Immune Tolerance Network

Protocol ITN028AI

Effect of Antithymocyte Globulin on Preserving Beta-cell Function in New-onset Type 1 Diabetes Mellitus

Short title: ATG for New-onset T1DM

Version 11.0 (March 1, 2012)

[IND# 12693]

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Protocol Chair

Stephen E. Gitelman, MD
Professor of Clinical Pediatrics
Department of Pediatrics
University of California, San Francisco
S-679, Box 0434
513 Parnassus Avenue
San Francisco, CA 94143
Phone: (415) 476-3748
Fax: (415) 476-8214
E-mail: sgitelma@peds.ucsf.edu

ITN Clinical Trial Physician

Mario Ehlers, MD, PhD
Deputy Director
Clinical Trials Group
Immune Tolerance Network
185 Berry St. Suite 3515
San Francisco, CA 94107
Phone: 415-353-4402
Fax: 415-353-4404
Email: mehlers@immunetolerance.org

NIAID Medical Monitor

Linna Ding, MD, PhD
Medical Officer
Division of Allergy, Immunology, and Transplantation, NIAID
6610 Rockledge Drive, Room 6802
Bethesda, MD, 20892-6601
Phone: 301- 402-6794
Fax: 301-402-2571
Email: linnad@niaid.nih.gov

ITN Manager of Clinical Operations

Audrey Plough, BSN, CCRC
Clinical Operations Manager
Immune Tolerance Network
185 Berry Street, Suite 3515
San Francisco, CA 94107
Phone: 415-514-8228
Fax: 415-353-4404
Email: aplough@immunetolerance.org

NIAID Project Manager

Peggy Lund Fitzgibbon, RN
Project Manager
Division of Allergy, Immunology, and Transplantation / NIAID
6610 Rockledge Drive, Room 6710
Bethesda, MD 20892-660
Tel: 301- 435-4417
Fax: 301- 402-2571
Email: fitzgibbonm@niaid.nih.gov

Protocol Approval

Protocol: ITN028AI	**Version and Date:** 11.0 March 1, 2012
IND: 12693	**Protocol Chair:** Stephen E. Gitelman, MD
Short Title: ATG for New-onset T1DM	

I have read protocol ITN028AI, and I approve it. As the principal investigator, I agree to conduct this protocol according to good clinical practices, which are delineated in the *International Conference on Harmonization (ICH) of Technical Requirements for Registration of Pharmaceuticals for Human Use, Guidance for Industry: E6 Good Clinical Practice Consolidated Guidance* (May 1996), and according to the criteria specified in this protocol.

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Principal Investigator (Print)

_____ _____

Principal Investigator (Signature) **Date**

Synopsis

Title	Effect of Antithymocyte Globulin on Preserving Beta-cell Function in New-onset Type 1 Diabetes Mellitus
Short Title	ATG for New-onset TIDM
Clinical Phase	II
IND Sponsor	NIAID
Conducted by	Immune Tolerance Network
Protocol Chair	Stephen E. Gitelman, MD
Study Design	Two-arm, 2:1 randomized, placebo-controlled, blinded, phase II trial
Accrual Objective	Initial: 66; Revised to 58 upon closure of enrollment on June 30, 2011 (2:1 randomization to Thymoglobulin® and placebo)
Study Duration and Pace of Enrollment	30 months recruitment; 24 months study; follow-up possibly up to 60 months Enrollment will be staggered in the beginning of the study. The first 10 participants enrolled in this study will be age 18-35. The Day 1 visit for these participants will occur at least 2 days apart. The last subject in this cohort will be followed until day 15 – at which time the adverse event experience in this group will be reviewed by the protocol chair, the NIAID medical monitor and the ITN clinical trial physician. If this review concludes that no significant safety concerns have been identified, enrollment to include pediatric participants will commence. The Day 1 visit for the first 10 pediatric participants will occur at least 2 days apart. (Enrollment of adult participants will also continue.) The 10th pediatric participant enrolled will be followed until day 15 and a safety review to include the first 10 pediatric participants enrolled will be conducted as described above. If this review concludes that no significant safety concerns have been identified, enrollment will continue until the study is fully enrolled. Study subjects will be recruited during an approximately 30-month accrual period. It is anticipated that because of the age restriction (section 4.1), enrollment of initial participants in the trial will be slower than subsequent enrollment.
Primary Endpoint	A 2-hour C-peptide area under the curve (AUC) in response to a mixed-meal tolerance test (MMTT) at month 12.
Secondary Endpoints	**Efficacy**: • A 4-hour C-peptide AUC in response to an MMTT at month 12. • Insulin use in units per kilogram body weight per day at months 12 and 24. • Proportion of subjects who are exogenous-insulin-free at 12, 18, and 24 months; possibly up to 60 months. • Major hypoglycemic events occurring from randomization to months 12 and 24.

- A 2-hour and 4-hour C-peptide AUC in response to an MMTT at month 24.
- HbA_{1C} levels at months 12 and 24.
- Changes of C-peptide AUC (2 and 4 hours) over time at months 12 and 24.

Safety:

- The rate of the following adverse events in patients receiving Thymoglobulin:
  - Infusion reactions
  - Cytokine release syndrome (CRS)
  - Opportunistic infections
  - Lymphopenia
  - CD4/CD8 ratio
  - Neutropenia
  - Thrombocytopenia
  - Serum sickness
- Frequency and severity of all adverse events in participants receiving Thymoglobulin or placebo
- Adverse event frequencies in the Thymoglobulin group will be compared to those in the control group.

Inclusion Criteria

1. Individuals 12–35 years of age who meet the American Diabetes Association standard type 1 diabetes mellitus (T1DM) criteria.[1] Note: The first 10 randomized participants will be 18–35 years of age.
2. Positive for at least one islet cell autoantibody (glutamate decarboxylase; insulin, if obtained within 10 days of the onset of insulin therapy; ICA 512 antibody, and/or ICA).
3. Diagnosis of T1DM within 100 days of enrollment.
4. Peak stimulated C-peptide level >0.4 pmol/mL or >1.2 ng/mL following an MMTT.
5. Serologic evidence of prior EBV infection.
6. Participants of childbearing age must agree to practice an effective form of birth control, which may include any one of the following: abstinence, barrier method, oral contraception, or surgery. For females, these contraceptive measures must be maintained throughout the study; for males, these measures must be followed for a minimum of 3 months following Thymoglobulin infusion.

Exclusion Criteria

1. Prior history of any significant cardiac disease, such as congestive heart failure, arrhythmia, or structural defects, or suspicion thereof.
2. Leukopenia (<3,000 leukocytes/μL), neutropenia (<1,500 neutrophils/μL), lymphopenia (<800 lymphocytes/μL), or thrombocytopenia (<125,000 platelets/μL).
3. Any sign of active infection (e.g., hepatitis, tuberculosis, EBV, CMV, or toxoplasmosis) or screening laboratory evidence consistent with active infection.
4. Positive for HIV, PPD, or HB_SAg at screening.
5. Prior treatment with rabbit ATG or known hypersensitivity or exposure to rabbit sera-derived products.
6. Ongoing use of diabetes medications other than insulin that affect glucose homeostasis, such as metformin, sulfonylureas, thiazolidinediones, or amylin.
7. Vaccination with a live virus within the last 6 weeks before enrollment.
8. Prior or current therapy that is known to cause a significant, ongoing change in the course of T1DM or immunologic status.
9. Evidence of liver dysfunction, with ALT or AST > 3 times the upper limit of normal.
10. Evidence of renal insufficiency as indicated by serum creatinine of > 2 times the upper limit of normal confirmed in a repeat test at least 1 week apart.
11. Females who are pregnant at the time of screening or unwilling to defer pregnancy during the 24-month study period.
12. Any condition that, in the investigator's opinion, may compromise study participation or may confound the interpretation of the study results.

Treatment Description

The Thymoglobulin group will receive a total of 6.5 mg/kg of Thymoglobulin divided into four doses as follows: day 1, 0.5 mg/kg; day 2, 2 mg/kg; day 3, 2 mg/kg; and day 4, 2 mg/kg. The placebo group will receive saline solution.

Glossary

AA	aplastic anemia
AE	adverse event
ALG	antilymphocyte globulin
ALS	antilymphocyte serum
ALT	alanine aminotransferase
APC	antigen-presenting cell
AST	aspartate aminotransferase
ATG	antithymocyte globulin
AUC	area under the curve
CFR	Code of Federal Regulations
CMV	cytomegalovirus
CRF	case report form
CTCAE	Common Terminology Criteria for Adverse Events
DC	dendritic cell
DCCT	Diabetes Control and Complications Trial research group
DSMB	Data and Safety Monitoring Board
EBV	Epstein-Barr virus
EDIC	Epidemiology of Diabetes Intervention and Complications
GCP	good clinical practice
G-CSF	granulocyte-colony stimulating factor
HbA_{1C}	glycosylated hemoglobin A_{1C}
HLA	human leukocyte antigen
ICH	International Conference on Harmonization
IND	investigational new drug
IRB	institutional review board
ITN	Immune Tolerance Network
IVRS	Interactive Voice Response System
MHC	major histocompatibility complex

MMTT	mixed-meal tolerance test
NCI	National Cancer Institute
NIAID	National Institute of Allergy and Infectious Diseases
NOD	nonobese diabetic mouse
PBMC	peripheral blood mononuclear cell
PCP	*Pneumocystis carinii* pneumonia
PCR	polymerase chain reaction
PIG	phosphatidylinositol glycan
PNH	paroxysmal nocturnal hemoglobinuria
PTLD	posttransplant lymphoproliferative disease
PSS	Product Safety Scientist
RATG	rabbit antithymocyte globulin
Rho Fed	**Rho Fed:** Rho Federal Systems Division, Inc. (Clinical Research Organization)
SAE	serious adverse event
SAP	statistical analysis plan
T_H	T-helper cell
T_{REG}	T-regulatory cell
VZV	varicella zoster virus

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1. BACKGROUND AND RATIONALE

1.1 RATIONALE FOR AMENDMENT

The trial opened in August 2007 and initially planned to enroll 66 participants. Enrollment was originally planned for 18 months but more than 24 months elapsed before enrollment and treatment of the first adult cohort of 10 subjects was completed, triggering a safety review to allow pediatric enrollment to commence. Enrollment was opened to pediatric participants in October 2009. By December 2010 a total of 45 participants had been enrolled. Given that enrollment had been open for 36 months, the ITN leadership decided to close enrollment on June 30, 2011, achieving a subsequent sample size of 58 participants.

1.2 BACKGROUND

Type 1 diabetes mellitus (T1DM) is a chronic autoimmune disease in which insulin-producing beta cells are completely destroyed, resulting in a dependence on exogenous insulin for life.[1-3] Current management of T1DM is not optimal. To avoid long-term complications, patients must maintain near-normal glycemic control by frequently monitoring glucose throughout the day and taking multiple daily doses of insulin (via injection or pump) adjusted for variations in diet and exercise.[4] Such strict glycemic control is rarely achieved with current T1DM management, and overly aggressive therapy can result in recurrent severe hypoglycemia. It is not possible to fully mimic beta-cell function, and no established treatments can prevent the beta cell's immunologic destruction.

Some studies are evaluating the use of genotype, autoantibodies, and metabolic markers in screening first-degree relatives at risk for the disease.[5, 6] However, it is still not possible to identify the majority of subjects in the general population who will develop T1DM in the subclinical phase of the disease. At present, interventions effective at the time of clinical presentation represent the most useful form of therapy. Even after clinical presentation, affected individuals often enter a honeymoon, or remission phase, when they are still able to make substantial amounts of insulin.[7-9] Nevertheless, endogenous insulin secretion continues to deteriorate over the first 1–2 years of disease, eventually becoming undetectable and necessitating increasing reliance on exogenous insulin. Past studies have demonstrated that preservation of even modest endogenous insulin secretion dramatically improves metabolic control of T1DM, which, in turn, is associated with reduced morbidity and mortality.[10, 11] The ultimate goal of this study is to identify a means of blocking further autoimmune destruction of the beta cells to retain endogenous insulin secretion and thereby improve metabolic control. Eventually, such a therapy may also be used at an even earlier stage to prevent T1DM.

Extensive prior studies demonstrate that beta-cell destruction stems from autoreactive T cells.[12, 13] Histology of the pancreas reveals T-cell infiltration of pancreatic islets before and at the time of diagnosis. In rodent models, adoptive transfer of purified T cells from an affected animal or a specific T-cell clone can induce T1DM in the unaffected recipient, whereas neonatal thymectomy can prevent T1DM.[14, 15]

A recent case report of T1DM developing in a patient with B-cell deficiency lends further support to the essential role of T cells in this process.[16] Both $CD4^+$ and $CD8^+$ islet-reactive T cells are implicated in this process. Pathogenesis appears to result, at least in part, from a deviation in a T-helper 1(T_H1)-mediated autoimmune response, whereas protection results from a T_H2- and/or T-regulatory (T_{REG}) cell response.[17] Thus, interventions that affect T-cell number and function are the logical targets to ameliorate further beta-cell destruction in those with new-onset T1DM.

Proof of this principle comes from studies that target T cells before or shortly after the onset of T1DM, the time when interventions in both rodent models and humans have been shown to prolong endogenous insulin secretion. In nonobese diabetic (NOD) mice, many monoclonal and polyclonal antibodies directed against T cells, including CD4, CD8, CD40L, major histocompatibility complex (MHC) class II, and TCR αβ antibodies, are able to prevent the onset of T1DM.[18, 19] However, only a few agents have been shown to reverse T1DM in animals with pre-existing disease. Anti-CD3 and -CD4 monoclonal antibodies and polyclonal antilymphoctye serum (ALS) are unique in their ability to induce a lasting remission when administered for a limited course to rodents with new-onset T1DM.[20-24]

No clinical trial has yet demonstrated a means to prevent T1DM. However, for those with new-onset T1DM, clinical trials that target T cells with cyclosporine[25, 26] and azathioprine, alone or in conjunction with glucocorticoids,[27, 28] have demonstrated the possibility of altering the natural course of T1DM. In these trials, cyclosporine and azathioprine prolonged endogenous insulin secretion, and some participants experienced complete remission. However, further and more widespread use of these broad immunosuppressants is limited by the potential complications and toxicities from ongoing therapy and by the transience of effects: the effect of these drugs wanes as therapy is continued or as they are withdrawn.[29-31] In the most promising clinical trial in T1DM to date, 75% of the patients treated with hOKT3γ1(Ala-Ala), a modified, nonmitogenic, humanized form of the anti-CD3 monoclonal antibody, maintained the same or improved endogenous insulin secretion 1 year after an initial 12-day therapy (as opposed to 25% in the control group).[32] The effect appears to have waned over time because insulin secretion declined in this cohort during the second year.[33]

T-cell activation requires a complex set of interactions between antigen-presenting cells (APC) and T cells in which signal 1 is generated by the interaction between the MHC and the antigen from the APC and the T-cell receptor, and signal 2 is generated by a variety of possible co-stimulatory molecules. Building on the initial limited success of the clinical trial with the anti-CD3 monoclonal antibody, we postulate that the broader specificity of the polyclonal agent antithymocyte globulin (ATG), specifically Thymoglobulin, will prove even more efficacious in disrupting autoreactive T-cell activation in T1DM and may induce tolerance, thereby prolonging endogenous insulin secretion.

1.3 INVESTIGATIONAL PRODUCT

1.3.1 Overview

This study will use Thymoglobulin® (Genzyme, Inc.), a rabbit anti-thymocyte globulin (RATG) (see section 5.1). Thymoglobulin is produced using thymocytes of pediatric patients whose thymuses have been removed during cardiac surgery. The donor tissue and blood are screened for viruses, and rabbits are immunized with a standardized quantity of purified human thymocytes. The rabbit blood is collected at three different time points and undergoes several purification and concentration steps. Further tests are performed to ensure viral safety, purity (anti-rabbit serum precipitation and immune electrophoresis, in-vivo antiplatelet activity in mice, anti-red blood cell (RBC) activity, and fibroblast toxicity), and potency (lymphocytotoxicity, E-rosette inhibition between human lymphocytes and sheep RBCs, and live primate skin-transplant model).

Possible mechanisms by which Thymoglobulin induces immunosuppression in vivo include T-cell clearance from the circulation and modulation of T-cell activation, homing, and cytotoxic activities. Thymoglobulin includes antibodies against T-cell markers (such as CD2, CD3, CD4, CD8, CD11a, CD18, CD25, CD44, CD45), human leukocyte antigen (HLA) class I heavy chains, HLA-DR subsets, and β2 microglobulin. Antibodies against various adhesion molecules (LFA, ICAM1, VLA4, α4β7 integrins, etc.) have also been described. In vitro, Thymoglobulin (concentrations > 0.1 mg/mL) mediates T-cell suppressive effects via inhibition of proliferative responses to several mitogens. In patients, T-cell depletion is usually observed within a day from ATG administration. The nature of

the T-cell repopulation may constitute an important aspect of Thymoglobulin function, with a persisting inversion in the $CD4^+$:$CD8^+$ ratio and the possible induction of a regulatory cell population.

Thymoglobulin may also affect APC cell function. It does not activate B cells; rather it prevents B-cell proliferation and differentiation, even in Epstein-Barr virus (EBV)-transformed B cells. Binding to dendritic cells has also been described in vitro.

1.3.2 T-cell Depletion

A single dose of ATG reduces the total lymphocyte count by more than 85%, and reductions are sustained throughout the daily dosing period and the course of treatment.[34] T-cell depletion may result from complement-dependent opsonization and cellular lysis, Fc-dependent opsonization, or Fas-mediated apoptosis; the latter mechanism may be operative at lower ATG concentrations, at which ATG exhibits preferential effects on preactivated, as opposed to nonactivated, T-cells.[35] T-cell depletion in peripheral blood persists for several days to several weeks following cessation of ATG administration. Recovery from treatment-induced lymphocyte depletion is gradual, and total lymphocyte counts usually return to normal within 2 months after ATG administration.[34]

1.3.3 T-cell Repopulation

The nature of T-cell repopulation following depletion may be an important aspect of ATG function. The ratio of $CD4^+$ T cells to $CD8^+$ T cells remains significantly and persistently lower in those who have received ATG or other T-cell-depleting drugs, with a sluggish increase in the number of $CD4^+$ cells and a robust increase in the number of $CD8^+$ cells.[36-40] This change has been shown to correlate with favorable outcomes in transplantation studies. From the phase I/II anti-CD3 study in T1DM, the single best predictor of those who responded to drug was the change in the ratio of $CD4^+$ cells to $CD8^+$ cells.[32] Similar findings were noted in preliminary studies with ATGAM®[41] and ATG-Fresenius in new-onset T1DM.[42] We postulate that the change in this ratio reflects an alteration in the autoreactive effector T-cell population and may result in the generation of T_{REG} cells.

Evidence exists for vital regulatory cells that mediate autoimmunity and that are both $CD4^+$ and $CD8^+$.[43, 44] In both mouse (NOD mouse) and human studies with anti-CD3, investigators have noted an increase in the $CD4^+$ and $CD25^+$ cell population, a subset of T_{REG} cells that has been associated with suppressor cell function.[45, 46] Preliminary evidence suggests that this cell population may be depleted in individuals with new-onset T1DM[47] or have abnormal function.[48] There are no reports to date documenting that ATG also has an effect similar to anti-CD3 on this T_{REG} cell population.

A CD8 regulatory cell population may also be present and induced upon exposure to ATG. Muller et al. noted that following renal transplants with ATG induction, the $CD8^+$ and $CD57^+$ T-cell population increased from 1%–15% at baseline to 70%–80%.[49] Others have noted related findings following solid organ and bone marrow transplants.[49-52] This subset of T cells has been noted following cytomegalovirus (CMV) infection, but its function in the setting of ATG administration remains unclear.[53, 54] Halwani and colleagues noted that $CD8^+$ and $CD57^+$ cells suppressed mixed lymphocyte culture responses as well as pokeweed mitogen–induced IgG secretion and that the supernatants from cell cultures were also suppressive.[51]

Recent attention has focused on the potential regulatory function of CD8+ and CD28− cells. This is potentially of great interest in the setting of ATG administration, because CD57+ and CD28− T cells usually exhibit reciprocal expression and because only ~1% of CD8+ T cells express both markers. Furthermore, others[38, 55] have noted an increase in CD8+ and CD28− cells after T-cell depletion in other settings that are comparable to the increase in CD8+and CD57+ cells noted by Muller.[39] Thus, we hypothesize that ATG administration may induce a regulatory population of CD8+, CD57+, and CD28− cells that is critical for selective downregulation of pathogenic self-reactive CD4+ T_H1 cells and induction of tolerance. In chronic experimental autoimmune encephalitis, in a mouse model of

autoimmunity, CD8+ CD28− T cells have been shown to function as regulatory cells. They seem to act through a direct cell-to-cell contact mechanism to inhibit upregulation of costimulatory molecules on APCs and thereby inhibit activation and clonal expansion of CD4+T_H1 cells.[56] Evidence for regulatory function of this cell population has also been noted in limited human studies.[43, 57] The mechanisms by which such a T_{REG} cell population may function remain under investigation. Possible mechanisms are direct interaction with CD4+ cells and by cytokine secretion.[58] Finally, preliminary studies suggest that hOKT3γ1(Ala-Ala) may also induce a CD8+ regulatory cell population.[46] In vitro studies with T cells from normal volunteers showed that treatment with this agent induced continuous proliferation of CD8+ cells, with either limited or no proliferation of CD4+ cells. The CD8+ cells appeared to regulate CD4+ proliferation, which was dependent on the presence of APCs.

1.3.4 Other Mechanisms of Antithymocyte Globulin Action

Antithymocyte globulin has been shown to induce changes in the functional properties of T cells by several additional mechanisms. In cynomolgus monkeys, Preville and colleagues[59] noted ATG coating of T cells and downregulation of surface expression of CD2, CD3, CD4, and CD8 molecules, along with impaired responses in mixed lymphocyte reactions. Merion and colleagues[60] exposed purified T cells taken from healthy volunteers to ATGAM in vitro and studied T cells from renal transplant recipients exposed to ATGAM in vivo. They found that ATGAM induces partial T-cell activation, which leads to an anergic state. Similar findings have been noted with anti-CD3 monoclonal antibody therapy in vitro.[61] In addition, antibodies to leukocyte adhesion molecules in ATG may interfere with T-cell migration to sites of inflammation.[62] In in-vitro studies, ATG interferes with the main leukocyte surface molecules involved in cellular adhesion and endothelial interactions, including dose-dependent downmodulation of cell surface β2 integrin.

Finally, in addition to these effects, ATG may also prevent costimulation of T cells by binding directly to APCs. Monti and colleagues[63] noted that ATG binds to human dendritic cells (DCs) in vitro and is able to induce complement-mediated lysis. Antigens recognized by ATG include CD86, CD32, CD4, CD11b, CD29, and CD51/61, some of which are shared by lymphoctyes and DCs. The ATG binding appears to be stronger in mature DCs and thus may affect T-cell activation in vivo. The authors speculate that ATG administration might lead to the lysis of mature DCs and the survival of immature DCs, which may be more tolerogenic. There is also evidence that ATG contains antibodies that cross-react with B-cell surface antigens and thereby induces activated B-cell apoptosis in vitro.[35, 64, 65] Because B cells may serve as APCs that affect T-cell activation,[66] this mechanism may represent yet another level for ATG action.

1.4 RATIONALE FOR USE OF THYMOGLOBULIN IN T1DM

The rationale for a trial using Thymoglobulin in new-onset T1DM stems from a variety of observations: the past positive experiences with ALS in animal models of diabetes mellitus (See below in section 1.5.1), the possible efficacy of ATG in an early human T1DM trial (see below in section 1.5.2.1), the successful outcomes with ATG in some human autoimmune conditions (see below in section 1.5.2.1), the large positive experience with ATG in transplantation and the possibility of tolerance induction in this setting (see below in section 1.5.2), and the fact that ATG does not appear to act via a single mechanism. Although recognized for its ability to induce lymphocyte depletion, ATG has also been shown to act at other levels, including modulation of T-cell activation, homing, and cytotoxic activities.

Because of the initial success of the anti-CD3 new-onset T1DM clinical study, the broader specificity of Thymoglobulin, a polyclonal agent, may prove to be more efficacious for induction of tolerance, thereby prolonging endogenous insulin secretion in new-onset T1DM.

1.5 PRECLINICAL AND CLINICAL EXPERIENCE

1.5.1 Preclinical Studies

It is very hard to evaluate Thymoglobulin in an animal model of diabetes because Thymoglobulin is a rabbit anti-human thymocyte preparation. To determine its efficacy in an animal model, one would need to prepare anti-thymocyte immunoglobulin against the specific animal being tested. Even then, it would not be clear whether T1DM in that particular animal model is truly representative of human disease.

Nonetheless, investigators have pursued this agent in a related manner, evaluating the efficacy of ALS in rodent models of T1DM. ALS treatment of NOD mice induced remission in 76% of affected animals for up to 220 days when treatment was within 14 days from the onset of T1DM.[23] In follow-up studies, Ogawa and colleagues noted that 40% of NOD mice with new-onset diabetes achieved complete remission when treated with a brief course of ALS alone, and 88% entered remission when treated with ALS plus exendin-4.[24] Similarly, ALS treatment of biobreeding rats induced remission in 36% of animals with recent onset T1DM for at least 60 days and prevented development of the disease in all rats receiving medication.[21] Recent studies with an anti-CD4–depleting monoclonal antibody in NOD mice have extended these initial observations:[22] a short course of this agent soon after T1DM-onset induced remission for more than 100 days and also prevented destruction of syngeneic islet grafts, whereas costimulatory blockade with anti-CD154 and CTLA4Ig was ineffective. The window of opportunity was quite narrow with anti-CD4 because treatment at more than 1 week after the onset of T1DM was ineffective, which may be of importance in evaluating the aforementioned ALS studies. A similar narrow window of efficacy was noted with anti-CD3 studies in NOD mice.[20] Thus, at the dosing schedule and timing used, the results with ALS in new-onset T1DM are encouraging in rodent models and support proof-of-principle for the use of Thymoglobulin in humans with new-onset T1DM.

Finally, extensive studies with ATG in a nonhuman primate model demonstrate its effectiveness as a single agent in T-cell depletion in blood and peripheral lymphoid organs, and in prolonging allograft transplantation of the skin and heart.[59]

1.5.2 Clinical Studies

1.5.2.1 *Antithymocyte Globulin in T1DM and Other Autoimmune Conditions*

Experience with ATG in human new-onset T1DM is limited. In the 1980s, two studies of ATG were performed in humans with new-onset T1DM. Eisenbarth and colleagues[41] noted that equine ATG showed promise in prolonging the honeymoon phase: Four of five treated subjects exhibited a lower HbA_{1C} on < 0.2 U/kg/day of insulin 100 or more days following ATG administration, and two subjects did not require exogenous insulin for more than 8 months.

Another trial in which four subjects with new-onset T1DM were treated with RATG for 2–4 weeks, plus a more protracted course of prednisone (mean duration of 10 weeks), did not appear to be efficacious.[67] However, subjects treated with RATG plus corticosteroids were noted to have enhanced T-cell suppressor activity in a co-culture system using T-cell-dependent pokeweed mitogen for in-vitro immunoglobulin secretion from B cells, and this effect lasted up to 3–6 months following therapy.

