C O N F I D E N T I A L

Autoimmunity Centers of Excellence (ACE)

Protocol APG01

A Phase I, Open-Label, Multicenter Trial Exploring the Safety and Tolerability of Autologous Polyclonal Regulatory T Cell Therapy in Adults with Active Pemphigus

Short Title: PolyTregs for Pemphigus

BB-IND 17351

Version 3.0 17 Dec 2018

IND Sponsor: Division of Allergy, Immunology, and Transplantation (DAIT)
National Institute of Allergy and Infectious Diseases (NIAID)
National Institutes of Health (NIH)

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Protocol: APG01 **Version/Date: v3.0/ 17 December 2018**

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Study Sponsor: The National Institute of Allergy and Infectious Diseases (NIAID)

INSTRUCTIONS: *The site Principal Investigator should print, sign, and date at the indicated location below. A copy should be kept for your records and the original signature page sent. After signature, please return the original of this form by surface mail to:*

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I confirm that I have read the above protocol in the latest version. I understand it, and I will work according to the principles of Good Clinical Practice (GCP) as described in the United States Code of Federal Regulations (CFR) – 45 CFR part 46 and 21 CFR parts 50, 56, and 312, and in the International Conference on Harmonization (ICH) document *Guidance for Industry: E6 (R2) Good Clinical Practice: Consolidated Guidance* dated March 2018. Further, I will conduct the study in keeping with local legal and regulatory requirements.

As the site Principal Investigator, I agree to carry out the study by the criteria written in the protocol and understand that no changes can be made to this protocol without the written permission of the IRB and NIAID.

____

Site Principal Investigator (Print)

____ ____________________

Site Principal Investigator (Signature) **Date**

PROTOCOL SYNOPSIS

Title of the Protocol: A Phase I, Open-Label, Multicenter Trial Exploring the Safety and Tolerability of Autologous Polyclonal Regulatory T Cell Therapy in Adults with Active Pemphigus

ACE Protocol Number: APG01

Protocol Chair(s): [redacted]

IND Holder: DAIT, NIAID

Objectives:

Primary Objective

- To evaluate significant events that occur when using PolyTregs and establish the dose recommended for further clinical investigation in subjects with pemphigus. A significant event will be defined as a National Cancer Institute-Common Terminology Criteria for Adverse Events (NCI-CTCAE) Grade 3 or higher adverse event (AE) which is related (possibly related or related) to study drug or any serious adverse event (SAE) which is related to study drug.

Secondary Objectives

- To characterize the safety of PolyTregs in subjects with pemphigus
- To provide preliminary data addressing the efficacy of PolyTregs
- To demonstrate the presence of and assess the persistence and function of transferred deuterium-labeled Tregs in peripheral blood and skin
- To assess disease specific and immunologic biomarkers in peripheral blood and skin
- To determine whether the PolyTregs proliferate in blood and/or skin

Study Cohorts:

Cohort	Subjects #	Target Cell Dose	Allowable Dose Range (-30% to +20%)	Volume (ml)	Concentration (cells/ml)	Allowable Concentration Range (-30% to +20%)
1	4-6	1.0×10^8	0.7×10^8 to 1.2×10^8	100	1.0×10^6	0.7×10^6 to 1.2×10^6
2	4-6	2.5×10^8	1.75×10^8 to 3.0×10^8	100	2.5×10^6	1.75×10^6 to 3.0×10^6

Study Design:

This phase I trial will be conducted as an open-label, dose-escalation, multicenter trial in adult patients with active pemphigus. We expect approximately three clinical sites to participate (UCSF and approximately 3 remote sites). The study will employ a sequential dose-escalation design with a maximum of twelve subjects receiving a single infusion of *ex vivo* selected and expanded autologous PolyTregs. The primary study endpoint is the number of significant events, defined as any related NCI-CTCAE Grade 3 or higher AE or any related SAE. Related is defined as being possibly, probably, or related to the *ex vivo* expanded autologous PolyTregs, as determined by the safety review committee (SRC, consisting of the protocol chair, representatives from the Statistical and Clinical Coordinating Center [DAIT-SACCC], and representatives of DAIT) that will review the laboratory data and clinical experience of all subjects in the cohort. Pemphigus relapses/flares will be assessed using the consensus definition of 3 or more new lesions a month that do not heal spontaneously within 1 week or by the extension of established lesions in a patient who has achieved disease control; however, only flare components assessed as AEs of NCI-CTCAE Grade 3 or higher or SAEs will contribute to the evaluation of significant events.

Two dosing groups will be employed in this trial: 1.0×10^8 and 2.5×10^8 PolyTregs. Each cohort will enroll 4-6 subjects with pemphigus. Each subject in cohorts 1 and 2 will receive an infusion and must reach Week 2 prior to the subsequent subject's blood donation. A schematic representation of an individual's participation is shown in Figure 1. The study dose can be escalated after the DSMB and SRC evaluates the safety data

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collected when the fourth subject in cohort 1 completes Week 8. After reviewing the data from the first four subjects, the DSMB and SRC will recommend adding additional subject(s) to the first cohort, advancing enrollment in the second cohort, or stopping the trial. A schematic representation of the study's cohort design is shown in Figure 2.

Since a significant event in this trial can occur from toxicity from the study drug or disease worsening (e.g., a pemphigus flare), the standard 3 + 3 design rules for dose escalation will not be employed. Therefore, if a Grade 3 or higher AE or any SAE occurs in a subject during his/her first year of study participation, enrollment will be suspended until the SRC can convene to review the event. If this occurs, the SRC may decide to proceed with enrollment, add additional subjects to the cohort, continue the suspended enrollment until the Data and Safety Monitoring Board (DSMB) can review the data, or place the trial on hold. The SRC will review all Grade 3 or higher AEs or any SAEs that occur throughout the study. However, since the likelihood of events being related to the investigational treatment diminishes over time, Grade 3 or higher AEs or any SAEs occurring during a subject's second or third year of the study will not result in an immediate suspension of enrollment, donation, and treatment, unless the SRC determines that such action is indicated. Provided the SRC can review the event within 5 business days of awareness, events judged to be significant will drive protocol management in terms of proceeding, suspending enrollment/treatment pending ad hoc DSMB input, or placing the study on hold pending further evaluation.

DSMB input will be required for the evaluation of some specific events. Description of these events and additional directives are outlined in Section 5.5.4, *Safety Stopping Guidance*.

Accrual of all subjects is expected to take approximately 1.5 years.

Endpoints:

Primary Safety Endpoint

- The number of significant events in each cohort through Week 52, defined as any related NCI-CTCAE Grade 3 or higher AE or any related SAE

Secondary Safety Endpoints

- Number of significant events in each cohort through Week 156
- All AEs through Week 156
- All NCI-CTCAE Grade 3-5 AEs through Week 52
- All NCI-CTCAE Grade 3-5 AEs through Week 156
- All SAEs through Week 156
- All infection related events through Week 156
- All infusion reactions, defined as any adverse reaction of NCI-CTCAE Grade 1 and higher within 24 hours of infusion

Secondary Efficacy Endpoints

- Change in pemphigus disease area index (PDAI) score from baseline to Weeks 1, 2, 8, 12, 26, 39, 52, 78, 104, 130, and 156
- Change in Desmoglein 1 and 3 titers by ELISA from baseline to Weeks 1, 2, 8, 12, 26, 39, 52, 78, 104, 130, and 156
- Time to relapse (flare)
- Number of participants on prednisone dose ≤10 mg/day at Weeks 12, 26, 39, 52, 78, 104, 130, and 156

Exploratory Safety Endpoints

- All pemphigus relapses/flares, as defined by consensus definition of 3 or more new lesions a month that do not heal spontaneously within 1 week or by the extension of established lesions in a patient who has achieved disease control through Week 156
- Absolute and change from baseline in clinical chemistry, hematology, and urinalysis after treatment

Exploratory Efficacy Endpoints

- Change in Skindex-29 scores (symptoms domain, emotions domain, functioning domain, overall score) from baseline to Weeks 1, 2, 8, 12, 26, 39, 52, 78, 104, 130, and 156
- Change in patient global assessment (PGA) from baseline to Weeks 1, 2, 8, 12, 26, 39, 52, 78, 104,

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130, and 156

- Change in physician global assessment (PhGA) from baseline to Weeks 1, 2, 8, 12, 26, 39, 52, 78, 104, 130, and 156
- Number of participants on prednisone dose 0 mg/day at Weeks 12, 26, 39, 52, 78, 104, 130, and 156
- Number of participants requiring an increase in prednisone dose relative to last recorded prednisone dose at any time prior to Weeks 12, 26, 39, 52, 78, 104, 130, and 156

