EFFECTS OF RITUXIMAB ON THE PROGRESSION OF TYPE 1 DIABETES IN NEW ONSET SUBJECTS

(Protocol TN-05)

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

PREFACE

The TrialNet Type 1 Diabetes Protocol TN-05, *Effects of Rituximab on the Progression of Type 1 Diabetes in New Onset Subjects*, describes the background, design, and organization of the study. The protocol will be maintained by the TrialNet Coordinating Center at the University of South Florida 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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1 INTRODUCTION

1.1 Study Overview

Title	EFFECTS OF RITUXIMAB ON THE PROGRESSION OF TYPE 1 DIABETES IN NEW ONSET SUBJECTS
IND Sponsor	TrialNet
Conducted By	TrialNet
Protocol Chair	Mark Pescovitz, MD
Accrual Objective	66 participants over two years
Study Design	The study is a two-arm, multicenter, randomized, double-masked, placebo-controlled clinical trial. Both groups will receive standard intensive diabetes treatment with insulin and dietary management.
Treatment Description	Rituximab (RITUXAN^R, Genentech and Biogen Idec) is a chimeric murine/human monoclonal antibody approved for the treatment of B cell non-Hodgkin's lymphoma. The antibody binds to the CD20 antigen on the surface of B cells and mediates B cell depletion. Participants randomly assigned to rituximab treatment will receive four doses of 375mg/m^2 IV each a week apart.
Study Duration	Total duration is approximately 4 years (2 years accrual and 2 years follow-up). Follow-up for up to 4 years will continue for those who have persistence of beta cell function at 2 years and/or detectable immunologic effects of treatment by descriptive analysis until the disappearance of detectable beta cell function or resolution of immunologic changes.
Objective	To assess the safety, efficacy, and mode of action of rituximab, anti-CD20 monoclonal antibody, for the treatment of individuals with new onset type 1 diabetes.
Primary Outcome	The primary statistical hypothesis to be assessed in this study is whether the mean C-peptide value for study subjects on rituximab differs significantly from the mean value for placebo subjects assessed at one year of follow-up.
Secondary Goals	The study will examine the effect of the proposed treatment on surrogate markers for immunologic effects, namely disease-specific outcomes and immunological outcomes.
Major Inclusion Criteria	Type 1 diabetes within past 3 months Age 8-45 years* At least one diabetes associated autoantibody

*Enrollment will be limited to subjects 12-45 years until DSMB review of the first 10 subjects treated with rituximab.

1.2 Statement of Purpose

This protocol describes the background, design, and organization of the *Effects of Rituximab on the Progression of Type 1 Diabetes in New Onset Subjects*. The protocol was written by Dr. Mark Pescovitz, Chair of the TrialNet Anti-CD20 Protocol Committee, the TrialNet Chairman's Office, and the TrialNet Coordinating Center at the University of South Florida . Significant 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) or the equivalent at international sites.

2 BACKGROUND AND SIGNIFICANCE

Type 1 diabetes mellitus is an immune-mediated disease in which insulin-producing beta cells are completely destroyed resulting in life-long dependence on exogenous insulin. While this beta cell destruction process begins before clinical onset and continues after development of hyperglycemia and diagnosis, at the time of diagnosis subjects retain a significant amount of beta cell function as measured by C-peptide responses to a mixed meal tolerance test. However, this beta cell function deteriorates after diagnosis with the presumed eventuality of absent function over time. As shown in the Diabetes Control and Complications Trial, the persistence of residual beta cell function has been associated with important clinical outcomes, specifically reduction in severe hypoglycemia and complications. Thus, an intervention that can enable continued endogenous insulin production would significantly improve the day-to-day management for subjects with diabetes and therefore reduce long-term complications.

2.1 Immune Dysfunction in Type I Diabetes

The pathophysiology of type I diabetes most likely requires the presentation of beta cell antigen(s) to T cells within lymph nodes (*1*). The antigen reactive T cells then migrate to the pancreas where autoimmune destruction of the beta cells occurs (*2*). Given the clear demonstration of the immunologic nature of type I diabetes, it is not surprising that many trials to prevent or treat the disease using immunosuppressive drugs have been attempted using agents such as prednisone, cyclosporine, azathioprine, anti-thymocyte globulin, plasmapheresis, ketotifen and indomethacin, mostly with inconclusive or negative results (*3-6*). In a murine system, anti-CD3 therapy was effective in inducing durable (>4 months) remissions in 60 to 80% of animals (*7*). The broad application of this therapy, however, is limited by the immunosuppressive toxicity of this nonspecific drug including severe first dose reactions (*8-10*), development of neutralizing human anti-murine antibodies that precludes long term dosing (*11*), viral infections and lymphomas (*12-14*). Newer-generation specifically modified antibodies such as humanized anti-CD3 may obviate some of the problems of neutralizing antibody formation and first dose reaction increasing the safety profile. Early clinical studies with humanized anti-CD3 have suggested at least a short-term benefit and repeat dosing studies are currently being planned *(82, 83, 86)*.

There is growing evidence that many T cell-mediated diseases including type 1 diabetes, multiple sclerosis and rheumatoid arthritis, may include a B cell/autoantibody component. A recent report suggests that B cells play a crucial role as antigen presenting cells (*15*). Activated autoreactive B cells can generate cryptic peptides that have not been previously presented to T cells to which the T cells are not tolerant (reviewed in (*16*)). By binding to and endocytosing multiple proteins, as might occur *in situ* in the pancreas, additional epitopes can be presented (epitope spreading). B cells are very efficient antigen presenting cells (*17*), expressing high levels of class II MHC antigens and stimulate response to tumor antigens (*18*), diabetes (*19*) and rheumatoid arthritis (*20, 21*). In a B cell deficient murine carotid artery allograft model of graft arteriosclerosis, smooth muscle proliferation was decreased compared to wild type mice suggesting that manipulation of B cell immunity may be important in preventing graft arteriosclerosis in clinical transplantation (*22*). Skin grafts performed across H-Y antigen rejected slower in B cell deficient mice than wild type suggesting that T cell responses might depend on B cell function (*23*).

In NOD (non-obese diabetic) mice depleted of B cells by deletion of Ig genes or by infusion of anti-mu-chain antibody, diabetes is inhibited (*15, 24, 25*). Late loss of B cells during the active phase in NOD abrogated islet cell destruction (personal communication, J. Bluestone). A case of type 1 diabetes in a child with X-linked agammaglobulinemia has been put forward to

demonstrate that B cells are not critical for human type 1 diabetes (*26*). While experiments of nature, such as this patient expand our knowledge of disease mechanisms, in a subject without B cells for their entire life, other cells maybe able to take over the role of B cells as antigen presenting cells. Such a role for other cells may not be dominant in a normal immune system where B cells were present from the beginning. As noted in the editorial to this case report by H. McDevitt, the fact that diabetes appeared somewhat late in this patient, suggests that B cells facilitate the development of the disease (*27*). He notes that this is similar to the situation in NOD mice without B cells. In such animals the incidence of diabetes decreases (but does not disappear) from 80% to 30% in females and disease develops later in the animal's life.

