TrialNet exists, participants who provide samples will have the option of having their stored samples destroyed and still be allowed to participate. However, once TrialNet is completed and data are de-identified, it will no longer be possible to destroy samples upon request.

Data collected for this study will be sent to the Coordinating Center at The George Washington University. After the study is completed, de-identified data will be stored at the NIDDK Repository Site, under the supervision of the NIDDK/NIH, for use by researchers outside of TrialNet.

When TrialNet is completed, samples will continue to be stored at the NIDDK Repository Sites. 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.

9.3.2 Risks and Benefits

The risks of this study are presented in the informed consent form and are described in Sections 2.4 and 6.1. There is no guaranteed benefit to subjects for their participation in the

study. It is hoped that the immunosuppressive intervention will preserve beta cell function, but there is no guarantee that this will occur.

9.4 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 center alone. All presentations and publications using TrialNet trial 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 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 should be approved by the Steering Committee before release.

10 Study Timeline

The study began enrolling subjects in July 2004. Enrollment will last for approximately two years. The study is projected to last for approximately four to five years.

Appendix 1 – Study Schedule

Week of Trial:	-1	0	1	2	3	4	6	10	Month	2	3	4	5	6	7	8	9	10	11	12	15	18	21	24	27	30	36	*42*	*48*
History		X																											
Physical exam		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	X	X	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	X	X	X	X
HLA determination		X																											
Serum for autoantibodies	X					X					X			X			X			X		X		X					
Daclizumab Administration		X		X																									
Mycophenolate mofetil dispensation		X									X			X			X			X	X	X	X						
Mycophenolate levels				X							X			X						X		X		X					
MMF Pharmacokinetic (PK) analysis						X																							
Daclizumab levels				X		X																							
Adverse Events Assessments			X	X	X	X				X	X			X			X			X	X	X	X	X	X	X	X	X	X
Hemoglobin A1c	X										X			X			X			X	X	X	X	X		X	X	X	X
Basal C-peptide		X																											
Mixed Meal Tolerance Test (4-hour)[1]	X[1]																							X					
Mixed Meal Tolerance Test (2-hour)											X			X						X		X				X	X	X	X
Glycemic excursion																								X[3]					
PPD Test (follow-up 48-72 hours later)	X																												
Insulin Dosing Assessment		X	X	X	X	X				X	X			X			X			X	X	X	X	X					
Hypoglycemia assessment		X	X	X	X	X				X	X			X			X			X	X	X	X	X					
Urine pregnancy test (if female)	X	X		X		X				X	X			X			X			X	X	X	X	X					
HIV, Hep B and C, Varicella screening	X																												
ECG Acquisition	X																			X				X					
Immune testing (CD4/CD25/apoptosis)		X	X	X		X				X	X			X						X				X			X		X
Rubella titers		X				X								X						X		X		X					
Viral flu titers		X				X								X						X		X		X					
T-cells (ELISPOT)		X									X			X						X		X		X					
RNA (stored)		X			X						X			X						X		X		X		X	X		X
T-cells (stored)[2]		X									X			X						X		X		X		X	X		X
Serum (stored)	X					X					X			X			X			X		X		X					
DNA (stored)		X																											

***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.**

X[1]: test completed on another day separate from other Week –1 screening samples.

T-cells (stored) [2]: plasma left over following extraction of T-cells will also be sent to the NIDDK Repository for storage.

Glycemic excursion[3]: two day record of 8 point glucose profile and/or five day record from continous glucose monitor. Note, for subjects who have passed their 24 month period prior to institution of this protocol change, the window for obtaining this data is 12 months.

Month 42 and 48: Post-treatment follow-up visits will only extend to this point for participants enrolled into the study in the first 6-months of recruitment

Appendix 2 – EBV and CMV Monitoring Schedule

Week of Trial:	-1	0	1	2	3	4	6	10	Month	2	3	4	5	6	7	8	9	10	11	12	15	18	21	24	27	30	36	*42*	*48*
Epstein-Barr Virus (EBV)																													
EBV PCR	X			X		X				X	X	X	X	X	X	X	X	X	X	X	X	X	X	X		X	X	X	X
EBV Serology[1]	X			X[1]		X[1]				X[1]	X[1]	X[1]	X[1]	X[1]	X[1]	X[1]	X[1]	X[1]	X[1]	X[1]	X[1]	X[1]	X[1]	X[1]		X[1]	X[1]	X[1]	X[1]
[1] Samples for EBV serology will be drawn at all of the same timepoints as EBV PCR, but will only be run immediately in EBV seronegative subjects. All other samples will only be run if there is clinical indication, the PCR is elevated or for purposes of retrospective analyses.																													
Cytomegalovirus (CMV)																													
CMV PCR	X					X				X	X			X			X			X	X	X	X	X					
CMV Serology[2]	X					X[2]				X[2]	X[2]			X[2]			X[2]			X[2]	X[2]	X[2]	X[2]	X[2]					
[2] Samples will be drawn at the same time as CMV PCR, but will only be run if there is clinical indication, the PCR (viral load) is increased, or for purposes of retrospective analyses																													

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