Reported interim data from an ongoing study show promising results for using ATG in patients with newly diagnosed T1DM.[42] In this randomized, placebo-controlled, single-blinded trial with RATG (ATG-Fresenius, Germany), participants 18–35 years of age are receiving a total dose of 18 mg/kg of ATG, administered in four infusions. Of the 17 study participants treated, 11 received drug, and 6 received placebo. Two treated subjects have achieved insulin remission (defined as no need for

insulin therapy for at least 1 month and a fasting glycemia below 7 mmol/L while on a diet that includes approximately 225–300 g of carbohydrates per day). There was a significant increase in glucagon-stimulated C-peptide levels, a lower insulin requirement, and lower glycosylated hemoglobin in the ATG group, but not in the placebo group, from baseline to 12 months. Adverse events in the treatment group have included fever, phlebitis at the infusion site, and fever with arthralgia 9–11 days after the first ATG dose, which is consistent with serum sickness. Patients were not pretreated with any medications before ATG administration, and all subjects improved spontaneously without glucocorticoid therapy. No adverse events occurred later than 1 month after study entry, and there has been no change in CMV antigen status. Data directly comparing efficacy of Thymoglobulin and ATG-Fresenius are not available. However, it is believed that ATG-Fresenius has several-fold lower activity than Thymoglobulin based in part on a flow cytometry–based assay that compared activities to specific CD antigens found on the surface of peripheral blood lymphocytes,[68] and based on other references mentioned below.[69-72]

Antithymocyte globulin has also been used to treat other autoimmune conditions, either alone or in combination with other agents. The positive experiences with ATG and related products include treatment of multiple sclerosis, rheumatoid arthritis, Wegner's granulomatosis, systemic lupus erythematosus, aplastic anemia, scleroderma, and myelodysplastic disorders.[73-84]

Although there are limited reports of long-term remissions for many different autoimmune conditions, the largest experience to date is with aplastic anemia. Forty percent to 70% of patients entered remission following a single course of ATG.[85] Although one third of these patients subsequently relapsed, a second course of ATG induced remission in up to 77% of them. ATG is now often used in combination with cyclosporine, and up to 80% of patients achieve remission.[86] Short-term results improve with this combination therapy, whereas long-term results, particularly overall survival, do not and are similar to those achieved by ATG alone.[42, 74] Long-term studies show that 38% of patients treated with ATG relapse by 11 years whether or not they receive cyclosporine.[74]

1.5.2.2 *Antithymocyte Globulin in Transplantation*

Rabbit ATG has enjoyed widespread use in organ transplantation (e.g., pancreas[87, 88] and some current islet transplantation protocols), and is now a routine component of therapy for both induction and rejection. Gaber and colleagues[89] noted that Thymoglobulin had a higher rejection reversal rate than equine ATG (ATGAM), and recurrent rejection occurred less frequently with Thymoglobulin (17%, versus 36% in the ATGAM group at 90 days after therapy). Brennan and colleagues[90] compared Thymoglobulin with ATGAM as an agent for initial induction immunosuppressive therapy for renal transplantation and found that 4% of Thymoglobulin-treated subjects, compared to 25% of ATGAM-treated subjects, experienced acute rejection. In addition, the Thymogloblin group had a lower rate of acute rejection and less severe rejection than the ATGAM group. Furthermore, the mean number of infections and severe adverse events was lower in the Thymoglobulin group. Significantly improved outcomes in the Thymoglobulin-treated group, as compared with the ATGAM-treated group, continue to be noted in this cohort, now out to 5 years. Thymoglobulin has been widely and successfully utilized in a number of other organ transplantation settings, including heart and lung, intestine, and bone marrow.[71, 87-109] Thymoglobulin has also been used for steroid-resistant, acute graft versus host disease.[110]

1.5.2.3 *Antithymocyte Globulin and Induction of Immune Tolerance*

Antithymocyte globulin has most often been used in combination with other agents for various indications, including transplantation, making it difficult to assess its potential role as a single agent in a clinical trial for new-onset T1DM. However, more recent studies in transplantation suggest that ATG may function at least as a partial tolerizing agent because only limited maintenance therapy is needed following induction therapy with ATG.[106, 107, 111]

Starzl and colleagues reported a protocol in which transplant recipients receive preconditioning with Thymogloblin, followed by twice-daily tacrolimus maintenance therapy, with attempts to later wean the tacrolimus to a more limited spaced-maintenance therapy. They noted that 43 of 72 recipients with surviving grafts (liver, kidney, pancreas, and intestine) could be weaned to a less frequent tacrolimus dosing interval. In fact, 11 recipients were weaned to an interval of once per week.[106]

In a larger study of 150 consecutive renal transplant patients treated according to this protocol, 63% of patients were successfully weaned to doses ranging from every other day to once a week.[111] Similar success was recently reported in pediatric kidney transplantation.[112]

1.5.2.4 *Antithymocyte Globulin in Pediatric Patients*

Although ATG has not been studied in the pediatric population in controlled clinical trials, it has been widely used in the pediatric population in transplant and autoimmune settings.

A review of 4,898 pediatric patients who received renal transplants between 1987 and 1996 shows that the polyclonal T-cell antibody ATG/ALG has been used as an induction regimen for up to 47% of cadaver donor and 37% of living-donor recipients.[113] Other published data on the use of ATG in pediatric patients are from studies in renal transplantation,[112, 114-119] liver transplantation,[118, 119] heart transplantation,[118-123] aplastic anemia,[124-126] and bone marrow transplantation.[127] A review of these publications shows that ATG has generally been well-tolerated and efficacious; 583 patients ranging in age from 12 days to 20 years received ATG in these studies. The dose range was from 0.5–15 mg/kg/day, and the duration of therapy was anywhere from 1 to 90 days. ATG was given to pediatric patients as part of an immunosuppressive regimen with other agents. No cases of anaphylactic reaction, serum sickness, or severe infusion reaction were reported. One case of lymphoma was reported in a cardiac transplant patient 5 months posttransplant.[122] Thirty cases of posttransplant lymphoproliferative disease (PTLD) were reported in a retrospective study of 241 pediatric patients who received prophylactic T-cell depletion by ATG or antilymphocyte globulin (ALG) before bone marrow transplantation.[127] In other studies, only one case of PTLD was reported in a cardiac transplant patient.[121, 123]

1.6 KNOWN AND POTENTIAL RISKS AND BENEFITS TO HUMANS

1.6.1 General

Antithymocyte globulin, and more specifically Thymoglobulin, has been widely used in transplantation and autoimmune disorders, and the medical community has extensive knowledge of its potential safety concerns. We expect that side effects will be minimized in this study because of the modest dose used, the absence of other immunosuppressive medications, and the lack of severe medical conditions other than T1DM. Although Thymoglobulin has been widely used in pediatric transplant and aplastic anemia patients, its safety and efficacy has not been established in T1DM pediatric patients in controlled clinical trials.

Moderate fever and chills, usually with the first infusion, are the most common adverse events observed after administration of ATG.[95] Immediate allergic responses are anaphylactic or anaphylactoid-type reactions, and delayed allergic responses are serum sickness or serum sickness-like symptoms (fever, pruritus, and rash associated with arthralgia or myalgia). Premedication with glucocorticoids, acetaminophen, and antihistamines is helpful. Malaise, dizziness, and leukopenia were the only adverse events shown to be significantly higher with Thymoglobulin than with ATGAM administration in a U.S. phase III controlled trial. Thrombocytopenia has also been seen but is not clinically significant. Although infection is expected with immunosuppressive therapy, it is rarely noted in spontaneous reporting. Reports in the literature include both high and low rates of infection for ATG, and lower rates of CMV for ATG than for OKT3.

Thymoglobulin can stimulate the production of antibodies that cross-react with rabbit immune globulins. Skin testing is not recommended for subjects who receive ATG because it poses the same risks as the treatment itself and thus does not offer any therapeutic or safety benefit.

The carcinogenic effects and the effect of Thymoglobulin on fertility have not been established.

1.6.2 Cytokine Release Syndrome

Thymoglobulin infusion, particularly the first dose, is often associated with cytokine release syndrome (CRS) and resultant transient inflammatory reaction with fever and chills. Common symptoms may include high fever, chills, rigors, headache, tremor, nausea, vomiting, diarrhea, abdominal pain, muscle and joint pain, and malaise. The incidence and severity of these adverse reactions may be decreased with premedication.[34] The most severe adverse reactions, such as life-threatening anaphylactic shock, have rarely been reported for Thymoglobulin.

1.6.3 Serum Sickness

Occasionally Thymoglobulin causes delayed allergic reactions such as serum sickness. Serum sickness is due to host immunization against rabbit protein and tends to occur 7–15 days after the onset of treatment. The symptoms include fever, skin rash, itching, hives, joint pain, malaise, and lymphadenopathy. The clinical outcome is favorable, resolving either spontaneously or with a brief course of corticosteroid therapy.

1.6.4 Leukopenia and Thrombocytopenia

Thymoglobulin, which contains a variety of antibodies that may cross-react with cell-surface markers on cell types other than lymphocytes, has been reported to cause leukopenia and thrombocytopenia. These side effects are dose-dependent and are mainly encountered with overdosage. The investigator's brochure notes that approximately 3% of Thymoglobulin-treated subjects developed severe thrombocytopenia.[34] However, these patients were receiving approximately twice the dose proposed for this study. Furthermore, thrombocytopenia in these subjects often occurred in a postoperative transplant setting and with other immunosuppressants, both of which could affect platelet count. Platelet transfusions were rarely required because the platelet count increased spontaneously after the dose was reduced or held. With the relatively low dose used in this trial and the lack of other immunosuppressants, thrombocytopenia would be an unlikely adverse event. Neutropenia, leukopenia, and thrombocytopenia will be closely monitored and are part of a select list of adverse events that comprise the major safety endpoint of the trial (see section 3.2.2.2).

1.6.5 Infection

Another potential risk from T-cell depletion by ATG is the increased risk for opportunistic infections. ATG induces marked and acute depletion of lymphocytes, and the circulating number of T cells gradually increases with cessation of therapy, usually reaching pretreatment levels by 2 months. The study participants may be vulnerable to opportunistic infection during this window of recovery and will require close surveillance.[128, 129] Please refer to sections 5.9 and 5.10 for prophylactic medications used in this trial.

1.6.6 Liver Toxicity

Transient abnormalities in liver function tests have been described in patients with aplastic anemia treated with Thymoglobulin or other ATG preparations.[86, 130] Such adverse events have not been noted in numerous other clinical settings in which Thymoglobulin or related agents have been used, and it is not clear if this is related to the underlying disease or to associated medications used in this particular

study rather than to Thymoglobulin itself. No hepatotropic virus or other infectious agent accounted for these problems.

1.6.7 EBV-associated Lymphoproliferative Disease

Immunosuppressive agents are associated with an increased risk of malignancy, particularly from EBV and PTLD. Two retrospective studies looking at the risk of PTLD in kidney transplant patients have found different results. Bustami and colleagues have found an increased risk of PTLD, while Cherikh and colleagues found no significant increase in risk of PTLD in patients receiving ATG in kidney transplantation from the United Network for Organ Sharing.[131, 132] It is difficult to resolve the differences from these studies, though they did have different inclusion criteria. It is important to note that limitations and potential bias may arise in retrospective registry evaluations. In particular, this registry does not include the actual dose of medications used, and only recently began collecting data on antiviral prophylaxis. Furthermore, the studies did not account for how rejection therapy might have factored into the PTLD risk, and neither study stratified its analysis by year of transplant (though Bustami and colleagues[131] clearly noted that the PTLD risk had been decreasing over time). Finally, it should be noted that the overall PTLD risk in this transplant population was low, ranging from 0.25%–0.85%, and that patients received a host of other immunosuppressive therapies and were on continuous immunosuppression over time.

However, the risk for EBV-associated lymphoproliferative disease is expected to be minimized in this trial for the following reasons:

- Unlike in the transplant setting, Thymoglobulin will be used as a single agent for a brief time without ongoing immunosuppression. No PTLD cases have been reported in patients receiving ATG alone, such as those with aplastic anemia (see section 1.6.8), or in the most recent studies by Starzl and colleagues,[106] where ATG has been used at lower doses (comparable to the dose proposed for this study) concomitantly with other immunosuppressive agents.
- ATG does not activate B cells but rather prevents B-cell proliferation and differentiation.[133] ATG has also been shown to exhibit a selective anti-proliferative effect towards most of the EBV-infected human lymphoblastoid and Burkitt's lymphoma cell lines.[133] Thymoglobulin has specifically been shown to induce apoptosis in EBV-infected B-cell lines.[34] This effect may serve to minimize rather than increase the risk of PTLD as is commonly observed with other immunosuppressive agents.[89, 90]
- Patients without evidence of prior EBV infection are excluded from this study. This will decrease the chances of EBV-associated lymphoproliferative disease in case patients become primarily infected by EBV during T-cell depletion.

1.6.8 Long-Term Safety of ATG in Aplastic Anemia

Long-term safety data is available from patients with aplastic anemia (AA) treated with ATG. AA is defined as pancytopenia with a hypocellular bone marrow, and thus at baseline these individuals are at significantly higher risk for infection, hemorrhage, and mortality than the subjects with T1DM who will enter this study. Previously treated patients with AA have usually received equine ATG, because this product received the primary indication for immunosuppressive therapy for AA. In more recent studies, many subjects also receive combination therapy with other agents (cyclosporine, danazol, and/or granulocyte-colony stimulating factor [G-CSF]). It is also notable that many such patients have abnormal cytogenetic clones at diagnosis, especially abnormalities of chromosome 5 and 7. The frequency of such abnormalities has been estimated to be 11%,[134] though the diagnosis may be difficult with a hypocellular bone marrow that has an insufficient number of cells in metaphase. Patients may also have a coexisting paroxysmal nocturnal hemoglobinuria (PNH) clone, with deficient expression of phosphatidylinositol glycan (PIG)-anchored proteins.

Treated AA patients have been noted to be at cumulative later risk of clonal disease: with up to an 11-year follow-up, between 5% and 10% develop myelodysplastic syndrome or acute myelogenous leukemia, and 10%–15% are noted to have paroxysmal nocturnal hemoglobinuria (PNH).[74, 79, 135-139] The 10-year actuarial risk for solid tumors has been reported to be 2%–11%.[74, 137, 139] Although one cannot exclude a primary effect of the immunosuppressive therapy on the later risk for cancer, many consider it most likely that these occurrences represent an emergence of one or more abnormal clone(s) following immunosuppressive therapy or that the increased survival time may unveil the natural history of aplastic anemia as a premalignant disease.[134, 135] The strongest potential risk factors for these complications, which have been identified by multivariate analysis, include the year of therapy, splenectomy, treatment with multiple courses of immunosuppressive therapy, and concurrent use of other agents, such as G-CSF and cyclosporine.[136, 138, 139] It should be noted that these specific issues with clonal disease have not been noted in other settings in which ATG has been used (such as transplantation or other autoimmune diseases). Moreover, it has also been observed in subjects who received alternate immunosuppressive therapy to treat AA.[140]

Long-term survival is variable, depending on the severity of disease at diagnosis, neutrophil count after immunosuppressive therapy, and year that treatment was initiated;[85, 141] a 5-year actuarial survival in the European cohort has been reported at 87%[141] and 54% at 11.3 years in the German cohort.[74] Many patients remain neutropenic and/or thrombocytopenic before and following immunosuppressive therapy for AA. Thus, infectious diseases and hemorrhage remain ongoing and potentially life-threatening concerns.

From extensive review of the AA literature, no reported cases of EBV-associated lymphoproliferative disease have been reported in patients treated with ATG.[74, 77, 78, 136-138, 140, 142-148] This evaluation represents a cumulative sum of 1,675 treated subjects, many receiving additional immunosuppressive agents, such as cyclosporine, over a longer time course. It is notable that most of these reports included children, and some were exclusively pediatric cohorts.

1.6.9 Benefits

In this study, all participants will receive intensive diabetes management aimed at achieving near-normal metabolic control per the standard American Diabetes Association guidelines. The Diabetes Control and Complications Trial (DCCT) research group documented that improved metabolic control lowers the risk for long-term complications.[4] The means to achieve this improved control in the DCCT has become the idealized standard of care, with clinical management and education provided by a diabetes specialty team. It should be noted that such care may not necessarily be available to those outside the study, and those who are not seen by a diabetes specialty team may have worse outcomes over time.[149, 150]

Improved metabolic control early in the course of T1DM will have a long-standing effect on lowering the risk for long-term complications for many years to follow; i.e., there appears to be a "metabolic memory" that influences later risk. This effect has been documented in the Epidemiology of Diabetes Interventions and Complications (EDIC) study, the long-term follow-up of the DCCT cohort.[4, 151-153] Although the conventional group (with less stringent metabolic control) and the intensive group (with near-normal metabolic control) have had comparable HbA_{1C} levels since the end of the formal DCCT study, the intensive group continues to have significantly lower risk for complications 10 years later.

An additional benefit that may be realized by all participants is that maintaining near-normal glycemic control through intensive diabetes management may by itself lead to the preservation of beta-cell function.[11, 154] The benefits of endogenous insulin secretion, even if one needs to continue exogenous insulin therapy, have been demonstrated in a number of studies, including the DCCT, where those subjects with residual C-peptide had improved metabolic control, with lower risk for severe hypoglycemia and less likelihood of microvascular complications.[4] Finally, the treatment

group may have significant benefits from participation even if Thymoglobulin therapy has only modest effects on the preservation of endogenous insulin secretion, which is what was noted in the new-onset T1DM study with anti-CD3 therapy.[32, 153]

1.7 RATIONALE FOR STUDY DESIGN

1.7.1 Overview

The clinical outcome variable proposed for clinical protocol ITN028AI is a serial assessment of endogenous insulin secretion, which is measured by C-peptide area under the curve (AUC) secreted in response to a 4-hour mixed-meal tolerance test (MMTT). The primary endpoint of clinical protocol ITN028AI (section 3.2.1) is in accord with an American Diabetes Association (ADA) workshop[155] and the TrialNet consensus guidelines for new-onset T1DM studies, following review of other new-onset T1DM trials,[32, 154, 156] and extensive discussion by the TrialNet steering committee. Other clinical outcome measures of efficacy include insulin use, HbA_{1C}, and major hypoglycemic events, all of which support the primary analysis. The safety of patients in this study will be closely monitored. Adverse events closely related to Thymoglobulin have been selected as the secondary endpoints for safety (section 3.2.2.2).

Participants in clinical protocol ITN028AI will be randomly assigned to either the placebo group or the Thymoglobulin group. The inclusion of a placebo group in this study will provide comparable data on the clinical and immunologic outcome variables.

For clinical protocol ITN028AI, we plan to recruit participants between 12 and 35 years of age. T1DM can occur at any age, but it occurs with dramatically increased incidence in children. The average age of children at presentation of this disease is approximately 13 years, and a great majority of individuals present when they are under the age of 18 years. The incidence of T1DM is increasing at approximately 3% per year, mainly in the younger population. Thymoglobulin has been safely and effectively used in children for transplantation and treatment of some autoimmune diseases. The dosing used in children, as well as the safety spectrum, appears to be similar to that used in adults. The collective clinical experience and safety information for Thymoglobulin is significantly greater than that for other agents currently under experimental use for treatment of new-onset T1DM in children, such as hOKT3γ1(Ala-Ala) and a related anti-CD3 monoclonal antibody used in Europe.

We will enroll participants in clinical protocol ITN028AI within 100 days of their diagnosis. Studies in animal models of diabetes, such as the NOD mouse model, suggest that early intervention is critical in effecting long-term remission.[20] It is unclear how such a timeline may translate into clinical studies. Past studies with cyclosporine in new-onset T1DM suggested that earlier initiation of drug therapy, at 6 weeks or earlier, is more likely to lead to clinical remission.[26, 156-158] In a Phase I/II study with hOKT3γ1 (Ala-Ala), Herold and colleagues noted that enrollment by 6 weeks and receipt of study drug by 8 weeks from diagnosis resulted in stabilization of endogenous insulin secretion in the active group participants as compared to controls.[32] Chatenoud and colleagues reported similar findings using treatment with a related monoclonal anti-CD3 monoclonal antibody by 4 weeks from diagnosis.[159] Finally, pilot study results with ATG-Fresenius suggest efficacy in preserving endogenous insulin secretion when drug is administered by 4 weeks from diagnosis.[42] However, other studies suggest that such a stringent window for intervention may not be necessary. Ludvigsson et al. noted that glutamate decarboxylase treatment offered within 6 months from the onset of T1DM diminished the rate of loss of endogenous insulin secretion.[160] Currently, according to study listings posted on ClinicalTrials.gov, all new onset type 1 diabetes trials conducted by TrialNet enroll patients within 3 months of diagnosis. Furthermore, no benefit has been noted among early versus later enrollees in the two trials completed to date with Mycophenolate Mofetil with or without Daclizumab and Rituximab (Gottlieb and Perscovitz, personal communications, manuscripts in preparation). Two large, pharmaceutical company sponsored, phase III trials with different anti-CD3 monoclonal

antibodies (ClinicalTrials.gov identifiers NCT00385697and NCT00678886) are now enrolling subjects within 3 months of diagnosis, and a third study (ClinicalTrials.gov identifier NCT00378508) is evaluating the efficacy of hOKT3γ1(Ala-Ala) for subjects enrolled from 4 to 12 months from T1DM diagnosis. From extensive studies of the natural history of progression to type 1 diabetes in first degree relatives, we now know that this autoimmune destruction is occurring over months, and sometimes 10-20 years[5] and thus one would not expect that a difference of several weeks would ultimately determine whether or not a therapy would be effective. Moreover, we must also balance our enthusiasm for early enrollment into these trials with the feasibility for early recruitment. Possible subjects may not hear about clinical trials in the early weeks, and may not be prepared to commit to a trial until they have had some initial time to adjust to the diagnosis. Extending the enrollment window will not preclude earlier trial enrollment, and in fact we will also eliminate the requirement that the screening MMTT occur no sooner than 21 days after diagnosis so that interested subjects can screen and enroll any time from their initial diagnosis. We will plan to conduct a sub-analysis to evaluate the role of time from diagnosis on efficacy.

It is important to consider whether the degree of C-peptide decline in the placebo group with optimal diabetes management is significant enough during the first year of diagnosis to detect a difference between the Thymoglobulin group and the placebo group. Steele and colleagues demonstrated that those with new-onset T1DM have steady, progressive loss in beta-cell function from the time of diagnosis, as assessed by C-peptide AUC in response to an MMTT, the measure to be employed in this study.[8] This finding has been corroborated by others, though some have noted no significant decline from diagnosis to 12 months but a subsequent decline from months 12 to 24.[161, 162] This consideration must be balanced by the most likely time for the Thymoglobulin effect from a single course of therapy. A 12-month primary endpoint has been utilized and appears suitable for comparable studies that may function by related mechanisms—namely, the hOKT3γ1(Ala-Ala) phase I/II study,[32] a related anti-CD3 monoclonal antibody study in Europe[159] and the ongoing study with ATG-Fresenius.[42] A new-onset T1DM study with the antigen heat shock protein 60 also demonstrated efficacy at 10 months in comparison to the control group.[163] With a primary endpoint at a significantly later time point, such as 24 months, there may be more issues with study dropouts over time.

Finally, clinical protocol ITN028AI remains an early attempt to evaluate both the safety and possible efficacy of Thymoglobulin. If we were to choose a later primary endpoint, such as 24 months, we would not have any insight into efficacy for at least 4-5 years (given the time to full enrollment and patient follow-up), which will delay plans for modifications and new trials in the near future. Although we are hopeful that Thymoglobulin will prove effective, we anticipate that it may ultimately need to be coupled with agents that work via alternate mechanisms, such as ATG plus a glucagon-like peptide-1 receptor agonist (which is extremely effective in inducing remission in NOD mice with new-onset T1DM)[24] or an antigen. We plan to follow subjects at 6-month intervals until 24 months from initial therapy, and for those who have measurable C-peptide, we may extend clinical protocol ITN028AI to do an on-going yearly evaluation thereafter for up to 5 years.

The tolerogenic effect of Thymoglobulin will also be assessed during the follow-up phase (months 12–24). Preservation of C-peptide levels at the end of the follow-up phase in the Thymoglobulin group will be an indication of whether Thymoglobulin induces tolerance. A host of immunologic assays performed throughout the study will also provide insight into the mechanism of Thymoglobulin action and its possible tolerogenic properties. Particular attention will be placed on the re-emerging population of T cells following initial depletion since one hypothesis is that the emerging population of T cells will be less autoreactive than the original population of T cells and have a higher proportion of T_{REG} cells. (Refer to section 7 for a complete discussion of immunologic assays.)

To minimize development of PTLD, participants who are seronegative for EBV will be excluded from clinical protocol ITN028AI. It is believed that more than 90% of adults are EBV seropositive,[164] and a recent study has shown that in the U.S. 64% of children 6 months to 17 years of age are seropositive for EBV and that this percentage increases with age.[165] Therefore, this exclusion criterion should have minimal effect on recruitment.

1.7.2 Rationale for Thymoglobulin Dose

The total dose of Thymoglobulin to be administered for clinical protocol ITN028AI is 6.5 mg/kg. This and higher doses have been effective and well-tolerated by subjects in other studies cited below, and is within the safety experience of the drug. In France, the package insert for rabbit anti-human thymocyte immunoglobulin (Thymoglobuline) allows for a cumulative dose of 12.5 to 17.5 mg/kg in aplastic anemia and 4.5 to 21.0 mg/kg in acute graft rejection.[110] The daily recommended dose is 1 to 1.5 mg/kg/day for transplantation and 2.5 to 3.5 mg/kg/day for aplastic anemia.[110] This dosing regimen is further supported by the positive experiences in human bone marrow transplantation, where the dosing schedule improved early tolerability of the drug in that setting.[94, 166] The total dose to be utilized is further supported by the experiences of the Starzl group in organ transplantation.[106, 111] In addition, this total dose is supported by studies with Thymoglobulin in a nonhuman primate model, in which a comparable dose resulted in T-cell depletion in blood and peripheral lymphoid organs.[59] Further, this dose resulted in functional alterations in the remaining T cells which were coated with antibody. Overall, this dose resulted in prolonged allograft survival.[59] Although some transplant protocols have incorporated ATG dose adjustment based on peripheral CD3 counts,[102, 104, 108, 167] this approach ignores the potential benefits of depletion in peripheral lymphoid tissues. Further, this approach has not been adequately assessed for efficacy, in particular not in autoimmune settings.

Although the recommended daily dosage of Thymoglobulin for treatment of acute renal graft rejection is 1.5 mg/kg, many investigators have routinely used higher daily doses safely and effectively in other settings. These experiences include 2.5 to 3.5 mg/kg or higher in aplastic anemia[110, 168, 169] and up to 6 mg/kg/day in myelodysplastic syndrome.[170]

Finally, the total proposed dose is comparable to the dose used by Saudek et al in their on-going trial with ATG-Fresenius, in which they have early pilot results suggesting efficacy.[42] The potency of ATG-Fresenius translates to roughly 2-4 times that of Thymoglobulin.[69, 70, 72, 171] Although both are rabbit ATG products, they are derived from different preparations, namely purified thymocytes for Thymoglobulin versus the Jurkat T-cell line for ATG-Fresenius. Thus, it is not surprising that these agents have differing activities, and potencies. Furthermore, the variations in dose and duration of ATG therapy between treatment centers and study protocols make it difficult to find an exact dose equivalence between Thymoglobulin and ATG-Fresenius.[70] Nonetheless, from the data available to us, the dose of 18mg/kg total dose of ATG-Fresenius in the Saudek et al study[42] compares favorably to our proposed dose of 6.5 mg/kg.

There is extensive experience using Thymoglobulin without glucocorticoid premedication.[95, 116, 172-175] It appears that side effects can be minimized by slow Thymoglobulin infusion and premedication with antihistamine and acetaminophen.

In the current trial the initial intent was to avoid glucocorticoids unless necessary in order to 1) minimize the effects on glycemic control (although this will be transient, and can be controlled with increases in exogenous insulin); 2) minimize potential negative effects on beta-cell function, and 3) most importantly, to minimize exposure to any concomitant medications that may interfere with tolerance induction

In this regard, it is important to note that the preclinical animal studies and the clinical trial with ATG-Fresenius in new-onset T1DM were *not* conducted in the presence of glucocorticoid premedication.[21, 24, 42, 59]

Furthermore, it is notable that in NOD mice with recent-onset T1DM, a short course of treatment with an anti-CD3 monoclonal antibody induces a lasting, durable remission; however, a single dose of cyclophosphamide blocks tolerance induction in the NOD mouse treated with anti-CD3 monoclonal antibody.[176]

However, experience with early participants in this trial led to a change in approach. Two early subjects had significant infusion reactions characterized by fever, nausea, rash, and headache. One subject had cytokine release syndrome graded as severe (grade 3). As specified in section 5.3.1.3 study medication was permanently discontinued. It was concluded that the use of rabbit ATG at the dose and schedule in this trial without steroid premedication was not sufficiently well-tolerated to be acceptable. For this reason premedication with steroids was added to the study regimen for participants randomly assigned to ATG.

1.7.3 Measures to Minimize Bias

Potential bias is minimized because of the following:

- The objective nature of the primary outcome variable.
- An unbiased assessment of C-peptide by laboratory personnel blinded to the source of C-peptide.
- Clinical study personnel remaining blinded to the C-peptide results.
- Participants and diabetes management teams will be blinded to study drug.

2. OBJECTIVES

2.1 PRIMARY OBJECTIVE

- To assess the effects of a single course of Thymoglobulin on preserving beta-cell function in T1DM.

2.2 SECONDARY OBJECTIVES

- To assess other diabetes measures of efficacy.
- To assess the safety of Thymoglobulin in patients with T1DM.
- To assess potential tolerogenic modifications of the immune response by Thymoglobulin.

3. STUDY DESIGN

3.1 DESCRIPTION

Clinical protocol ITN028AI is a multicenter, double-arm, blinded, placebo-controlled, 2:1 randomized, phase II clinical trial in individuals with new-onset T1DM.

Participants will be blinded and randomly assigned to receive either Thymoglobulin or placebo. The Thymoglobulin group will receive one course of Thymoglobulin, the placebo group will receive one course of matching saline solution.