2.2 Rituximab, Chimeric Anti-Human CD20 Antibody

The ability to selectively deplete a sub-population of B cells in humans is now possible with the anti-CD20 drug rituximab. Rituximab (Rituxan^R, Genentech and Biogen Idec), a molecularly engineered, chimeric murine/human monoclonal antibody, was approved for the treatment of B cell non-Hodgkin's lymphoma. It binds to the CD20 antigen on the surface of B cells and mediates B cell depletion (*28*). The CD20 (human B-lymphocyte-restricted differentiation antigen, Bp35), is a hydrophobic transmembrane protein with a molecular weight of approximately 35 kD expressed on pre-B and mature B lymphocytes but is not found on hematopoietic stem cells, pro-B cells, normal plasma cells or other normal tissues (*29*). Although the CD20 expression is decreased or absent on plasma cells (*30-32*), reducing their susceptibility to anti-CD20-mediated depletion and it is debated as to whether plasma cells are long or short lived (*33*), neither of these is relevant to the study proposed here, where B cells are targeted not as a source of antibody but as antigen presenting cells.

There are several proposed mechanisms for rituximab-mediated B cell depletion including complement-dependent cytotoxicity (CDC) (*34*), antibody-dependent cellular cytotoxicity (ADCC) (*28*) and apoptosis (*35*). Polymorphisms of Fc receptors gamma RIIIa (CD16) and Fc gamma RIIa (CD32) have been shown to be associated with anti-tumor efficacy in follicular lymphoma patients suggesting that ADCC play an important role (*36*). However, in a study with B cell chronic lymphocytic leukemia patients treated with rituximab, Fc gamma RIIIa and Fc gamma RIIa polymorphisms were not predictive of response to the treatment suggesting that ADCC is less important than other mechanisms (*87*). FcR genotype will be studied as part of the pharmacogenomics objectives of the Protocol (*36*).

2.3 Rituximab for Lymphoma

Administration of rituximab at a dose of 375 mg/m^2 as an intravenous (IV) infusion for four doses to lymphoma patients resulted in a rapid and sustained depletion of circulating and tissue based B cells. Lymph node biopsies performed 14 days after therapy showed a decrease in the percentage of B cells in seven of eight patients who had received single doses of rituximab ≥ 100 mg/m^2. Among the 166 patients with lymphoma in the pivotal study (*37*), circulating B cells (measured as CD19+ cells) were depleted within the first three doses with sustained depletion for up to 6 to 9 months post-treatment in 83% of patients. B cell recovery began at approximately six months following completion of treatment. Median B cell levels returned to normal by twelve months following completion of treatment. There were sustained and statistically significant reductions in both IgM and IgG serum levels observed from 5 through 11 months following rituximab administration. However, only 14% of patients had reductions in IgG and/or IgM serum levels resulting in values below the normal range.

2.4 Rituximab for Human Autoimmune Diseases

While rituximab is only labeled for treatment of non-Hodgkin's lymphoma, it has been used successfully and with minimal toxicity in many different antibody-mediated or antibody-associated diseases such as chronic refractory idiopathic thrombocytopenia (ITP) (*39*), myasthenia gravis (*40*), rheumatoid arthritis (*41, 42*) and chronic cold agglutinin disease (*43*). Recent data suggest that even classically considered antibody-mediated diseases, such as ITP, might be T cell mediated, where the action of rituximab would be elimination of antigen presentation by B cells (*44*). The typical dose used in these studies was 4 weekly doses of 375/m^2, however, in a recently reported phase 3 trial in rheumatoid arthritis, the dose was 1000 mg every other week for 2 doses *(81)*. Typically, there were few reported clinical problems, including infections, in these patients.

For this proposal, the two most relevant diseases that have been successfully treated with rituximab are rheumatoid arthritis (RA) and ANCA-positive vasculitis. Both are T cell mediated diseases with established autoreactive T cell repertoires analogous to the situation with type 1 diabetes with an identified associated circulating antibody. Both cellular (T cell) and humoral (B cell) immune responses are thought to play key roles in the immunopathogenesis of RA (*20, 21*). B cells probably play multiple roles in RA immunopathogenesis including direct T-B cell interaction driven by B cell antigen presentation leading to T cell activation and self-perpetuation of the B cells themselves (*45, 46*). A pilot trial of rituximab use (4 weekly doses of 375 mg/m^2) to treat 22 rheumatoid arthritis patients showed good efficacy with clinical improvement in 50% patients. Rituximab was well tolerated in this group (*41, 42*). In a larger phase II study with 40 subjects in each of three arms given two 1000 mg doses of rituximab, the response (ACR50 at week 24) in the rituximab arms (33%) was higher than in the control (methotrexate alone, 13%) arm (p=0.059) *(81)*. Of great interest, the response was maintained beyond 26 weeks, after the time that circulating B cells had recovered. Rituximab treatment was well tolerated with an adverse event rate less than when it was used for treatment of malignancy. The major adverse event noted in the rituximab treated groups was mild hypotension (30% vs. 18% in the controls). This was graded as mild with only one subject requiring fluid therapy and the incidence was reduced by half during the second treatment. Other common symptoms were cough, rash, and pruritus. There were six serious infections through 48 weeks of treatment in those receiving rituximab, including fatal bronchopneumonia in a subject with a preexisting cardiac condition. Nonetheless, there was no overall increase in infections as compared to methotrexate control group. Additionally, there was no effect on antitetanus antibody titers *(81)*.

The use of rituximab for ANCA was first reported as a single case (*47*). The patient was given 4 infusions of 375 mg/m^2 of rituximab and high-dose glucocorticoids. Complete remission was associated with the disappearance of B cells and cANCA. Glucocorticoid treatment was then discontinued. After 11 months, the cANCA recurred, and rituximab therapy was repeated, without glucocorticoids. At 8 months after the second course of rituximab (18 months after the first course), the patient has remained in complete remission. Based on these results, 11 additional patients have been treated and 3 of them appear to have become "tolerant". B cells have returned, without reappearance of disease or ANCA *(74)*.

2.5 Rituximab Treatment in Allosensitized Dialysis Patients

The use of rituximab has been studied in patients with renal failure to evaluate the effect of rituximab on preformed alloantibody *(75-78)*. The primary study is a single dose, dose escalation study of rituximab in 9 (age 44±10; 5 females, 4 males) adult HLA sensitized (PRA > 50%) dialysis patients awaiting transplant. The subjects were given a single intravenous dose of 50, 150 or 375 mg/m^2 of rituximab. Subjects were followed for safety as the primary endpoint.