Exploratory Mechanistic Endpoints

- Determine the relative frequency of adoptively transferred Tregs in blood and skin by quantifying transferred deuterium-labeled Tregs in peripheral blood and skin from Week 1 to 12. In addition, T Cell Receptor (TCR) sequencing may be performed on sorted Tregs from either skin or blood
- Perform whole transcriptome RNA sequencing to determine the effects of PolyTreg infusion on inflammatory and immunoregulatory pathways in the target tissue (skin)
- Quantify inflammatory cell subsets and immunologic biomarkers in blood and skin, including changes in lymphocyte subsets, cytokine profile, and autoantibody production
- Changes in disease specific and immunologic biomarkers in peripheral blood including lymphocyte subsets, cytokine profiles, polyclonal and antigen-specific Treg function (flow cytometry with MHC II-tetramer staining), and autoantibody production from baseline to Weeks 1, 2, 8, and 12
- Changes in disease specific and immunologic biomarkers in skin including lymphocyte subsets, polyclonal and antigen-specific Treg function (flow cytometry with MHC II-tetramer staining), and cytokine profiles from baseline to Weeks 1 and 12

Key Entry Criteria:

Key Inclusion Criteria:

1. Ability to provide informed consent
2. Age 18-75 years at Screening Visit
3. Diagnosis of pemphigus vulgaris (PV) or pemphigus foliaceus (PF), defined by H&E and direct immunofluorescence staining of skin biopsy at any time prior to enrollment
4. Pemphigus treated with systemic corticosteroids within the 2 years prior to screening (historic or current) or treated with rituximab at least 12 months prior
5. Presence of:
    a. anti-Dsg3 antibodies (>20.0 U/ml) at screening visit consistent with diagnosis of pemphigus vulgaris, or
    b. anti-Dsg1 antibodies (>20.0 U/ml) at screening visit consistent with diagnosis of pemphigus foliaceus
6. Active PV or PF as defined by PDAI overall activity score 3-10 at screening visit, and PDAI overall activity score 1-12 at baseline visit
7. Positive test for Epstein-Barr virus (EBV) antibody
8. Adequate venous access to support draw of 400 ml whole blood and infusion of investigational therapy
9. An absolute Treg count of ≥42 cells/μL within 6 weeks prior to whole blood collection at Week -2

Key Exclusion Criteria:

1. Initiation of systemic corticosteroid therapy, prednisone dose > 25 mg/d (or equivalent) or change in prednisone dose within 4 weeks prior to screening
2. Addition of a new medication, or change in the dose of any background medication used to treat any aspect of pemphigus within the timeframes listed below. Specifically:
    a. methotrexate, mycophenolate mofetil, mycophenolic acid, azathioprine, cyclosporine or dapsone within the 6 weeks prior to screening or in the time between screening and study drug infusion
    b. intravenous immunoglobulin (IVIG) within 12 weeks prior to screening or in the time

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between screening and study drug infusion (subjects on IVIG must be on stable dose for at least 12 weeks prior to screening)

c. treatment with cyclophosphamide within 12 weeks prior to screening or in the time between screening and study drug infusion

3. Doses of background medications at screening:
   a. methotrexate > 25 mg/week
   b. mycophenolate mofetil > 3000 mg/d
   c. mycophenolic acid > 1080 mg/bid
   d. azathioprine > 200 mg/d
   e. cyclosporine > 2 mg/kg/d
   f. dapsone >250 mg/d
   g. IVIG > 4mg/kg IV monthly
4. Use of rituximab within the 12 months prior to screening
5. Change in dosing frequency, concentration, or applied surface area of topical steroids and/or topical calcineurin inhibitors within 2 weeks prior to screening

Sample Size: No formal power analyses were conducted since the study objectives require no hypothesis testing. However, the ability to detect at least 1 significant event at different frequencies was considered. Table 8.1 illustrates the probability of observing at least one significant event assuming different example scenarios.

The number of subjects to be enrolled will depend upon the observed safety profile and SRC review, which will determine the number of subjects per dose level, the number of dose escalations, and the number of cohorts.

Data Analyses: Due to the exploratory nature of this study, no confirmatory inferential analyses are planned. Descriptive statistics (such as medians, quartiles, and ranges for continuous data and percentages for categorical data) will be used to summarize patient characteristics, safety, efficacy, and mechanistic parameters. These summaries will be presented overall and separately for the subjects in the different dosing groups.

Lay Summary:

T cells, a type of white blood cell called a lymphocyte, play an important role in the immune system. One subtype, the regulatory T cell (Treg) helps to regulate the immune system and may provide protection against the development of autoimmune disease. The hope is that these naturally occurring Treg cells can be utilized for the treatment of autoimmune disease and potentially replace the use of chronic immunosuppressive therapies that are associated with multiple side effects. There has been a small study showing safe administration of Tregs with decreased disease activity in patients with insulin-dependent diabetes, and it is being studied in lupus, cancer and organ transplantation. The purpose of this study is to test the safety and effect of Treg therapy in patients with active pemphigus.

Up to 12 adults between the ages of 18 & 75 who have been diagnosed with pemphigus and meet all other entry criteria will be enrolled to receive one infusion of their own expanded Tregs at one of the following doses (1.0×10^8or 2.5×10^8 PolyTregs). Safety, disease activity, and mechanism of action will be assessed over a three year period, using specimens from blood and skin. Study therapy administration will occur during an overnight stay, followed by 2 weekly visits, then monthly visits

from Week 8 to Week 12, then quarterly visits from Week 26 to Week 52, then twice a year visits until Week 156.