Thymoglobulin administration can elicit adverse reactions during infusion that may potentially unblind patients and caregivers. To correct for this possibility, the study design includes two sequential patient-care teams, an unblinded study-drug administration team (for the first 8 weeks), and a blinded diabetes management team (for the remainder of the study). Both teams will meet the participants prior to random assignment. The study-drug administration team will care for participants during their hospital stay and drug infusions through week 8 of the study and will remain involved in

non-diabetes-related issues thereafter. After week 8, the diabetes management team will assume care of the participants' diabetes care and the study drug administration team will no longer be involved in diabetes management. The study drug administration team will continue to receive and review all laboratory studies not directly related to the diabetes care beyond week 8, and will manage any possible issues related to drug administration. After week 8, if participants develop serious infectious complications, the study drug administration team will evaluate and manage the participant and will be free to consult with infectious disease experts as necessary.

Both treatment and placebo groups will undergo identical procedures and will be followed for 24 months; if they preserve any beta-cell function, they will be followed longer for up to 60 months. The primary endpoint will be measured at 12 months. Safety, diabetes control, beta-cell function, and immune function will be assessed for 24 months. Participants will be asked to contact the study site for a period of up to 5 years after study entry in case of secondary malignancies. Both groups will receive intensive diabetes management.

A major goal of clinical protocol ITN028AI is to obtain immunologic evidence that supports a state of clinical and immunologic tolerance, i.e. to determine if a short-term drug therapy results in the halting or elimination of autoimmune destruction of beta cells without ongoing therapy. During the follow-up phase, participants will undergo clinical and immunologic assessments similar to the ones they underwent during the first 12 study months. Possible mechanisms of Thymoglobulin action will also be assessed. (Refer to section 1.7 for more discussion regarding the study design.)

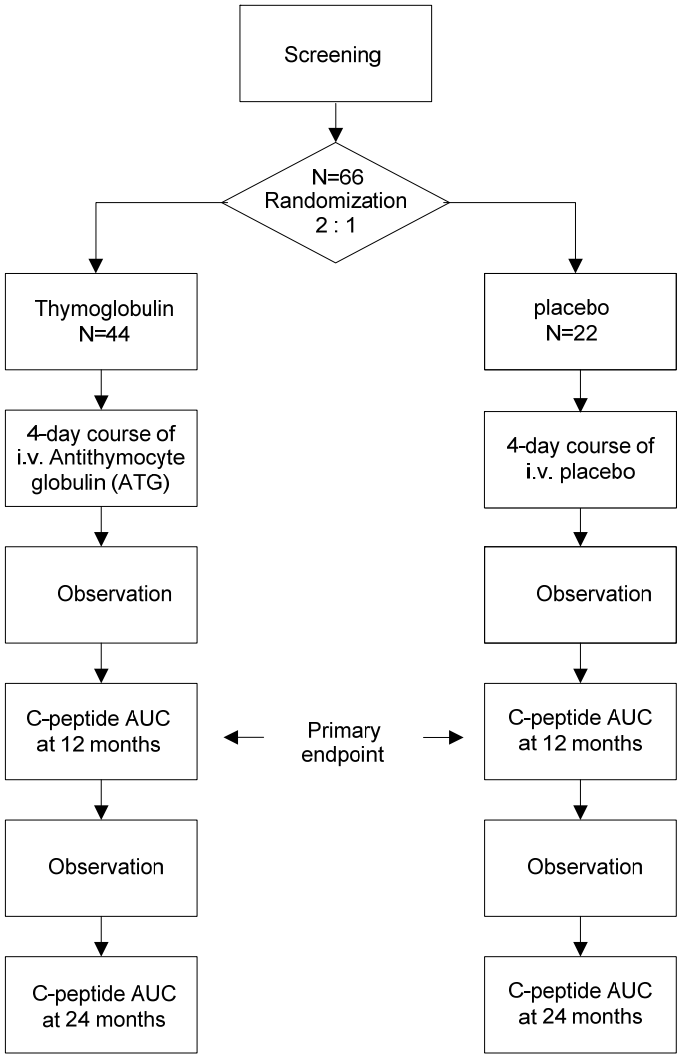

Figure 1. Trial scheme

3.2 STUDY ENDPOINTS

3.2.1 Primary Endpoint

- A 2-hour C-peptide AUC in response to an MMTT at month 12.

3.2.2 Secondary Endpoints

3.2.2.1 *Efficacy*

- A 4-hour C-peptide AUC in response to an MMTT at month 12.
- Insulin use in units per kilogram body weight per day at months 12 and 24.
- Proportion of subjects who are exogenous-insulin-free at 12, 18, and 24 months; possibly up to 60 months.
- Major hypoglycemic events occurring from randomization to months 12 and 24.
- A 2-hour and 4-hour C-peptide AUC in response to an MMTT at month 24.

- HbA_{1C} levels at months 12 and 24.
- Changes of C-peptide AUC (2 and 4 hours) over time at months 12 and 24.

3.2.2.2 *Safety*

- The rate of the following adverse events in participants receiving Thymoglobulin or placebo:
    - Infusion reactions
    - CRS
    - Opportunistic infections
    - Lymphopenia
    - CD4/CD8 ratio
    - Neutropenia
    - Thrombocytopenia
    - Serum sickness
- The frequency and severity of all adverse events in participants receiving Thymoglobulin or placebo.
- Adverse event frequencies in the Thymoglobulin group will be compared with those in the control group.

3.2.3 Stopping Rules for Premature Termination of the Study

Enrollment in clinical protocol ITN028AI will be suspended and study drug will be halted if any one of the following occurs:

- The NIAID Data and Safety Monitoring Board (DSMB) requests termination of the study upon review of safety data.
- Any death related to study therapy.
- Two or more of the first 10 treated participants experience an adverse event resulting in the permanent discontinuation of study treatment, as defined in section 5.6.
- A case of significant EBV-associated lymphoproliferative disease defined as
    - lymphadenopathy (focal or diffuse), or hepatosplenomegaly, or organ infiltration, with or without fever, of 1 month or greater duration, and
    - confirmation of EBV by tissue biopsy or viral load.
- A sign or symptom of serum sickness of ≥ grade 3 severity, as defined in section 8.3.3, that does not resolve or improve to at least grade 1 severity 2 weeks after glucocorticoid treatment (see section 5.3.3).
- Grade 2 or higher renal dysfunction associated with serum sickness, as defined in section 8.3.3, that does not resolve or improve to at least grade 1 severity in 2 weeks.

The DSMB will be immediately notified of any such event. Resumption of enrollment is contingent upon a favorable DSMB review.

3.3 PACE OF ENROLLMENT AND STUDY DURATION

Enrollment will be staggered in the beginning of the study. The first 10 participants enrolled in this study will be age 18-35. These participants will be enrolled at least 2 days apart. The last subject in this cohort will be followed until day 15 – at which time the adverse event experience in this group will be reviewed by the protocol chair, the NIAID medical monitor and the ITN clinical trial physician. If this review concludes that no significant safety concerns have been identified, enrollment to include pediatric participants will commence. The first 10 pediatric participants will be enrolled at least 2 days apart. (Enrollment of adult participants will also continue.) The 10^{th} pediatric

participant enrolled will be followed until day 15 and a safety review to include the first 10 pediatric participants enrolled will be conducted as described above. If this review concludes that no significant safety concerns have been identified, enrollment will continue until the study is fully enrolled.

Study subjects will be recruited during an approximately 30-month accrual period. It is anticipated that because of the age restriction (section 4.1), enrollment of initial participants in the trial will be slower than subsequent enrollment.

Each participant will be in the study for 24 months to 60 months.

4. SELECTION AND WITHDRAWAL OF PARTICIPANTS

4.1 INCLUSION CRITERIA

1. Individuals 12–35 years of age who meet the American Diabetes Association standard T1DM criteria.[1] Note: the first 10 randomized participants will be 18–35 years of age.
2. Positive for at least one islet cell autoantibody (glutamate decarboxylase; insulin, if obtained within 10 days of the onset of insulin therapy; ICA 512-antibody, and/or ICA).
3. Diagnosis of T1DM within 100 days of enrollment.
4. Peak stimulated C-peptide level >0.4 pmol/mL or >1.2 ng/mL following an MMTT.
5. Serologic evidence of prior EBV infection.
6. Participants of childbearing age must agree to practice an effective form of birth control, which may include any one of the following: abstinence, barrier method, oral contraception, or surgery. For females, these contraceptive measures must be maintained throughout the study; for males, these measures must be followed for a minimum of 3 months following Thymoglobulin infusion.

4.2 EXCLUSION CRITERIA

1. Prior history of any significant cardiac disease such as congestive heart failure, arrhythmia, or structural defects or suspicion thereof.
2. Leukopenia (<3,000 leukocytes/μL), neutropenia (<1,500 neutrophils/μL), lymphopenia (<800 lymphocytes/μL) or thrombocytopenia (<125,000 platelets/μL).
3. Any sign of active infection (e.g., hepatitis, tuberculosis, EBV, CMV, or toxoplasmosis), or screening laboratory evidence consistent with active infection.
4. Positive for HIV, PPD, or HB_SAg at screening.
5. Prior treatment with RATG or known hypersensitivity or exposure to rabbit sera–derived products.
6. Ongoing use of diabetes medications other than insulin that affect glucose homeostasis, such as metformin, sulfonylureas, thiazolidinediones, or amylin.
7. Vaccination with a live virus within the last 6 weeks before enrollment.
8. Prior or current therapy that is known to cause a significant, ongoing change in the course of T1DM or immunologic status.
9. Evidence of liver dysfunction, with alanine aminotransferase (ALT) or aspartate aminotransferase (AST) > 3 times the upper limit of normal.
10. Evidence of renal insufficiency as indicated by serum creatinine of >2 times the upper limit of normal confirmed in a repeat test at least 1 week apart.
11. Females who are pregnant at the time of screening or unwilling to defer pregnancy during the 24-month study period.
12. Any condition that, in the investigator's opinion, may compromise study participation or may confound the interpretation of the study results.

4.3 PREMATURE TERMINATION FROM THE STUDY

Participants may be prematurely terminated from clinical protocol ITN028AI if they withdraw consent from all future study activities, including follow-up (months 13–24 or possibly 60), or if they are "lost to follow-up" (i.e., no further follow-up is possible because attempts to reestablish contact with the participants have failed). The ITN clinical trial physician and the NIAID medical monitor must be promptly informed of such events.

5. STUDY MEDICATIONS

5.1 INVESTIGATIONAL PRODUCT: THYMOGLOBULIN AND PLACEBO (SALINE SOLUTION)

5.1.1 Formulation, Packaging, Labeling, and Storage

The investigational product, Thymoglobulin® (antithymocyte globulin [rabbit], Genzyme Inc.), is a purified, pasteurized, gamma-immune globulin, obtained by immunizing rabbits with human thymocytes. Thymoglobulin contains cytotoxic antibodies directed against antigens expressed on human T lymphocytes. It is supplied as a sterile, freeze-dried product for intravenous administration after reconstitution with sterile water for injection, USP.

Thymoglobulin should be stored in a refrigerator between 2 ºC and 8 ºC (36 ºF and 46 ºF), protected from light, and should not be frozen. Reconstituted vials should be used within 4 hours. Infusion solutions of Thymoglobulin must be used immediately, and any unused drug remaining after infusion must be discarded. For reconstitution and dilution guidelines refer to the Thymoglobulin package insert (Attachment 1). Dosage and administration should be carried out as described below (Sections 5.1.2 and 5.1.3). If, in the judgment of the attending clinical staff, the flow rate is not sufficient to maintain the infusion line open, the staff may piggyback the bag containing the study drug into an infusion line with a separate bag of saline that is running at a faster rate. The volume of the saline bag should be no larger than 250mL. If a piggyback is used it should be noted in the source documentation.

A designated drug distributor under contract to NIAID will package and label Thymoglobulin and distribute it to the clinical study sites. Placebo for Thymoglobulin consists of saline solution and will be provided by the clinical study sites.

5.1.2 Dosage

5.1.2.1 *Thymoglobulin Group*

Participants will receive four doses of Thymoglobulin including 1000 U heparin and 20 mg hydrocortisone in the IV bag over 4 days.

Day 1: 0.5 mg/kg

Day 2: 2 mg/kg

Day 3: 2 mg/kg

Day 4: 2 mg/kg

5.1.2.2 *Placebo Group*

Participants will receive four doses of placebo (saline solution only) for Thymoglobulin IV over 4 days.

5.1.3 Administration

All participants will be admitted to the hospital for the duration of the infusions and will be discharged no sooner than 24 hours after the last infusion. Body weight at baseline will be used in calculating the doses for all infusions.

Thymoglobulin or placebo-Thymoglobulin will be infused via a peripheral vein using a regular or high-flow peripheral catheter and a 0.22 mm in-line filter, as follows:

Thymoglobulin group. To minimize the risk for thrombophlebitis, we will include 1000 U heparin and 20 mg hydrocortisone in the infusion bag, and the catheter will be changed after two drug doses.

Placebo group. Because the risk for thrombophlebitis with placebo is only minimal, heparin and hydrocortisone will not be included in the infusion bag. However, the catheter will be changed after two drug doses to ensure blinding of the participant.

Either group. Alternatively, Thymoglobulin or placebo may be infused via a peripherally inserted or regular central catheter and a 0.22 mm in-line filter. Because the risk for thrombophlebitis with a central catheter is only minimal, heparin or hydrocortisone will not be included in the infusion bag in this case.

The first dose will be infused over a minimum of 12 hours, and subsequent doses over a minimum of at least 8 hours. Subsequent doses should be given no less than 12 and no more than 36 hours after the previous dose, unless problems arise that require drug modification or discontinuation (see sections 5.3 and 5.6). Vital signs will be checked every 15 minutes for the first 4 hours of the first two study drug infusions and thereafter at 30-minute intervals. For the last two infusions, vital signs will be assessed at 30-minute intervals. Blood for laboratory testing should be drawn at least 6 hours after completion of each infusion in order to obtain reliable and comparable results.

5.2 PREMEDICATIONS

5.2.1 Glucocorticoid

Thymoglobulin group. To reduce the risk of adverse reactions:

a. Days 1, 2, and 3: Methylprednisolone 0.5 mg/kg IV will be given no less than 30 minutes before each infusion and 0.25 mg/kg IV will be given 12 hours (±15 min) after the start of each infusion.

b. Day 4: Methylprednisolone 0.25 mg/kg IV will be given no less than 30 minutes before each infusion and if necessary 0.25 mg/kg IV 12 hours (±15 min) after the start of the infusion.

Placebo group. Because the risk for adverse reactions with placebo is only minimal, on days 1, 2, 3, and 4, a placebo (saline) infusion similar in appearance will be given no less than 30 minutes before each infusion and at 12 hours (±15 min) after the start of each infusion.

Methylprednisolone and placebo (saline) infusions must be prepared by the investigational pharmacy in order to preserve blinding.

5.2.2 Antihistamine and Acetaminophen

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

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

Diphenhydramine and acetaminophen may be supplied by the clinical pharmacy.

5.3 MANAGEMENT OF ADVERSE EVENTS

5.3.1 Cytokine Release Syndrome

With Thymoglobulin infusion, the subject may experience CRS. The signs and symptoms can span a wide clinical spectrum.

Medications used to manage CRS may be supplied by the clinical pharmacy.

5.3.1.1 *Mild Reactions*

For mild (grade 1) reactions per the NCI-CTCAE for CRS, and/or the participant is experiencing difficulty tolerating the infusion, the investigator may consider one or more of the following actions, depending on the type of the reaction:

1. Administer additional doses of antihistamine and acetaminophen.
2. Reduction of the rate of infusion by 50% or more, or interruption of the infusion.
3. For chills and rigors, meperidine may be considered.
4. For nausea and vomiting, Zofran (Ondansetron hydrochloride) may be considered.
5. If necessary, glucocorticoids can be given every 6 hours at a dose of 0.25 to 0.5 mg/kg of methyprednisolone or equivalent.

5.3.1.2 *Moderate Reactions*

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

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

5.3.1.3 *Severe Reactions*

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

5.3.2 Allergic Reactions

5.3.2.1 *Hypersensitivity*

In rare cases, patients may experience hypersensitivity, which refers to immediate allergic, IgE-mediated reactions to Thymoglobulin.[34] Such patients primarily develop skin rash and respiratory distress early in the course of the infusion (usually within the first hour). In very rare cases patients may develop a grade 4 allergic reaction (anaphylaxis).

For such reactions, the investigator shall take one or more of the following actions:

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

5.3.2.2 *Mild to Severe Reactions*

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

5.3.2.3 *Life-threatening Reactions*

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

5.3.3 Serum Sickness and Associated Renal Dysfunction

Serum sickness from host immunization against rabbit protein may occur 7–15 days after the first dose of ATG. In addition to performing the per-protocol safety assessments (e.g., CBC with differentials, urinalysis, and serum chemistries including blood urea nitrogen, creatinine, and liver function tests), we will also assess C3, C4, and CH50 complement levels during the course of the serum sickness. The patient may require glucocorticoid treatment for supportive care. The dose will depend on the severity of signs and symptoms (see grading criteria in section 8.3.3). For grade 3 symptoms and signs, we recommend a maximum dose of 1.5 mg/kg prednisone PO per day for 3 days with rapid tapering such that the total treatment course is given over 7 days (or less, depending on the subject's symptomatology). For persisting signs and symptoms of serum sickness, the course of glucocorticoid therapy may need to be extended until serum sickness has resolved, at the discretion of the study team. If serum sickness does not resolve or improve to at least grade 1 within 2 weeks of initiating glucocorticoid treatment, the study medication will be stopped for all participants and enrolment will be halted pending DSMB review.

Renal dysfunction may occur as part of the pathology of serum sickness. Of particular note are proteinuria, hematuria, and increased serum creatinine, which will be graded as described in section 8.3.3. If renal dysfunction grade 2 or higher does not resolve or improve to at least grade 1 within 2 weeks the study medication will be stopped for all participants and enrollment will be halted pending DSMB review.

5.4 DRUG ACCOUNTABILITY

Under Title 21 of the Code of Federal Regulations (21CFR §312.62), the investigator is required to maintain adequate records of the disposition of the investigational agent, including the date and quantity of the drug received, to whom the drug was dispensed (participant-by-participant accounting), and a detailed accounting of any drug accidentally or deliberately destroyed.

Records for receipt, storage, use, and disposition will be maintained by the study site. A drug-dispensing log will be kept current for each participant. This log will contain the identification of each participant and the date and quantity of drug dispensed.

All records regarding the disposition of the investigational product will be available for inspection by the clinical trial monitor.

5.5 ASSESSMENT OF COMPLIANCE WITH STUDY DRUG

Participants will be admitted to the hospital for administration of Thymoglobulin; therefore, compliance will be assessed by recording the time, amount, and duration of the infusion on the appropriate source document and case report form (CRF).

5.6 MODIFICATION OR DISCONTINUATION OF STUDY DRUG

Doses may need to be reduced by 50% or more, temporarily held, or discontinued according to the guidelines listed in section 5.6.1 and below. However, the last dose of Thymoglobulin must be administered by the end of day 7 if any dose adjustments occur. For example, if on study day 4 the fourth dose must be held, then the subject may receive the fourth dose on day 5 or day 6 if he or she has recovered and meets the criteria listed below. No doses are given after day 7.

5.6.1 Modification

The study medication dose will be reduced by 50% if a participant's platelet count falls between 50,000 and 75,000 cells/mm^3, or if the neutrophil count falls to a value >800 but <1,200 cells/mm^3.

Although the Thymoglobulin package insert recommends reducing the dose by 50% if a patient has a total white blood cell (WBC) count of between 2000 and 3000 cells/mm^3, it has been decided not to reduce the dose in this protocol because of the following reasons:

- Because the primary focus of this study is initial T-cell depletion, premature or unnecessary reduction of the Thymoglobulin dose may result in reduced efficacy.
- If the WBC count falls to a significantly lower level, i.e. <2000 cells/mm^3, then Thymoglobulin will be held (see section 5.6.2).

Participants will be closely monitored for infectious disease risk and offered prophylaxis as warranted to minimize their risk (see sections 5.9–5.10.3).

5.6.2 Modification and Discontinuation

The study medication will be discontinued if any one of the following is observed:

- A total WBC count <2,000 cells/mm^3 that persists in repeat tests up to 48 hours after the time of the planned infusion.
- A neutrophil count <800 cells/mm^3 that persists in repeat tests up to 48 hours after the time of the planned infusion.
- A platelet count <50,000 cells/mm^3 that persists in repeat tests up to 48 hours after the time of the planned infusion.
- The participant experiences exacerbation of CRS twice consecutively despite the use of glucocorticoids, as outlined in section 5.3.

If the repeat tests show acceptable values (WBC count >2,000 cells/mm^3, neutrophil count >1,200 cells/mm^3, and platelet count >75,000 cells/mm^3), Thymoglobulin will be resumed at the full dose. Participants will receive a 50% dose if they meet the criteria outlined in section 5.6.1.

In addition to the guidelines in section 5.3.1, the study medication will also be permanently discontinued if the investigator believes that the study treatment is no longer in the best interest of the participant. Participants who prematurely discontinue study treatment will remain in the study and undergo all efficacy and safety assessments.

5.7 CONCOMITANT MEDICATIONS AND INSULIN USE

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

5.8 PROHIBITED MEDICATIONS

The following medications are contraindicated during participation in this study:

- Agents that influence insulin sensitivity or secretion (sulfonylureas, metformin, diphenylhydantoin, thiazide, or other potassium-depleting diuretics, beta-adrenergic blockers, niacin).
- Live vaccination 6 weeks before enrollment and throughout study participation. Live vaccines include varicella, measles, mumps, rubella, cold-attenuated intranasal influenza vaccine, Bacillus Calmette-Guérin, and smallpox.
- Any medication that may result in immunosuppression or immunomodulation, including systemic glucocorticoids (unless required during Thymoglobulin administration or for the treatment of cytokine release syndrome or serum sickness).

If participants receive, or if the investigator believes that participants must receive, any of the above medications, the case must be immediately discussed with the NIAID medical monitor and ITN clinical trial physician. The use of prohibited medications must be documented on the source document and CRF, and a protocol deviation must be requested. A decision regarding continuation of the participant in the trial will be made by the protocol chair, the NIAID medical monitor, and the ITN clinical trial physician.

5.9 PROPHYLAXIS FOR PCP

Immediately after Thymoglobulin treatment, the resultant T-cell depletion may render treated participants vulnerable to opportunistic infections. All participants treated with Thymoglobulin should receive prophylaxis for *Pneumocystis carinii* pneumonia (PCP). The standard regimen is one single-strength trimethoprim-sulfamethoxazole tablet (80 mg–400 mg) orally daily (Attachment 2). For sulfa-allergic patients, inhaled pentamidine may be used for PCP-prophylaxis once per month: adults and children 5 years of age and older may be administered 300 mg every 4 weeks (Attachment 4). Treatment should begin on the day following Thymoglobulin infusion, at hospital discharge. Treatment should continue for a minimum of 3 months or until the patient's CD4 count is greater than 200 cells/mm^3, whichever comes later.[59, 128, 129] To maintain blinding, the study drug administration team will decide when PCP prophylaxis can be stopped and will inform the participant independently of the diabetes management team. The study drug administration team therefore will collaborate with the diabetes management team, who will be blinded to the CD4 counts. If other changes in PCP prophylaxis are deemed necessary by the investigator because of adverse reactions, they should be discussed with the medical monitor.

The placebo group will receive placebo-trimethoprim-sulfamethoxazole or placebo-pentamidine-inhalation as described above. All antimicrobial placebos will be manufactured and distributed by a designated drug distributor under contract to NIAID. If necessary to prevent unblinding, the drug distributor under contract to NIAID will over encapsulate actual antimicrobial medication.

5.10 MONITORING AND TREATMENT OF EBV AND CMV

5.10.1 Cytomegalovirus

Both seropositive and seronegative subjects will undergo regularly scheduled monitoring for CMV viremia by quantitative PCR. No prophylaxis will be given.

5.10.1.1 *CMV-seropositive Subjects*

5.10.1.2 Monitoring

- If quantitative PCR for CMV is > 1,000 copies/mL, the subject will have weekly PCR performed.
- If quantitative PCR for CMV is > 1,000 and < 10,000 copies/mL, the subject will be treated if concomitant symptoms, signs, or laboratory abnormalities consistent with CMV infection are present (e.g., fever [>100.4 °F], malaise, weakness, lymphadenopathy, hepatomegaly, splenomegaly, leukopenia, thrombocytopenia, and elevated AST and ALT.)
- If quantitative PCR for CMV is > 10,000 copies/mL, the subject will be treated for CMV reactivation.

5.10.1.3 Treatment

CMV prophylaxis after the first 10 participants: The decision to use preemptive therapy rather than universal prophylaxis for CMV-seropositive subjects is based upon several factors:

- The relatively transient nature of lymphopenia expected and the absence of additional immunosuppression
- The experience that many solid-organ transplant recipients spontaneously resolve low-level CMV reactivation
- Long-standing experience with the use of ATGAM plus cyclosporin in patients with aplastic anemia who rarely, if ever, experience significant CMV reactivation.

Therefore, the risk of serious CMV disease is thought to be low, and the potential benefits of prophylaxis in a small number of subjects may well be outweighed by adverse events with universal prophylaxis. However, if treatment is required for CMV for three or more of the first 10 CMV-seropositive patients treated with Thymoglobulin, then universal prophylaxis will be reconsidered for subsequent seropositive enrollees.

5.10.1.4 *CMV-seronegative Subjects*

5.10.1.5 Monitoring

A positive PCR on a second sample from a previously seronegative subject indicates primary infection. Because primary infection during Thymoglobulin-induced lymphopenia confers higher risk for tissue invasive disease than reactivation in seropositive subjects, the threshold for treatment of CMV in previously seronegative subjects is lower. Once the PCR is below the limit of detection, monthly monitoring may be resumed. These subjects should have repeat CMV serology performed to document seroconversion:

- If quantitative PCR is > 1,000 copies/mL, the subject will have weekly PCR performed.

- If quantitative PCR is two-fold compared with the previous measurement or >2,000 copies/mL will be treated for primary CMV infection.

5.10.1.6 Treatment

For adult subjects (> 18 years of age):

- Induction treatment: 900 mg valganciclovir PO 2 times daily, or adjusted for renal function for 2 weeks.
- Maintenance treatment: 900 mg valganciclovir PO per day, or adjusted for renal function for 2 weeks or until PCR is undetectable.

For children: Valganciclovir is not approved for use in children, and dosing regimens have not been established. Therefore, CMV reactivation in children will be treated with an induction course of IV ganciclovir (5 mg/kg q 12 hours) for 2 weeks followed by maintenance dosing for 2 weeks (5 mg/kg qd) or until PCR is undetectable. At the discretion of each site investigator in consultation with local experts in pediatric infectious disease, more mature children may be treated with oral valganciclovir rather than IV ganciclovir. Once the PCR is below the detection limit, regularly scheduled monitoring may be resumed.

5.10.2 Epstein-Barr Virus

EBV-seronegative subjects will be excluded from clinical protocol ITN028AI. EBV-seropositive subjects will be monitored in two ways:

- At each visit post treatment, during which adverse events are reviewed, the subjects will be questioned regarding signs or symptoms suggestive of EBV reactivation, e.g., fever (>100.4 °F), lymphadenopathy, pharyngitis or tonsillar swelling, skin rash, weight loss, malaise, or fatigue.
- Quantitative PCR will be performed for EBV viremia beginning at visit 6 (day 10).
- In case of symptoms or signs suggestive of EBV reactivation at any time, a PCR will be performed as follows:
- If quantitative PCR > 1,000 copies/mL on a screening test or on a test performed for symptoms will then have PCR performed one week later and weekly thereafter.
- If quantitative PCR is persistently positive or rising, subjects will be thoroughly examined for evidence of EBV-related disease, including fever, lymphadenopathy, pharyngitis, hepatomegaly, splenomegaly, liver function abnormalities, atypical lymphocytosis, or leukopenia.
- If quantitative PCR is > 10,000 copies/mL, strong consideration for treatment with valacyclovir (1 g PO tid for adults, adjusted for children weighing less than 50 kg) should be prompted. However, a recent study with an anti-CD3 monoclonal antibody showed that EBV reactivation was cleared spontaneously without intervention.[159] Therefore, individual site investigators may choose to closely observe those with no or mild clinical signs and symptoms without treatment but will continue to monitor weekly PCR results and will maintain a low threshold for initiation of antiviral therapy.