Subjects were premedicated with acetaminophen and diphenhydramine; but no other immunosuppression was administered. All 9 patients tolerated the rituximab infusion. There were four adverse events. Two patients, who had a previous history of such infections, developed subsequent infections of their peritoneal dialysis catheter sites. One patient developed presumed histoplasmosis at 18 weeks with resolution of symptoms (chest pain, and shortness of breath) after an empiric course of itraconazole. One patient developed fever during rituximab infusion that resolved after administration of additional oral acetaminophen. No significant change was seen in WBC, hemoglobin, platelet count, chemistry, liver enzymes, or pneumococcal or CMV IgG titers. Two of 9 subjects developed anti-human chimeric antibodies (HACA). Positive antibodies were detected at only 6 months post treatment (5.06 ng/ml) in one patient and at 3 months post treatment (12.4 ng/ml), and at 6 months (12.9 ng/ml) but had converted to negative at 12 months in another patient. Neither of these HACA showed cross reactivity to another chimeric antibody, basiliximab, indicating idiotypic specificity of the HACA *(75, 76)*.

2.5.1 Peripheral B cell Depletion and Recovery

As early as two days post therapy, there was nearly complete depletion of CD19+ (pre: 181±137 vs. 12±6, p=0.006) and CD20+ cells (pre: 205±116 vs. 11±12, p=0.001) (Figure 1) *(77)*. There was a drop in CD3+ cells at day 2 (pre: 1075±510 vs. 847±439, p=0.002) but no difference at six months. At 6 months, B cells were still reduced compared to baseline for CD19+ and CD20+cells respectively (65±43 p=0.004; 78±41 p<0.001). The B cells that repopulated the subjects were selectively depleted of CD19+/CD27+ memory B cells (Figure 2). In the first 3 months after rituximab treatment the percentage of CD19 cells that were CD27+ was constant, indicating that both CD27+ and CD27- B cells were equally depleted by rituximab. After 3 months, however, the percent of CD27+ B cells decreased, indicating that repopulation was primarily with a naïve population of CD27- B cells. This percentage is roughly that seen in a 10-year-old child (*49*).

Figure 1

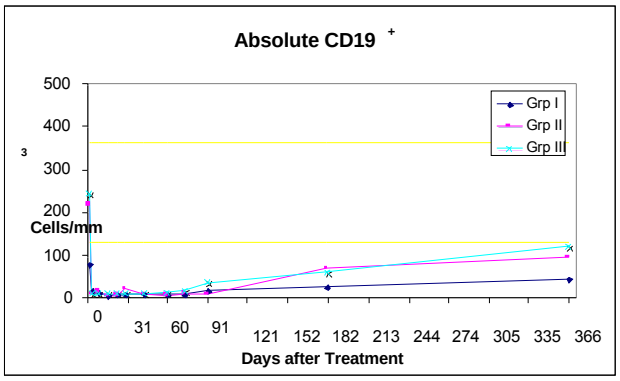
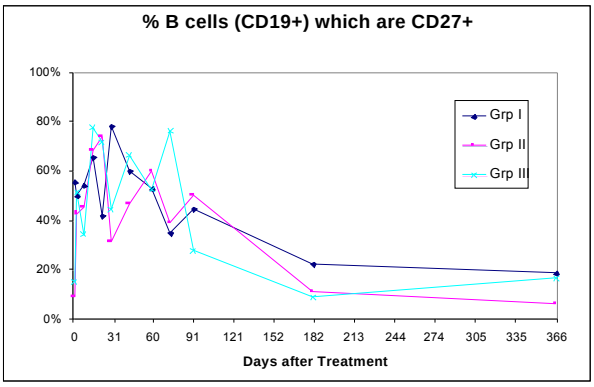


Figure 2



Dosing: Group I - 50 mg/m^2, Group II - 150 mg/m^2, Group III - 375 mg/m^2

A similar pattern of recovery of CD20 cells has been reported in rheumatoid arthritis patients treated with rituximab *(81)*.

2.5.2 Primary and Recall Response to Immunization

Eighteen chronic renal failure subjects were immunized intravenously with bacteriophage phiX174 (2x10^9 pfu/kg) at 2 weeks after rituximab therapy with eight of nine patients receiving a booster immunization at 8 weeks. Blood samples were drawn prior to and at 1, 2 and 4 weeks after each immunization. Phage-neutralization by antibody occurs in an exponential fashion and

the rate of inactivation of an *E. coli* assay is reported as a first order kinetic constant, Kv (expressed as geometric mean after primary and secondary immunizations (Table 1)). Data were compared to data from 46 normal, non-immunosuppressed historical controls. Statistical analysis of IgM to IgG switch was performed using Chi-square and Kv using Student's t test on log transformed data *(78)*.

Table 1 – Antibody Response to PhiX174 (Geometric Mean of K_v)

	Primary Response			Secondary Response		
Day post immune	CRF Patients n=9	Controls n=47	Rituximab Patients n=9	CRF Patients n=8	Controls n=47	Rituximab Patients n=8
7	3.2 (0.26-163)	9.1 (1.4-57.4)	0.09 (0.01-4.68)	186 (80-548)	539 (138-2544)	0.09 (0.00-4.10)
14	22 (0.91-182)	119 (5.9-1168)	0.22 (0.01-11.8)	191 (108-448)	349 (118-1848)	0.20 (0.01-5.57)
28	17 (1.54-80.4)	67 (7.3-641)	0.07 (0.01-5.0)	105 (29.2-255)	175 (58-880)	0.36 (0.00-10.6)
Peak K_v	30 (1.40-182)	128 (10.6-1168)	0.15 (0.01-11.8)	224 (110-548)	537 (138-2544)	0.47 (0.01-10.6)

Data presented are Geometric Mean of K_v with low to high range displayed in parenthesis.

Only 2 of 9 rituximab treated patients exhibited a weak detectable isotype switch compared to all of the chronic renal failure and normal controls ($p<0.001$). In summary, all rituximab treated patients showed a severely depressed primary and secondary response to a neoantigen suggesting a profound effect on *in vivo* B cell function. While the response in the chronic renal failure patients was depressed somewhat compared to normals, the decrease in rituximab treated was profound. These results indicate that administration of a single dose of rituximab has a dramatic effect on *in vivo* neoantigen antibody responses. Three of the nine rituximab treated patients were available for further study at more than 2 years post rituximab dose when total CD20 counts had returned to baseline. Two of the subjects were also immunized with tetanus toxoid as a recall antigen (one of the three had an allergy to tetanus and was not immunized). The results (IU/ml) were for baseline, 1 week, 2 weeks and 6 weeks for patient #1 and #2 respectively were: 1.53, >20, >20, >20 and 0.63, 0.61, 3.03, 5.13, both normal recall responses. All three long-term subjects were also re-immunized twice with phiX174 and 0 and 6 weeks. One of these responded to re-immunization with a classic “primary response” with lower titered IgM (Kv = 405) followed by higher titered IgG “secondary response” (Kv=1020). The second demonstrated a classic “tertiary” response with IgG detectable after both immunizations (Kv = 2618 and 278, respectively). The last subject (#1 above, baseline CD20 306 cells/μl; 2 year CD20 415 cells/μl) was hypo-responsive with only a very weak response to the third (Kv = 3.87) and forth boosts (Kv = 16.5), all of which was IgM. This latter subject then demonstrated apparent phiX174-specific hypo-responsiveness. The data from this study and two others indicate that after B cell recovery, immune responsiveness to both recall and de novo antigens are intact *(79, 80)*.