FLOW DIAGRAMS OF PROTOCOL

Figure 1, Subject Participation

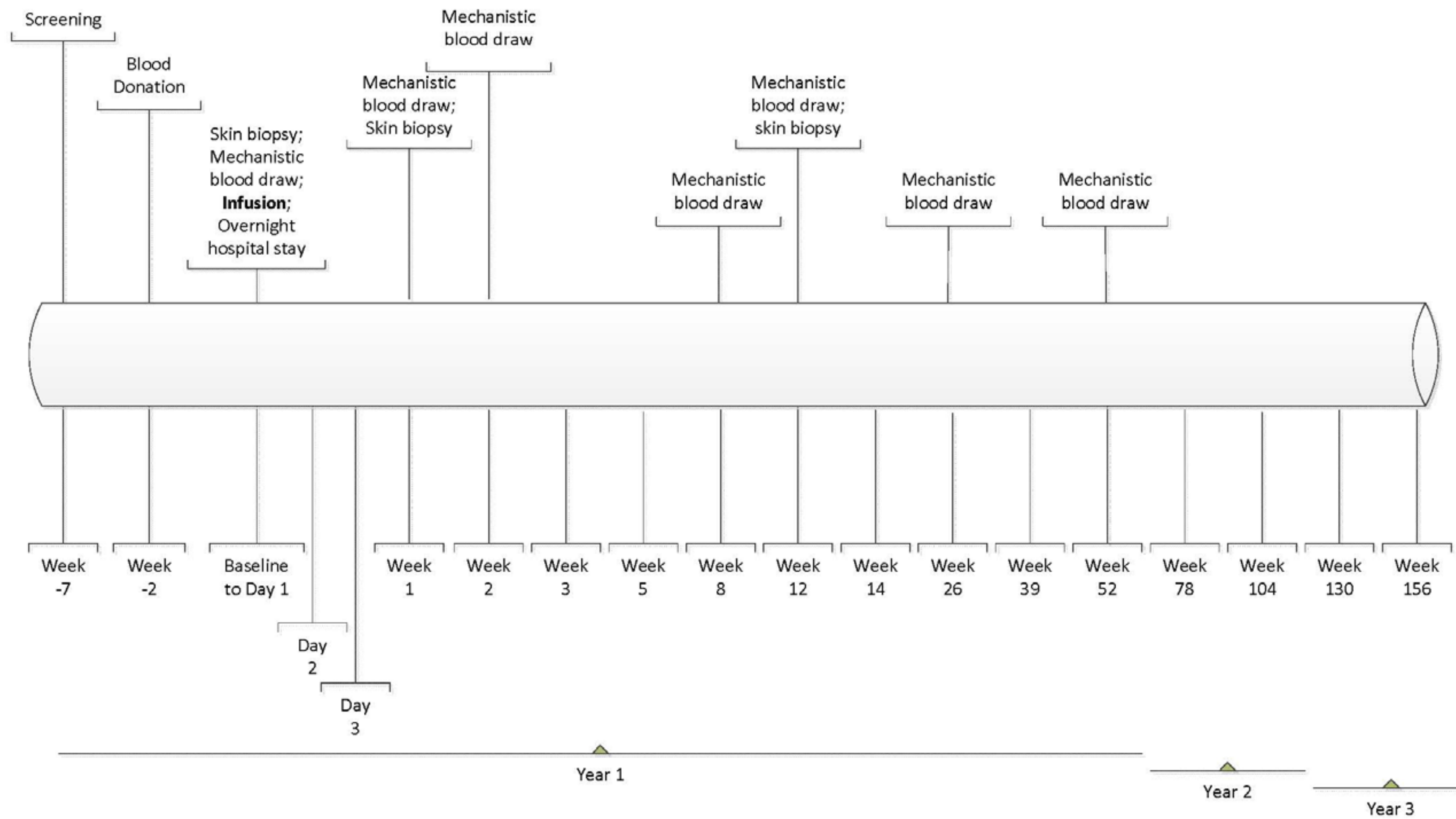
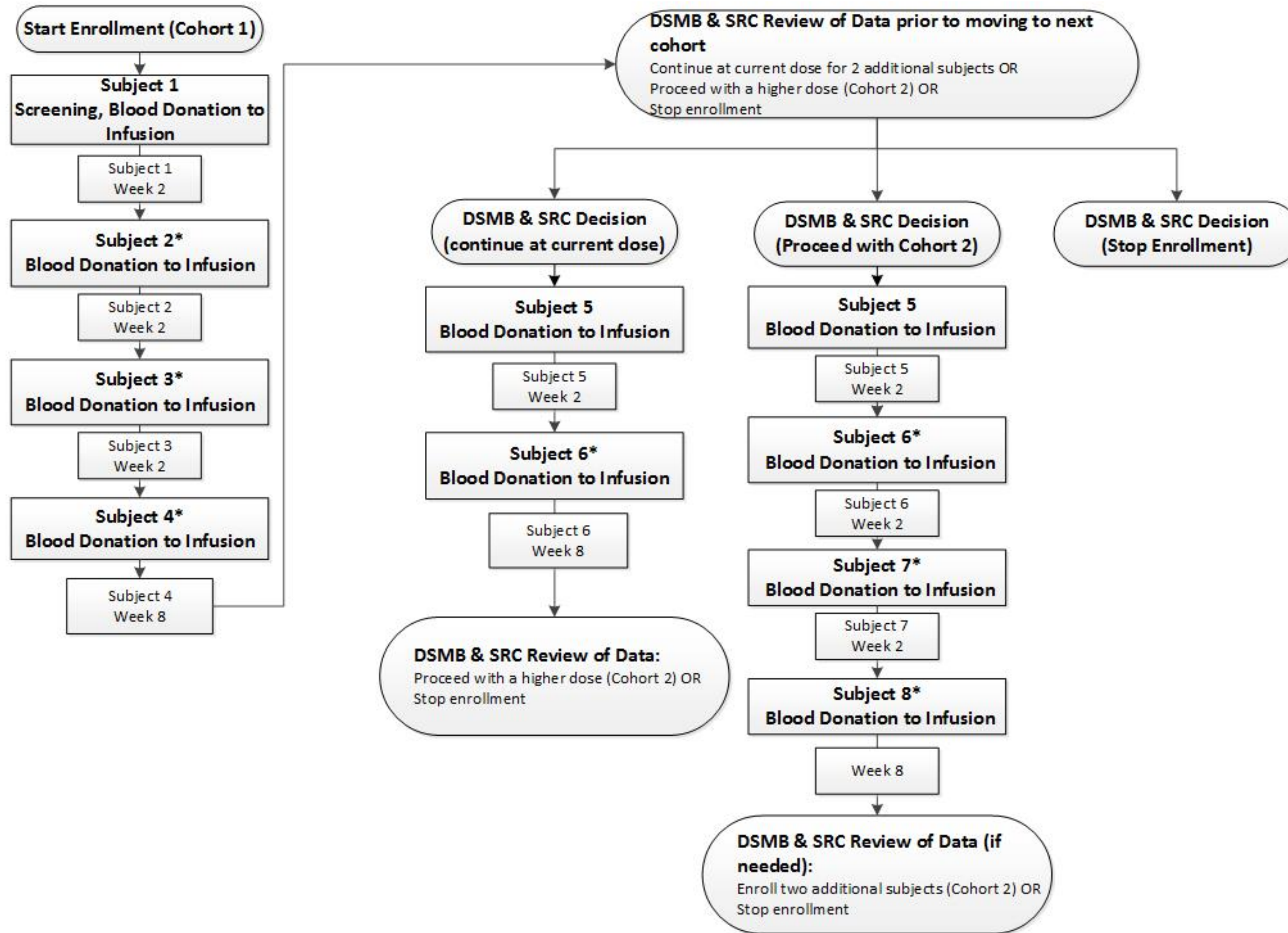


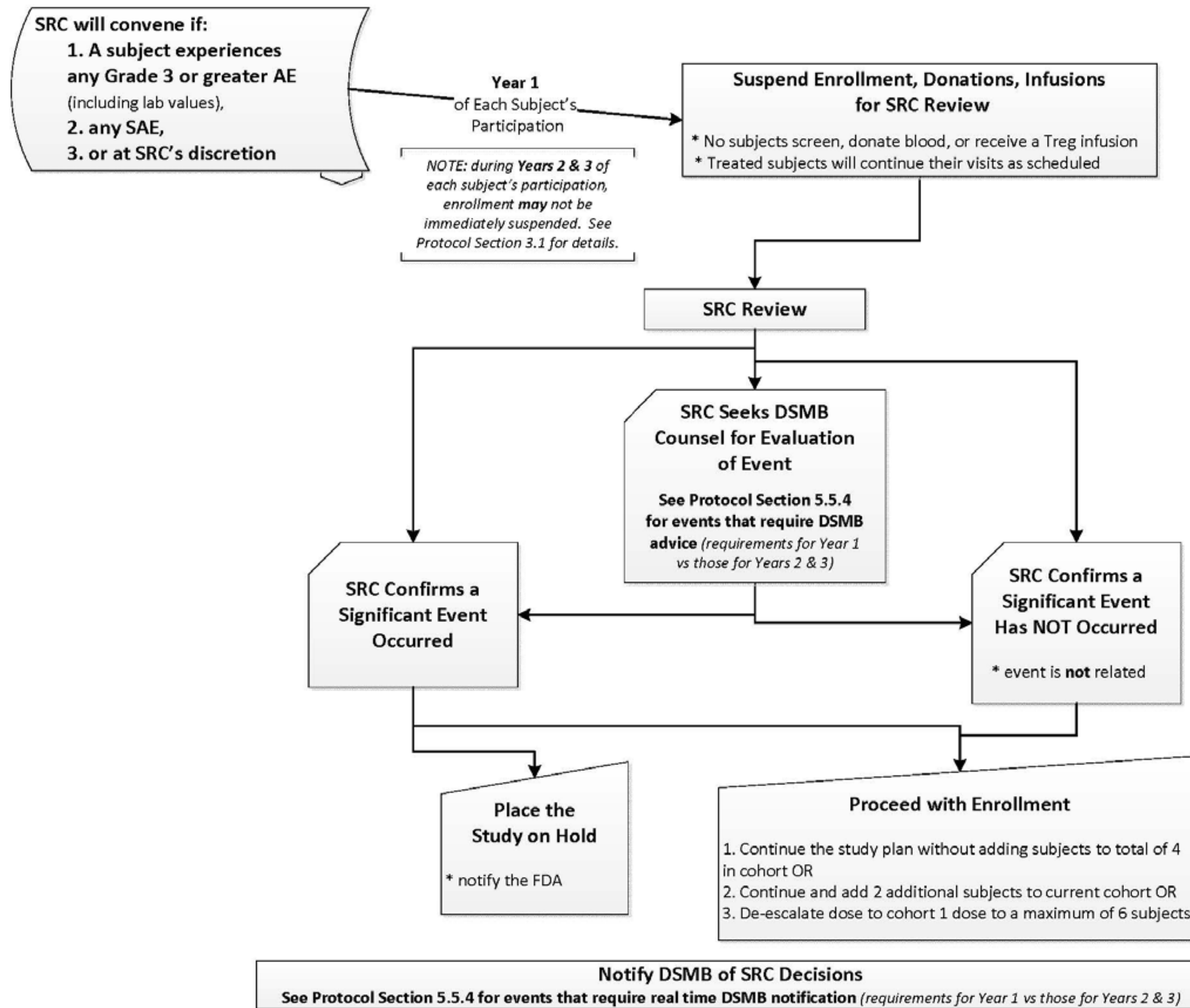
Figure 2, Study Design



***Cohorts 1 and 2: Each subject that receives an infusion must reach Week 2 prior to the subsequent subject's blood donation.**

See Section 3.1.1. *Management Plan for Out of Range PolyTreg Doses* for subjects in cohort 2 that fall short of the PolyTreg target range.