Persons whose symptoms do not resolve spontaneously over 4–6 weeks or who display progression will prompt further evaluation. Persons with localized or diffuse lymphadenopathy should undergo lymph-node biopsy with appropriate histologic and immunohistochemical evaluation for EBV genomes and gene products. Further evaluation may include a CT scan of the chest, abdomen, and pelvis to investigate possible visceral lymphadenopathy, liver and spleen size and homogeneity, and masses in the lungs or abdominal cavity. Any such event is believed to be highly unlikely, but if it occurs, consultation with an oncologist should be obtained for consideration of further diagnostic and

treatment options (e.g., liver biopsy, characterization of the clonality and origin of lymphoid proliferation, and B-cell ablation with rituximab).

5.10.3 Herpes Simplex 1,2 and Varicella Zoster

Subjects will be screened for serologic evidence of prior infection with herpes simplex virus (HSV1 and HSV2) and varicella zoster virus (VZV). Subjects treated with Thymoglobulin who are seropositive for prior infection with HSV1, HSV2, or VZV will receive oral acyclovir prophylaxis for a minimum of 3 months or until their CD4 count is greater than 200 cells/mm^3, whichever comes later (Attachment 3). Subjects who weigh 50 kg or more should receive 400 mg acyclovir PO twice daily; those who weigh less than 50 kg should receive 200 mg PO twice daily. Treatment should begin on the day following completion of Thymoglobulin infusion, at hospital discharge.

Subjects who are VZV seronegative will be cautioned to avoid any contact with individuals who have chickenpox or shingles during the 3 months following treatment with Thymoglobulin. These subjects are at significant risk for severe varicella, particularly if they are not receiving acyclovir prophylaxis for HSV. Participants who are eligible will receive VariZIG™. Participants will be counseled to report any such contact immediately in order to receive the vaccine within 96 hours of exposure. The placebo group will receive placebo-acyclovir as described above.

Placebo-acyclovir will be manufactured and distributed by a designated drug distributor under contract to NIAID. To prevent unblinding, the drug distributor under contract to NIAID will overencapsulate drug-containing medication.

6. STUDY PROCEDURES

6.1 INFORMED CONSENT

The potential participant will sign an informed consent form, in which the research trial will be explained in layman's language, before undergoing any study-specific screening procedures.

6.2 SCREENING

The screening visit cannot occur until the participant has signed the informed consent. At the screening visit, the participant will undergo the following assessments: physical examination with height and weight, medical history, blood draw for serum chemistries, CBC, and other clinical and immunologic tests (see Appendix 1), and, if female and of childbearing potential, a pregnancy test.

6.3 RANDOMIZATION AND BLINDING

Participants who meet the inclusion and exclusion criteria will be randomly assigned in a 2:1 fashion to either the Thymoglobulin group or the placebo group within 35 days (5 weeks) from their screening MMTT. Participants will receive first dose of study drug no later than 100 days (~14 weeks) after T1DM diagnosis.

Each randomized participant will be given a unique participant number. Participants will be randomly assigned to their treatment groups utilizing an Interactive Voice or Web-based Response System or a phone-based response system whereby sites contact Rho Fed support staff by telephone for treatment assignments. Randomization will be stratified by study site.

A designated drug distributor under contract to NIAID will prepare and ship study drug in bulk supply to individual sites. Since the pharmacist will be unblinded, there is no need for study drug kits in this study.

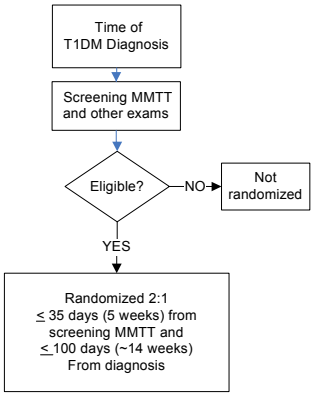

Figure 2. Screening, randomization, and first infusion (study day -1 through study day 1)

6.4 UNBLINDING

Before emergency unblinding of individual participants, the medical monitor will be notified. The site investigator will inform the protocol chair of the unblinding event. The medical monitor will inform the study management team. All unblinding information will be recorded and reported to the DSMB. Any unblinding event will require a full written account including the name of the medical monitor who was notified, date, time, and reason for the unblinding of treatment for an individual participant. During site visits, the site monitor must verify that the medical monitor of the trial was notified and that a written account was completed. The reasons for unblinding each individual participant's treatment will be included in the final study report. ITN and NIAID approval is required for unblinding of any participant or subgroups of participants for possible interim analyses to support DSMB reviews and final analysis.

6.5 CLINICAL PROCEDURES

A 4-hour MMTT will be administered to all participants at screening and at 6-month intervals throughout the 24-month study. Glucose, C-peptide levels, and total insulin levels for each test will be obtained at time 0, and at 15, 30, 60, 90, 120, 150, 180, 210, and 240 minutes after oral ingestion of Boost High Protein Nutritional Energy Drink® (Mead-Johnson) 6 kcal/kg @ 1 kcal/mL of mixed meal, to a maximum of 360 mL. If a participant has a known food allergy to one or more components of Boost, an equivalent substitution may be used. Specific details for conduct of the MMTT are provided in Appendix 2.

HbA_{1C} will be assessed every 3 months throughout the study to optimize diabetes management. Insulin use and hypoglycemic episodes will be recorded on appropriate case report forms (CRFs).

Participants will periodically undergo blood draws for both clinical and immunologic tests. Blood volumes drawn will be based on participant body weight and may be modified to limit the total blood volume drawn at any one visit.

Participants are required to have a CBC performed after each infusion in a timely manner so that the results are available before the next infusion. Please refer to section 5.6 for the modification of study treatment based on laboratory results. (For the complete schedule of events, please see Appendix 1.)

6.6 INTENSIVE DIABETES MANAGEMENT

6.6.1 Guidelines

During the study period, all participants will receive "intensive" management of their diabetes, and HbA_{1C} will be assessed every 3 months to evaluate metabolic control. The goal of treatment will be to maintain the HbA_{1C} level as close to normal as possible, without frequent occurrence of hypoglycemia. All individuals should strive for targets in accordance with current American Diabetes Association recommendations,[177] with HbA_{1C} levels of $\leq$ 7% in adults and $\leq$ 8% in adolescents (age 13-16 years), and with preprandial glucose levels of 90–130 mg/dL (plasma), postprandial levels of <180 mg/dL, and bedtime levels of 110–150 mg/dL.[177] During the hospitalization, more frequent glucose monitoring will occur, and insulin dosing will be adjusted as necessary in order to maintain glycemic control. All participants will be expected to take a sufficient number of daily insulin injections to meet the glycemic targets. In general, the expectation is that all participants will receive at least three injections of insulin daily, including short- and long-acting insulin preparations, or will utilize continuous subcutaneous insulin infusion (CSII insulin pump). Glucose levels should be checked at least four times daily. After reviewing these records, the diabetes management team will contact the treating physician about possible adjustments in the insulin regimen, referral to a registered dietitian, or other approaches that the diabetes management team believes would improve the glucose control if necessary. Records of glucose measurements and communication with the participant will be kept as source documentation.

Participants will be contacted by the diabetes educator every 2 weeks between visits to assess their diabetes. In addition, insulin use and hypoglycemic events will be captured at each 3-month visit on the appropriate CRFs. Participants will be required to record the type and amount of insulin they have used during the 5-day period immediately preceding each study visit. Insulin use logs will be provided to participants at each study visit and collected at the next visit. These logs will serve as the source documents.

Participants who fail to achieve an HbA_{1C} level within the stated goals will not be excluded from the study, but additional measures will be instituted to improve the glycemic control. Any episodes of severe hypoglycemia (i.e., unconsciousness, seizure, or needing the assistance of another individual to correct the hypoglycemia) will prompt a review of the cause of the episode and adjustment of insulin dosing, diet, and exercise as deemed appropriate.

6.6.2 Hypoglycemia

For the purposes of this study, hypoglycemic events will be recorded as follows:

- Minor hypoglycemia will be defined as a glucose concentration of <65–55 mg/dL.
- Major hypoglycemia will be defined as a glucose concentration <55 mg/dL (grades 2–5, NCI-CTCAE version 3.0), or clinically: involving seizure or involving loss of consciousness (coma), or requiring assistance from another individual in order to recover.

All episodes of hypoglycemia that require hospitalization and/or emergency care will be reported to the DSMB as described in Sections 8.1 and 8.4.3.1.

6.6.3 Discontinuing Insulin

Exogenous insulin will be continued for all participants unless they reach an insulin-free endpoint, as defined in the IDS guidelines for new-onset trials; that is, normal HbA_{1C} levels on two occasions 3 months apart. [178] Subjects should then have documentation of a normal standard oral glucose tolerance test after omitting insulin for 3 consecutive days. The subjects will continue to be followed closely in the context of the study, with repeated HbA_{1C} and further glucose tolerance tests at 3 month

intervals, and reinstitution of insulin if abnormalities in metabolism are present as per standard ADA guidelines.[177]

6.7 STUDY VISITS

Participants will have biweekly contact with the study staff by means of scheduled study visits, unscheduled visits, telephone calls, or email.

Participants may make an unscheduled visit for diabetes management or for safety reasons. At a minimum, participants should be assessed for adverse events, insulin use, and concomitant medications during an unscheduled visit.

An early termination visit will be required for those who withdraw consent and decide not to continue their participation in the study. All the assessments listed for month 12 will be performed for participants who withdraw from the study before month 12. All assessments listed for month 24 will be performed for participants who withdraw between months 12 and 24.

6.8 VISIT WINDOWS

Study visits should take place within the time limits listed in Table 1.

Table 1. Study visit windows

Visit	Target Day	Visit Window
-1	Screening	
0	Baseline	Randomization ≤ 35 days from screening MMTT and ≤ 100 days from T1DM diagnosis
1*	Day 1 (infusion 1)	Infusion must start ≤ 100 days from T1DM diagnosis
2*	Day 2 (infusion 2)	No sooner than 12 hours before, or 36 hours after, planned infusion*
3*	Day 3 (infusion 3)	No sooner than 12 hours before, or 36 hours after, planned infusion*
4*	Day 4 (infusion 4)	No sooner than 12 hours before, or 36 hours after, planned infusion*
5	Day 5	Within 36 hours of 4th infusion
6	Day 10	± 2 days of target day
7	Day 15	± 2 days of target day
8	Month 1	± 3 days of target day
9	Month 2	± 3 days of target day
10	Month 3	± 3 days of target day
12–18	Months 6–24 (every 3 months)	± 7 days of target day

*Refer to section 5.6 for possible schedule modification that may need to occur during Thymoglobulin infusion. No Thymoglobulin may be administered beyond 7 full days from the start of the initial infusion. In the event of a low WBC, platelet, or neutrophil count, a subsequent dose can be delayed for up to 48 hours from the planned infusion time.

6.9 FOLLOW-UP FOR MALIGNANCIES AND/OR LYMPHOPROLIFERATIVE DISORDERS

At their final visit, study participants will be provided with a wallet card indicating that they were enrolled in the current study. This card will include a phone number for the participant or their physician to call in case of malignancies and/or lymphoproliferative disorders.

Moreover, at their final visit, study participants will be reminded that the site will contact them by telephone every 6 months for up to 60 months.

6.10 STUDY PERSONNEL

The NIAID medical monitor, ITN clinical trial physician, and protocol chair will oversee the overall conduct of clinical protocol ITN028AI and will interact with the site investigators. The protocol chair will also function as a site investigator.

Each investigator is responsible for conducting clinical protocol ITN028AI at his or her site according to GCP and according to the protocol. The site investigator may share certain responsibilities with a site co-investigator.

The site investigator may appoint a study coordinator and/or study nurse to assist in coordinating study activities, including patient care, study administration, AE assessment, specimen collection, and administration of questionnaires.

The unblinded study drug administration team will include a physician and a study coordinator. The blinded diabetes management team will include a physician and a certified diabetes educator.

7. TOLERANCE ASSAYS

7.1 OVERVIEW

The proposed immunologic mechanistic studies are designed to determine how Thymoglobulin may alter the natural progression of T1DM through the deletion and modulation of autoreactive T cells, induction of T_{REG} cell populations, shift in T_H1 population towards T_H2, change in autoantibody titer, and alteration in APC function.

The following specimens may be used in future assays to re-evaluate biologic responses as research tests are developed over time. Study participants will be informed that they may be approached regarding additional clinical evaluations for other studies that have received the full approval of the ITN. If additional evaluations are determined to be desirable, this protocol (and other study documents as appropriate, e.g. informed consent, statistical analysis plan) will be amended and submitted to the appropriate regulatory authorities, ethics committees, and IRBs for approval. Each participant's signature will be obtained on the revised informed consent before additional tests are performed.

7.2 INDUCTION OF CLINICAL TOLERANCE

Evidence of clinical tolerance will be examined 12 months after the infusion with Thymoglobulin. All participants will be followed for a total of 24 months, with additional immunologic assays out to this time.

Preservation of beta-cell function at 12 months, in conjunction with a competent immune response as assessed by immunologic assays, will determine the induction of clinical tolerance. Specifically, patients are considered clinically tolerant at month 12 if they have the following:

- Preserved their endogenous insulin secretion.
- A normal T-cell population.
- Competent immune response.

They may also have changes in some of the immunologic assays described in detail in the sections to follow, such as significantly lower levels of autoantibodies than those in the placebo group.

7.3 WHOLE BLOOD–FLOW CYTOMETRY PANEL STAINING

The effect of Thymoglobulin on the depletion of T cells and the repopulation of PBMCs will be assessed over time in drug-treated and control subjects using flow cytometry. These studies will be performed on peripheral blood and will assess change in various T-cell subsets (including CD4, CD8, CD25, CD45RA, and CD45RO), as well as other immune cell populations. As discussed previously, from review of past Thymoglobulin and anti-CD3 studies,[32, 49] the ratio of $CD4^+$ to $CD8^+$ cells is of particular interest. The relative abundance of RA^+ and RO^+ cells is also important since the repopulation of PBMCs in adult subjects following T-cell depletion is significantly more rapid with RO^+ cells than with RA^+ cells.[49] This effect is evident for $CD4^+$ and RA^+ cells, which are thought to reflect age-dependent limitations in thymic function. Flow cytometry will also determine which fraction of the reconstituted immune system are potential T_{REG} cells.

7.4 SERUM–SECRETED CYTOKINES

The collection of serum will be taken at regular intervals during the study. Levels of various immune cytokines will be measured in patient serum to determine if cytokine levels correlate with clinical adverse effects and efficacy. Other studies using immune cell depleting therapies have indicated that clinical adverse effects, such as headache, fever, vomiting, and diarrhea correlate with serum elevations in IL-2, TNFα, and IFNγ. Preliminary data obtained in previous trials evaluating hOKT3γ1 (Ala-Ala) suggest that increases in the levels of serum IL-10 after hOKT3γ1 (Ala-Ala) treatment are possibly related to efficacy.[32]

7.5 FROZEN PBMC–T-CELL ASSAYS

PBMCs from participants in this study will be collected and cryopreserved before and after therapy administration. We are working on the development of different T-cell assays. One of the assays is to assess T-cell reactivity, measured by the number of T cells secreting select cytokines in response to the autoantigens GAD65 and insulin, using peptide libraries derived from those antigens. A diminished response, as measured by fewer T cells secreting cytokines in response to antigen stimulation, will be used as a potential marker for tolerance induction. In addition, changes in T-cell cytokine profiles in response to GAD65 or insulin peptides could be indicative of a shift in the type of T-cell response. The threshold for T-cell activation may be modulated by Thymoglobulin, which can be assessed by measuring the responsiveness of T cells to anti-CD3 stimulation in the absence or presence of costimulation through CD28. A new assay to identify phosphorylation profiles of signaling molecules within individual cells may also be a new way to distinguish two cells with similar surface markers but a different internal activation status. If differences are identified, we may be able to explain why some individuals respond better to the proposed therapy. Finally, functional assays that address the effects of potential regulatory T cells on effector T-cell responses may be measured in a T-cell suppression assay to further explore the effects of Thymoglobulin therapy on islet-reactive CD4+ cells. The MHC tetramer technology utilized in the laboratory of Gerald Nepom (Virginia Mason) will be used to assess the presence or absence of autoreactive T cells in subjects in

the Thymoglobulin and placebo groups. Such cells have previously been detected in individuals at risk for or having pre-existing T1DM but not in healthy volunteers.[179] The assay will enable us to enumerate more directly the number of autoreactive T cells present and specific for a given peptide or MHC combination. All study participants will need to be HLA-typed before the assay is run. Studies will be performed with DR4-GAD, DR4-IA-2, and DR3-proinsulin to assess the markers displayed by patient T-cell populations.

7.6 WHOLE BLOOD–GENE EXPRESSION PROFILING

To further elucidate possible changes in cytokine and cellular profiles, gene-expression profiling analysis will be performed on RNA isolated from peripheral blood using microarray or high-throughput real-time polymerase chain reaction (RT-PCR). RT-PCR will be used to compare the expression of several genes reported to play a role in T1DM including IFN-γ, TGF-β, and IL-4, and microarray will employ a survey of most known human genes. The goal of these assays is to identify differences between a tolerant versus non-tolerant state and to find new genes that could serve as potential markers of disease. These types of analyses may also explain why some individuals respond better to this treatment or elucidate mechanisms resulting in adverse responses to treatment. This assay has proven informative in characterizing unique genes that determine the clinical course in systemic lupus erythematosus, and preliminary studies with this technology have enabled us to determine a distinct subset of genes that are either up- or downregulated in those with new-onset T1DM as opposed to controls in other studies.

7.7 WHOLE BLOOD–DNA-HLA GENOTYPES

Peripheral blood will be collected from all participants and utilized to make both high and low molecular weight DNA. These samples will be useful in identifying genetic predictors of a clinical response to Thymoglobulin, specifically identifying HLA class II genes. Because of the important role of HLA in antigen presentation, different types present unique peptides that may influence our analysis.

7.8 WHOLE BLOOD–QUANTITATIVE PCR FOR CMV/EBV REACTIVATION AND IMMUNIZATIONS

CMV and EBV viral infections will be closely monitored since patients receiving Thymoglobulin will be partially immunocompromised for a short period after drug infusion.

As Thymoglobulin may affect B cell function, we will also evaluate the possible change over time in response to previous immunizations, including tetanus and hepatitis B. Tetanus and hepatitis B titers will be obtained at baseline and 1 year after study enrollment. At 1 year, subjects will receive a rechallenge with both tetanus and hepatitis B vaccines, with titers drawn 3 months later (month 15). Subjects will also be evaluated for their response to a hepatitis A neoantigen, administered at 1 year. Hepatitis A titers will be drawn at 1 year and at month 15.

7.9 DNA–TREC

To determine the effects of Thymoglobulin on T-cell depletion and to monitor the subsequent repopulation of T cells, we may use the T-cell receptor excision circles (TREC) assay. TREC uses PCR amplification of rearrangement circles derived from TCRα/β rearrangements to assess recent thymic emigrants (RTEs). The excised DNA remains in T cells as circular, nonreplicated episomes, and during each subsequent cell division, these episomes are diluted since only one of the daughter cells retains the circles. Previous studies in patients with new-onset T1DM have shown a surprisingly large number of RTEs, well above levels seen in age-matched controls (HM Dosch, unpublished data). The number of RTEs in peripheral blood will be determined periodically to determine the

relationship between these cells and other immunologic markers. The RTEs may have an altered repertoire post treatment versus pretreatment, suggesting a general effect of the mAb-induced depletion.

7.10 ANTI-ATG ANTIBODIES

Rabbit and horse anti-human thymocyte globulins are effective immunosuppressive drugs. However, the antibody-mediated immune response that develops against these infused foreign proteins limits their prolonged use. Anti-horse antibody developed in 40% of the subjects, and was associated both with early renal graft failure and decreased levels of circulating equine immunoglobulin levels.[180] Early studies showed little immune response to infused rabbit ALS with only 4 of 66 of patients developing anti-rabbit Ig antibodies, but showed an association of serum sickness with those patients who did develop antibodies.[181] Another study showed a rate of sensitization of patients treated with RATG of 83–87%.[182] Both solid-phase enzyme-linked immunosorbent assay (ELISA) and flow cytometric assays have been used to detect human antibodies to foreign proteins.[183-185] In this study, an ELISA assay will be used to test for human anti-RATG antibodies (see Appendix 1).

8. SAFETY MONITORING

8.1 DATA AND SAFETY MONITORING BOARD

The NIAID Data and Safety Monitoring Board (DSMB) for autoimmune trials will monitor the study and will have the authority to suspend clinical protocol ITN028AI if it determines that the risks to individuals exceed the originally described risks and/or that modifications to the protocol are needed to minimize the risks. The DSMB will include or consult with experts in infectious diseases since some of the issues surrounding the administration of Thymoglobulin may require such expertise.

The DSMB will receive and review safety data during their regular reviews for the first 10 randomized participants aged 18–35 and for the first 10 randomized participants aged 12-17. The DSMB will also receive periodic safety reports, and will be informed immediately if one of the criteria for stopping the trial (section 3.2.3) is met.

8.2 DEFINITIONS

8.2.1 General

Adverse events that are classified as serious according to the definition set forth by the health authorities must be reported promptly to ITN, NIAID, Rho Fed, health authorities, investigators, and institutional review boards (IRBs). This section defines the types of adverse events and outlines the procedures for appropriately collecting, grading, recording, and reporting them. Information in this section complies with *ICH Guideline E2A: Clinical Safety Data Management: Definitions and Standards for Expedited Reporting* and *ICH E6: Guideline for Good Clinical Practice,* and applies the standards set forth in the National Cancer Institute (NCI), *Common Terminology Criteria for Adverse Events version 3.0* (June 10, 2003).

8.2.2 Adverse Event

An adverse event is any occurrence or worsening of an undesirable or unintended sign, symptom (including an abnormal laboratory finding), or disease that is temporally associated with the use of a medicinal product whether considered related to the medicinal product or not.

8.2.3 Adverse Reaction and Suspected Adverse Reaction

An adverse reaction means any adverse event caused by a drug. Adverse reactions are a subset of all suspected adverse reactions for which there is reason to conclude that the drug caused the event.

Suspected adverse reaction (SAR) means any adverse event for which there is a reasonable possibility that the drug caused the adverse event. For the purposes of safety reporting, 'reasonable possibility' means there is evidence to suggest a causal relationship between the drug and the adverse event. A suspected adverse reaction implies a lesser degree of certainty about causality than adverse reaction, which means any adverse event caused by a drug (21 CFR 312.32(a)).

Grade 4 and below lymphopenia and/or CD4 counts will be recorded on the AE CRF. However, because they are deemed "expected", grade 4 and below lymphopenia and/or CD4 counts that occur within the first 30 days after completion of study drug administration, and which do not require medical intervention and are not associated with clinical findings, will not be recorded as SAEs.

Adverse events and treatment-emergent laboratory abnormalities, such as aminotransferase elevations, rises in BUN and/or creatinine, and abnormalities in Hb, WBC count and differential, and/or platelets should be followed until they resolve or stabilize.

8.2.4 Serious Adverse Event

An AE or SAR is considered "serious" if, in the view of either the investigator or DAIT/NIAID, it results in any of the following outcomes (21 CFR 312.32(a)):

- Death. A death that occurs during the study or that comes to the attention of the investigator during the protocol-defined follow-up after the completion of therapy must be reported whether it is considered to be treatment-related or not.
- A life-threatening event: An AE or SAR is considered "life-threatening" if, in the view of either the investigator or DAIT/NIAID, its occurrence places the subject at immediate risk of death. It does not include an AE or SAR that, had it occurred in a more severe form, might have caused death.
- . Inpatient hospitalization or prolongation of existing hospitalization.
- Persistent or significant incapacity or substantial disruption of the ability to conduct normal life functions.
- An event that required intervention to prevent permanent impairment or damage. An important medical event that may not result in death, be life threatening, or require hospitalization may be considered serious when, based upon appropriate medical judgment, it may jeopardize the participant and may require medical or surgical intervention to prevent one of the outcomes listed above.
- Congenital anomaly or birth defect.
- Other conditions specified in the protocol.

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

8.2.5 Unexpected Adverse Reaction

A SAR is considered "unexpected" if it is not listed in the investigator's brochure or is not listed at the specificity or severity that has been observed (21 CFR 312.32(a)).

8.3 ADVERSE EVENTS

8.3.1 Collecting Procedure

Adverse events will be collected from the time the participant signs the informed consent until the time an event is resolved or until 30 days after the participant completes study treatment, whichever comes first.

Adverse events may be discovered through any of these methods:

- Observing the participant.
- Questioning the participant in an objective manner.
- Receiving an unsolicited complaint from the participant.

An abnormal value or result from a clinical or laboratory evaluation (e.g., a radiograph, an ultrasound, or an electrocardiogram) can also indicate an adverse event. If this is the case, then the evaluation that produced the value or result should be repeated until the value or result returns to normal or can be explained and the participant's safety is not at risk. If an abnormal value or result is determined by the investigator to be clinically significant, it must be recorded as an adverse event on the appropriate laboratory evaluation form(s).

8.3.2 Recording Procedure

Throughout the study the investigator will record adverse events on the appropriate adverse event CRF regardless of their severity or relation to study medication or study procedure. All adverse events will be recorded on the adverse event CRFs except those that are expected following treatment and described in Section 8.2.2. The investigator will treat participants experiencing adverse events appropriately and observe them at suitable intervals until their symptoms resolve or their status stabilizes.

8.3.3 Grading and Attribution of Serum Sickness and Associated Renal Dysfunction

The severity of serum sickness will be evaluated and interpreted by the study sites according to the criteria set forth in the National Cancer Institute's *Common Terminology Criteria for Adverse Events Version 4.0* (published May 28, 2009 and updated September 15, 2009). This document is found at this website: http://evs.nci.nih.gov/ftp1/CTCAE/CTCAE_4.02_2009-09-15_QuickReference_5x7.pdf

Serum sickness will be graded according to the following standards in NCI-CTCAE manual v4.02:

Grade 1 = Asymptomatic; clinical or diagnostic observations only; intervention not indicated.
Grade 2 = Moderate arthralgia, fever, rash, urticaria; antihistamines indicated.
Grade 3 = Severe arthralgia or arthritis; extensive rash; steroids or IV fluids indicated.
Grade 4 = Life-threatening consequences; pressor or ventilator support indicated.
Grade 5 = Death.

Renal dysfunction associated with serum sickness will be graded as follows: Proteinuria and serum creatinine will be graded according to NCI-CTCAE manual v4.02. Hematuria will be graded based on the following criteria described in Bielory et al[186]:

Grade 1 = 5–9 cells/hpf.
Grade 2 = 10–19 cells/hpf.
Grade 3 = 20–39 cells/hpf.
Grade 4 = ≥ 40 cells/hpf.

The relationship, or attribution, of serum sickness or related renal dysfunction to the investigational product will be determined by the site investigator according to definitions in section 8.3.4.2.

8.3.4 Grading and Attribution of All Other Adverse Events

8.3.4.1 *Grading Criteria*

The study site will grade the severity of adverse events experienced by ITN study participants according to the criteria set forth in the National Cancer Institute's *Common Terminology Criteria for Adverse Events Version 3.0* (published June 10, 2003 and updated August 9, 2006). (http://ctep.cancer.gov/protocolDevelopment/electronic_applications/docs/ctcaev3.pdf)

This document provides a common language to describe levels of severity, to analyze and interpret data, and to articulate the clinical significance of all adverse events.

Adverse events will be graded on a scale from 1 to 5 according to the following standards in the NCI-CTCAE manual:

Grade 1 = Mild adverse event.
Grade 2 = Moderate adverse event.
Grade 3 = Severe and undesirable adverse event.
Grade 4 = Life threatening or disabling adverse event.
Grade 5 = Death.

All adverse events will be reported and graded whether they are or are not related to disease progression or treatment.

8.3.4.2 *Definition of Attribution*

Adverse events will be categorized for their relation to thymoglobulin. The principal investigator will do the initial determination of the relation, or attribution, of an AE to study participation and will record the initial determination on the appropriate eCRF and/or SAE reporting form. The relation of an AE to study participation will be determined using definitions inTable 2. Final determination of attribution for safety reporting will be decided by DAIT/NIAID.

Table 2. NCI-CTCAE attribution of adverse events

Code	Descriptor	Relationship (to primary investigational product and/or other concurrent mandated study therapy)
Unrelated Categories		
1	Unrelated	The adverse event is clearly not related.
2	Unlikely	The adverse event is unlikely related.
Related Categories		
3	Possible	The adverse event has a reasonable possibility to be related; there is evidence to suggest a causal relationship.
4	Probable	The adverse event is likely related.
5	Definite	The adverse event is clearly related.

8.4 REPORTING SERIOUS ADVERSE EVENTS

8.4.1 Collecting Procedure

Serious adverse events will be collected from the time the participant begins study treatment until 30 days after he or she completes study treatment or until 30 days after he or she prematurely withdraws from the study.