2.6 Rituximab in Children with Type 1 Diabetes

Most reports are abstracts of data presented at meetings and report rituximab use for treatment of lymphoma (*50-54*) (and as reviewed in (*55*)). The usual dose of rituximab employed in children has been that approved for use in adults (375mg/m^2 for 4 doses). In the largest retrospective review, 13 children with a median age of 8.5 years, were treated for CD20-positive, EBV-positive, lymphoma 30 months after organ transplant (*56*). One to 4 doses of rituximab were administered at 375 mg/m^2/dose (one patient received 125 mg/m^2/dose). There was complete resolution in 11 of these cases although one required a second course. At a median follow-up of 12 months, 11 of the 13 patients were alive but one had returned to dialysis. Rituximab therapy was associated with mild to moderate infusion-related events. The two deaths were from failure to control the lymphoma. Other smaller series including children as young as 19 months also typically used 375 mg/m^2 weekly for 4 weeks with generally good

responses and minimal toxicity (*57-66*). Most investigators have reported clinical responses to rituximab (*57, 59-62*) but also with occasional reported failures (*58*). As with adults, autoimmune hemolytic anemia (*67, 68*) and refractory idiopathic thrombocytopenia purpura (*69*) have been successfully treated in children (as young as 7 months) with rituximab. Two deaths have been reported however after using rituximab for hemolytic anemia (*70, 71*). Both of these deaths occurred after recurrence of primary disease, and in the patients who had undergone a bone marrow or stem cell transplant.

The most recent report of rituximab in children demonstrates its safety and efficacy in 4 patients with multisystem autoimmune diseases. Four children, ages 5, 7, 15, and 17, received rituximab at 375mg/m^2 once weekly for 4 doses. Each patient had improvement in his or her clinical autoimmune pathology. Two of the patients had insulin-dependent diabetes mellitus as part of their clinical spectrum diagnosed 5 years and 2 years, respectively, before rituximab treatment. Neither child neither suffered adverse events from the rituximab treatment nor were problems reported with insulin dosing after rituximab treatment. While neither child had improvement in their diabetes, this is not surprising because of the long-standing diabetes. Neither child would have met inclusion criteria for this proposed study based on this delay in treatment. Furthermore, careful assessment of change in stimulated C-peptide was not included in this report. Nevertheless, the experience of these two subjects does suggest that rituximab can be safely given to children with type 1 diabetes (*72*).

3 STUDY DESIGN

3.1 Overview

Type I diabetes results from immune mediated destruction of pancreatic islet beta cells. While typically thought of as a T cell mediated disease, the data reviewed above support the hypothesis that B cells play a major role in the pathophysiology of type I diabetes, perhaps through their function as antigen presenting cells. We hypothesize that rituximab, by eliminating B cells, would be effective at halting beta cell destruction thereby preventing the progression of diabetes.

3.2 Summary of Inclusion/Exclusion Criteria

Participants must meet all entry criteria for the protocol as outlined below.

3.2.1 Inclusion Criteria

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

1. Be between the ages of 8 and 45 years*
2. Be within 3-months (100 days) of diagnosis of type 1 diabetes based on American Diabetes Association (ADA) criteria
3. Must have at least one diabetes–related autoantibody present
4. Must have stimulated C-peptide levels $\geq$ 0.2 pmol/ml measured during a mixed meal tolerance test (MMTT) conducted at least 21 days from diagnosis of diabetes and within one month (37 days) of randomization
5. If participant is female with reproductive potential, she must be willing to avoid pregnancy and have a negative pregnancy test
6. At least one month from last immunization received
7. Must be willing to comply with intensive diabetes management
8. Must weigh at least 25 kg at study entry

*Enrollment will be limited to subjects 12-45 years until DSMB review of the first 10 subjects treated with rituximab.

3.2.2 Exclusion Criteria

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

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

3.3 Informed Consent

The process of assuring that individuals (and parent/guardian if less than 18 years of age) are making an informed decision about participating in this study includes both verbal and written

communication. Written material includes a Patient Handbook and written consent forms. There are two consent forms for this study. One is a screening consent form that describes the procedures, risks, and benefits, and determines eligibility for the study. The second is the intervention consent form, which describes the procedures, risks, and benefits for the remainder of the study. The consent form will be reviewed with participants (and their guardian in the case of participants under 18 years of age) and the participant will be given time to review the written consent form and ask questions. An assent form has also been developed for participants less than 18 years of age (unless local IRB requirements differ in procedure).

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

Additional consent for testing for reportable conditions such as HIV or Hepatitis C will be obtained as required by individual institutions. If participants 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 according to local regulations.

3.4 Description of Treatment Groups

This protocol will enroll a total of 66 participants who will be randomly assigned to the following two groups:

- 44 participants will receive active rituximab (anti-CD20 monoclonal antibody) as an intravenous infusion, with 4 administrations at weeks 0, 1, 2, and 3 at a dose of 375mg/m^2.
- 22 participants will receive placebo given as an intravenous infusion with 4 administrations at weeks 0, 1, 2, and 3.

3.5 Treatment Assignment and Double Masking

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 receive either rituximab or placebo.

Participants will be randomized in a 2:1 ratio of active treatment to placebo. The randomization method will be stratified by TrialNet study site. This approach ensures that study site will not be a potential confounder.

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.

Since rituximab can induce infusion related reactions, all subjects will receive acetaminophen and diphenhydramine prior to dosing with study medication. Flow cytometry results, which would lead to unblinding based on change of cell phenotypes particularly B cells, will not be reported to the clinicians caring for the subject unless required for management of a serious adverse event.

3.6 Study Assessments

During the course of the study, participants will frequently undergo assessments of their insulin production, immunologic status, overall health and well being and diabetes care (see schedule of assessments in Appendix A). The participant's insulin production will be measured by a series of mixed meal glucose tolerance tests (MMTT) conducted regularly during the study. The participant's diabetes control will be evaluated by measuring glycosylated hemoglobin (HbA1c) every three months.

During the course of the study, samples will be drawn for storage in the National Institute for Diabetes and Digestive and Kidney Disease (NIDDK) Repository and at TrialNet Sites for future analysis. These samples will be collected only with the subject's permission. Subjects who decline consent for these sample collections will still be eligible to participate in this study (see Section 10.4).

3.7 Quality Assurance

During the study, duplicate collections of blood samples for assays will be obtained in a small sample of subjects for the purpose of external quality surveillance of the performance of the central laboratories. These additional collections will only be taken from subjects aged 18 years and older who weigh at least 110 lbs (50 kg).