Figure 3, Safety Review Committee Process



ABBREVIATIONS

ACE	Autoimmunity Centers of Excellence
ACS	American Cancer Society
ADCT	Autoimmune Diseases Clinical Trials
AE	Adverse event
APGAR	Appearance, pulse, grimace, activity, and respiration
ALK	Alkaline Phosphatase
ALT	Alanine Aminotransferase
ANA	Anti-Nuclear Antibody
AST	Aspartate Aminotransferase
AUC	Area Under the Curve
BCG	Bacille Calmette Guerin
BID	Bis in Die (twice a day)
BUN	Blood Urea Nitrogen
CBC	Complete Blood Count
CDC	Centers for Disease Control and Prevention
CFDA-SE	Carboxyfluorescein Diacetate Succinimidyl Ester
CFR	Code of Federal Regulations
CFSE	Carboxyfluorescein Succinimidyl Ester
cGMP	Current Good Manufacturing Practice
CLE	Cutaneous Lupus
CMV	Cytomegalovirus
CoA	Certificate of Analysis
CRF	Case Report Form
CRS	Cytokine Release Syndrome
CTCAE	Common Terminology Criteria for Adverse Events
d	Day
dA	Deoxyadenosine
DAIT	Division of Allergy, Immunology, and Transplantation
DAIT RMC at PPD	DAIT Regulatory Management Center at Pharmaceutical Product Development, Inc.
DAIT-SACCC	DAIT Statistical and Clinical Coordinating Center
dG	Deoxyguanosine
DHHS	Department of Health and Human Services
dl	Deciliter
DNA	Deoxyribonucleic Acid
dR	Deoxyribose
Dsg	Desmoglein
DSMB	Data and Safety Monitoring Board
EBV	Epstein–Barr virus
eCRF	Electronic Case Report Form
eGFR	Estimated Glomerular Filtration Rate
ELISA	Enzyme-Linked Immunosorbent Assay

FACS	Fluorescence-Activated Cell Sorting
FDA	Food and Drug Administration
FSC	Forward Scatter
FWA	Federal Wide Assurance
GCP	Good Clinical Practice
GMP	Good Manufacturing Practice
GvHD	Graft vs Host Disease
HICTF	Human Islet and Cellular Transplantation Facility
HIV	Human Immunodeficiency Virus
HSV	Herpes Simplex Virus
IB	Investigator's Brochure
ICH	International Conference on Harmonization
IgG	Immunoglobulin G
IL	Interleukin
IND	Investigational New Drug Application
IPEX	Immunodysregulation Polyendocrinopathy Enteropathy X-linked syndrome
IRB	Institutional Review Board
ITT	Intention-to-Treat or Intent-to-Treat
IU	International Units
IV	Intravenous
IVIG	Intravenous Immunoglobulin
kg	Kilogram
L	Liter
mAb	Monoclonal Antibody
mITT	Modified Intention-to-Treat or Intent-to-Treat
mg	Milligram
ml	Milliliter
MM	Medical Monitor
MMF	Mycophenolate Mofetil
MMTT	Mixed-Meal Tolerance Test
NCI	National Cancer Institute
NIAID	National Institute of Allergy and Infectious Diseases
NIH	National Institutes of Health
NK	Natural Killer
NSAID	Non-Steroidal Anti-Inflammatory Drug
OHRP	Office of Human Research Protection
PBMC	Peripheral Blood Mononuclear Cell
PCR	Polymerase Chain Reaction
PDAI	Pemphigus Disease Area Index
PF	Pemphigus Foliaceus
PGA	Patient's Global Assessment
PhGA	Physician's Global Assessment
PI	Principal Investigator
POC	Point of Care

PolyTregs	*Ex Vivo* Expanded Autologous CD4+CD127lo/-CD25+ Polyclonal Regulatory T Cells
PP	Per Protocol
PPD	Purified Protein Derivative
PV	Pemphigus Vulgaris
QA	Quality Assurance
QoL	Quality of Life
qPCR	Quantitative Polymerase Chain Reaction
REMS	Risk Evaluation and Mitigation Strategy
RhoFED	Rho Federal Systems Division, Inc.
SAE	Serious Adverse Event
SAP	Statistical Analysis Plan
SAR	Suspected Adverse Reaction
SLE	Systemic Lupus Erythematosus
SMT	Study Management Team
SOP	Standard Operating Procedure
SP	Safety Population
SRC	Safety Review Committee, consisting of the protocol chair, representatives from the Statistical and Clinical Coordinating Center (DAIT-SACCC), and representatives of DAIT that will review the laboratory data and clinical experience of all subjects in the cohort
SSC	Side Scatter
SUSAR	Serious and Unexpected Suspected Adverse Reaction
T1DM	Type 1 Diabetes Mellitus
TCR	T Cell Receptor
Tdap	Tetanus, Diphtheria, Pertussis
Teff	Effector T Cell
TGFβ	Transforming Growth Factor Beta
T_H	T Helper Cell
Treg	Regulatory T Cell
TSDR	Treg Specific Demethylation Region
UCSF	University of California at San Francisco
ul	Microliter
ULN	Upper Limit of Normal
UVA	Ultra Violet A
UVB	Ultra Violet B
WBC	White Blood Cell

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1 BACKGROUND INFORMATION AND SCIENTIFIC RATIONALE

Pemphigus is a life-threatening autoimmune disease of the skin characterized by loss of epidermal cell-cell adhesion due to autoantibodies against desmosomal molecules, desmoglein (Dsg)3 and Dsg1 [1]. While broad immunosuppressive regimens have significantly improved management of pemphigus over the past several decades [2], current therapeutic strategies are associated with significant side effects and toxicities that limit effective management [3-9]. As autoantibodies are critical in pemphigus pathogenesis, therapeutic strategies aimed at limiting autoantibody production hold great promise for effective disease management with minimal adverse effects [10, 11]. Biologic therapy targeting B lymphocytes with anti-CD20 monoclonal antibodies has led to successful induction of disease remission [12], however, prolonged B cell depletion with rituximab has been associated with increased infection risk. An alternate strategy for limiting autoantibody production lies in augmenting immune regulation. Autoreactive helper T cells and autoreactive B cells play major roles in driving autoantibody formation [13], and both populations are controlled by regulatory T cells (Tregs) [14]. Furthermore, recent evidence demonstrates reduced peripheral Tregs in pemphigus vulgaris (PV) patients as compared with healthy controls regardless of disease status, suggesting an important role for Tregs in pemphigus pathogenesis [15, 16]. Taken together, these data suggest that a therapeutic strategy targeting Treg depletion may be effective in managing pemphigus by re-establishing stable immune tolerance with low toxicity and few side effects.

The discovery of the importance of regulatory T cells (Tregs) in the effective regulation of the basic processes that maintain tolerance has opened an important new opportunity for therapeutic intervention in immunology [17]. Tregs have unique and multiple mechanisms of action. While requiring specific T cell receptor-mediated activation to develop regulatory activity, their effector function appears to work by bystander suppression, regulating local inflammatory responses through a combination of cell-cell contact, metabolic effects on immunity, and suppressive cytokine production.

The therapeutic potential of Tregs is now well-established in animal models, but experience in humans is very limited. In a pilot study of 23 patients, Brunstein *et al.* [18] infused *ex vivo* expanded Tregs in leukemia and lymphoma patients who were undergoing transplantation with umbilical cord blood. No infusion-related toxicities were observed. Compared with 108 identically treated historical controls without Treg therapy, there was a lower incidence of acute graft-versus-host disease (GvHD). More recently, Marek-Trzonkowska *et al.* [19] infused *ex vivo* expanded Tregs into 10 children within 2 months of the diagnosis of type I diabetes mellitus (T1DM). These investigators also reported no short-term toxicity associated with Treg therapy. Compared with matched T1DM controls, there was an increase in Tregs in the peripheral blood of the treated children, lower plasma C-peptide levels, and a reduction in insulin requirement. These limited but encouraging experiences contribute to the rationale for this trial.

Investigators involved in this project have pioneered the use of Tregs as therapeutic agents [20-25]. Some of this work has been conducted with support from the ACE program with the intent of laying a sound scientific foundation for the innovative use of Tregs in the treatment of autoimmune diseases. The details of the work accomplished to date are included in the next section. In brief summary, the following pre-clinical work has been completed:

- demonstrated that *ex vivo* expanded PolyTregs can ameliorate autoimmune disease in murine models for systemic lupus erythematosus (SLE) and T1DM [20-22]
- developed the technology for the production of large quantities of purified PolyTregs that meet the rigorous standards for infusion into humans [23]
- preliminary experience in patients with T1DM that demonstrates the feasibility of the proposed trial, including the feasibility of conducting the trial in a multicenter fashion if necessary with Treg expansion performed at Univerisity of California, San Francisco (UCSF)

Treg therapy is unlike any other form of therapy currently under investigation for pemphigus. Although the development of an antigen-specific approach to Treg therapy is the subject of active investigation at UCSF, there is not yet a robust way to expand antigen-specific Tregs without risking the simultaneous expansion of potentially autoreactive Teffs. Therefore, at present, the use of PolyTregs provides the most feasible and safe approach to Treg therapy. PolyTregs have broad self-antigen reactivity, which is a good match for the broad loss of self-tolerance that occurs in pemphigus. The pathogenesis of this classic autoimmune disease and associated disease effector cells provide strong rationale for study of this novel therapeutic strategy in pemphigus.