8.4.2 Reporting SAEs to the IND Sponsor

The following process for reporting an SAE ensures compliance with 21CFR 312 and ICH guidelines. After learning that a participant has experienced an SAE, the principal investigator or designee will report the SAE via the electronic SAE report form (SAE eCRF) within 24 hours of becoming aware of the event. Initial SAE eCRF should include as much information as possible, but at a minimum must include the following:

- AE term.
- Study drug.
- Relationship to study medications.
- Reason why the event is serious.
- Supplementary eCRF pages that are current at the time of SAE reporting: medical history, concomitant medications, demographics, study drug administration, death.

As additional details become available, the SAE eCRF should be updated and submitted. Every time the SAE eCRF is submitted, it should be electronically signed by the principal investigator or subinvestigator.

For additional information regarding SAE reporting, contact Rho Product Safety:

Rho Product Safety
6330 Quadrangle Drive, Suite 500
Chapel Hill, NC 27517
Toll-free: 1-888-746-7231
SAE Fax Line: 1-888-746-3293
Email: rho_productsafety@rhoworld.com

8.4.3 Reporting SAEs to Health Authorities

After the SAE has been reported by the principal investigator and assessed by the IND sponsor, the IND sponsor must report an event to the appropriate health authorities using one of these two options:

- **Standard reporting (report in the IND annual report).** This option applies if the AE is classified as one of the following:
  - Serious, expected, suspected adverse reaction (see Section 8.2.3, *Adverse and Suspected Adverse Reaction* and *Section 8.2.5, Unexpected Adverse Reaction).*
  - Serious and not a suspected adverse reaction (see *Section 8.2.3, Adverse and Suspected Adverse Reaction*).
- **Expedited Safety Report.** This option applies if the adverse event is classified as one of the following:

- Serious and unexpected suspected adverse reaction (see *Section 8.2.3, Adverse and Suspected Adverse Reaction* and Section *8.2.5, Unexpected Adverse Reaction).*
- Aggregate analysis of serious adverse events that suggests a causal relationship to the study drug.
- Any findings from clinical or epidemiological studies, analysis of data pooled across multiple studies, published or unpublished scientific papers, or from animal or in vitro testing that would result in a safety-related change in the protocol, informed consent, investigator brochure or other aspects of the overall conduct of the trial will be reported.

- Safety Reports must be reported by DAIT/NIAID to the appropriate health authorities within 15 calendar days; fatal or immediately life-threatening serious, unexpected, suspected adverse reactions must be reported within 7 calendar days.

All principal investigators must report SAEs to their respective IRBs as mandated by them.

8.4.3.1 *Notifying the Data and Safety Monitoring Board*

The NIAID will provide the DSMB with listings of all SAEs on an ongoing basis. Furthermore, the DSMB will be informed of expedited reports of SAEs at the same time as health authorities.

8.4.3.2 *Reporting Pregnancy*

Any pregnancy that occurs during a clinical study that is using an investigational drug must be reported to Rho using the AE page in the electronic data capture (EDC) system. This form is *for tracking purposes only*. All pregnancies that are identified during the study must be followed to conclusion and the outcome of each must be reported. The investigator should be informed immediately of any pregnancy and should instruct a pregnant participant to stop taking study medication. The investigator should report all pregnancies within 24 hours (as described in sections 8.3 and 8.4) using the electronic data capture (EDC) system. The investigator should counsel the participant and discuss the risks of continuing with the pregnancy and the possible effects on the fetus. Monitoring of the participant should continue until the conclusion of the pregnancy, and a follow-up pregnancy report form detailing the outcome of the pregnancy should be submitted.

8.4.4 Updating Source Documentation

Documents describing the safety profile of a drug, such as the investigator's brochure, will be amended as needed by the study drug manufacturer to ensure that the description of safety information adequately reflects any new clinical findings. Until these documents are updated, expedited reporting will be required for additional occurrences of a reaction.

9. Statistical Considerations and Analytical Plan

9.1 ANALYSIS SAMPLES

Intent-to-treat (ITT) sample will include all randomized participants who received any dose of study treatment. The efficacy analyses will be based on ITT sample according to the group to which the participants are assigned.

The safety sample will include all participants who received any degree of study treatment. Safety analyses will be based on actual treatment the participants receive.

The per-protocol (PP) sample will include all participants in the ITT sample with no major protocol deviations that impact efficacy assessments, who have completed at least 4 mg/kg of study drug, and who have the Month 12 MMTT assessment for C-peptide. The reported major deviations will be reviewed during a masked data review at the end of the study to determine which participants should be excluded from the per-protocol population.

Statistical presentation and analysis of all patient data will be further defined in the statistical analysis plan (SAP).

9.2 STUDY ENDPOINT ASSESSMENT

9.2.1 Analysis of Primary Endpoint

The null hypothesis proposes that there will be no difference when results from the 2-hour C-peptide AUC at month 12—after adjusting for baseline values— are compared between the treatment and control groups. The alternative hypothesis proposes that there will be a difference when results of the 2-hour C-peptide AUC at month 12 —after adjusting for baseline values— are compared between the treatment and control groups. All statistical analyses of the 2-hour C-peptide AUC will be actually performed using the transformed variable mean 2-hour C-peptide ln (AUC+1).

The primary endpoint (section 3.2.1) will be analyzed using the ANCOVA model adjusted for baseline 2-hour C-peptide AUC from a 4-hour MMTT. For all primary and secondary analyses of C-peptide AUC from the MMTT, all values of the C-peptide response will be analyzed as ln(AUC + 1).

As a secondary analysis of the primary endpoint, we will model the mean 2-hour C-peptide ln(AUC+1) with other covariates such as age, sex, baseline HbA_{1C}, in addition to the baseline mean 2-hour C-peptide ln(AUC+1), in the ANCOVA model.

For a participant who misses the entire week 52 MMTT assessment, we will impute the missing AUC values using the following conservative approach for the primary analysis of the primary endpoint, which employs a pessimistic estimate when imputing in treatment group and an optimistic estimate when imputing in the placebo group:

- If the subject's last observed AUC value is 0, the missing week 52 AUC will be imputed as zero.

- If the subject's last observed AUC value is >0, then the missing AUC at week 52 will be imputed using data observed over the same time interval among subjects in the same group who had non-zero AUC values at the start of the interval. Specifically a linear regression line and 95% confidence bands will be fit where observed week 52 AUC are regressed on AUC values at the start of the interval. For example, if a subject's last observed value AUC was at week 32, then, using data from other subjects in the same treatment arm, week 52 AUC values would serve as the outcome in a regression model where week 32 AUC values are the predictors.

- In the treatment group, a missing week 52 AUC value will be imputed as the value predicted from the estimated lower limit of a 95% confidence band about the linear regression line.

- In the placebo group, a missing week 52 AUC value will be imputed as the value predicted from the estimated upper limit of a 95% confidence band about the linear regression line.

Sensitivity analyses will be included as secondary analyses. These will include:

- Using imputed values for missing week 52 AUCs as described above using a conservative approach. In the treatment arm a missing week 52 AUC value will be imputed as the value predicted from the estimated upper limit of a 95% confidence band about the linear regression line. In the placebo group, a missing week 52 AUC value will be imputed as the value predicted from the estimated lower limit of a 95% confidence band about the linear regression line.

- Using imputed values for missing week 52 AUCs as described above but using a "best guess" approach. In each arm, a missing week 52 AUC value will be imputed as the value predicted from the linear regression line.

- Using observed data only (i.e. without imputing any missing week 52 AUCs).

9.2.2 Analysis of Secondary Endpoints

The secondary endpoints are defined in section 3.2.2. The null hypothesis proposes that there is no difference in the secondary endpoint (measured either as means or proportions) between study groups. The alternative hypothesis proposes the opposite; that there is a difference in the secondary endpoints between the treatment and control groups.

- An analysis similar to the analysis of primary endpoint will be performed on the corresponding
  2-hour C-peptide AUC at month 24 and 4-hour C-peptide AUC at months 12 and 24.
- ANCOVA model adjusted for baseline will be used to analyze insulin use and HbA1C level at months 12 and 24.
- Fisher's exact test will be used to compare proportions of participants who are exogenous-insulin free and have major hypoglycemic events between the two groups.
- As exploratory analysis, we plan to use the mixed effects model to compare the Thymoglobulin group and the placebo group for C-peptide in response to MMTT, HbA1C, required dose of insulin, and major hypoglycemic events over time, from baseline to month 24.

9.2.3 Analysis of Safety

Safety evaluation will be performed in the safety sample. Safety parameters presented for each treatment group will include adverse events, changes in vital signs, and changes in laboratory values between baseline and end of study.

Adverse events will be coded using the MedDRA dictionary. The number of events and incidence of adverse events by system organ class and preferred term will be summarized. Adverse events by maximum severity and relationship to study drug will be compared between treatment groups. Separate summaries will be provided for serious adverse events and adverse events leading to study discontinuation.

9.3 PATIENT AND DEMOGRAPHIC DATA

9.3.1 Baseline Characteristics and Demographics

- Summary descriptive statistics for baseline and demographic characteristics will be provided for all enrolled participants. Demographic data will include age, race, sex, body weight, and height; these data will be presented in the following manner:
- Continuous data (i.e., age, body weight, and height) will be summarized descriptively by mean, standard deviation, median, and range.

- Continuous data specific to diabetes including basal, peak, and AUC of C-peptide in response to MMMT; HbA1c; and insulin dose in U/kg (e.g., the mean dose during the 5 days prior to first study drug administration) will be summarized descriptively, by treatment group, by mean, standard deviation, median, and range.
- Categorical data (i.e., sex and race) will be presented as enumeration and percentages.

9.3.2 Medical History

Medical history, including the existence of current signs and symptoms and clinical significance for each body system, will be collected and presented by group.

9.3.3 Use of Medications

All medications used will be coded using the World Health Organization (WHO) drug dictionary. The number and percentage of participants receiving concomitant medications or therapies will be presented.

9.3.4 Study Completion

The percentage of participants who complete the study, loss to follow-up, time to loss of follow-up, and reasons for discontinuation will be presented.

9.4 SAMPLE SIZE AND POWER CALCULATIONS

Based on data from the Herold trial,[32] the 12-month mean 2-hour C-peptide AUC after adjusting for baseline is assumed to be 0.462 pmol/mL in the Thymoglobulin group. For analysis, we use the TrialNet transformation ln(AUC+1). After transformation, the value is ln(0.462 + 1) = 0.380. Also based on the Herold trial,[32] this variable is assumed to have a root mean square error of 0.164.

Using a method similar to that used by TrialNet,[155] the true decrease in the 12-month mean 2-hour C-peptide AUC after adjusting for baseline is assumed to be 38% in the placebo group. The value is thus assumed to be 62% of 0.462 pmol/mL = 0.286 pmol/mL in the placebo group. After transformation, the value is ln(0.286+1) = 0.252. This variable is assumed to have the same root mean square error as it has in the treatment group.

With 2:1 Thymoglobulin versus placebo (saline solution) random assignment and a two-sided t-test with a significance level of 5%, a sample size of 60 provides an 80% power to detect this difference. To allow for a 10% dropout rate, a total of 66 patients will be enrolled; that is, 44 in the treatment group and 22 in the placebo group.

If the true decrease in the placebo group is only 30%, the power to detect such a difference would be 59%. On the other hand, if the true decrease in the placebo group is as high as 45%, the power would be 91%.[32]

Enrollment for this trial was stopped at 58 participants and did not meet the planned sample size of 66 participants. See section 1 for more details..

9.5 REPLACEMENT OF DISCONTINUED PARTICIPANTS

Randomly assigned participants shall not be replaced. The primary analysis is ITT.

9.6 INTERIM ANALYSIS

The DSMB will receive periodic safety reports on enrolled participants along with all study treatment discontinuation cases. The DSMB may request modifications to the protocol based on its review of the findings. No formal interim efficacy analysis is planned for this study.

9.7 REPORTING DEVIATIONS FROM THE ORIGINAL STATISTICAL PLAN

The principal features of the study design and the plan for statistical analysis of the data are outlined in this protocol and in the subsequent SAP. Any changes in these principal features will require a protocol or an SAP amendment, which would be subject to review by the independent DSMB, the study sponsor, and the health authorities. These changes will be described in the final report as appropriate.

10. IDENTIFICATION AND ACCESS TO SOURCE DATA

10.1 IDENTIFYING SOURCE DATA

The investigator is required to keep accurate records to ensure that the conduct of the study is fully documented (see section 11). The results of all clinical and clinical laboratory evaluations will be maintained in the participant's medical records and the data will be transferred to clinical CRFs.

Safety data will be recorded on CRFs specifically designed for this purpose. All the SAEs will be reported on an SAE report form as well as on individual CRFs. All data will be reviewed periodically by the DSMB and IRB. The DSMB and/or the IRB have the authority to withdraw any participants and/or terminate the study because of safety findings.

10.2 PERMITTING ACCESS TO SOURCE DATA

The investigational site participating in this study will maintain the highest degree of confidentiality permitted for the clinical and research information obtained from the participants in this clinical trial. 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 health authorities to examine (and when required by applicable law, to copy) clinical records for the purpose of quality assurance reviews, audits, and evaluations of the study safety and progress. Unless required by the laws that permit copying of records, only the coded identity associated with documents or with other participant data may be copied (and all personally identifying information must be obscured). Authorized representatives as noted above are bound to maintain the strict confidentiality of medical and research information that is linked to identify individuals. The investigational site will normally be notified before auditing visits occur.

11. QUALITY CONTROL AND QUALITY ASSURANCE

The investigator is required to keep accurate records to ensure that the conduct of the study is fully documented.

The sponsor is responsible for regularly reviewing the conduct of the trial, for verifying adherence to the protocol, and for confirming the completeness, consistency, and accuracy of all documented data.

11.1 DATA HANDLING

The investigator is required to keep accurate records to ensure that the conduct of the study is fully documented. The investigator is required to ensure that all CRFs are completed for every participant entered in the trial. The sponsor is responsible for regular inspection of the conduct of the trial, for verifying adherence to the protocol, and for confirming the completeness, consistency, and accuracy of all documented data. The CRFs will be completed online via a Web-based electronic data capture (EDC) system that has been validated and is compliant with Part 11 Title 21 of the Code of Federal Regulations. Study staff at the site will enter information into the electronic CRFs, and the data will

be stored remotely at a central database. Data quality will be ensured through the EDC system's continuous monitoring of data and real-time detection and correction of errors. All elements of data entry (i.e., time, date, verbatim text, and the name of the person performing the data entry) will be recorded in an electronic audit trail to allow all changes in the database to be monitored and maintained in accordance with federal regulations.

12. ETHICAL CONSIDERATIONS AND COMPLIANCE WITH GOOD CLINICAL PRACTICE

12.1 STATEMENT OF COMPLIANCE

Clinical protocol ITN028AI will be conducted using good clinical practice (GCP), as delineated in *Guidance for Industry: E6 Good Clinical Practice Consolidated Guidance*, and according to the criteria specified in this study protocol. Before study initiation, the protocol and the informed consent documents will be reviewed and approved by an appropriate EC or IRB. Any amendments to the protocol or to the consent materials must also be approved before they are implemented.

12.2 INFORMED CONSENT AND ASSENT

The informed consent form is a means of providing information about the trial to a prospective participant and allows for an informed decision about participation in the study. All participants (or their legally acceptable representative) must read, sign, and date a consent form before entering the study, taking study drug, or undergoing any study-specific procedures. Consent materials for participants who do not speak or read English must be translated into the participants' appropriate language.

The informed consent form must be revised whenever important new safety information is available, whenever the protocol is amended, and/or whenever any new information becomes available that may affect participation in the trial.

A copy of the informed consent will be given to a prospective participant for review. The attending physician, preferably in the presence of a witness, will review the consent and answer questions. The prospective participant will be told that being in the trial is voluntary and that he or she may withdraw from the study at any time, for any reason.

12.3 PRIVACY AND CONFIDENTIALITY

A participant's privacy and confidentiality will be respected throughout the study. Each participant will be assigned a sequential identification number, and these numbers rather than names will be used to collect, store, and report participant information.

13. PUBLICATION POLICY

The ITN policy on the publication of study results will apply to this trial. Authorized participants can find details of the policy statement on the ITN website at http://www.immunetolerance.org.

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Appendix 1. Schedule of Events

	Day									Month (ET = early termination)										
Time point[1]	**Scr**	**0**	**1**	**2**	**3**	**4**	**5**	**10**	**15**	**1**	**2**	**3**	**6**	**9**	**12/ ET**	**15**	**18**	**21**	**24/ ET**	**25–60**
Visit number	**-1**	**0**	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**	**16**	**17**	
General Assessments																				
Randomization		X																		
Informed consent	X	X																		
Inclusion/exclusion criteria	X																			
Medical history	X																			
Adverse events	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	
Concomitant medications	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	
Physical examination (includes height and weight)	X		X	X	X	X	X			X		X	X		X		X		X	
Telephone call to monitor malignancies every 6 months																				X
Local Laboratory Assessments																				
Serum chemistries[2]	X		X	X[3]	X	X	X	X	X	X	X		X[3]		X[3]					
CBC (with differential, platelets) [4]			X	X	X	X														
CBC (with differential, T-cell subsets, platelets)	X						X	X	X	X	X	X	X		X		X		X	
PPD, hepatitis B and C, HIV, Toxoplasmosis, HSV1 IgG, HSV2 IgG, VZV, EBV, CMV IgG (serology)	X																			
C3, C4, and CH50 complement levels		X					X	X	X	X	X									
Immunoglobulin A (IgA)[5]		X																		
Urine pregnancy test	X	X	X[6]																	
Urinalysis	X						X	X	X	X	X									
ITN Core Assessments (Clinical)																				

[1] See section 6.8 for visit windows.
[2] Electrolytes (sodium, potassium, chloride, total CO_2), urea nitrogen (BUN), creatinine, and liver panel (AST, ALT, alkaline phosphate, direct and total bilirubin).
[3] Liver panel only (AST, ALT, alkaline phosphate, direct bilirubin, and total bilirubin).
[4] Must be performed at least 6 hours after each Thymoglobulin infusion, with results available before the next infusion.
[5] Perform only if participant was VZV seronegative at screening.
[6] Perform only if visit 1 occurs later than 7 days after visit 0.

	Day									Month (ET = early termination)										
Time point[1]	**Scr**	**0**	**1**	**2**	**3**	**4**	**5**	**10**	**15**	**1**	**2**	**3**	**6**	**9**	**12/ ET**	**15**	**18**	**21**	**24/ ET**	**25–60**
Visit number	**-1**	**0**	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**	**16**	**17**	
Whole blood–quantitative PCR for EBV/CMV reactivation[7]	X							X	X	X	X	X	X	X	X	X	X	X	X	
Serum–autoantibody analysis	X											X	X		X		X		X	
Medications																				
Premedication[8]			X	X	X	X														
Thymoglobulin or placebo (saline solution) infusion[9]			X	X	X	X														
Trimethoprim-sulfamethoxazole[10] or placebo-trimethoprim-sulfamethoxazole							X	X	X	X	X	X								
Acyclovir[11] or placebo-acyclovir							X	X	X	X	X	X								
Diabetes Assessments																				
MMTT[12]	X												X		X		X		X	
HbA_{1C}		X										X	X	X	X	X	X	X	X	
Insulin use (U/kg body weight/day)		X										X	X	X	X	X	X	X	X	
Hypoglycemia events		X										X	X	X	X	X	X	X	X	

[7] If PCR results >1,000 copies per milliliter, weekly PCR is indicated. (For details, see sections 5.10.1 and 5.10.2.)
[8] Refer to section 5.2 for the list of premedications.
[9] If the Thymoglobulin dose is adjusted, the last infusion must be administered by the end of day 7. Refer to section 5.1.2 and 5.6 for details.
[10] Refer to section 5.9 for dosing information and alternatives.
[11] For patients who are HSV1, HSV2, or VZV seropositive. Refer to section 5.10.3 for dosing information.
[12] See Appendix 2.

	Day									Month (ET = early termination)										
Time Point [1]	**Scr**	**0**	**1**	**2**	**3**	**4**	**5**	**10**	**15**	**1**	**2**	**3**	**6**	**9**	**12 /ET**	**15**	**18**	**21**	**24 /ET**	**25-60**
Visit Number	**-1**	**0**	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**	**16**	**17**	
ITN Core Laboratory Assessments (Mechanistic)																				
Hepatitis B and tetanus antibody titers[13,14,,15]		X													X[13,14]	X[15]				
Hepatitis B and tetanus vaccine/booster[14]															X[14]					
Hepatitis A neoantigen vaccine[14]															X[14]					
Hepatitis A antibody titers[13,14,15]															X[13,14]	X[15]				
Serum–secreted cytokines			X[16]	X[16]	X[16]					X										
Frozen PBMC–T-cell assay		X										X	X		X		X		X	
Whole blood–gene expression profiling		X											X			X			X	
Whole blood–flow cytometry panel staining		X		X		X			X	X		X	X		X		X		X	
Whole blood DNA–HLA and SNP genotypes														X						
Frozen PBMC–DNA TREC		X							X	X	X	X	X	X	X					
Anti-ATG antibodies		X							X	X	X	X								

[13] Must be drawn before the vaccine/booster is given.
[14] May be performed at visit 14 (month 15).
[15] If the Hepatitis B, tetanus, and /or Hepatis A neoantigen vaccines are administered at visit 14 (month 15), then the post vaccine titers should be collected at visit 15 (month 18).
[16] Before and 3 hours after completion of each infusion.

Appendix 2. Procedures for Performing the Mixed-Meal Tolerance Test

The mixed-meal tolerance test (MMTT) is performed in the morning (between 7:00 a.m. and 10:00 a.m.), which means that administration must begin within this time. It is recommended that the tests be scheduled early in the morning (7:00–7:30 am) because blood glucose will be more likely to be within the target range at that time.

The mixed meal used in this protocol will be the Boost High Protein Nutritional Energy Drink® (Mead-Johnson). If a participant has a known food allergy to one or more components of Boost, an equivalent substitution may be used. The MMTT should take 250 minutes to perform.

Dietary Guidelines and Pretest Instructions

Carbohydrates (CHO) should not be restricted from the diet before the test. A general guideline is that preadolescent participants should consume at least 25 kcal (6.25 g) CHO/kg/day and adolescent and adult participants should consume at least 15 kcal (3.75 g) CHO/kg/day for 3 days before the test. These are minimum amounts of CHO; most diets will include greater amounts of CHO. There is no need to alter the participant's diet unless he or she has been on a CHO-restricted diet.

In preparation for the visit, each participant should:

- Fast for at least 10 hours (but not more than 16 hours) before the test. Fasting should start the night before the test, and should continue up until the start of the test. Participants should not eat or drink anything except water. This means no coffee, tea, soda, cigarettes, alcohol, or chewing gum during the fasting period.
- Refrain from vigorous exercise during the fasting period.
- Refrain from working the night before the morning of the test.
- Discontinue taking any prescription medications that must be taken daily.

Glucose and Insulin Before the Test

- Short-acting insulin analogues (such as lispro or l-aspart) may be administered up to 2 hours before the test.
- Regular insulin may be administered up to 6 hours before the test.
- Intermediate-acting insulin (such as NPH) may be administered on the evening before the MMTT, but not on the morning of the test. Participants managed with intermediate-acting insulin (NPH or Lente) should administer their usual dose on the evening before the MMTT, but not on the morning of the test.
- Long-acting basal (such as glargine) insulin or continuous subcutaneous insulin infusion may be administered before, during and after the test as usual. Participants on glargine may take their usual injection at the appropriate time, and those on continuous subcutaneous insulin infusion may continue with their usual basal settings.

Target Glucose Level at the Start of Test

The target glucose level at the start of the test is between 70 and 200 mg/dL. Regular insulin or short acting insulin analogues may be used up to 6 and 2 hours before the test, respectively, to achieve the desired glucose level. The investigator and the study participant should discuss the individual situation for insulin administration to attain the goal of meter capillary glucose values within the range of 70–200 mg/dL at the start of the test. For example, as a practical matter, participants may be instructed to check their blood glucose by meter at home 2 hours before the start of the test so that marked hyperglycemia can be treated with a short-acting insulin analogue. Alternatively, participants

who arrive at the research unit with elevated blood glucose can receive additional short-acting insulin analogues at the time of their arrival, if the test itself does not start until at least 2 hours after insulin administration and occurs before 10 a.m.

IV Placement During the Test

- The IV should be in place for the duration of the test and must be flushed after each draw with saline solution or heparin flush.
- The participant should remain sitting or resting in bed quietly throughout the test and until the test is completed. However, he or she may engage in quiet, nonstrenuous activities, such as reading, playing cards, or watching TV. The participant may walk to the bathroom between blood draws if necessary.

Testing Instructions

Time Point −10 minutes

- The first sample should be taken at least 10 minutes after establishing the line(s) and when the participant is calm and relaxed (if possible, depending on age) - this is the "−10 minute" sample.
- Draw one 2-mL sample into the purple-top tube for C-peptide and transfer the sample to the laboratory. At the laboratory, spin the tube in a tabletop centrifuge (1000–1300 g) for 10 minutes. Tubes must be spun within 15 minutes of blood draw. Freeze the sample at −0 °C.
- Draw one 2-mL sample into the gray-top tube for glucose. Refrigerate the sample until centrifugation.

Time Point 0 minutes

- The second sample should be taken just before the participant drinks the Boost; this is the "0-minute" sample.
- Then the MMTT dose should be given with 6 kcal/kg @ 1 kcal/mL of mixed meal, to a maximum of 360 mL. The participant should consume the MMTT dose in no more than 5 minutes.

Time Points 15, 30, 60, 90, 120, 150, 180, 210, and 240 minutes

- Draw one 2-mL sample into the gray-top tube for glucose at each of the time points specified.
- Draw one 2-mL sample into the purple-top tube for C-peptide and insulin levels. Refrigerate the sample or put on ice until centrifugation. Please check blood glucose by meter.
- At the conclusion of the test, please check blood glucose by glucometer, and administer insulin as per participant's standard insulin plan.

Tube-Processing Instructions

- Spin the gray- and purple-top tubes. Then collect the serum into individual vials. Please make sure that each vial is properly identified with a label that indicates the time point.
- Freeze the samples for glucose and C-peptide / insulin levels.
- Ship the specimens to the laboratory where the assays will be performed.

A clogged line, missed sample, or other deviation from the protocol must be noted on the Comments section of the MMTT specimen transmittal form.

Time (minutes)	Glucose Sample Taken	C-peptide/Insulin Sample Taken
–10	X	X
0	X	X
Participant drinks boost		
15	X	X
30	X	X
60	X	X
90	X	X
120	X	X
150	X	X
180	X	X
210	X	X
240	X	X

Attachment 1. Package Insert for Thymoglobulin

Thymoglobulin®
Anti-thymocyte Globulin (Rabbit)

Sterile Lyophilized Preparation
For Intravenous Use Only

R_X only

Thymoglobulin® should only be used by physicians experienced in immunosuppressive therapy for the management of renal transplant patients.

DESCRIPTION

Thymoglobulin® [Anti-thymocyte Globulin (Rabbit)] is a purified, pasteurized, gamma immune globulin, obtained by immunization of rabbits with human thymocytes. This immunosuppressive product contains cytotoxic antibodies directed against antigens expressed on human T-lymphocytes.

Thymoglobulin is a sterile, freeze-dried product for intravenous administration after reconstitution with sterile Water for Injection, USP (SWFI).

Each 10 mL vial contains 25 mg anti-thymocyte globulin (rabbit) as well as 50 mg glycine, 50 mg mannitol, and 10 mg sodium chloride.

After reconstitution with 5 mL SWFI, each vial of reconstituted product contains approximately 5 mg/mL of Thymoglobulin, of which >90% is rabbit gamma immune globulin (IgG). The reconstituted solution has a pH of 7.0 ± 0.4. Human red blood cells are used in the manufacturing process to deplete cross-reactive antibodies to non-T-cell antigens. The manufacturing process is validated to remove or inactivate potential exogenous viruses. All human red blood cells are from US registered or FDA licensed blood banks. A viral inactivation step (pasteurization, i.e., heat treatment of active ingredient at 60°C/10 hr) is performed for each lot. Each Thymoglobulin lot is released following potency testing (lymphocytotoxicity and E-rosette inhibition assays), and cross-reactive antibody testing (hemagglutination, platelet agglutination, anti-human serum protein antibody, antiglomerular basement membrane antibody, and fibroblast toxicity assays on every fifth lot).