3.8 Study Feasibility

A total of 66 subjects will be enrolled for this study over two years. Subjects will be followed for two years. There will be additional follow-up for up to 4 years for those subjects who have persistence of beta cell function at 2 years and/or detectable immunologic effects of treatment. There will be complete ascertainment of dosing non-compliance, since the study drug is given intravenously at the clinical site.

4 PATIENT MANAGEMENT

4.1 Screening

After informed consent, subjects will undergo assessments to determine if they meet eligibility criteria. This screening process will occur between 3 weeks and 3 months of the date of diagnosis of type 1 diabetes. Documentation of the subjects understanding of the risks and benefits of the study will be collected through the volunteer quiz.

4.2 Randomization

Eligible study participants will be randomized at the clinical sites at the baseline visit, and will be assigned a study randomization number corresponding to the treatment group assignment. The subject will receive the initial dose of rituximab or placebo at the baseline visit.

4.3 Intensive Diabetes Management

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

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

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

4.4 Administration of Rituximab/Placebo

Rituximab/placebo will be given by IV infusion for anywhere between 3-8 hours at a dose of 375mg/m^2 on four visits a week apart. Vital signs will be routinely monitored prior to and during infusion.

4.4.1 Doses and Dose Changing Rules

Pre-medication, consisting of acetaminophen and diphenhydramine will be given before each infusion of rituximab/placebo to attenuate infusion-related events and may be repeated every four hours as needed. Since transient hypotension may occur during infusion, anti-hypertensive medications, if any, may be withheld 12 hours prior to infusion. This will be decided by the investigators on a case-by-case basis based on the antihypertensive therapy and clinical status of the patient.

The rituximab/placebo solution for infusion should be administered intravenously at an initial rate of 50 mg/hr. Rituximab should not be mixed or diluted with other drugs. If hypersensitivity or infusion-related events do not occur, escalate the infusion rate in 50 mg/hr increments every

30 minutes, to a maximum of 400 mg/hr. If the subject tolerates the first infusion then the subsequent infusions can start at 100 mg/hr and then be increased by 100 mg/hr every 30 minutes until 400 mg/hr.

If mild hypersensitivity or an infusion related event develops, the infusion should be temporarily interrupted. Bronchodilators and or saline infusions may be used if indicated for bronchospasm or mild hypotension. Subjects may be redosed with acetaminophen and diphenhydramine. The infusion can continue or be resumed at one-half the previous rate upon improvement of patient symptoms.

Patients with moderate or severe hypersensitivity or infusion reactions, such as those who require pressor support for hypotension or treatment with epinephrine for bronchospasm will not be restarted on therapy and will not receive any subsequent doses.

4.5 Primary and Recall Responses

As supported by data obtained in renal failure patients receiving rituximab (Section 2.5.2), we hypothesize that rituximab penetrates into effected sites of antibody production but selectively allows the repopulation of CD19+/CD27- naïve cells to allow a primary antibody response only once the B cells have returned. This will be tested by administration of the neoantigen bacteriophage phiX174 at 3 and 9 weeks after the last rituximab/placebo treatment with measurement of responses at 1, 2, and 4 weeks post immunization. These responses will be compared to those from a second round of phiX174 immunizations following B cell reconstitution at one year. Due to the additional visits required to administer and assess responses to phiX174 immunization, subjects may decline to participate in this aspect of the protocol while remaining in the main study. Those who do participate will receive compensation for their time and effort.

At the time of B cell reconstitution one year after rituximab/placebo administration, subjects will receive a single tetanus (standard Td) immunization and a hepatitis A immunization course consisting of two doses of the vaccine separated by at least 6 months. Responses to these immunizations will be determined by titer post immunization.

5 STUDY VISIT ASSESSMENTS

The schedule of evaluations and laboratory studies is presented in Appendix 1. A summary of assessments for the Protocol is given below.

5.1 General Assessments

General assessments for this Protocol will include:
- Informed consent
- Inclusion/exclusion criteria
- Medical history
- Physical examination
- Neurologic assessment
- Concomitant medications
- Adverse events

5.2 Laboratory Assessments

The following laboratory assessments will be performed during the study:
- Chemistry (sodium, potassium, chloride, CO2, glucose, urea, creatinine)
- Liver function tests (ALT, AST, alkaline phosphatase, total protein, albumin, total and direct bilirubin)
- Hematology (complete blood count with differential and platelets)
- Purified protein derivative (PPD) test
- Urine pregnancy test
- Serum IgG and IgM levels
- Autoantibodies (GAD65, ICA512, IAA)
- Antibodies to HIV, hepatitis B (HbcAg, HbsAg), hepatitis C (HCV)
- Cytomegalovirus (CMV IgG) and viral load
- Epstein-Barr Virus (EBV IgG and IgM) and viral load
- Samples for virology and other immunization titers

5.3 Mechanistic Outcome Assessments

Mechanistic assessments will consist of:
- HLA and FcγR genotyping
- B and T cell function and subpopulations measured by flow cytometry
- Stored PBMC, RNA, plasma, and serum samples
- Response to immunizations
- DNA

5.4 Metabolic Outcome Assessments

Metabolic assessments will consist of:
- Glucose records and reports of hypoglycemia
- Insulin dose
- HbA1c
- Mixed meal tolerance test (MMTT)

5.5 Laboratory Measures Related to Rituximab Administration

Laboratory tests to measure drug level and immune response to the drug:

- Rituximab pharmacokinetic (PK) analysis
- Anti-rituximab (HACA) levels

5.6 Visit Windows

The initial treatment should begin within 100 days from the day of diagnosis and within 37 days from the screening MMTT. The subsequent treatment visits should be within 2 days on either side of the targeted date to be permissible and not less than 96 hours from the start of the previous infusion.

6 ADVERSE EVENT REPORTING AND SAFETY MONITORING

6.1 Adverse Event Definitions

6.1.1 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.

6.1.2 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.

6.1.3 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.

6.1.4 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.

6.2 Adverse Event Reporting and Monitoring

Study personnel will assess adverse events and the use of concomitant medications throughout the study. All adverse events will be reported to the TrialNet Coordinating Center as described

below. They will be graded as to severity according to common toxicity criteria or study-specific criteria and the investigator will make a determination as to the relation to therapy. Events will be assessed and reported in accordance with the ICH Guideline For Good Clinical Practice and per the guidance of the DHHS Office for Human Research Protections (OHRP).

The adverse event case report form for the protocol must be completed for all adverse events (AE). 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.