1.1 Disease Background

Pemphigus is a severe intraepithelial autoimmune blistering disease resulting in painful erosions of the skin and mucous membranes (mouth, genitals, or esophagus). The two classic forms of pemphigus are pemphigus foliaceus (PF) and pemphigus vulgaris (PV) [26]. The diagnosis of pemphigus can be made by characteristic findings on skin or mucosal histopathology and direct immunofluorescence microscopy. Pathogenic anti-desmoglein 3 (anti-Dsg3) in PV and anti-desmoglein 1(anti-Dsg1) antibodies in PF play a central role in these classic, autoantibody-mediated conditions [27]. Clinically, PF is considered a more superficial variant of pemphigus because it is limited to body sites where Dsg1 is expressed, principally in the granular layer of the epidermis, and does not involve the mucous membranes. In contrast, PV is characterized by involvement of body sites where Dsg3 is expressed, specifically in the basal and immediate suprabasal layer of the epidermis and throughout the oral mucosa. Supporting the significant overlap in features and pathogenesis between PV and PF, about one-half to two-thirds of sera from PV patients also contain antibodies against Dsg1 [28-32]. In both disease variants, autoantibodies have been shown to react with the same Dsg1 ectodomain and share similar binding and disulfide linkage properties, supporting that PV and PF are variants of the same disease [33]. Binding of anti-Dsg antibodies to keratinocytes results in breakdown of intercellular desmosomal bridges and failure of the skin barrier. The production of pathogenic anti-Dsg autoantibodies in pemphigus requires an orchestrated effort between autoreactive B cells and autoreactive T cells [14, 34], implicating dysfunction of both humoral and cell mediated immunity in this condition. Furthermore, both quantitative and qualitative dysfunction of Tregs is implicated in the pathogenesis of PV [16, 35, 36].

In the last 40 years, the availability of broadly immunosuppressive regimens has converted pemphigus from a fatal disease to a serious chronic condition. However, patients remain at high risk of severe complications and death as a direct result of treatment. Chronic systemic corticosteroids are still generally required as a pillar of the therapeutic regimen in pemphigus

and are associated with a host of adverse effects (including but not limited to hyperglycemia, hypertension, infection risk, glucocorticoid induced osteoporosis, and cataracts) [2, 37]. Potent immunosuppressants such as mycophenolic acid and azathioprine have a steroid sparing effect but are associated with cytopenias and infection risk. Azathioprine in particular may increase cancer risk. While rituximab can induce remission, it results in prolonged B cell depletion, which may persist for many years with subsequent associated increased risk of adverse events [38, 39]. Intravenous immunoglobulin (IVIG) can be used alone or with rituximab for nonresponders [37]; however, the drawbacks of this agent include hypercoagulable state, fluid shifts, and a risk for tachyphylaxis over time. Pemphigus patients tend to be younger (40-60 years old) than patients with other autoimmune bullous disorders (e.g., bullous pemphigoid) and may require decades of potentially toxic therapy. Thus, there is a critical need for more effective, less toxic approaches to treat this disease.

1.2 Scientific Rationale

An appealing alternative to chronic immunosuppressive therapy rests on the hope that it may be possible to harness naturally-occurring regulatory mechanisms to restore self-tolerance in people with pemphigus. Under normal circumstances, the immune system relies on multiple mechanisms to control untoward immune responses. Recent studies have focused particular attention on a small subpopulation of $CD4^+$ T cells, termed Tregs, that play an essential role in maintaining self-tolerance [17]. The importance of Tregs is exemplified by an autoimmune syndrome called immune dysregulation, polyendocrinopathy, enteropathy, X-linked (IPEX) syndrome [40]. IPEX is an inherited Treg-deficiency disease that causes severe multiorgan autoimmunity and that, left untreated, leads to death within the first few years of life. Conversely, recent studies have shown that Tregs can be isolated, expanded, functionally defined and, when administered in small animal models of autoimmune disease, provide lasting disease-specific therapy in the absence of adjuvant immunosuppression [20-22, 41]. These findings suggest that *ex vivo* expanded autologous Tregs may have the potential to stably restore immune homeostasis in the setting of autoimmunity.

Recent studies in people with other diseases support the feasibility and potential of Treg therapy. Indirect evidence in support of this strategy comes from two studies in which treatment with low-dose interleukin 2 (IL-2) increased Tregs and ameliorated disease in patients with hepatitis C virus-induced vasculitis [42] and in patients with GvHD [43]. More direct evidence comes from two trials in which Tregs were harvested from patients, expanded *ex vivo*, and then reinfused. In patients undergoing stem cell transplant for leukemia, treatment with Tregs prevented GvHD, promoted lymphoid reconstitution, and reduced the frequency of opportunistic infection without weakening the graft-vs-leukemia effect [44]. In children with recent-onset T1DM, this approach reduced the insulin requirement in treated patients relative to patients who did not receive Tregs [19]. These observations demonstrate that the technology now exists to seriously consider autologous Treg therapy in patients with pemphigus.

Against this background, UCSF has devoted considerable effort in recent years to develop an alternative approach for the treatment of autoimmune diseases that is based on augmenting the beneficial effect of autologous Tregs. The details of this innovative approach are described in the following sections of this protocol. In brief, we have developed a Current Good Manufacturing Practice (cGMP) process for obtaining $>2 \times 10^9$ PolyTregs via efficient isolation and in vitro-based culture-expansion of Tregs sorted from peripheral blood

lymphocytes from a single donor, for the purpose of performing adoptive cell therapy. We have used this strategy successfully in mice to treat autoimmune diseases, including murine models for diabetes mellitus [22] and systemic lupus erythematosus (SLE) [20, 21]. In addition, phase I trials of adoptively transferred PolyTregs in patients with T1DM and SLE have occurred. This preliminary experience has established the feasibility of adoptive Treg therapy in people and has provided reassuring preliminary information regarding safety. This protocol extends this line of investigation to patients suffering from pemphigus, including the incorporation of recent approaches designed to enrich for Dsg-specific Tregs during the expansion process. By so doing, this trial will be addressing a great unmet need for better and safer treatments for patients with pemphigus. This protocol extends this line of investigation to patients suffering from pemphigus using polyclonal expansion of autologous Tregs, with the intent to follow with a subsequent study using adoptively transferred enriched antigen-specific Tregs.

Pemphigus is an ideal disease in which to test this novel approach because: (i) the autoantigens driving this disease have been extensively characterized, which will allow "head-to-head" eventual assessment of adoptively transferred enriched antigen-specific Tregs compared to PolyTregs; (ii) the pathophysiology of pemphigus is well-studied and involves aberrant autoreactivity in both the humoral and cell-mediated arms of the immune system [14, 34, 45]; (iii) pemphigus is a potentially fatal disease with major shortcomings and toxicities of current therapies [2, 37, 46]; (iv) both murine and human data highlight the importance of Tregs in both disease pathogenesis and suppression [14, 16, 35, 36, 47]; (v) the tissues (i.e., skin and mucous membranes) targeted by this disease are highly accessible, allowing for comprehensive cellular and molecular analyses; and (vi) there are clinically robust biomarkers (i.e., anti-Dsg3 & anti-Dsg1 Enzyme-Linked Immunosorbent Assays [ELISAs]) of disease activity [48].

1.3 Summary of Pre-Clinical and Clinical Studies

1.3.1 Pre-Clinical Studies

1.3.1.1 Treg Therapy in Murine Pemphigus Vulgaris

Currently, mouse models that perfectly recapitulate human PV are lacking. However, in one of the best-accepted PV mouse models, Tregs have been shown to significantly attenuate disease. Using an adoptive transfer approach to generate pathogenic anti-Dsg3 antibodies, Yomoyama and colleagues [35] have shown that co-adoptive transfer of PolyTregs resulted in reduced anti-Dsg3 antibodies in serum and reduced disease activity as measured by weight loss and PV clinical score (Figure 4). In addition to this study, there are numerous reports providing evidence that Tregs potently suppress humoral immune responses in mice [14]. Taken together, this data provides a strong scientific rationale to explore the potential of adoptive Treg therapy for human PV.

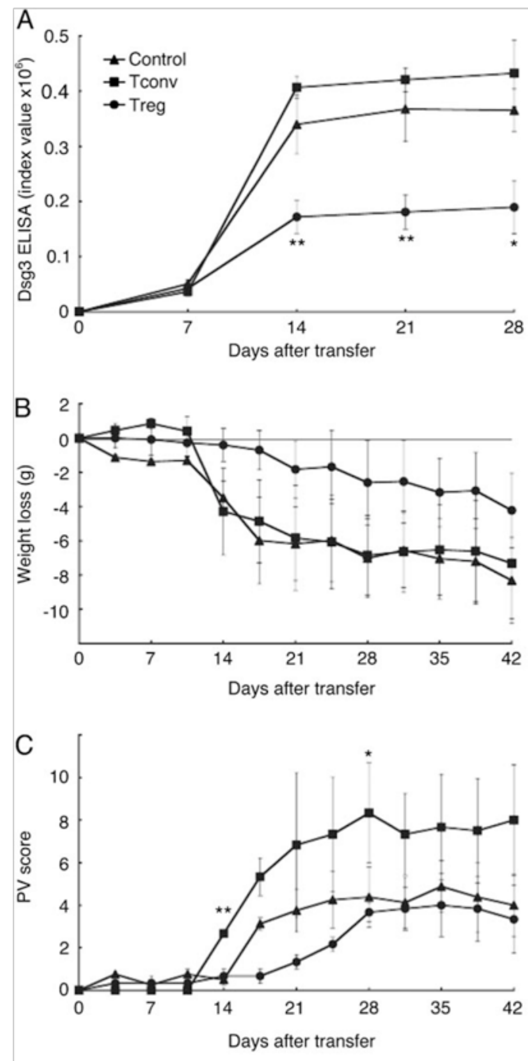


Figure 4, Tregs suppress anti-Dsg3 autoantibody production and clinical disease in a PV mouse model

Production of anti-Dsg3 IgG in Rag2-/- recipient mice that were adoptively transferred with immunized Dsg3-/- splenocytes together with Tregs or Tconvs obtained from wild type mice. The time course of changes in the titer of anti-Dsg3 IgG examined by Dsg3 ELISA assay score (A), body weight loss (B) and PV score (C) are shown [35].