PHARMACOLOGY

Mechanism of Action

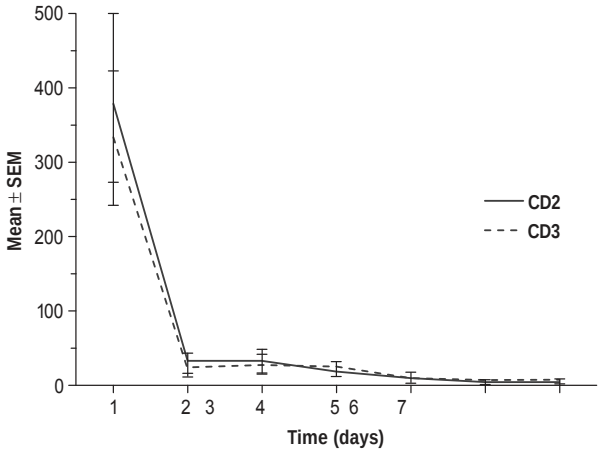
The mechanism of action by which polyclonal anti-lymphocyte preparations suppress immune responses is not fully understood. Possible mechanisms by which Thymoglobulin may induce immunosuppression in vivo include: T-cell clearance from the circulation and modulation of T-cell activation, homing, and cytotoxic activities. Thymoglobulin includes antibodies against T-cell markers such as CD2, CD3, CD4, CD8, CD11a, CD18, CD25, CD44, CD45, HLA-DR, HLA Class I heavy chains, and β2 microglobulin. In vitro, Thymoglobulin (concentrations >0.1 mg/mL) mediates T-cell suppressive effects via inhibition of proliferative responses to several mitogens. In patients, T-cell depletion is usually observed within a day from initiating Thymoglobulin therapy. Thymoglobulin has not been shown to be effective for treating antibody (humoral) mediated rejections.

Pharmacokinetics and Immunogenicity

After an intravenous dose of 1.25 to 1.5 mg/kg/day (over 4 hours for 7–11 days) 4–8 hours post-infusion, Thymoglobulin levels were on average 21.5 µg/mL (10–40 µg/mL) with a half-life of 2–3 days after the first dose, and 87 µg/mL (23–170 µg/mL) after the last dose. During the Thymoglobulin* Phase III randomized trial, of the 108 of 163 patients evaluated, anti-rabbit antibodies developed in 68% of the Thymoglobulin-treated patients, and anti-horse antibodies developed in 78% of the Atgam®**-treated patients (p=n.s.). No controlled studies have been conducted to study the effect of anti-rabbit antibodies on repeat use of Thymoglobulin. However, monitoring the lymphocyte count to ensure that T-cell depletion is achieved upon retreatment with Thymoglobulin is recommended.

Based on data collected from a limited number of patients (Clinical study Phase III, n=12), T-cell counts are presented in the chart below. These data were collected using flow cytometry (FACSCAN, Becton-Dickinson).

Mean T-Cell Counts Following Initiation of Thymoglobulin Therapy



Clinical Trials

US Phase III Study

A controlled, double-blind, multicenter, randomized clinical trial comparing Thymoglobulin and Atgam was conducted at 28 US transplant centers in renal transplant patients (n=163) with biopsy-proven Banff Grade II (moderate), Grade III (severe), or steroid-resistant Grade I (mild) acute graft rejection. This clinical trial rejected the null hypothesis that Thymoglobulin was more than 20% less effective in reversing acute rejection than Atgam. The overall weighted estimate of the treatment difference (Thymoglobulin–Atgam success rate) was 11.1% with a lower 95% confidence bound of 0.07%. Therefore, Thymoglobulin was at least as effective as Atgam in reversing acute rejection episodes.

*Thymoglobulin is a registered trademark of Genzyme Corporation, Cambridge, MA 02142

**Atgam is a registered trademark of Pfizer Inc, New York, NY 10017

In the study, patients were randomized to receive 7 to 14 days of Thymoglobulin (1.5 mg/kg/day) or Atgam (15 mg/kg/day). For the entire study, the two treatment groups were comparable with respect to donor and recipient characteristics. During the trial, the FDA approved new maintenance immunosuppressive agents (tacrolimus and mycophenolate). Off-protocol use of these agents occurred during the second half of the study in some patients without affecting the overall conclusions (Thymoglobulin 22/43, Atgam 20/37; p=0.826). The results, however, are presented for the first and second halves of the study (Table 1). In Table 1, successful treatment is presented as those patients whose serum creatinine levels (14 days from the diagnosis of rejection) returned to baseline and whose graft was functioning on day 30 after the end of therapy.

Table 1. Response to Study Treatment by Rejection Severity and Study Half

Success / n	Total		First Half		Second Half	
	Thymoglobulin	Atgam	Thymoglobulin	Atgam	Thymoglobulin	Atgam
Risk Factor:						
Baseline						
Rejection Severity:						
Mild	9/10 (90.0%)	5/8 (62.5%)	5/5 (100%)	1/3 (33.3%)	4/5 (80.0%)	4/5 (80.0%)
Moderate	44/58 (75.5%)	41/58 (70.7%)	22/26 (84.6%)	22/32 (68.8%)	22/32 (68.8%)	19/26 (73.1%)
Severe	11/14 (71.6%)	8/14 (57.1%)	6/8 (75.0%)	3/8 (37.5%)	5/6 (83.3%)	5/6 (83.3%)
Overall	64/82 (78.0%)	54/80 (67.5%)	33/39 (84.6%)	26/43 (60.5%)	31/43 (72.1%)	28/37 (75.7%)
Weighted estimate of difference (Thymoglobulin – Atgam)	11.1%[a]		19.3%		–3.2%	
Lower one-sided 95% confidence bound	0.07%		4.6%		–19.7%	
p Value[b]	0.061[1]		0.008[2]		0.625[2]	

1. one-sided stratified on rejection severity and study half
2. one-sided stratified on rejection severity
a. across rejection severity and study half
b. under null hypothesis of equivalence (Cochran-Mantel-Haenszel test)

There were no significant differences between the two treatments with respect to (i) day 30 serum creatinine levels relative to baseline, (ii) improvement rate in post-treatment histology, (iii) one-year post-rejection Kaplan-Meier patient survival (Thymoglobulin 93%, n=82 and Atgam 96%, n=80), (iv) day 30 and (v) one-year post-rejection graft survival (Thymoglobulin 83%, n=82; Atgam 75%, n=80).

INDICATIONS AND USAGE

Thymoglobulin is indicated for the treatment of renal transplant acute rejection in conjunction with concomitant immunosuppression.

CONTRAINDICATIONS

Thymoglobulin is contraindicated in patients with history of allergy or anaphylaxis to rabbit proteins, or who have an acute viral illness.

WARNINGS

Thymoglobulin should only be used by physicians experienced in immunosuppressive therapy for the treatment of renal transplant patients. Medical surveillance is required during Thymoglobulin infusion. In rare instances, anaphylaxis has been reported with Thymoglobulin use. In such cases, the infusion should be terminated immediately. Medical personnel should be available to treat patients who experience anaphylaxis. Emergency treatment such as 0.3 mL to 0.5 mL aqueous epinephrine (1:1000 dilution) subcutaneously and other resuscitative measures including oxygen, intravenous fluids, antihistamines, corticosteroids, pressor amines, and airway management, as clinically indicated, should be provided. Thymoglobulin or other rabbit immunoglobulins should not be

PRECAUTIONS

General

Thymoglobulin infusion may produce fever and chills. To minimize these, the first dose should be infused over a minimum of 6 hours into a high- flow vein. Also, premedication with corticosteroids, acetaminophen, and/or an antihistamine and/or slowing the infusion rate may reduce reaction incidence and intensity (see DOSAGE AND ADMINISTRATION).

Prolonged use or overdosage of Thymoglobulin in association with other immunosuppressive agents may cause over-immunosuppression result- ing in severe infections and may increase the incidence of lymphoma or post-transplant lymphoproliferative disease (PTLD) or other malignancies. Appropriate antiviral, antibacterial, antiprotozoal, and/or antifungal prophylaxis is recommended.

Laboratory Tests

During Thymoglobulin therapy, monitoring the lymphocyte count (i.e., total lymphocyte and/or T-cell subset) may help assess the degree of T-cell depletion (see Pharmacokinetics and Immunogenicity). For safety, WBC and platelet counts should also be monitored (see DOSAGE AND ADMINISTRATION).

Drug Interactions

- Because Thymoglobulin is administered to patients receiving a standard immunosuppressive regimen, this may predispose patients to over-immunosuppression. Many transplant centers decrease maintenance administered again for such patients. Thrombocytopenia or neutrope- nia may result from cross-reactive antibodies and is reversible following dose adjustments.

immunosuppression therapy during the period of antibody therapy.

- Thymoglobulin can stimulate the production of antibodies which cross-react with rabbit immune globulins. (See Pharmacokinetics and Immunogenicity.)

Drug/Laboratory Test Interactions

Thymoglobulin has not been shown to interfere with any routine clinical laboratory tests which do not use immunoglobulins. Thymoglobulin may interfere with rabbit antibody-based immunoassays and with cross-match or panel-reactive antibody cytotoxicity assays.

Carcinogenesis, Mutagenesis, Impairment of Fertility

The carcinogenic and mutagenic potential of Thymoglobulin and its potential to impair fertility have not been studied.

Pregnancy: Pregnancy Category C

Animal reproduction studies have not been conducted with Thymoglobulin. It is also not known whether Thymoglobulin can cause fetal harm or can affect reproduction capacity. Thymoglobulin should be given to a pregnant woman only if clearly needed.

Nursing Mothers

Thymoglobulin has not been studied in nursing women. It is not known whether this drug is excreted in human milk. Because many drugs are excreted in human milk, caution should be exercised when Thymoglobulin is administered to a nursing woman.

Pediatric Use

The safety and effectiveness of Thymoglobulin in pediatric patients has not been established in controlled trials. However, the dose, efficacy, and adverse event profile are not thought to be different from adults based on limited European studies and US compassionate use.

ADVERSE REACTIONS

Thymoglobulin adverse events are generally manageable or reversible. In the US Phase III controlled clinical trial (n = 163) comparing the efficacy and safety of Thymoglobulin and Atgam, there were no significant differ- ences in clinically significant adverse events between the two treatment groups (Table 2). Malignancies were reported in 3 patients who received Thymoglobulin and in 3 patients who received Atgam during the one-year follow-up period. These included two PTLDs in the Thymoglobulin group and two PTLDs in the Atgam group.

Infections occurring in both treatment groups during the 3-month follow-up are summarized in Table 3. No significant differences were seen between the Thymoglobulin and Atgam groups for all types of infections, and the incidence of cytomegalovirus (CMV) infection was equivalent in both groups. (Viral prophylaxis was by the center's discretion during antibody treatment, but all centers used gancyclovir infusion during treatment.)

Table 2. Frequently Reported and Significant Adverse Events*

Preferred Term	Thymoglobulin n=82 No. of Patients	(%)	Atgam n=81 No. of Patients	(%)	p Value†
Frequently Reported Events					
Fever	52	(63.4)	51	(63.0)	1.0
Chills	47	(57.3)	35	(43.2)	0.086
Leukopenia	47	(57.3)	24	(29.6)	<0.001
Pain	38	(46.3)	35	(43.2)	0.753
Headache	33	(40.2)	28	(34.6)	0.518
Abdominal pain	31	(37.8)	22	(27.2)	0.181
Diarrhea	30	(36.6)	26	(32.1)	0.622
Hypertension	30	(36.6)	23	(28.4)	0.316
Nausea	30	(36.6)	23	(28.4)	0.316
Thrombocytopenia	30	(36.6)	36	(44.4)	0.341
Peripheral edema	28	(34.1)	28	(34.6)	1.0
Dyspnea	23	(28.0)	16	(19.8)	0.271
Asthenia	22	(26.8)	26	(32.1)	0.495
Hyperkalemia	22	(26.8)	15	(18.5)	0.262
Tachycardia	22	(26.8)	19	(23.5)	0.719
Significant Events§					
Leukopenia	47	(57.3)	24	(29.6)	<0.001
Malaise	11	(13.4)	3	(3.7)	0.047
Dizziness	7	(8.5)	20	(24.7)	0.006

* Treatment Emergent Adverse Events (TEAE) are summarized. Frequently reported adverse events are those reported by more than 25% of patients in a treatment group; significant adverse events are those where the incidence rate differed between treatment groups by a significance level of ≤0.05.

† p Value comparing treatment groups using Fisher's exact test.

§ Statistically significant differences in the AEs.

Table 3. Infections

	Thymoglobulin n=82			Atgam n=81			
BODY SYSTEM Preferred Term	No. of Patients	Total (%)	Reports	No. of Patients	Total (%)	Reports	p Value†
BODY AS A WHOLE	30	(36.6)	36	22	(27.2)	29	0.240
Infection	25	(30.5)	26	19	(23.5)	21	0.378
Other	14	(17.1)	15	11	(13.6)	12	0.665
CMV	11	(13.4)	11	9	(11.1)	9	0.812
Sepsis	10	(12.2)	10	7	(9.6)	7	0.610
Moniliasis	0	(0.0)	0	1	(1.2)	1	0.497
DIGESTIVE	5	(6.1)	5	3	(3.7)	3	0.720
Gastrointestinal moniliasis	4	(4.9)	4	1	(1.2)	1	0.367
Oral moniliasis	3	(3.7)	0	2	(2.5)	1	0.497
Gastritis	1	(1.2)	1	0	(0.0)	0	1.000
RESPIRATORY	0	(0.0)	0	1	(1.2)	1	0.497
Pneumonia	0	(0.0)	0	1	(1.2)	1	0.497
SKIN	4	(4.9)	4	0	(0.0)	0	0.120
Herpes simplex	4	(4.9)	4	0	(0.0)	0	0.120
UROGENITAL	15	(18.3)	15	22	(29.2)	22	0.195
Urinary tract infection	15	(18.3)	15	21	(25.9)	21	0.262
Vaginitis	0	(0.0)	0	1	(1.2)	1	0.497
NOT SPECIFIED	0	(0.0)	0	2	(2.5)	2	0.245

†p Value comparing treatment groups using Fisher's exact test.

OVERDOSAGE

Thymoglobulin overdosage may result in leukopenia or thrombocytopenia, which can be managed with dose reduction. (See DOSAGE AND ADMINISTRATION).

DOSAGE AND ADMINISTRATION

The recommended dosage of Thymoglobulin for treatment of acute renal graft rejection is 1.5 mg/kg of body weight administered daily for 7 to 14 days. The recommended route of administration is intravenous infusion using a high-flow vein. Thymoglobulin should be infused over a minimum of 6 hours for the first infusion and over at least 4 hours on subsequent days of therapy.

Thymoglobulin should be administered through an in-line 0.22 µm filter.

Thymoglobulin is supplied as a 10 mL vial containing lyophilized (solid) Thymoglobulin (25 mg).

For vial reconstitution, dilution in infusion solution and infusion procedure. (See Preparation for Administration) Investigations indicate that Thymoglobulin is well tolerated and less likely to produce side effects when administered at the recommended rate. Administration of antiviral prophylactic therapy is recommended. Premedication with corticosteroids, acetaminophen, and/or an antihistamine 1 hour prior to the infusion is recommended and may reduce the incidence and intensity of side effects during the infusion. (See PRECAUTIONS: General) Medical personnel should monitor patients for adverse events during and after infusion. Monitoring T-cell counts (absolute and/or subsets) to assess the level of T-cell depletion is recommended. Total white blood cell and platelet counts should be monitored.

Overdosage of Thymoglobulin may result in leukopenia and/or thrombocytopenia. The Thymoglobulin dose should be reduced by one-half if the WBC count is between 2,000 and 3,000 cells/mm^3 or if the platelet count is between 50,000 and 75,000 cells/mm^3. Stopping Thymoglobulin treatment should be considered if the WBC count falls below 2,000 cells/mm^3 or platelets below 50,000 cells/mm^3.

Preparation for Administration

Reconstitution

After calculating the number of vials needed, using aseptic technique, reconstitute each vial of Thymoglobulin with 5 mL of sterile Water for Injection, USP (SWFI). Reconstituted Thymoglobulin is physically and chemically stable for up to 24 hours at room temperature; however, room temperature storage is not recommended. As Thymoglobulin contains no preservatives, reconstituted product should be used immediately.

1. Allow Thymoglobulin vials to reach room temperature before reconstituting the lyophilized product.
2. Aseptically remove caps to expose rubber stoppers.
3. Clean stoppers with germicidal or alcohol swab.
4. Aseptically reconstitute each vial of Thymoglobulin lyophilized powder with the 5 mL of SWFI.
5. Rotate vial gently until powder is completely dissolved. Each reconstituted vial contains 25 mg or 5 mg/mL of Thymoglobulin.
6. Inspect solution for particulate matter after reconstitution. Should some particulate matter remain, continue to gently rotate the vial until no particulate matter is visible. If particulate matter persists, discard this vial.

Dilution

1. Transfer the contents of the calculated number of Thymoglobulin vials into the bag of infusion solution (saline or dextrose). Recommended volume: per one vial of Thymoglobulin use 50 mL of infusion solution (total volume usually between 50 to 500 mL).
2. Mix the solution by inverting the bag gently only once or twice.

Infusion

1. Follow the manufacturer's instructions for the infusion administration set. Infuse through a 0.22-micron filter into a high-flow vein.
2. Set the flow rate to deliver the dose over a minimum of 6 hours for the first dose and over at least 4 hours for subsequent doses.

HOW SUPPLIED

Thymoglobulin is available as sterile, lyophilized powder to be reconstituted with sterile Water for Injection, USP (SWFI). Each package contains a 10 mL vial of freeze-dried Thymoglobulin (25 mg) NDC# 58468-0080-1.

Storage

- Store in refrigerator between +2°C to +8°C (36°F to 46°F).
- Protect from light.
- Do not freeze.
- Do not use after the expiration date indicated on the label.
- Reconstituted Thymoglobulin is physically and chemically stable for up to 24 hours at room temperature; however, room temperature storage is not recommended. As Thymoglobulin contains no preservatives, recon- stituted product should be used immediately.
- Infusion solutions of Thymoglobulin must be used immediately.
- Any unused drug remaining after infusion must be discarded.

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Manufactured for:
Genzyme Corporation
500 Kendall Street
Cambridge, MA 02142 USA

By:
Genzyme Polyclonals, S.A.S.
Marcy L'Etoile, France
US License No. 1596

Issued: 07/06

genzyme

Attachment 2. Package Insert for Sulfamethoxazole and Trimethoprim

Source: http://dailymed.nlm.nih.gov/dailymed/fdaDrugXsl.cfm?id=2945&type=display

sulfamethoxazole and trimethoprim (Sulfamethoxazole and Trimethoprim) tablet
[Mutual Pharmaceutical Company, Inc.]

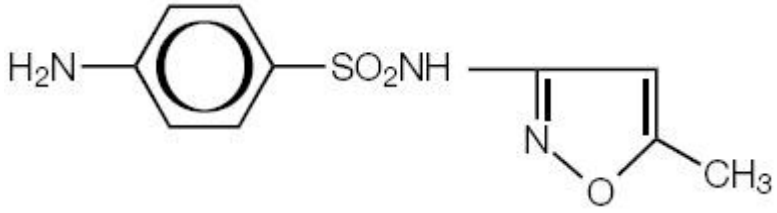
Rx only

To reduce the development of drug-resistant bacteria and maintain the effectiveness of sulfamethoxazole and trimethoprim tablets and other antibacterial drugs, sulfamethoxazole and trimethoprim tablets should be used only to treat or prevent infections that are proven or strongly suspected to be caused by bacteria.

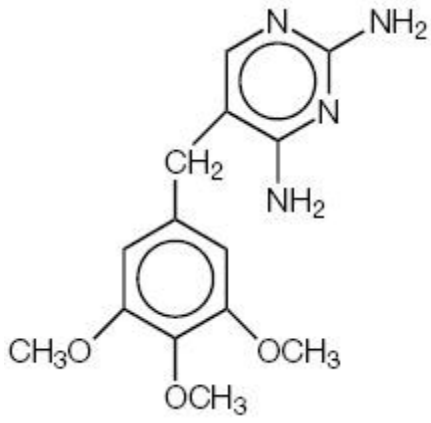
DESCRIPTION

Sulfamethoxazole and trimethoprim is a synthetic antibacterial combination product available in DS (double strength) tablets, each containing 800 mg sulfamethoxazole and 160 mg trimethoprim; in tablets, each containing 400 mg sulfamethoxazole and 80 mg trimethoprim for oral administration.

Sulfamethoxazole is N1 - (5-methyl-3-isoxazolyl)sulfanilamide; the molecular formula is C10H11N3O3S. It is almost white, odorless, tasteless compound with a molecular weight of 253.28 and the following structural formula:



Trimethoprim is 2,4-diamino-5-(3,4,5-trimethoxybenzyl)pyrimidine; the molecular formula is C14H18N4O3. It is a white to light yellow, odorless, bitter compound with a molecular weight of 290.3. It has the following structural formula:



Inactive ingredients: Docusate sodium 85%, sodium benzoate 15%, sodium starch glycolate, magnesium stearate and pregelatinized starch.

CLINICAL PHARMACOLOGY

Sulfamethoxazole and trimethoprim is rapidly absorbed following oral administration. Both sulfamethoxazole and trimethoprim exist in the blood as unbound, protein-bound and metabolized forms; sulfamethoxazole also exists as the conjugated form. The metabolism of sulfamethoxazole occurs predominately by N4-acetylation, although the glucuronide conjugate has been identified. The principal metabolites of trimethoprim are the 1- and 3-oxides and the 3'- and 4'- hydroxy derivatives. The free forms of sulfamethoxazole and trimethoprim are considered to be the therapeutically active forms. Approximately 70% of sulfamethoxazole and 44% of trimethoprim are bound to plasma proteins. The presence of 10 mg percent sulfamethoxazole in plasma decreases the protein binding of trimethoprim by an insignificant degree; trimethoprim does not influence the protein binding of sulfamethoxazole.

Peak blood levels for the individual components occur 1 to 4 hours after oral administration.

The mean serum half-lives of sulfamethoxazole and trimethoprim are 10 and 8 to 10 hours, respectively. However, patients with severely impaired renal function exhibit an increase in the half-lives of both components, requiring dosage regimen adjustment (see DOSAGE AND ADMINISTRATION section). Detectable amounts of sulfamethoxazole and trimethoprim are present in the blood 24 hours after drug administration. During administration of 800 mg sulfamethoxazole and 160 mg trimethoprim b.i.d., the mean steady-state plasma concentration of trimethoprim was 1.72 µg/mL. The steady-state mean plasma levels of free and total sulfamethoxazole were 57.4 µg/mL and 68.0 µg/mL, respectively. These steady-state levels were achieved after three days of drug administration.1

Excretion of sulfamethoxazole and trimethoprim is primarily by the kidneys through both glomerular filtration and tubular secretion. Urine concentrations of both sulfamethoxazole and trimethoprim are considerably higher than are the concentrations in the blood. The average percentage of the dose recovered in urine from 0 to 72 hours after a single oral dose of sulfamethoxazole and trimethoprim is 84.5% for total sulfonamide and 66.8% for free trimethoprim. Thirty percent of the total sulfonamide is excreted as free sulfamethoxazole, with the remaining as N4-acetylated metabolite.2 When administered together as sulfamethoxazole and trimethoprim, neither sulfamethoxazole nor trimethoprim affects the urinary excretion pattern of the other.

Both sulfamethoxazole and trimethoprim distribute to sputum, vaginal fluid and middle ear fluid; trimethoprim also distributes to bronchial secretion, and both pass the placental barrier and are excreted in human milk.

Geriatric Pharmacokinetics

The pharmacokinetics of sulfamethoxazole 800 mg and trimethoprim 160 mg were studied in 6 geriatric subjects (mean age: 78.6 years) and 6 young healthy subjects (mean mean age: 29.3 years) using a non-US approved formulation. Pharmacokinetic values for sulfamethoxazole in geriatric subjects were similar to those observed in young adult subjects. The mean renal clearance of trimethoprim was significantly lower in geriatric subjects compared with young adult subjects (19 mL/h/kg vs. 55 mL/h/kg). However, after

normalizing by body weight, the apparent total body clearance of trimethoprim was on average 19% lower in geriatric subjects compared with young adult subjects.3

Microbiology

Sulfamethoxazole inhibits bacterial synthesis of dihydrofolic acid by competing with paraaminobenzoic acid (PABA). Trimethoprim blocks the production of tetrahydrofolic acid from dihydrofolic acid by binding to and reversibly inhibiting the required enzyme, dihydrofolate reductase. Thus, sulfamethoxazole and trimethoprim blocks two consecutive steps in the biosynthesis of nucleic acids and proteins essential to many bacteria.

In vitro studies have shown that bacterial resistance develops more slowly with both sulfamethoxazole and trimethoprim in combination than with either sulfamethoxazole or trimethoprim alone.

Sulfamethoxazole and trimethoprim have been shown to be active against most strains of the following microorganisms, both in vitro and in clinical infections as described in the INDICATIONS AND USAGE section.

Aerobic gram-positive microorganisms:

- Streptococcus pneumoniae

Aerobic gram-negative microorganisms:

- Escherichia coli (including susceptible enterotoxigenic strains implicated in traveler's diarrhea)
- Klebsiella species
- Enterobacter species
- Haemophilus influenzae
- Morganella morganii
- Proteus mirabilis
- Proteus vulgaris
- Shigella flexneri
- Shigella sonnei

Other Organisms:

- Pneumocystis carinii

Susceptibility Testing Methods

DILUTION TECHNIQUES

Quantitative methods are used to determine antimicrobial minimum inhibitory concentrations (MICs). These MICs provide estimates of the susceptibility of bacteria to antimicrobial compounds. The MICs should be determined using a standardized procedure. Standardized procedures are based on a dilution method5 (broth or agar) or equivalent with standardized inoculum concentrations and standardized concentrations of sulfamethoxazole/trimethoprim powder. The MIC values should be interpreted according to the following criteria:

For testing Enterobacteriaceae:

MIC (µg/mL)	**Interpretation**
≤ 2/38	Susceptible (S)
≥ 4/76	Resistant (R)

When testing either Haemophilus influenzae* or Streptococcus pneumoniae†:

MIC (µg/mL)	**Interpretation†**
≤ 0.5/9.5	Susceptible (S)
1/19-2/38	Intermediate (I)
≥ 4/76	Resistant (R)

*
These interpretative standards are applicable only to broth microdilution susceptibility tests with Haemophilus influenzae using Haemophilus Test Medium (HTM)[5].

†
These interpretative standards are applicable only to broth microdilution susceptibility tests using cation-adjusted Mueller-Hinton broth with 2% to 5% lysed horse blood[5].

A report of "Susceptible" indicates that the pathogen is likely to be inhibited if the antimicrobial compound in the blood reaches the concentrations usually achievable. A report of "Intermediate" indicates that the result should be considered equivocal, and, if the microorganism is not fully susceptible to alternative, clinically feasible drugs, the test should be repeated. This category implies possible clinical applicability in body sites where the drug is physiologically concentrated or in situations where high dosage of drug can be used. This category also provides a buffer zone which prevents small uncontrolled technical factors from causing major discrepancies in interpretation. A report of "Resistant" indicates that the pathogen is not likely to be inhibited if the antimicrobial compound in the blood reaches the concentrations usually achievable; other therapy should be selected.

QUALITY CONTROL

Standardized susceptibility test procedures require the use of laboratory control microorganisms to control the technical aspects of the laboratory procedures. Standard sulfamethoxazole/trimethoprim powder should provide the following range of values:

Microorganism			**MIC (µg/mL)**
Escherichia coli	ATCC	25922	≤ 0.5/9.5
Haemophilus influenzae*	ATCC	49247	0.03/0.59 – 0.25/4.75
Streptococcus pneumoniae†	ATCC	49619	0.12/2.4 – 1/19

*

Microorganism	MIC (µg/mL)
This quality control range is applicable only to Haemophilus influenzae ATCC 49247 tested by broth microdilution procedure using Haemophilus Test Medium (HTM)5. †	
This quality control range is applicable to tests performed by the broth microdilution method only using cation-adjusted Mueller-Hinton broth with 2% to 5% lysed horse blood5.	

DIFFUSION TECHNIQUES

Quantitative methods that require measurement of zone diameters also provide reproducible estimates of the susceptibility of bacteria to antimicrobial compounds. One such standardized procedure6 requires the use of standardized inoculum concentrations. This procedure uses paper disks impregnated with 1.25/23.75 µg of sulfamethoxazole/trimethoprim to test the susceptibility of microorganisms to sulfamethoxazole/trimethoprim.

Reports from the laboratory providing results of the standard single-disk susceptibility test with a 1.25/23.75 µg of sulfamethoxazole/trimethoprim disk should be interpreted according to the following criteria:.

For testing either Enterobacteriaceae or Haemophilus influenzae*:

Zone Diameter (mm)	Interpretation
≥ 16	Susceptible (S)
11 – 15	Intermediate (I)
≤ 10	Resistant (R)

When testing Streptococcus pneumoniae*:

Zone Diameter (mm)	Interpretation
≥ 19	Susceptible (S)
16 – 18	Intermediate (I)
≤ 15	Resistant (R)

*
These zone diameter standards are applicable only for disk diffusion testing with Haemophilus influenzae and Haemophilus Test Medium (HTM)[6].