The study will be put on clinical hold for review of safety if the following occurs in more than 2 subjects, in the first 10 subjects: Grade 3 opportunistic infections associated with greater than Grade 2 lymphopenia (requiring IV antibiotics, antiviral, or antifungal with radiologic and/or surgical intervention); Grade 4 lymphopenia (less than 200/mm3); Grade 3 thrombocytopenia (25-50,000 platelets/mm3); Grade 3 conduction abnormalities, supra-ventricular and ventricular arrhythmias (incompletely controlled medically or controlled with device, with symptoms); Grade 3 myocarditis (CHF responsive to medications); Grade 3 hypoxia (decreased oxygen saturation at rest, with continuous oxygen indicated); or Grade 3 cytokine release syndrome (prolonged, not responding rapidly and recurring post initial treatment, requiring hospitalization). There will be a prompt halt to the study for review of safety if any one subject has a Grade 3 hemorrhage (pulmonary, GI, or GU), requiring 2 units of packed red blood cells and/or radiologic, endoscopic, or surgical intervention. Review of the SAE will be conducted by the TrialNet Medical Monitor, TrialNet Safety Monitoring Committee, TrialNet research staff, and the DSMB to determine if changes in the protocol are warranted before the clinical hold is lifted.

6.3 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.

All infusions will take place in a facility that has resuscitation capabilities.

Subjects will be counseled by study personnel and requested to avoid pregnancy for 1 year following the last study infusion.

7 PARTICIPANT SAFETY

7.1 Expected Side Effects and Adverse Events

7.1.1 Infusion Reactions

In subjects treated with rituximab for autoimmune disease, mild to moderate hypotension (up to 30%), cough, rash, and pruritus were the most common side effects. In contrast, fever (43%) was the most commonly reported side effect of rituximab treatment in a phase I trial in lymphoma patients by Maloney et al (34). Bronchospasm (8%) and hypotension (10%) also occurred, but none of these events was severe. Other adverse events in this population included chills/rigors, headache, nausea, vomiting, rhinitis, and mild hypotension, occurred primarily during rituximab infusions and typically responded to an interruption of the infusion and resumption at a slower rate. Additional adverse events included neutropenia, thrombocytopenia, asthenia, lymphopenia, leukopenia, and anemia.

Patients with preexisting cardiac conditions, including arrhythmia and angina, have had recurrences of these cardiac events during rituximab infusions. In rare cases, severe and fatal cardiopulmonary events, including hypoxia, pulmonary infiltrates, acute respiratory distress syndrome, myocardial infarction, and cardiogenic shock, have occurred. Nearly all-fatal infusion-related events occurred in association with the first infusion in patients who had a previous cardiac history. No such deaths have been reported in subjects treated for autoimmune disease.

These risks will be mitigated by pre-treatment of subjects with acetaminophen and diphenhydramine, careful monitoring during and post infusion, and modification of infusion rate and dose as described above (Section 4.4.1).

7.1.2 Infectious Adverse Events

As with all immunosuppressive drugs, use of rituximab has been associated with infections, some of which have been serious or fatal. Causality however, is not clear.

Of the approximately 1 million people treated with rituximab, 25 fatal cases of progressive multifocal leukoencephalopathy (PML) have been reported. These individuals also received other drugs that compromised their immune system. Two cases occurred in people with SLE and the remainder in people with malignancies. According to estimates provided by the FDA, up to 10,000 SLE patients have received this drug. In addition, there are reports of 24 SLE patients with PML who never received rituximab.

In the recently reported trial of rituximab in subjects with rheumatoid arthritis, there were six serious infections occurring during the 48 weeks of treatment in those receiving rituximab, including fatal bronchopneumonia in a subject with a preexisting cardiac condition. Nonetheless, there was no overall increase in infections as compared to methotrexate control group. Other infections have also been reported in subjects treated with rituximab for non-autoimmune indications (chronic renal failure, lymphoma, etc).

This risk will be mitigated in this study by careful neurological exams as well as having subjects report even mild illness between study visits including neurological symptoms. They will be specifically asked about infectious adverse events during the study visit, and they will be monitored regularly for infections and appropriate anti-infective therapy will be instituted. All infectious adverse events will be reviewed by the TrialNet Medical Monitor and DSMB, if serious.

CMV and EBV serology will be determined at baseline. In order to assess whether rituximab treatment has any impact on viral activation, subjects will routinely have samples obtained at regular intervals for viral load determination as a retrospective measure unless there is a clinical need to measure the responses earlier.

7.1.3 Immunoglobulin Levels

While typically there is no change in serum IgG level in patients who have received rituximab as part of clinical trials, there have been a few reported cases of hypogammaglobulinemia, particularly in the setting for treatment of lymphoma. In the rheumatoid arthritis study, hypogammaglobulinemia has not been reported. Because of this concern, serum IgG levels will be obtained before and during the trial. In the unlikely event of hypogammaglobulinemia, treatment will be performed as per standard of care. Serum IgG levels less than 500 mg/dl would be considered an adverse event (normal 1000 mg/dl by age 6 years). A level of less than 300 mg/dl would be considered a serious adverse event and would be an indication for treatment.

7.1.4 Immunizations

Preliminary data *(78,79,80,81)* suggests little or no impact of rituximab treatment on either primary and recall responses, or level of protection from previous immunizations after B cell recovery. As a precautionary measure, during the first year after treatment subjects will be instructed not to receive any routine immunizations outside of the study.

To address the question of rituximab's impact on immunizations after reconstitution of B cells, individuals will receive Td and the two-dose hepatitis A immunization series starting 1-year post treatment with titers determined pre and post immunization.

To address the question of rituximab's impact on previous immunization protection, serum will be obtained at regular intervals for determinations of changes in titers.

7.1.5 Other Reported Adverse Events

The following serious adverse events have been rarely reported (<0.1%) in patients following completion of rituximab infusions for lymphoma: arthritis, disorders of blood vessels (vasculitis, serum sickness and lupus like syndrome), lung disorders including pleuritis and scarring of the lung (bronchiolitis obliterans), eye disorders (uveitis and optic neuritis), and severe bullous skin reactions (including toxic epidermal necrolysis and pemphagus) that may result in fatal outcomes. Patients may have these symptoms alone or in combination with rash and polyarthritis.

7.2 Pregnancy

Pregnant and lactating women will not be included in the study. Females must have a negative pregnancy test prior to enrolling in the study and will be required to use birth control during the study. At every study visit the sexual activity of female participants of reproductive age will be re-assessed. If a subject who was previously sexually inactive becomes sexually active, she will be counseled about the need to use a reliable form of birth control. Female subjects will also be required to undergo urine pregnancy tests at regular intervals and with weekly infusions over 4 weeks. Subjects will be requested to avoid pregnancy for 1 year following the last study infusion and instructed to use birth control.

8 STATISTICAL CONSIDERATIONS AND ANALYSIS PLAN

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

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 one-year visit. The AUC is computed using the trapezoidal rule that is a weighted sum of the C-peptide values over the 120 minutes. By the mean value theorem of integral calculus, the weighted mean C-peptide in pmol/mL is simply AUC/120. The primary statistical hypothesis to be assessed in the study is whether the mean C-peptide value for study subjects on rituximab 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 log transformation is used to allow for zero values in the mean C-peptide measure. 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)* (88).