1.3.1.2 Expansion of Tregs from Individual Patients

A robust selection and expansion method for Tregs from individual patients has been established [23]. Preclinical and clinical studies have been in agreement in showing that expression of the transcription factor, FOXP3, is found in Tregs. The combined use of three markers (CD4, CD25, and CD127) identifies the majority of FOXP3^{+} Tregs present in the peripheral blood as illustrated in Figure 5. The fluorescence-activated cell sorting (FACS) plots illustrate the selection strategy in which the lymphocyte population is gated based on forward scatter vs. side scatter (upper left panel), followed by CD4^{+} T cells (upper right panel) and subsequently on CD127$^{lo/-}$CD25^{+} T cells (lower left panel). A representative histogram of percent FOXP3 expression (lower right panel) is shown for the final

$CD4^{+}127^{lo/-}25^{+}$ Treg population (green gate – lower left panel) isolated per the described flow cytometry-based sorting procedure (Figure 5).

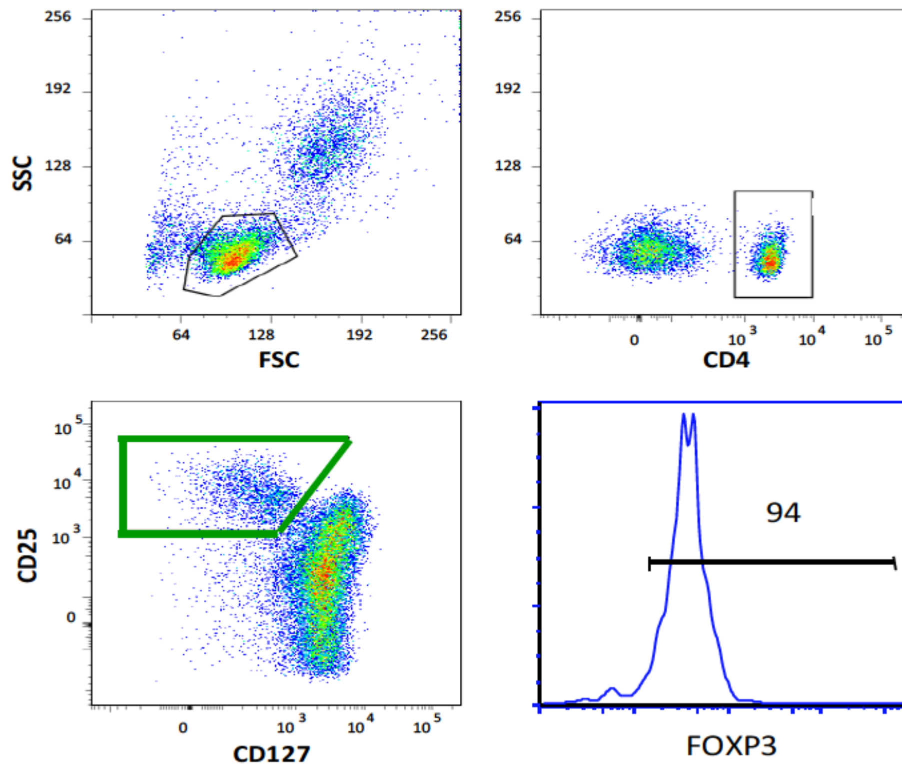


Figure 5, Gating strategy for separating $CD4^{+}127^{lo/-}25^{+}$ Tregs

The four panels show the sequential gating by flow cytometry. Upper left: gating on lymphocytes on the basis of forward (FSC) and side scatter (SSC). Upper right: Gating on $CD4^{+}$ T cells within the lymphocyte gated subpopulation. Lower left: Expression of CD25 and CD127 on $CD4^{+}$ lymphocytes. Lower right: Expression of FOXP3 on the $CD25^{+}CD127^{lo/-}$ subpopulation. Ninety-four percent of the gated cells are positive for FOXP3.

The 'stable' protein expression of FOXP3 has emerged as the most reliable correlate of functional Tregs [49, 50]. Therefore, two approaches have been utilized to evaluate FOXP3 expression among the expanded Tregs. First, by flow-cytometry, expanded cells have been shown to retain FOXP3 expression after the 14-day expansion period. Overall, the expanded cells from 11 independent expansions had a mean of 87.3% $FOXP3^{+}$ cells with a range of 79.2% to 98.4%. The majority of the $FOXP3^{lo/-}$ cells are likely to have been derived from an initial $FOXP3^{+}$ subset and not from a contaminating non-Treg population based on evidence that these expanded cells still express high levels of CD4, CD25 and low levels of CD127 and demonstrate suppressive activity in *in vitro* suppression assays.

Second, the epigenetic modifications of the FOXP3 locus have been evaluated. This measure has been shown to be one of the most robust measures of Treg lineage [49, 50]. Whereas transcription factor protein levels may be transiently increased in response to activation signals and extracellular cues, Deoxyribonucleic Acid (DNA) methylation state is thought to reflect a durable commitment to a specific cell lineage. Figure 6 shows the comparison between protein levels of FOXP3 (by flow cytometry) compared to DNA methylation status from the same cells. Each bar represents cells expanded from individual subjects, with

protein expression in blue and percent demethylation in red. The top panel represents expanded Effector T Cell (Teffs) compared to the bottom panel illustrating expanded Tregs. FOXP3 protein levels correlate very well with overall methylation status at the FOXP3 Treg Specific Demethylation Region (TSDR) indicating overall stability of the expanded Tregs compared to the Teffs.

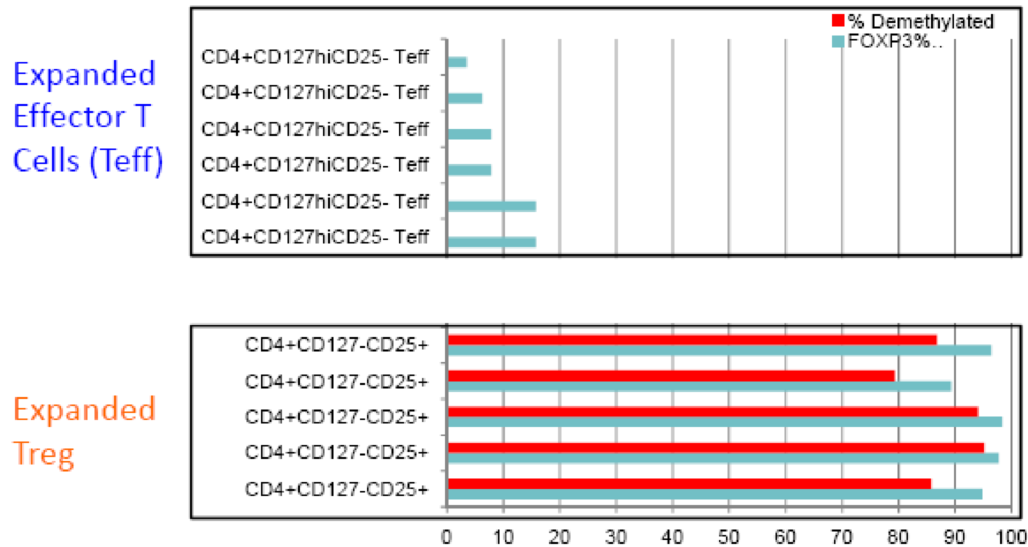


Figure 6, Correlation of protein expression and methylation patterns of FOXP3 in Teff and Tregs

Expanded effector T cells (Teff, top panel) and expanded Tregs (bottom panel) were analyzed for protein levels of FOXP3 by flow cytometry and DNA demethylation. FOXP3 expression is shown in blue bars as the present of cells positive of all CD4+ cells in culture. Demethylation is shown in red bars as the percentage of demethylation seen in the FOXP3 locus. Representative plots from both autoimmune patients and healthy controls are shown, where autoimmune subjects are the second and third sets of bars and all other samples are from healthy controls.