Interpretation should be as stated above for results using dilution techniques. Interpretation involves correlation of the diameter obtained in the disk test with the MIC for sulfamethoxazole/trimethoprim.

QUALITY CONTROL

As with standardized dilution techniques, diffusion methods require the use of laboratory control microorganisms that are used to control the technical aspects of the laboratory procedures. For the diffusion technique, the 1.25/23.75 µg sulfamethoxazole/trimethoprim

disk[1] should provide the following zone diameters in these laboratory test quality control strains:

Microorganism			Zone Diameter Ranges (mm)
Escherichia coli	ATCC	25922	24 – 32
Haemophilus influenzae*	ATCC	49247	24 – 32
Streptococcus pneumoniae†	ATCC	49619	20 – 28

*
This quality control range is applicable only to Haemophilus influenzae ATCC 49247 tested by a disk diffusion procedure using Haemophilus Test Medium (HTM)6.

†
This quality control range is applicable only to tests performed by disk diffusion using Mueller-Hinton agar supplemented with 5% defibrinated sheep blood when incubated in 5% CO26.

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[1] Mueller-Hinton agar should be checked for excessive levels of thymidine or thymine. To determine whether Mueller-Hinton medium has sufficiently low levels of thymidine and thymine, an Enterococcus faecalis (ATCC 29212 or ATCC 33186) may be tested with sulfamethoxazole/trimethoprim disks. A zone of inhibition ≥20 mm that is essentially free of fine colonies indicates a sufficiently low level of thymidine and thymine.

INDICATIONS AND USAGE

To reduce the development of drug-resistant bacteria and maintain the effectiveness of sulfamethoxazole and trimethoprim tablets and other antibacterial drugs, sulfamethoxazole and trimethoprim should be used only to treat or prevent infections that are proven or strongly suspected to be caused by susceptible bacteria. When culture and susceptibility information are available, they should be considered in selecting or modifying antibacterial therapy. In the absence of such data, local epidemiology and susceptibility patterns may contribute to empiric selection of therapy.

Urinary Tract Infections

For the treatment of urinary tract infections due to susceptible strains of the following organisms: Escherichia coli, Klebsiella species, Enterobacter species, Morganella morganii, Proteus mirabilis and Proteus vulgaris. It is recommended that initial episodes of uncomplicated urinary tract infections be treated with a single effective antibacterial agent rather than the combination.

Acute Otitis Media

For the treatment of acute otitis media in pediatric patients due to susceptible strains of Streptococcus pneumoniae or Haemophilus influenzae when in the judgment of the physician sulfamethoxazole and trimethoprim offers some advantage over the use of other antimicrobial agents. To date, there are limited data on the safety of repeated use of sulfamethoxazole and trimethoprim in pediatric patients under two years of age. Sulfamethoxazole and trimethoprim is not indicated for prophylactic or prolonged administration in otitis media at any age.

Acute Exacerbations of Chronic Bronchitis in Adults

For the treatment of acute exacerbations of chronic bronchitis due to susceptible strains of Streptococcus pneumoniae or Haemophilus influenzae when in the judgment of the physician sulfamethoxazole and trimethoprim offers some advantage over the use of a single antimicrobial agent.

Shigellosis

For the treatment of enteritis caused by susceptible strains of Shigella flexneri and Shigella sonnei when antibacterial therapy is indicated.

Pneumocystis Carinii Pneumonia

For the treatment of documented Pneumocystis carinii pneumonia and for prophylaxis against Pneumocystis carinii pneumonia in individuals who are immunosuppressed and considered to be at an increased risk of developing Pneumocystis carinii pneumonia.

Traveler's Diarrhea in Adults

For the treatment of traveler's diarrhea due to susceptible strains of enterotoxigenic E. coli.

CONTRAINDICATIONS

Sulfamethoxazole and trimethoprim is contraindicated in patients with a known hypersensitivity to trimethoprim or sulfonamides and in patients with documented megaloblastic anemia due to folate deficiency. Sulfamethoxazole and trimethoprim is also contraindicated in pregnant patients and nursing mothers, because sulfonamides pass the placenta and are excreted in the milk and may cause kernicterus. Sulfamethoxazole and trimethoprim is contraindicated in pediatric patients less than 2 months of age. Sulfamethoxazole and trimethoprim is also contraindicated in patients with marked hepatic damage or with severe renal insufficiency when renal function status cannot be monitored.

WARNINGS

FATALITIES ASSOCIATED WITH THE ADMINISTRATION OF SULFONAMIDES, ALTHOUGH RARE, HAVE OCCURRED DUE TO SEVERE REACTIONS, INCLUDING STEVENS-JOHNSON SYNDROME, TOXIC EPIDERMAL NECROLYSIS, FULMINANT HEPATIC NECROSIS, AGRANULOCYTOSIS, APLASTIC ANEMIA AND OTHER BLOOD DYSCRASIAS.

SULFONAMIDES, INCLUDING SULFONAMIDE-CONTAINING PRODUCTS SUCH AS SULFAMETHOXAZOLE/TRIMETHOPRIM, SHOULD BE DISCONTINUED AT THE FIRST APPEARANCE OF SKIN RASH OR ANY SIGN OF ADVERSE REACTION. In rare instances, a skin rash may be followed by a more severe reaction, such as Stevens-Johnson syndrome, toxic epidermal necrolysis, hepatic necrosis, and serious blood disorders (see PRECAUTIONS).

Clinical signs such as rash, sore throat, fever, arthralgia, pallor, purpura or jaundice may be early indications of serious reactions.

Cough, shortness of breath, and pulmonary infiltrates are hypersensitivity reactions of the respiratory tract that have been reported in association with sulfonamide treatment.

The sulfonamides should not be used for the treatment of group A β-hemolytic streptococcal infections. In an established infection, they will not eradicate the streptococcus and, therefore, will not prevent sequelae such as rheumatic fever.

Pseudomembranous colitis has been reported with nearly all antibacterial agents, including sulfamethoxazole/ trimethoprim, and may range in severity from mild to life-threatening. Therefore, it is important to consider this diagnosis in patients who present with diarrhea subsequent to the administration of antibacterial agents.

Treatment with antibacterial agents alters the normal flora of the colon and may permit overgrowth of clostridia. Studies indicate that a toxin produced by Clostridium difficile is one primary cause of "antibiotic-associated colitis".

After the diagnosis of pseudomembranous colitis has been established, therapeutic measures should be initiated. Mild cases of pseudomembranous colitis usually respond to drug discontinuation alone. In moderate to severe cases, consideration should be given to management with fluids and electrolytes, protein supplementation and treatment with an antibacterial drug effective against C. difficile.

PRECAUTIONS

General

Prescribing sulfamethoxazole and trimethoprim tablets in the absence of a proven or strongly suspected bacterial infection or a prophylactic indication is unlikely to provide benefit to the patient and increases the risk of the development of drug-resistant bacteria.

Sulfamethoxazole and trimethoprim should be given with caution to patients with impaired renal or hepatic function, to those with possible folate deficiency (e.g., the elderly, chronic alcoholics, patients receiving anticonvulsant therapy, patients with malabsorption syndrome and patients in malnutrition states) and to those with severe allergies or bronchial asthma. In glucose-6-phosphate dehydrogenase deficient individuals, hemolysis may occur. This reaction is frequently dose-related (see CLINICAL PHARMACOLOGY and DOSAGE AND ADMINISTRATION).

Cases of hypoglycemia in non-diabetic patients treated with sulfamethoxazole and trimethoprim are seen rarely, usually occurring after a few days of therapy. Patients with renal dysfunction, liver disease, malnutrition or those receiving high doses of sulfamethoxazole and trimethoprim are particularly at risk.

Hematological changes indicative of folic acid deficiency may occur in elderly patients or in patients with preexisting folic acid deficiency or kidney failure. These effects are reversible by folinic acid therapy.

Trimethoprim has been noted to impair phenylalanine metabolism, but this is of no significance in phenylketonuric patients on appropriate dietary restriction.

As with all drugs containing sulfonamides, caution is advisable in patients with porphyria or thyroid dysfunction.

Use in the Treatment of and Prophylaxis for Pneumocystis Carinii Pneumonia in Patients with Acquired Immunodeficiency Syndrome (AIDS)

AIDS patients may not tolerate or respond to sulfamethoxazole and trimethoprim in the same manner as non-AIDS patients. The incidence of side effects, particularly rash, fever, leukopenia and elevated aminotransferase (transaminase) values, with sulfamethoxazole and trimethoprim therapy in AIDS patients who are being treated for Pneumocystis carinii pneumonia has been reported to be greatly increased compared with the incidence normally associated with the use of sulfamethoxazole and trimethoprim in non-AIDS patients. The incidence of hyperkalemia appears to be increased in AIDS patients receiving sulfamethoxazole and trimethoprim. Adverse effects are generally less severe in patients receiving sulfamethoxazole and trimethoprim for prophylaxis. A history of mild intolerance to sulfamethoxazole and trimethoprim in AIDS patients does not appear to predict intolerance of subsequent secondary prophylaxis.7 However, if a patient develops skin rash or any sign of adverse reaction, therapy with sulfamethoxazole and trimethoprim should be reevaluated (see WARNINGS).

High dosage of trimethoprim, as used in patients with Pneumocystis carinii pneumonia, induces a progressive but reversible increase of serum potassium concentrations in a substantial number of patients. Even treatment with recommended doses may cause hyperkalemia when trimethoprim is administered to patients with underlying disorders of potassium metabolism, with renal insufficiency, or if drugs known to induce hyperkalemia are given concomitantly. Close monitoring of serum potassium is warranted in these patients.

During treatment, adequate fluid intake and urinary output should be ensured to prevent crystalluria. Patients who are "slow acetylators" may be more prone to idiosyncratic reactions to sulfonamides.

Information for Patients

Patients should be counseled that antibacterial drugs, including sulfamethoxazole and trimethoprim tablets should only be used to treat bacterial infections. They do not treat viral infections (e.g., the common cold). When sulfamethoxazole and trimethoprim tablets are prescribed to treat bacterial infection, patients should be told that although it is common to feel better early in the course of therapy, the medication should be taken exactly as directed. Skipping doses or not completing the full course of therapy may (1) decrease the effectiveness of the immediate treatment and (2) increase the likelihood that bacteria will develop resistance and will not be treatable by sulfamethoxazole and trimethoprim tablets or other antibacterial drugs in the future.

Patients should be instructed to maintain an adequate fluid intake in order to prevent crystalluria and stone formation.

Laboratory Tests

Complete blood counts should be done frequently in patients receiving sulfamethoxazole and trimethoprim; if a significant reduction in the count of any formed blood element is noted, sulfamethoxazole and trimethoprim should be discontinued. Urinalyses with careful microscopic examination and renal function tests should be performed during therapy, particularly for those patients with impaired renal function.

Drug Interactions

In elderly patients concurrently receiving certain diuretics, primarily thiazides, an increased incidence of thrombocytopenia with purpura has been reported.

It has been reported that sulfamethoxazole and trimethoprim may prolong the prothrombin time in patients who are receiving the anticoagulant warfarin. This interaction should be kept in mind when sulfamethoxazole and trimethoprim is given to patients already on anticoagulant therapy, and the coagulation time should be reassessed.

Sulfamethoxazole and trimethoprim may inhibit the hepatic metabolism of phenytoin. Sulfamethoxazole and trimethoprim, given at a common clinical dosage, increased the phenytoin half-life by 39% and decreased the phenytoin metabolic clearance rate by 27%. When administering these drugs concurrently, one should be alert for possible excessive phenytoin effect.

Sulfonamides can also displace methotrexate from plasma protein binding sites and can compete with the renal transport of methotrexate, thus increasing free methotrexate concentrations.

There have been reports of marked but reversible nephrotoxicity with coadministration of sulfamethoxazole and trimethoprim and cyclosporine in renal transplant recipients.

Increased digoxin blood levels can occur with concomitant sulfamethoxazole and trimethoprim therapy, especially in elderly patients. Serum digoxin levels should be monitored.

Increased sulfamethoxazole blood levels may occur in patients who are also receiving indomethacin.

Occasional reports suggest that patients receiving pyrimethamine as malaria prophylaxis in doses exceeding 25 mg weekly may develop megaloblastic anemia if sulfamethoxazole and trimethoprim is prescribed.

The efficacy of tricyclic antidepressants can decrease when coadministered with sulfamethoxazole and trimethoprim.

Like other sulfonamide-containing drugs, sulfamethoxazole and trimethoprim potentiates the effect of oral hypoglycemics.

In the literature, a single case of toxic delirium has been reported after concomitant intake of sulfamethoxazole/trimethoprim and amantadine.

In the literature, three cases of Hyperkalemia in elderly patients have been reported after concomitant intake of trimethoprim/sulfamethoxazole and angiotensin converting enzyme inhibitor.8,9

Drug/Laboratory Test Interactions

Sulfamethoxazole and trimethoprim, specifically the trimethoprim component, can interfere with a serum methotrexate assay as determined by the competitive binding protein technique (CBPA) when a bacterial dihydrofolate reductase is used as the binding protein. No interference occurs, however, if methotrexate is measured by a radioimmunoassay (RIA).

The presence of sulfamethoxazole and trimethoprim may also interfere with the Jaffé alkaline picrate reaction assay for creatinine, resulting in overestimations of about 10% in the range of normal values.

Carcinogenesis, Mutagenesis, Impairment of Fertility

CARCINOGENESIS

Long-term studies in animals to evaluate carcinogenic potential have not been conducted with sulfamethoxazole and trimethoprim.

MUTAGENESIS

Bacterial mutagenic studies have not been performed with sulfamethoxazole and trimethoprim in combination. Trimethoprim was demonstrated to be nonmutagenic in the

Ames assay. No chromosomal damage was observed in human leukocytes cultured in vitro with sulfamethoxazole and trimethoprim alone or in combination; the concentrations used exceeded blood levels of these compounds following therapy with sulfamethoxazole and trimethoprim. Observations of leukocytes obtained from patients treated with sulfamethoxazole and trimethoprim revealed no chromosomal abnormalities.

IMPAIRMENT OF FERTILITY

No adverse effects on fertility or general reproductive performance were observed in rats given oral dosages as high as 350 mg/kg/day sulfamethoxazole plus 70 mg/kg/day trimethoprim. These doses are 10.9-fold higher than the recommended human dose for sulfamethoxazole and trimethoprim.

Pregnancy

TERATOGENIC EFFECTS

Pregnancy Category C

In rats, oral doses of 533 mg/kg sulfamethoxazole (16.7-fold higher than the recommended human dose) or 200 mg/kg trimethoprim (31.3-fold higher than the recommended human dose) produced teratologic effects manifested mainly as cleft palates.

The highest dose which did not cause cleft palates in rats was 512 mg/kg sulfamethoxazole (16-fold higher than the recommended human dose) or 192 mg/kg trimethoprim (30-fold higher than the recommended human dose) when administered separately. In two studies in rats, no teratology was observed when 512 mg/kg of sulfamethoxazole (16-fold higher than the recommended human dose) was used in combination with 128 mg/kg of trimethoprim (20-fold higher than the recommended human dose). In one study, however, cleft palates were observed in one litter out of 9 when 355 mg/kg of sulfamethoxazole (11.1-fold higher than the recommended human dose) was used in combination with 88 mg/kg of trimethoprim (13.8-fold higher than the recommended human dose).

In some rabbit studies, an overall increase in fetal loss (dead and resorbed and malformed conceptuses) was associated with doses of trimethoprim 6 times the human therapeutic dose.

While there are no large, well-controlled studies on the use of sulfamethoxazole and trimethoprim in pregnant women, Brumfitt and Pursell,10 in a retrospective study, reported the outcome of 186 pregnancies during which the mother received either placebo or sulfamethoxazole and trimethoprim. The incidence of congenital abnormalities was 4.5% (3 of 66) in those who received placebo and 3.3% (4 of 120) in those receiving sulfamethoxazole and trimethoprim. There were no abnormalities in the 10 children whose mothers received the drug during the first trimester. In a separate survey, Brumfitt and Pursell also found no congenital abnormalities in 35 children whose mothers had received oral sulfamethoxazole and trimethoprim at the time of conception or shortly thereafter.

Because sulfamethoxazole and trimethoprim may interfere with folic acid metabolism, sulfamethoxazole and trimethoprim should be used during pregnancy only if the potential benefit justifies the potential risk to the fetus.

Nonteratogenic Effects

See CONTRAINDICATIONS section.

Nursing Mothers

See CONTRAINDICATIONS section.

Pediatric Use

Sulfamethoxazole and trimethoprim is not recommended for pediatric patients younger than 2 months of age (see INDICATIONS and CONTRAINDICATIONS sections).

Geriatric Use

Clinical studies of sulfamethoxazole and trimethoprim did not include sufficient numbers of subjects aged 65 and over to determine whether they respond differently from younger subjects.

There may be an increased risk of severe adverse reactions in elderly patients, particularly when complicating conditions exist, e.g., impaired kidney and/or liver function, possible folate deficiency, or concomitant use of other drugs. Severe skin reactions, generalized bone marrow suppression (see WARNINGS and ADVERSE REACTIONS sections), a specific decrease in platelets (with or without purpura), and hyperkalemia are the most frequently reported severe adverse reactions in elderly patients. In those concurrently receiving certain diuretics, primarily thiazides, an increased incidence of thrombocytopenia with purpura has been reported. Increased digoxin blood levels can occur with concomitant sulfamethoxazole and trimethoprim therapy, especially in elderly patients. Serum digoxin levels should be monitored. Hematological changes indicative of folic acid deficiency may occur in elderly patients. These effects are reversible by folinic acid therapy. Appropriate dosage adjustments should be made for patients with impaired kidney function and duration of use should be as short as possible to minimize risks of undesired reactions (see DOSAGE AND ADMINISTRATION section). The trimethoprim component of sulfamethoxazole and trimethoprim may cause hyperkalemia when administered to patients with underlying disorders of potassium metabolism, with renal insufficiency or when given concomitantly with drugs known to induce hyperkalemia, such as angiotensin converting enzyme inhibitors. Close monitoring of serum potassium is warranted in these patients. Discontinuation of sulfamethoxazole and trimethoprim treatment is recommended to help lower potassium serum levels. Sulfamethoxazole and trimethoprim tablets contain 1.8 mg sodium (0.08 mEq) of sodium per tablet. Sulfamethoxazole and trimethoprim DS tablets contain 3.6 mg (0.16 mEq) of sodium per tablet.

Pharmacokinetics parameters for sulfamethoxazole were similar for geriatric subjects and younger adult subjects. The mean maximum serum trimethoprim concentration was higher and mean renal clearance of trimethoprim was lower in geriatric subjects compared with younger subjects (see CLINICAL PHARMACOLOGY: Geriatric Pharmacokinetics).

ADVERSE REACTIONS

The most common adverse effects are gastrointestinal disturbances (nausea, vomiting, anorexia) and allergic skin reactions (such as rash and urticaria). FATALITIES ASSOCIATED WITH THE ADMINISTRATION OF SULFONAMIDES, ALTHOUGH RARE, HAVE OCCURRED DUE TO SEVERE REACTIONS, INCLUDING STEVENS-JOHNSON SYNDROME, TOXIC EPIDERMAL NECROLYSIS, FULMINANT HEPATIC NECROSIS, AGRANULOCYTOSIS, APLASTIC ANEMIA AND OTHER BLOOD DYSCRASIAS (SEE WARNINGS SECTION).

Hematologic: Agranulocytosis, aplastic anemia, thrombocytopenia, leukopenia, neutropenia, hemolytic anemia, megaloblastic anemia, hypoprothrombinemia, methemoglobinemia, eosinophilia.

Allergic Reactions: Stevens-Johnson syndrome, toxic epidermal necrolysis, anaphylaxis, allergic myocarditis, erythema multiforme, exfoliative dermatitis, angioedema, drug fever, chills, Henoch-Schoenlein purpura, serum sickness-like syndrome, generalized allergic reactions, generalized skin eruptions, photosensitivity, conjunctival and scleral injection, pruritus, urticaria and rash. In addition, periarteritis nodosa and systemic lupus erythematosus have been reported.

Gastrointestinal: Hepatitis (including cholestatic jaundice and hepatic necrosis), elevation of serum transaminase and bilirubin, pseudomembranous enterocolitis, pancreatitis, stomatitis, glossitis, nausea, emesis, abdominal pain, diarrhea, anorexia.

Genitourinary: Renal failure, interstitial nephritis, BUN and serum creatinine elevation, toxic nephrosis with oliguria and anuria, crystalluria and nephrotoxicity in association with cyclosporine.

Metabolic and Nutritional: Hyperkalemia (see PRECAUTIONS: Use in the Treatment of and Prophylaxis for Pneumocystis Carinii Pneumonia in Patients with Acquired Immunodeficiency Syndrome (AIDS).

Neurologic: Aseptic meningitis, convulsions, peripheral neuritis, ataxia, vertigo, tinnitus, headache.

Psychiatric: Hallucinations, depression, apathy, nervousness.

Endocrine: The sulfonamides bear certain chemical similarities to some goitrogens, diuretics (acetazolamide and the thiazides) and oral hypoglycemic agents. Cross-sensitivity may exist with these agents. Diuresis and hypoglycemia have occurred rarely in patients receiving sulfonamides.

Musculoskeletal: Arthralgia and myalgia. Isolated cases of rhabdomyolysis have been reported with sulfamethoxazole and trimethoprim, mainly in AIDS patients.

Respiratory: Cough, shortness of breath, pulmonary infiltrates (see WARNINGS).

Miscellaneous: Weakness, fatigue, insomnia.

OVERDOSAGE

Acute

The amount of a single dose of sulfamethoxazole and trimethoprim that is either associated with symptoms of overdosage or is likely to be life-threatening has not been reported. Signs and symptoms of overdosage reported with sulfonamides include anorexia, colic, nausea, vomiting, dizziness, headache, drowsiness and unconsciousness. Pyrexia, hematuria and crystalluria may be noted. Blood dyscrasias and jaundice are potential late manifestations of overdosage.

Signs of acute overdosage with trimethoprim include nausea, vomiting, dizziness, headache, mental depression, confusion and bone marrow depression.

General principles of treatment include the institution of gastric lavage or emesis, forcing oral fluids, and the administration of intravenous fluids if urine output is low and renal function is normal. Acidification of the urine will increase renal elimination of trimethoprim. The patient should be monitored with blood counts and appropriate blood chemistries, including electrolytes. If a significant blood dyscrasia or jaundice occurs, specific therapy should be instituted for these complications. Peritoneal dialysis is not effective and hemodialysis is only moderately effective in eliminating sulfamethoxazole and trimethoprim.

Chronic

Use of sulfamethoxazole and trimethoprim at high doses and/or for extended periods of time may cause bone marrow depression manifested as thrombocytopenia, leukopenia and/or megaloblastic anemia. If signs of bone marrow depression occur, the patient should be given leucovorin 5 to 15 mg daily until normal hematopoiesis is restored.

DOSAGE AND ADMINISTRATION

Not recommended for use in pediatric patients less than 2 months of age.

Urinary Tract Infections and Shigellosis in Adults and Pediatric Patients, and Acute Otitis Media in Pediatric Patients

ADULTS

The usual adult dosage in the treatment of urinary tract infections is 1 sulfamethoxazole and trimethoprim DS (double strength) tablet or 2 sulfamethoxazole and trimethoprim tablets every 12 hours for 10 to 14 days. An identical daily dosage is used for 5 days in the treatment of shigellosis.

PEDIATRIC PATIENTS

The recommended dose for pediatric patients with urinary tract infections or acute otitis media is 40 mg/kg sulfamethoxazole and 8 mg/kg trimethoprim per 24 hours, given in two

divided doses every 12 hours for 10 days. An identical daily dosage is used for 5 days in the treatment of shigellosis. The following table is a guideline for the attainment of this dosage:

Pediatric Patients 2 months of age or older:

Weight		Dose – every 12 hours
lb	kg	Tablets
22	10	-
44	20	1
66	30	1 1/2
88	40	2 or 1 DS tablet

For Patients with Impaired Renal Function

When renal function is impaired, a reduced dosage should be employed using the following table:

Creatinine Clearance (mL/min)	Recommended Dosage Regimen
Above 30	Usual standard regimen
15-30	1/2 the usual regimen
Below 15	Use not recommended

Acute Exacerbations of Chronic Bronchitis in Adults

The usual adult dosage in the treatment of acute exacerbations of chronic bronchitis is 1 sulfamethoxazole and trimethoprim DS (double strength) tablets or 2 sulfamethoxazole and trimethoprim tablets every 12 hours for 14 days.

Pneumocystis Carinii Pneumonia

TREATMENT

Adults and Pediatric Patients

The recommended dosage for treatment of patients with documented Pneumocystis carinii pneumonia is 75 to 100 mg/kg sulfamethoxazole and 15 to 20 mg/kg trimethoprim per 24 hours given in equally divided doses every 6 hours for 14 to 21 days.11 The following table is a guideline for the upper limit of this dosage.

Weight		Dose – every 6 hours
lb	kg	Tablets
18	8	-
35	16	1
53	24	1 1/2

Weight		Dose – every 6 hours
lb	**kg**	**Tablets**
70	32	2 or 1 DS tablet
88	40	2 1/2
106	48	3 or 1 1/2 DS tablets
141	64	4 or 2 DS tablets
176	80	5 or 2 1 /2 DS Tablets

For the lower limit dose (75 mg/kg sulfamethoxazole and 15 mg/kg trimethoprim per 24 hours) administer 75% of the dose in the above table.

PROPHYLAXIS

Adults

The recommended dosage for prophylaxis in adults is 1 sulfamethoxazole and trimethoprim DS (double strength) tablet daily.12

Pediatric Patients

For pediatric patients, the recommended dose is 750 mg/m2/day sulfamethoxazole with 150 mg/m2/day trimethoprim given orally in equally divided doses twice a day, on 3 consecutive days per week. The total daily dose should not exceed 1600 mg sulfamethoxazole and 320 mg trimethoprim.12 The following table is a guideline for the attainment of this dosage in pediatric patients:

Body Surface Area	Dose – every 12 hours
(m2)	**Tablets**
0.26	-
0.53	1/2
1.06	1

Traveler's Diarrhea in Adults

For the treatment of traveler's diarrhea, the usual adult dosage is 1 sulfamethoxazole and trimethoprim DS (double strength) tablets or 2 sulfamethoxazole and trimethoprim tablets every 12 hours for 5 days.

HOW SUPPLIED

SULFAMETHOXAZOLE AND TRIMETHOPRIM TABLETS, USP are supplied as follows: Sulfamethoxazole 400 mg and trimethoprim 80 mg tablets, white, round, scored, debossed MP 81

Bottles of 100	NDC 53489-145-01
Bottles of 500	NDC 53489-145-05

Sulfamethoxazole 800 mg and trimethoprim 160 mg tablets, double strength, white, oval shaped, scored, debossed MP 85

Bottles of 100	NDC 53489-146-01
Bottles of 500	NDC 53489-146-05

Store at 20° to 25°C (68° to 77°F). [See USP Controlled Room Temperature]

DISPENSE IN TIGHT, LIGHT-RESISTANT CONTAINER.

REFERENCES

1. Kremers P, Duvivier J, Heusghem C. Pharmacokinetic Studies of Co-Trimoxazole in Man after Single and Repeated Doses. J Clin Pharmacol. Feb-Mar 1974; 14:112-117.

2. Kaplan SA, et al. Pharmacokinetic Profile of Trimethoprim-Sulfamethoxazole in Man. J Infect Dis. Nov 1973; 128 (Suppl): S547-S555.

3. Varoquaux O, et al Pharmacokinetics of the trimethoprim-sulfamethoxazole combination in the elderly. Br J Clin Pharmacol 1985;20;575-581

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8. Marinella Mark A. 1999. Trimethoprim-induced hyperkalemia: An analysis of reported cases. Gerontol. 45:209-212.