The primary intent-to-treat analysis will include all subjects who received the initial infusion, regardless of whether the subject received subsequent infusions, except in the rare instance where infusions were halted due to safety considerations based on information received from outside of the study. The latter such subjects should continue follow-up, if consenting, and may be included in secondary analyses but would not be included in the primary intent-to-treat analysis. Further, such subjects should be replaced by an equal number of additional subjects so as to meet the sample size goal of 66 subjects total who will contribute to the primary intent-to-treat analysis.

Additional analyses will include:

- a log rank test of the difference in the hazard function between groups in the incidence of the loss of the 2 hour peak C-peptide < 0.2 pmol/ml on a semi-annual MMTT (90), and
- longitudinal analyses (89) using mixed effects models with a random intercept and slope of the C-peptide values over the post-treatment period, adjusted for the baseline level of C-peptide. The average intercept and slope will be compared between groups adjusting for age, gender and the baseline *log(C-peptide+1)*.
- Analyses will also be conducted to adjust for baseline C-peptide and HbA1c levels, and by gender and race/ethnicity, as appropriate.
- Analyses will also be conducted using all available data on all subjects randomized, including those who are excluded from the primary intent-to-treat analysis above.

8.2 Secondary Outcomes and Analyses

The secondary objectives are to examine how rituximab affects the following:

- Mean area under the stimulated C-peptide curve (AUC) curve at 2 years

- Mean area under the stimulated C-peptide curve (AUC) over 4 hours at 2 years
- HbA1c
- Insulin dose (units/kg)
- Total number of hypoglycemic events
- Number of major hypoglycemic events
- Number and severity of adverse events

The mean area under the stimulated C-peptide curve (AUC) is computed over the first 2 hours of the 4-hour mixed meal glucose tolerance test conducted at the two-year visit. This outcome will be analyzed in the same manner as the primary outcome.

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. 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. Both sets of analyses will be adjusted for age, gender, baseline *log(C-peptide+1)* and baseline HbA1c.

8.3 Additional Outcomes and Analyses

The goal of the mechanistic studies will be to distinguish rituximab treated from placebo patients during and after rituximab therapy. With the exception of islet autoantibodies, there are very limited assays identified to date that can distinguish normal from individuals with type 1 diabetes, and diabetes autoantibodies have not yet proved useful in previous immunotherapy trials to serve as a marker of treatment effect. However, due to the nature of action of rituximab, we anticipate that differences in autoantibody titer and isotype will be observed between rituximab and placebo subjects, and we will test whether these changes correlate with changes in T cell activity. These studies are exploratory in nature.

Our working hypothesis is that rituximab not only causes generalized B cell depletion, including elimination of auto-reactive and antigen-presenting B cells, but that it may indirectly effect the mobilization of a sub-population of antigen-specific T cells and may even induce regulatory T cells or a shift in the ratio of Th1 to Th2 lymphocytes upon B cell re-population. In this regard, we propose to compare changes in T cell function in placebo and rituximab-treated subjects, and evaluate the effects of treatment on T cell depletion and subsequent re-population of PBMCs, assessing T cell number and/or function such as CD4+CD25+ cells, that may modulate autoimmunity. We plan to determine if there is a change in the number of B cell subsets in drug-treated versus control subjects.

These studies will lead to an understanding of the effects of rituximab therapy, and allow correlation between immune markers, disease progression, and response to drug therapy. As developed, other mechanistic assays will be included if there is adequate blood volume.

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 type 1 diabetes could be at increased risk.

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.

8.4 Sample Size and Power Calculations

The primary analysis will compare the difference between groups 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 (73). 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. Using standard equations for the comparison of two means (90), it was determined that a total sample size of 66 subjects would provide power of 85% to detect a 65% increase in the geometric-like mean C-peptide relative to the placebo group using a test at the 0.05 level (one-sided), with 10% loss to follow-up and a 2:1 allocation to treatment versus control.

There will be 44 subjects in the active treatment and 22 subjects in the placebo group (66 total), who will be followed for two years post treatment. For those who continue to have persistence of beta cell function at 2 years and/or detectable immunologic effects of treatment will be followed for descriptive analysis until absence of detectable beta cell function or resolution of immunologic changes for up to 4 years.

When the 66th evaluable subject has been randomized into the study, the screening of new subjects will be closed, and all subjects who had initiated screening evaluations prior to that time will be allowed to be randomized if still both consenting and eligible.

8.5 Treatment Assignment

The sample of 66 participants will be randomly assigned to the following two treatment groups:

Active: Participants will receive active rituximab (anti-CD20) as an intravenous infusion, with 4 administrations at weeks 0, 1, 2, and 3 at a dose of 375mg/m^2.

Placebo: Participants will receive placebo given as an intravenous infusion with 4 administrations at weeks 0, 1, 2, and 3.

8.6 Interim Monitoring Plan

Interim analyses will be conducted periodically during the study and will be reviewed by the TrialNet DSMB for assessment of effectiveness and safety. The Lan-DeMets spending function with an O'Brien-Fleming boundary will be used to protect the type I error probability (84) and to assess the significance of the interim results that emerge during the trial. The monitoring plan will allow for early termination based on the treatment effects on C-peptide values at 1 year and also at 2 years of follow-up. DSMB reports will also include conditional power analyses conducted both under the study hypotheses and under the current trend of the data (85) to allow

early termination due to futility – i.e. lack of beneficial treatment effect. Additional analysis will assess potential adverse outcomes of treatment and will assess the incidence of all severe adverse events (see Section 7.1).

9 ETHICAL CONSIDERATIONS AND COMPLIANCE WITH GOOD CLINICAL PRACTICE

9.1 Statement of Compliance

This study will be conducted in compliance with the protocol and current Good Clinical Practices (GCP), adopting the principles of the Declaration of Helsinki, and all applicable regulatory requirements.

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

9.2 Participating Centers

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

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

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

9.3 Informed Consent

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

The informed consent form must be updated or revised whenever important new safety information is available, when indicated for a protocol amendment, and/or whenever any new

information becomes available that may affect a patients' participation in the study.

9.4 Study Subject Confidentiality

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

Study subject data, which is for reporting purposes, will be stored at the University of South Florida Coordinating Center. Case report forms sent to the Coordinating Center will identify participants by the unique TrialNet Identification Number. The data entry system at the Coordinating Center is a secured, password protected computer system. At the end of the study, all study databases will be archived at the Coordinating Center, and the data collection forms will be electronically scanned and saved in electronic format for long-term storage. All paper copies of the forms will ultimately be destroyed after the data is transferred.

HLA genotyping is for research purposes only. The HLA genotyping result will not be made available to the participant and his or her physician. DNA will be stored for future use with the permission of the study subject.

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

9.5 Risks and Benefits

The risks of this study are presented in the informed consent form and are described in Chapter 7. There is no guaranteed benefit to subjects for their participation in the study. This study will examine whether immunosuppressive intervention will preserve beta cell function, but there is no guarantee that this will occur.