1.3.1.3 Expansion of Tregs from Patients with Autoimmune Disease

Overall, the expansion technique has been tested in healthy controls, organ transplant recipients, and in patients with autoimmune diseases, including SLE and T1DM; the current trial is the first experience in pemphigus patients. Among subjects with autoimmune disease, the sorted population expansion has ranged from 30-fold to greater than 2000-fold in 14 days. The mean expansion observed in patients with SLE and T1DM is approximately 650-fold; among two patients with pemphigus the mean expansion is 43-fold. Based on this experience, a dose has been selected that is believed to be achievable in patients with pemphigus.

1.3.1.4 **Documentation of the Functional Capacity of Expanded Tregs**

Extensive analyses of *ex vivo* expanded Tregs have been conducted in healthy subjects and in patients with autoimmune diseases (T1DM and SLE). To assess the capacity of expanded Tregs to suppress proliferation of $CD8^+$ T cells (responders) when stimulated with anti-CD3 and anti-CD28, carboxyfluorescein succinimidyl ester (CFSE)-labeled responders were incubated with varying ratios of Tregs. A representative example of the suppression of proliferation of $CD8^+$ T cells by an expanded Treg population as assessed by CFSE assay is shown in Figure 7, left panel. The right panel of Figure 7 illustrates the mean percent suppression from 12 individuals with decreasing ratios of Tregs to responders as measured by ^{3}[H]-thymidine incorporation. Overall, expanded Tregs suppressed responder proliferation within a small range of activity with approximately 50% suppression at a 1:8 (Treg:responder) ratio.

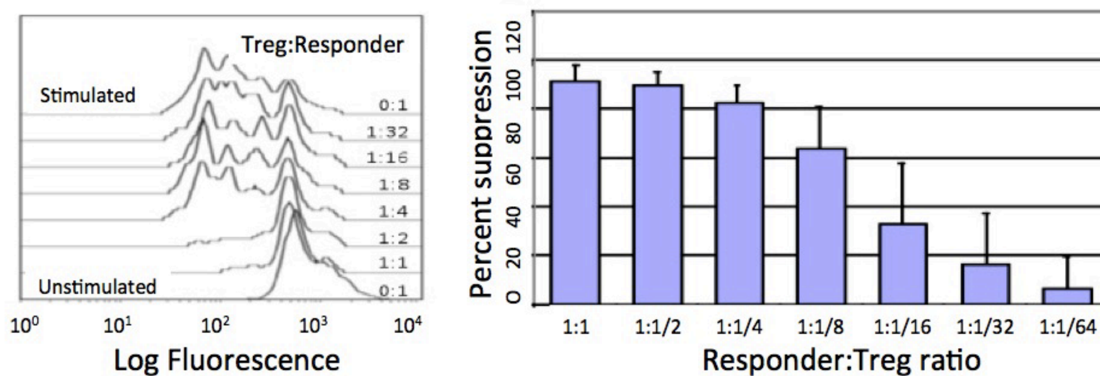


Figure 7, Functional Treg suppression assays

Suppression was assessed by the capacity of expanded Treg to suppress proliferation of CFSE-labeled CD8+ T cells (responders) when stimulated with anti-CD3 and anti-CD28. (Left Panel) A representative example of suppression by expanded Tregs from a subject with autoimmunity, where CFSE florescence is plotted on the x-axis. Overlaid is a titration of Treg to CD8+ responder rations, as labeled. The top histogram (0:1) indicates proliferation of responders when incubated alone in the presence of anti-CD3/anti-CD28 whereas the bottom histogram represents unstimulated responders. Decreased proliferation of responders with increasing ratio of Treg is shown. (Right Panel) The mean percent suppression from 12 individuals (non-diabetic healthy controls and T1DM subjects within one year of diagnosis) with decreasing ratios of Tregs to responders as measured by 3[H]-thymidine incorporation following stimulation with anti-CD3/anti-CD28.

In summary, Tregs from individual subjects, including subjects with active autoimmune disease, have been shown to be able to be expanded to sufficient numbers for *in vivo* therapy. Importantly, although the percentage of $FOXP3^+$ cells at the end of culture ranged from as low as 79.2% upward to 98.4% in these experiments, all the cultures of expanded Tregs suppressed *in vitro*, were anergic and retained low levels of CD127 expression, suggesting that these cells were not contaminated with significant numbers of Teffs. Furthermore, it has been illustrated that FOXP3 expression by FACS directly correlates with percent demethylation indicating that the $FOXP3^+$ expression in these cells is stable following a 14-day *ex vivo* expansion period. Last, we have demonstrated the suppressive function of these expanded Tregs. Together, these results suggest that the expansion protocol of $CD4^+127^{lo/-}25^+$ FACS-sorted T cells, expanded with anti-CD3/anti-CD28-coated beads (1:1) plus IL-2 (300 U/ml) and restimulated on day 9 in X-vivo media/10% human sera led to efficient

expansion of FOXP3$^+$ T cells with demonstrated regulatory function *in vitro* via functional suppression assays.

1.3.1.5 **Functional Analysis of Tregs in Tissues**

A new protocol for comprehensively studying leukocyte populations isolated from human skin has recently been developed and optimized at UCSF. Traditional approaches have relied on culturing skin biopsy specimens for 10-20 days in the presence of growth factors (i.e., recombinant IL-2), to allow infiltrating leukocytes to migrate out of the skin and expand in response to these factors. It is becoming increasingly appreciated that lymphocytes cultured for this length of time can alter their functional capabilities and may not accurately reflect *in vivo* differentiation and function. Using a single 4mm punch biopsy of freshly isolated human skin, lymphocytes and monocytes may be isolated and assessed for their functional activities after a 12-hour culture in the absence of growth factors (Figure 8). A 13-color flow cytometry is performed on these specimens, allowing for the quantification of multiple cytokine, chemokine, and cell surface and intracellular differentiation/activation markers (Figure 8A-C). This technique is extremely sensitive, as a biopsy specimen from normal skin (having no appreciable inflammatory infiltrate by routine histology) shows a defined memory T cell population capable of producing specific effector cytokines (Figure 8C).

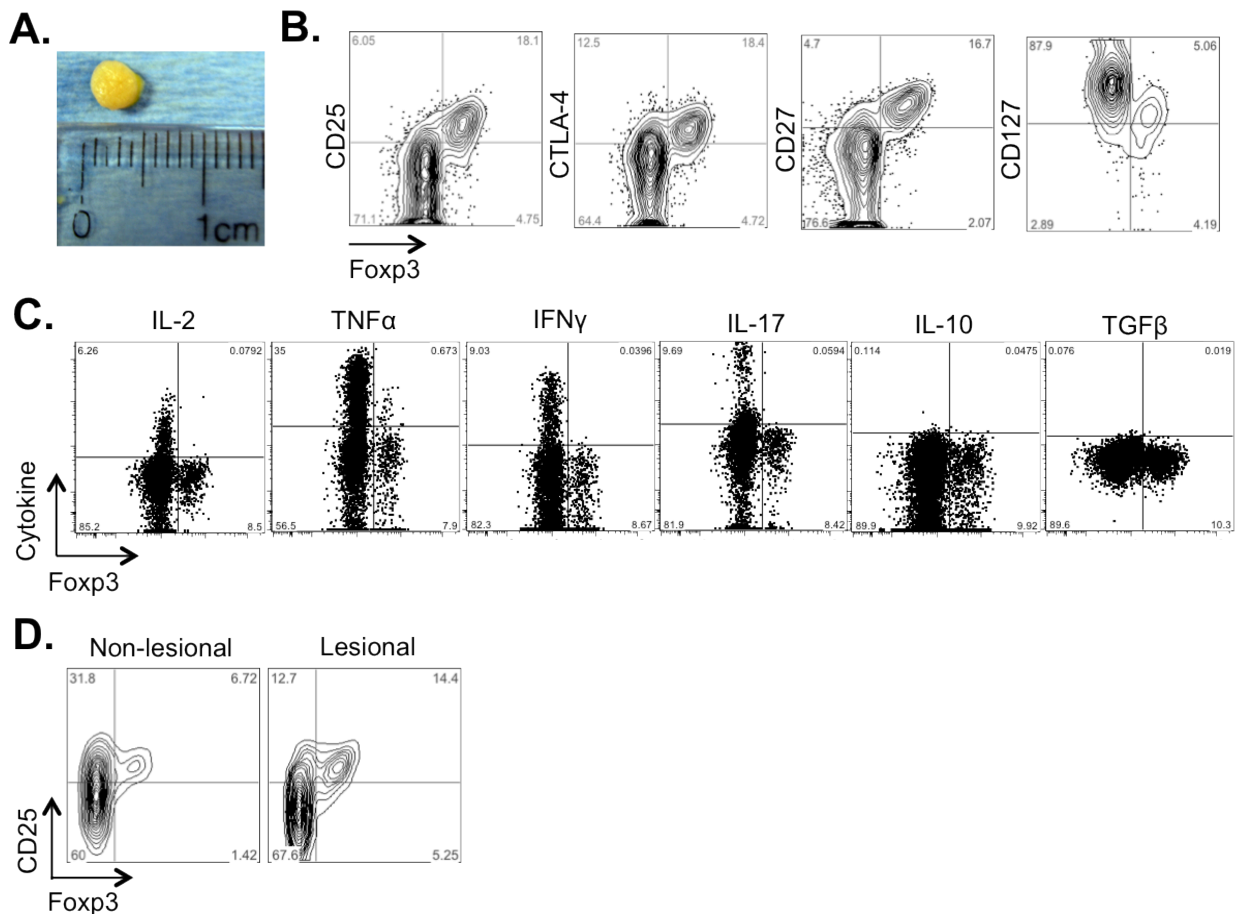


***Figure 8*, Functional immunoflow cytometry of human skin**

(A) A single 4mm punch biopsy was obtained from normal skin. Tissue was minced and digested for 12hrs in a digestion cocktail. Cutaneous T cells were assayed for Foxp3 expression and cell surface

markers (B) as well as intracellular cytokine production after stimulation with PMA/ionomycin (C). Panels B and C are gated on live CD3+ T cells. (D) This approach was used on 4mm skin biopsies from a patient with pemphigus (lesional and non-lesional skin). Cells in panel D are pre-gated on live $CD3^{+}CD4^{+}$cells [51].