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13. CDC Guidelines for prophylaxis against Pneumocystis carinii pneumonia for children infected with human immunodeficiency virus. MMWR. 1991; 40(RR-2):1-13

Manufactured By:
MUTUAL PHARMACEUTICAL COMPANY, INC.
Philadelphia, PA 19124 USA

Rev: December 2004Ch

Sulfamethoxazole and Trimethoprim (Sulfamethoxazole and Trimethoprim)

PRODUCT INFO

Product Code	53489-145	Dosage Form	TABLET
Route Of Administration	ORAL	DEA Schedule	

INGREDIENTS

Name (Active Moiety)	Type	Strength
Trimethoprim (Trimethoprim)	Active	400 MILLIGRAM In 1 TABLET
Sulfamethoxazole (Sulfamethoxazole)	Active	80 MILLIGRAM In 1 TABLET
Docusate sodium	Inactive	
sodium benzoate	Inactive	
sodium starch glycolate	Inactive	
magnesium stearate	Inactive	
pregelatinized starch	Inactive	

IMPRINT INFORMATION

Characteristic	Appearance	Characteristic	Appearance
Color	WHITE	Score	2
Shape	ROUND	Symbol	false
Imprint Code	MP;81	Coating	false
Size	11mm		

PACKAGING

#	NDC	Package Description	Multilevel Packaging
1	53489-145-01	100 TABLET In 1 BOTTLE, PLASTIC	None
2	53489-145-05	500 TABLET In 1 BOTTLE, PLASTIC	None

Sulfamethoxazole and Trimethoprim (Sulfamethoxazole and Trimethoprim)

PRODUCT INFO

Product Code	53489-146	Dosage Form	TABLET
Route Of Administration	ORAL	DEA Schedule	

INGREDIENTS

Name (Active Moiety)	Type	Strength
Trimethoprim (Trimethoprim)	Active	800 MILLIGRAM In 1 TABLET
Sulfamethoxazole (Sulfamethoxazole)	Active	160 MILLIGRAM In 1 TABLET
Docusate sodium	Inactive	
sodium benzoate	Inactive	
sodium starch glycolate	Inactive	
magnesium stearate	Inactive	
pregelatinized starch	Inactive	

IMPRINT INFORMATION

Characteristic	Appearance	Characteristic	Appearance
Color	WHITE	Score	2
Shape	OVAL	Symbol	false
Imprint Code	MP;85	Coating	false
Size	19mm		

PACKAGING

#	NDC	Package Description	Multilevel Packaging
1	53489-146-01	100 TABLET In 1 BOTTLE, PLASTIC	None
2	53489-146-05	500 TABLET In 1 BOTTLE, PLASTIC	None

Revised: 01/2007

Attachment 3. Package Insert for Acyclovir

Source: http://dailymed.nlm.nih.gov/dailymed/fdaDrugXsl.cfm?id=2748&type=display

acyclovir (Acyclovir) capsule
acyclovir (Acyclovir) tablet
[Genpharm Inc.]

DESCRIPTION

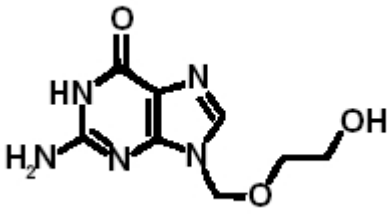
Acyclovir is a synthetic nucleoside analogue active against herpes viruses. Acyclovir Capsules and Tablets are formulations for oral administration. Each capsule of acyclovir contains 200 mg of acyclovir and the inactive ingredients pregelatinized starch, lactose monohydrate, magnesium stearate, and sodium lauryl sulfate. The capsule shell consists of gelatin, FD&C Blue No. 1, titanium dioxide, silicon dioxide and sodium lauryl sulphate. Printed with edible black ink. The contents of the edible black ink are synthetic black iron oxide, FD&C Blue No. 2 Aluminum Lake, FD&C Red No. 40 Aluminum Lake, FD&C Blue No. 1 Aluminum Lake, and D&C Yellow No. 10 Aluminum Lake.

Each 800 mg tablet of acyclovir contains 800 mg of acyclovir and the inactive ingredients FD&C Blue No.2 Aluminum Lake, magnesium stearate, microcrystalline cellulose, povidone, and sodium starch glycolate.

Each 400 mg tablet of acyclovir contains 400 mg of acyclovir and the inactive ingredients magnesium stearate, microcrystalline cellulose, povidone, and sodium starch glycolate.

Acyclovir is a white crystalline powder with the molecular formula C8H11N5O3 and a molecular weight of 225. The maximum solubility in water at 37°C is 2.5 mg/mL. The pka's of acyclovir are 2.27 and 9.25.

The chemical name of acyclovir is 2-amino-1,9-dihydro-9-[(2-hydroxyethoxy)methyl]6-H-purin-6-one; it has the following structural formula:



Chemical formula: C8H11N5O3

Virology

MECHANISM OF ANTIVIRAL ACTION

Acyclovir is a synthetic purine nucleoside analogue with in vitro and in vivo inhibitory activity against herpes simplex virus types 1 (HSV-1), 2 (HSV-2), and varicella-zoster virus (VZV).

The inhibitory activity of acyclovir is highly selective due to its affinity for the enzyme thymidine kinase (TK) encoded by HSV and VZV. This viral enzyme converts acyclovir into acyclovir monophosphate, a nucleotide analogue. The monophosphate is further converted into diphosphate by cellular guanylate kinase and into triphosphate by a number of cellular enzymes. In vitro, acyclovir triphosphate stops replication of herpes viral DNA. This is accomplished in three ways: 1) competitive inhibition of viral DNA polymerase, 2) incorporation into and termination of the growing viral DNA chain, and 3) inactivation of the viral DNA polymerase. The greater antiviral activity of acyclovir against HSV compared to VZV is due to its more efficient phosphorylation by the viral TK.

ANTIVIRAL ACTIVITIES

The quantitative relationship between the in vitro susceptibility of herpes viruses to antivirals and the clinical response to therapy has not been established in humans, and virus sensitivity testing has not been standardized. Sensitivity testing results, expressed as the concentration of drug required to inhibit by 50% the growth of virus in cell culture (IC50), vary greatly depending upon a number of factors. Using plaquereduction assays, the IC50 against herpes simplex virus isolates ranges from 0.02 to 13.5 mcg/mL for HSV-1 and from 0.01 to 9.9 mcg/mL for HSV-2. The IC50 for acyclovir against most laboratory strains and clinical isolates of VZV ranges from 0.12 to 10.8 mcg/mL.

Acyclovir also demonstrates activity against the Oka vaccine strain of VZV with a mean IC50 of 1.35 mcg/mL.

Drug Resistance

Resistance of HSV and VZV to acyclovir can result from qualitative or quantitative changes in the viral TK or DNA polymerase. Clinical isolates of HSV and VZV with reduced susceptibility to acyclovir have been recovered from immunocompromised patients, especially with advanced HIV infection.

While most of the acyclovir-resistant mutants isolated thus far from immunocompromised patients have been found to be TK-deficient mutants, other mutants involving the viral TK gene (TK partial and TK altered) and DNA polymerase have been isolated. TK-negative mutants may cause severe disease in infants and immunocompromised adults. The possibility of viral resistance to acyclovir should be considered in patients who show poor clinical response during therapy.

CLINICAL PHARMACOLOGY

Pharmacokinetics

The pharmacokinetics of acyclovir after oral administration have been evaluated in healthy volunteers and in immunocompromised patients with herpes simplex or varicella-zoster virus infection. Acyclovir pharmacokinetic parameters are summarised in Table 1.

Table 1: Acyclovir Pharmacokinetic Characteristics (Range)

Parameter	Range
Plasma protein binding	9% to 33%
Plasma elimination half-life	2.5 to 3.3 h
Average oral bioavailability	10% to 20%*

*Bioavailability decreases with increasing dose.

In one multiple-dose, cross-over study in healthy subjects (n=23), it was shown that increases in plasma acyclovir concentrations were less than dose proportional with increasing dose, as shown in Table 2. The decrease in bioavailability is a function of the dose and not the dosage form.

Table 2: Acyclovir Peak and Trough Concentrations at Steady State

Parameter	200 mg	400 mg	800 mg
CSSmax	0.83 mcg/mL	1.21 mcg/mL	1.61 mcg/mL
CSStrough	0.46 mcg/mL	0.63 mcg/mL	0.83 mcg/mL

There was no effect of food on the absorption of acyclovir (n=6); therefore, Acyclovir Capsules and Tablets may be administered with or without food.

The only known urinary metabolite is 9-[(carboxymethoxy)methyl]guanine.

Special Populations

ADULTS WITH IMPAIRED RENAL FUNCTION

The half-life and total body clearance of acyclovir are dependent on renal function. A dosage adjustment is recommended for patients with reduced renal function (see DOSAGE AND ADMINISTRATION).

GERIATRICS

Acyclovir plasma concentrations are higher in geriatric patients compared to younger adults, in part due to age-related changes in renal function. Dosage reduction may be required in geriatric patients with underlying renal impairment (see PRECAUTIONS: Geriatric Use).

PEDIATRICS

In general, the pharmacokinetics of acyclovir in pediatric patients is similar to that of adults. Mean half-after oral doses of 300 mg/m2 and 600 mg/m2 in pediatric patients ages 7 months to 7 years was 2.6 hours (range 1.59 to 3.74 hours).

Drug Interactions

Co-administration of probenecid with intravenous acyclovir has been shown to increase the mean acyclovir half-life and the area under the concentration-time curve. Urinary excretion and renal clearance were correspondingly reduced.

Clinical Trials

INITIAL GENITAL HERPES

Double-blind, placebo-controlled studies have demonstrated that orally administered acyclovir significantly reduced the duration of acute infection and duration of lesion healing. The duration of pain and new lesion formation was decreased in some patient groups.

RECURRENT GENITAL HERPES

Double-blind, placebo-controlled studies in patients with frequent recurrences (six or more episodes per year) have shown that orally administered acyclovir given daily for 4 months to 10 years prevented or reduced the frequency and/or severity of recurrences in greater than 95% of patients.

In a study of patients who received acyclovir 400 mg twice daily for 3 years, 45%, 52%, and 63% of patients remained free of recurrences in the first, second, and third years, respectively. Serial analyses of the 3-month recurrence rates for the patients showed that 71% to 87% were recurrence free in each quarter.

HERPES ZOSTER INFECTIONS

In a double-blind, placebo-controlled study of immunocompetent patients with localized cutaneous zoster infection, acyclovir (800 mg five times daily for 10 days) shortened the times to lesion scabbing, healing, and complete cessation of pain, and reduced the duration of viral shedding and the duration of new lesion formation.

In a similar double-blind, placebo-controlled study, acyclovir (800 mg five times daily for 7 days) shortened the times to complete lesion scabbing, healing, and cessation of pain, reduced the duration of new lesion formation, and reduced the prevalence of localized zoster-associated neurologic symptoms (paresthesia, dysesthesia, or hyperesthesia).

Treatment was begun within 72 hours of rash onset and was most effective if started within the first 48 hours.

Adults greater than 50 years of age showed greater benefit.

CHICKENPOX

Three randomized, double-blind, placebo-controlled trials were conducted in 993 pediatric patients ages 2 to 18 years with chickenpox. All patients were treated within 24 hours after the onset of rash. In two trials, acyclovir was administered at 20 mg/kg four times daily (up to 3200 mg per day) for 5 days. In the third trial, doses of 10, 15, or 20 mg/kg were administered four times daily for 5 to 7 days. Treatment with acyclovir shortened the time to 50% healing, reduced the maximum number of lesions, reduced the median number of vesicles, decreased the median number of residual lesions on day 28, and decreased the proportion of patients with fever, anorexia, and lethargy by day 2. Treatment with acyclovir did not affect varicella-zoster virus-specific humoral or cellular immune responses at 1 month or 1 year following treatment.

INDICATIONS AND USAGE

Herpes Zoster Infections

Acyclovir is indicated for the acute treatment of herpes zoster (shingles).

Genital Herpes

Acyclovir is indicated for the treatment of initial episodes and the management of recurrent episodes of genital herpes.

Chickenpox

Acyclovir is indicated for the treatment of chickenpox (varicella).

CONTRAINDICATIONS

Acyclovir is contraindicated for patients who develop hypersensitivity or intolerance to acyclovir or valacyclovir.

WARNINGS

Acyclovir Capsules and Tablets are intended for oral ingestion only. Renal failure, in some cases resulting in death, has been observed with acyclovir therapy (see ADVERSE REACTIONS, Observed DuringClinical Practice and OVERDOSAGE). Thrombotic thrombocytopenic purpura/hemolytic uremic syndrome (TTP/HUS), which has resulted in death, has occurred in immunocompromised patients receiving acyclovir therapy.

PRECAUTIONS

Dosage adjustment is recommended when administering acyclovir to patients with renal impairment (see DOSAGE AND ADMINISTRATION). Caution should also be exercised when administering acyclovir to patients receiving potentially nephrotoxic agents since this may increase the risk of renal dysfunction and/or the risk of reversible central nervous system symptoms such as those that have been reported in patients treated with intravenous acyclovir. Adequate hydration should be maintained.

Information for Patients

Patients are instructed to consult with their physician if they experience severe or troublesome adverse reactions, they become pregnant or intend to become pregnant, they intend to breastfeed while taking orally administered acyclovir, or they have any other questions. Patients should be advised to maintain adequate hydration.

HERPES ZOSTER

There are no data on treatment initiated more than 72 hours after onset of the zoster rash. Patients should be advised to initiate treatment as soon as possible after a diagnosis of herpes zoster.

GENITAL HERPES INFECTIONS

Patients should be informed that acyclovir is not a cure for genital herpes. There are no data evaluating whether acyclovir will prevent transmission of infection to others. Because genital herpes is a sexually transmitted disease, patients should avoid contact with lesions or intercourse when lesions and/or symptoms are present to avoid infecting partners.

Genital herpes can also be transmitted in the absence of symptoms through asymptomatic viral shedding. If medical management of a genital herpes recurrence is indicated, patients should be advised to initiate therapy at the first sign or symptom of an episode.

CHICKENPOX

Chickenpox in otherwise healthy children is usually a self-limited disease of mild to moderate severity. Adolescents and adults tend to have more severe disease. Treatment was initiated within 24 hours of the typical chickenpox rash in the controlled studies, and there is no information regarding the effects of treatment begun later in the disease course.

Drug Interactions

See CLINICAL PHARMACOLOGY, Pharmacokinetics.

Carcinogenesis, Mutagenesis, Impairment of Fertility

The data presented below include references to peak steady-state plasma acyclovir concentrations observed in humans treated with 800 mg given orally five times a day (dosing

appropriate for treatment of herpes zoster) or 200 mg given orally five times a day (dosing appropriate for treatment of genital herpes).

Plasma drug concentrations in animal studies are expressed as multiples of human exposure to acyclovir at the higher and lower dosing schedules (see CLINICAL PHARMACOLOGY, Pharmacokinetics).

Acyclovir was tested in lifetime bioassays in rats and mice at single daily doses of up to 450 mg/kg administered by gavage. There was no statistically significant difference in the incidence of tumors between treated and control animals, nor did acyclovir shorten the latency of tumors. Maximum plasma concentrations were three to six times human levels in the mouse bioassay and one to two times human levels in the rat bioassay.

Acyclovir was tested in 16 in vivo and in vitro genetic toxicity assays. Acyclovir was positive in 5 of the assays.

Acyclovir did not impair fertility or reproduction in mice (450 mg/kg/day, p.o.) or in rats (25 mg/kg/day, s.c.). In the mouse study, plasma levels were 9 to 18 times human levels, while in the rat study, they were 8 to 15 times human levels. At higher doses (50 mg/kg/day, s.c.) in rats and rabbits (11 to 22 and 16 to 31 times human levels, respectively) implantation efficacy, but not litter size, was decreased. In a rat peri- and postnatal study at 50 mg/kg/day, s.c., there was a statistically significant decrease in group mean numbers of corpora lutea, total implantation sites, and live fetuses.

No testicular abnormalities were seen in dogs given 50 mg/kg/day, i.v. for 1 month (21 to 41 times human levels) or in dogs given 60 mg/kg/day orally for 1 year (six to 12 times human levels).

Testicular atrophy and aspermatogenesis were observed in rats and dogs at higher dose levels.

Pregnancy

TERATOGENIC EFFECTS

Pregnancy Category B

Acyclovir administered during organogenesis was not teratogenic in the mouse (450 mg/kg/day, p.o.), rabbit (50 mg/kg/day, s.c. and i.v.), or rat (50 mg/kg/day, s.c.). These exposures resulted in plasma levels 9 and 18 , 16 and 106, and 11 and 22 times, respectively, human levels.

There are no adequate and well-controlled studies in pregnant women. A prospective, epidemiological registry of acyclovir use during pregnancy was established in 1984 and completed in April 1999. There were 749 pregnancies followed in women exposed to systemic acyclovir during the first trimester of pregnancy resulting in 756 outcomes. The occurrence rate of birth defects approximates that found in the general population. However, the small size of the registry is insufficient to evaluate the risk for less common defects or to permit reliable or definitive conclusions regarding the safety of acyclovir in pregnant women

and their developing fetuses. Acyclovir should be used during pregnancy only if the potential benefit justifies the potential risk to the fetus.

NURSING MOTHERS

Acyclovir concentrations have been documented in breast milk in two women following oral administration of acyclovir and ranged from 0.6 to 4.1 times corresponding plasma levels. These concentrations would potentially expose the nursing infant to a dose of acyclovir up to 0.3 mg/kg/day. Acyclovir should be administered to a nursing mother with caution and only when indicated.

PEDIATRIC USE

Safety and effectiveness of oral formulations of acyclovir in pediatric patients younger than 2 years of age have not been established.

GERIATRIC USE

Of 376 subjects who received acyclovir in a clinical study of herpes zoster treatment in immunocompetent subjects greater than or equal to 50 years of age, 244 were 65 and over while 111 were 75 and over. No overall differences in effectiveness for time to cessation of new lesion formation or time to healing were reported between geriatric subjects and younger adults subjects. The duration of pain after healing was longer in patients 65 and over. Nausea, vomiting, and dizziness were reported more frequently in elderly ubjects. Elderly patients are more likely to have reduced renal function and require dose reduction. Elderly patients are also more likely to have renal or CNS adverse events. With respect to CNS adverse events observed during clinical practice, somnolence, hallucinations, confusion, and coma were reported more frequently in elderly patients (see CLINICAL PHARMACOLOGY, ADVERSE REACTIONS,Observed During Clinical Practice, and DOSAGE AND ADMINISTRATION).

ADVERSE REACTIONS

Herpes Simplex

SHORT-TERM ADMINISTRATION

The most frequent adverse events reported during clinical trials of treatment of genital herpes with acyclovir 200 mg administered orally five times daily every 4 hours for 10 days were nausea and/or vomiting in 8 of 298 patient treatments (2.7%). Nausea and/or vomiting occurred in 2 of 287 (0.7%) patients who received placebo.

LONG-TERM ADMINISTRATION

The most frequent adverse events reported in a clinical trial for the prevention of recurrences with continuous administration of 400 mg (two 200 mg capsules) two times daily for 1 year in 586 patients treated with acyclovir were nausea (4.8%) and diarrhea (2.4%). The 589

control patients receiving intermittent treatment of recurrences with acyclovir for 1 year reported diarrhea (2.7%), nausea (2.4%), and headache (2.2%).

Herpes Zoster

The most frequent adverse event reported during three clinical trials of treatment of herpes zoster (shingles) with 800 mg of oral acyclovir five times daily for 7 to 10 days in 323 patients was malaise (11.5%). The 323 placebo recipients reported malaise (11.1%).

Chickenpox

The most frequent adverse event reported during three clinical trials of treatment of chickenpox with oral acyclovir at doses of 10 to 20 mg/kg four times daily for 5 to 7 days or 800 mg four times daily for 5 days in 495 patients was diarrhea (3.2%). The 498 patients receiving placebo reported diarrhea (2.2%).

Observed During Clinical Practice

In addition to adverse events reported from clinical trials, the following events have been identified during post-approval use of acyclovir. Because they are reported voluntarily from a population of unknown size, estimates of frequency cannot be made. These events have been chosen for inclusion due to either their seriousness, frequency of reporting, potential causal connection to acyclovir, or a combination of these factors:

• General: anaphylaxis, angioedema, fever, headache, pain, peripheral edema.

• Nervous: aggressive behavior, agitation, ataxia, coma, confusion, decreased consciousness, delirium, dizziness, dysarthria, encephalopathy, hallucinations, paresthesia, psychosis, seizure, somnolence, tremors. These symptoms may be marked, particularly in older adults or in patients with renal impairment (see PRECAUTIONS).

• Digestive: diarrhea, gastrointestinal distress, nausea.

• Hematologic and Lymphatic: anemia, leukocytoclastic vasculitis, leukopenia, lymphadenopathy, thrombocytopenia.

• Hepatobiliary Tract and Pancreas: elevated liver function tests, hepatitis, hyperbilirubinemia, jaundice.

• Musculoskeletal: myalgia.

• Skin: alopecia, erythema multiforme, photosensitive rash, pruritus, rash, Stevens-Johnson syndrome, toxic epidermal necrolysis, urticaria.

• Special Senses: visual abnormalities.

• Urogenital: renal failure, elevated blood urea nitrogen, elevated creatinine, hematuria (see WARNINGS).

OVERDOSAGE

Overdoses involving ingestion of up to 100 capsules (20 g) have been reported. Adverse events that have been reported only in association with overdosage include agitation, coma, seizures, and lethargy. Precipitation of acyclovir in renal tubules may occur when the solubility (2.5 mg/mL) is exceeded in the intratubular fluid. Overdosage has been reported following bolus injections or inappropriately high doses and in patients whose fluid and electrolyte balance were not properly monitored. This has resulted in elevated BUN and serum creatinine and subsequent renal failure. In the event of acute renal failure and anuria, the patient may benefit from hemodialysis until renal function is restored (see DOSAGE ANDADMINISTRATION).

DOSAGE AND ADMINISTRATION

Acute Treatment of Herpes Zoster

800 mg every 4 hours orally, five times daily for 7 to 10 days.

Genital Herpes

TREATMENT OF INITIAL GENITAL HERPES

200 mg every 4 hours, five times daily for 10 days.

CHRONIC SUPPRESSIVE THERAPY FOR RECURRENT DISEASE

400 mg two times daily for up to 12 months, followed by re-evaluation. Alternative regimens have included doses ranging from 200 mg three times daily to 200 mg five times daily.

The frequency and severity of episodes of untreated genital herpes may change over time. After 1 year of therapy, the frequency and severity of the patient's genital herpes infection should be re-evaluated to assess the need for continuation of therapy with acyclovir.

INTERMITTENT THERAPY

200 mg every 4 hours, five times daily for 5 days. Therapy should be initiated at the earliest sign or symptom prodrome) of recurrence.

Treatment of Chickenpox

CHILDREN (2 YEARS OF AGE AND OLDER)

20 mg/kg per dose orally four times daily (80 mg/kg/day) for 5 days. Children over 40 kg should receive the adult dose for chickenpox.

ADULTS AND CHILDREN OVER 40 KG

800 mg four times daily for 5 days.

Intravenous acyclovir is indicated for the treatment of varicella-zoster infections in immunocompromised patients.

When therapy is indicated, it should be initiated at the earliest sign or symptom of chickenpox. There is no information about the efficacy of therapy initiated more than 24 hours after onset of signs and symptoms.

PATIENTS WITH ACUTE OR CHRONIC RENAL IMPAIRMENT

In patients with renal impairment, the dose of Acyclovir Capsules or Tablets should be modified as shown in Table 3.

Table 3: Dosage Modifications for Renal Impairment

Normal Dosage Regimen	Creatinine Clearance (mL/min/1.73m2)	Adjusted Dosage Regimen Dose (mg)	Dosing Interval
200 mg every 4 hours	> 10	200	Every 4 hours, 5 x daily
	0 – 10	200	Every 12 hours
400 mg every 12 hours	> 10	400	Every 12 hours
	0 – 10	200	Every 12 hours
800 mg every 4 hours	> 25	800	Every 4 hours, 5 x daily
	10 - 25	800	Every 8 hours
	0 - 10	800	Every 12 hours

HEMODIALYSIS

For patients who require hemodialysis, the mean plasma half-life of acyclovir during hemodialysis is approximately 5 hours. This results in a 60% decrease in plasma concentrations following a 6-hour dialysis period. Therefore, the patient's dosing schedule should be adjusted so that an additional dose is administered after each dialysis.

PERITONEAL DIALYSIS

No supplemental dose appears to be necessary after adjustment of the dosing interval.

BIOEQUIVALENCE OF DOSAGE FORMS

Acyclovir suspension was shown to be bioequivalent to acyclovir capsules (n=20) and one acyclovir 800 mg tablet was shown to be bioequivalent to four acyclovir 200 mg capsules (n=24).

HOW SUPPLIED

Acyclovir Capsules (blue, opaque cap and body) containing 200 mg of acyclovir and printed with “G” on the cap and “0034” on the body.

- bottle of 100 (NDC 55567-034-18)

- bottle of 500 (NDC 55567-034-25)

- unit dose pack of 100 (NDC 55567-034-06).

Store at 15° to 25°C (59° to 77°F). Protect from light and moisture.

Acyclovir Tablets (blue, oval, unscored tablet) containing 800 mg acyclovir and engraved with “G” on one side and “0037” on the other.

- bottle of 100 (NDC 55567-037-18)

- bottle of 500 (NDC 55567-037-25)

- unit dose pack of 100 (NDC 55567-037-06).

Store at 15° to 25°C (59° to 77°F). Protect from light and moisture.

Acyclovir Tablets (white, 5 sided, unscored tablet) containing 400 mg acyclovir and engraved with “G” over “0036” on one side.

- bottle of 100 (NDC 55567-036-18)

- bottle of 500 (NDC 55567-036-25)

Store at 15° to 25°C (59° to 77°F). Protect from light and moisture.

Printed in Canada.

003-786 REV.#10 (P2) March 2006

Manufactured by:

Genpharm Inc.

Toronto, Ontario

Canada M8Z 2S6

1-800-661-7134

Acyclovir (Acyclovir)

PRODUCT INFO

Product Code	55567-034	Dosage Form	CAPSULE
Route Of Administration	ORAL	DEA Schedule	

INGREDIENTS

Name (Active Moiety)	Type	Strength
Acyclovir (Acyclovir)	Active	200 MILLIGRAM In 1 CAPSULE
D&C Yellow No. 10 Aluminum Lake	Inactive	
FD & C Blue No. 1	Inactive	
FD & C Blue No. 1 Aluminum Lake	Inactive	
FD & C Blue No. 2 Aluminum Lake	Inactive	
FD & C Red No. 40 Aluminum Lake	Inactive	
Gelatin	Inactive	
Lactose monohydrate	Inactive	
Magnesium stearate	Inactive	
Pregelatinized starch	Inactive	
Silicon dioxide	Inactive	
Sodium lauryl sulfate	Inactive	
Synthetic black iron oxide	Inactive	
Titanium dioxide	Inactive	

IMPRINT INFORMATION

Characteristic	Appearance	Characteristic	Appearance
Color	BLUE	Score	1
Shape	CAPSULE (CAPSULE)	Symbol	false
Imprint Code	G;0034	Coating	false
Size	19mm		

PACKAGING

# NDC	Package Description	Multilevel Packaging
1 55567-034-25	500 CAPSULE In 1 BOTTLE	None
2 55567-034-18	100 CAPSULE In 1 BOTTLE	None
3 55567-034-06	100 TABLET In 1 BLISTER PACK	None

Acyclovir (Acyclovir)

PRODUCT INFO

Product Code	55567-036	Dosage Form	TABLET
Route Of Administration	ORAL	DEA Schedule	

INGREDIENTS

Name (Active Moiety)	Type	Strength
Acyclovir (Acyclovir)	Active	400 MILLIGRAM In 1 TABLET
Magnesium stearate	Inactive	
Microcrystalline cellulose	Inactive	
Povidone	Inactive	
Sodium starch glycolate	Inactive	

IMPRINT INFORMATION

Characteristic	Appearance	Characteristic	Appearance
Color	WHITE	Score	1
Shape	PENTAGON (5 sided) (PENTAGON (5 sided))	Symbol	false
Imprint Code	G;0036	Coating	false
Size	10mm		

PACKAGING

#	NDC	Package Description	Multilevel Packaging
1	55567-036-25	500 TABLET In 1 BOTTLE	None
2	55567-036-18	100 TABLET In 1 BOTTLE	None

Acyclovir (Acyclovir)

PRODUCT INFO

Product Code	55567-037	Dosage Form	TABLET
Route Of Administration	ORAL	DEA Schedule	

INGREDIENTS

Name (Active Moiety)	Type	Strength
Acyclovir (Acyclovir)	Active	800 MILLIGRAM In 1 TABLET
FD & C Blue No. 2 Aluminum Lake	Inactive	
Magnesium stearate	Inactive	
Microcrystalline cellulose	Inactive	
Povidone	Inactive	
Sodium starch glycolate	Inactive	

IMPRINT INFORMATION

Characteristic	Appearance	Characteristic	Appearance
Color	BLUE	Score	1
Shape	OVAL (OVAL)	Symbol	false
Imprint Code	G;0037	Coating	false
Size	19mm		

PACKAGING

#	NDC	Package Description	Multilevel Packaging
1	55567-037-06	100 TABLET In 1 BLISTER PACK	None
2	55567-037-25	500 TABLET In 1 BOTTLE	None
3	55567-037-18	100 TABLET In 1 BOTTLE	None

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