10 STUDY ADMINISTRATION

10.1 Organizational Structure

This study is part of type 1 diabetes TrialNet, which is funded by the National Institutes of Health 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. Genentech will provide Rituxan (rituximab) and matched placebo free of charge for the participant's entire length of treatment.

10.2 Groups and Committees

10.2.1 TrialNet Coordinating Center

The TrialNet Coordinating Center (TNCC) will provide overall leadership to the TrialNet study group to include protocol and manual preparation, development of statistical design for each study, and analysis of study results. The TNCC will also coordinate interactions among the participating TrialNet clinical centers, test laboratories including TrialNet core laboratories and other subcontract laboratories, NIDDK, and other sponsoring agencies.

10.2.2 Clinical Sites

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

10.2.3 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 and reporting of adverse events (AEs) and serious adverse events (SAEs).

10.2.4 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. The DSMB will also evaluate data after the first 10 subjects are treated with active rituximab and notify the TrialNet study group if enrollment of subjects between ages 8-11 can proceed.

10.3 Partnering with Industry

The proposed study drug, rituximab (Rituxan^{R}, Genentech or Biogen Idec) is commercially available. Genentech is providing the study drug rituximab and placebo and partial support (laboratory and financial) for this study.

10.4 Sample and Data Storage

Samples to be stored for research purposes will be located at the NIDDK Repository and at TrialNet 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.

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

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.

10.5 Preservation of the Integrity of the Study

The scientific integrity of the trial dictates that results be reported on a study-wide basis; thus, an individual center will not report the data collected from its 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.

10.6 Participant Reimbursement and Compensation

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

11 STUDY TIMELINE

It is anticipated that patient enrollment will occur during the first two years of the trial. Subjects will be followed until two years after initial treatment. The projected study duration is four to five years. Additional follow-up for up to 4 years will continue for those who have persistence of beta cell function at 2 years and/or detectable immunologic effects of treatment.

12 STUDY ENDPOINTS AND LONG TERM FOLLOW-UP

Analysis of the primary endpoint has demonstrated that rituximab treatment was effective in preserving beta cell function. It is unknown how long the effect of treatment will persist and further whether there are any long term adverse events associated with drug use in this population of individuals with Type 1 diabetes. As such, after the conclusion of the primary study (when all subjects have reached their 24 month secondary endpoint and the dataset for those visits is verified), subjects will be debriefed about their participation to date. They will be informed about the overall study results, their treatment group assignment and their individual beta cell function test results. Prior to the debriefing, subjects will be asked participate in the completion of a questionnaire evaluating their experience with the study.

Subjects will also be asked to participate in long-term follow up. Whenever feasible, subjects will be contacted annually to ascertain health and diabetes status. If significant adverse changes in health status are reported, requests for release of appropriate medical records may be made for additional information. Samples for mechanistic studies and to ascertain diabetes control may also be obtained, with blood volumes in total not to exceed 3 mg/kg body weight for children. Subjects who have persistence of beta cell function may undergo MMTT no more than every six months. Subjects will indicate their willingness to participate in long-term follow-up through written informed consent The consent form will be reviewed with participants (and their guardian in the case of participants under 18 years of age) and the participant will be given time to review the written consent form and ask questions. An assent form has also been developed for participants less than 18 years of age (unless local IRB requirements differ in procedure).

APPENDIX 1 - Schedule of Assessments

~Month of Trial									2		3			4	5	6	9	12			13					15	18	21	24
Week of Trial:	**-1[6]**	**0**	**1**	**2**	**3**	**5**	**6**	**7**	**8**	**10**	**12**	**13**	**14**	**16**	**19**	**26**	**39**	**52**	**53**	**54**	**56**	**58**	**59**	**60**	**62**	**65**	**78**	**91**	**104**
History and Physical Exam[7]		X*[5]	X	X	X	X					X					X*	X	X*									X*		X*
Adverse Events Assessments			X	X	X	X				X	X				X	X	X	X									X		X
CBC with Differential	X	X	X	X	X	X				X	X					X	X	X									X		X
Chemistries	X										X					X		X											X
PPD Test	X																												
HIV, Hep B and C	X																												
Urine Pregnancy Test	X	X	X	X	X						X					X		X									X		X
Serum for Autoantibodies	X					X					X					X	X	X									X		X
Rituximab Administration		X	X	X	X																								
Rituximab Pharmacokinetic (PK) Analysis		X														X	X												
Anti-rituximab (HACA) Levels		X														X	X												
Hemoglobin A1c	X										X					X	X	X									X		X
MMTT (4-hour)	X																	X											X
MMTT (2-hour)											X					X											X		
HLA Determination	X																												
FcR Genotype Testing	X																												
EBV/CMV PCR		X		X		X					X				X	X	X				X						X		X
EBV/CMV Viral Serology		X		X		X					X				X	X	X				X						X		X
Serology[4]		X														X					X						X		X
B and T cell Assay (fresh)		X				X										X		X											X
B and T cells (frozen)		X				X					X					X		X									X		X
Flow Cytometry		X				X					X					X		X											X
RNA (frozen)		X				X					X					X		X									X		X
Tetanus Immunization Course[1]																		X*			X								
Hepatitis A Immunization Course[2]																		X*			X						X*		X*
PhiX174 Immunization Course[3]							X*	X	X	X	X*	X	X	X				X*	X	X	X	X*	X	X	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.

Tetanus Immunization Course[1]: The tetanus immunization is a single intramuscular immunization designated by * with titers obtained prior to and 4 weeks after immunization.
Hepatitis A Immunization Course[2]: Hepatitis A immunization course consists of two intramuscular immunizations designated by * administered >6 months apart. Therefore the second immunization can be done at either month 18 or month 24 visit. Titers may be obtained before and after the immunizations.
PhiX174 Immunization Course[3]: PhiX immunization course consists of two intravenous immunizations designated by * separated by 6 weeks. Blood samples are drawn prior to and at 1, 2, and 4 weeks after each immunization.
Serology[4]: Antibody titers to other childhood immunizations and illnesses will be measured on these samples.
Physical Exam[5]: Prior to administration of drug.
Screening Visit[6]: May take place several weeks prior to Baseline Visit. Screening MMTT must be within one month (37 days) of randomization.
Physical Exam[7]: Including a neurologic assessment at visits designated by *.

Follow-Up After 24 Months:

Visits may be conducted approximately every 6 months and assessments may include:
History and Physical Exam, Adverse Events Assessments, CBC with Differential, Serum for Autoantibodies and Immunization Response, Hemoglobin A1c, MMTT (2-hour), Serology, B and T cells (fresh or frozen), Flow Cytometry, and RNA (frozen).

Long term follow up:
Whenever feasible, subjects will be contacted annual to ascertain health and diabetes status. Samples for mechanistic studies and to ascertain diabetes control may also be obtained with blood volumes not to exceed 3 mg/kg body weight for children. The samples may include serum, PBMC, and RNA. Subjects who have persistence of beta cell function will undergo MMTT no more than every six months.

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