This technique is being used to define the major inflammatory pathways active in the skin of patients with diverse inflammatory and autoimmune diseases as well as to functionally characterize Tregs in both normal and diseased skin, including pemphigus (Figure 8D). Using this approach UCSF has discovered that Tregs are activated, proliferate, and secrete small amounts of interleukin-17 (IL-17) in lesions of psoriasis compared to non-lesional skin from the same individual. In contrast, absolute numbers and percentages of Tregs are reduced in both non-lesional and lesional skin in patients with systemic sclerosis. Isolation of leukocytes and assessment of their *in vivo* function using this approach will for the first time allow both Treg and Teff cell function in inflamed tissue before and after adoptive Treg therapy to be functionally characterized.

1.3.2 Clinical Studies

1.3.2.1 Prior Experience Using Polyclonal $CD4^{+}CD127^{lo/-}CD25^{+}$ Tregs in T1DM

1.3.2.1.1 Enrollment

UCSF and Yale have completed the study "A Phase 1 Safety Trial of $CD4^{+}CD127^{lo/-}CD25^{+}$ Polyclonal Treg Adoptive Immunotherapy for the Treatment of Type 1 Diabetes" conducted under IND 14462 (NCT01210664) [52]. In this study, we tested the safety and feasibility of treating adults with recent onset (within 3-24 months of diagnosis) T1DM with a single infusion of Polyclonal Tregs and obtained data on the pharmacokinetics of the transferred cells. A total of 26 potential participants signed consent and underwent screening assessments. Sixteen subjects were determined to be eligible and were enrolled. Two of these sixteen subjects were withdrawn from the study prior to infusion due to failure of PolyTreg product to meet release criteria. The other fourteen subjects were treated with a single dose of Polyclonal Tregs. Three subjects were treated in each of the first and second dosing cohorts with $0.05x10^{8}$ and $0.4x10^{8}$ cells, respectively, and four subjects were treated in each of the third and fourth dosing cohorts with approximately $3.2x10^{8}$ and 26 $x10^{8}$ cells, respectively. The highest dose represents approximately 20% of the total number of Tregs (13 x 10^{9}) that are predicted to exist in normal individuals [53]. At the time of this update, all 14 subjects have completed follow-up up to 5 years.

1.3.2.1.2 Manufacturing Summary

Our experience in this dose escalation trial has shown that we can consistently expand Polyclonal Tregs in 14 days, averaging 748-fold for cohorts 3 and 4 combined and 555-fold overall. Fourteen of 16 cell products met release criteria for identity, purity, viability, and sterility.

The two subjects who were withdrawn from the study were withdrawn due to failure of the manufactured Treg product to meet release criteria. The first failure was due to unanticipated expression of CD8 on a small subset of CD4+FOXP3+ cells. This subset was pre-existing in this patient prior to expansion and the proportion was not markedly altered by the ex vivo

expansion. Further investigation demonstrated that this subset expressed both CD4 and CD8 markers but had all other features of Tregs and was equally suppressive in vitro when compared with the subset that did not express CD8. The second product did not pass specified FOXP3 release criteria: 20.9% (less than 60%) of the cells were FOXP3+ by flow cytometry. This was ultimately due to a suboptimal anti-CD127 antibody staining for sorting the initial Treg population on the clinical sorter. This resulted in the contamination of non-Treg CD4+ T cells in the initial sort leading to selective expansion of the contaminating cells.

1.3.2.1.3 **Safety Summary**

At the conclusion of the study, 166 adverse events were reported in 15 subjects since the beginning of the trial. All 14 treated subjects reported at least one Adverse Event (AE). One subject who underwent phlebotomy but was withdrawn before treated, reported one adverse event before withdrawal from the trial. One hundred and five events were judged as mild in severity, 48 were judged as moderate, 11 were judged as severe, and 2 were judged as life-threatening. Thirty events were judged to be possibly related, 40 unlikely related and 96 unrelated to study therapy.

The most common SOC affected was “Infections and Infestations” followed by “Gastrointestinal Disorders” and “General Disorders and Administration Site Conditions.” Of 36 infections recorded, 24 were upper respiratory infections. Of those, 19 were judged grade 1 (CTCAE category: Infections and Infestations Other, Specify) and 5 were judge grade 2 in severity (CTCAE designation; Upper Respiratory Infection). One infection, initially reported as grade 2 pharyngitis, was subsequently demonstrated to reflect a new cytomegalovirus (CMV) infection that occurred prior to treatment with Tregs.

Of the 11 AEs judged as severe (grade 3), all were judged unlikely related or unrelated to the investigational agent. Two occurred prior to Treg infusion and were judged unrelated to the investigational agent. Four events were hypoglycemia, of which three were judged unrelated and one judged unlikely related to the investigational agent. One event was syncope occurring 35 weeks after Treg infusion. One event was hyperglycemia occurring 10 weeks after Treg infusion. One was depressed level of consciousness due to inebriation. One was vomiting occurring 156 weeks after Treg infusion. One was elevated D-dimers occurring 104 weeks after Treg infusion.

Five serious adverse events (SAE) have been reported since the beginning of the trial. Three severe (grade 3) hypoglycemic SAEs, one judged unlikely related and two judged unrelated to the investigational product have been reported. One severe (grade 3) hyperglycemic SAE judged unrelated to the investigational product has been reported. One severe (grade 3) vomiting SAE judged unrelated to the investigational product has been reported.

Two events were life-threatening (grade 4) events of hypoglycemia in one subject occurring 59 and 62 weeks after Treg infusion. Grade 4 hypoglycemia is defined with a glucose < 30 mg/dL. Both of these events were judged unrelated to the investigational product.

1.3.2.1.4 **Pharmacokinetics**

After infusion of the product, the team used stable isotope labeling to track $CD4^{+}CD127^{lo/-}CD25^{+}$ polyclonal Tregs in the peripheral vascular space in humans. During *ex vivo* expansion, the ^{2}H label from deuterated glucose ($^{2}H_2$-glucose) contained in the cell culture medium is incorporated into the deoxyribose moiety in replicating DNA through the *de novo* purine nucleotide synthesis pathway. Following infusion of stable isotope-labeled Tregs, the total number of Tregs in peripheral blood can be measured by flow cytometry and stable-isotope enrichment in purified Tregs can be determined. Following isolation and hydrolysis of genomic DNA, the isotopic enrichment of the purine deoxyribonucleosides in Tregs sorted from whole blood can be assessed by gas chromatography/mass spectrometry. The change in ^{2}H enrichment in the total Treg pool can be assessed at specified time points post-infusion.

Three subjects were treated with a target dose of $3.2x10^{8}$ cells and 4 subjects were treated with a target dose of $2.6x10^{9}$ that were approximately 60% enriched for the ^{2}H-label in the study "A Phase 1 Safety Trial of $CD4^{+}CD127^{lo/-}CD25^{+}$ Polyclonal Treg Adoptive Immunotherapy for the Treatment of Type 1 Diabetes" conducted under IND 14462. ^{2}H-labeled Tregs were detectable in the peripheral blood up to 365 days post-infusion in cohort 4 patients as shown in Figure 9. A peak enrichment of 6% indicates that approximately 0.2% Tregs of the total Treg pool were ex vivo expanded infused Tregs. The delayed peak enrichments in several individuals suggest that the infused Tregs may initially migrate to the tissues. The majority of the tissue-blood exchange and/or cell death occurs by day 28, after which the kinetics appear stable: based on a two-phase decay curve, the average half-life of the fast decay phase is about 19.6 days (range 4.7 to 32.5 days) and the second slow decay phase has a half-life of a year or more in 4 of 7 patients studied (Figure 9).

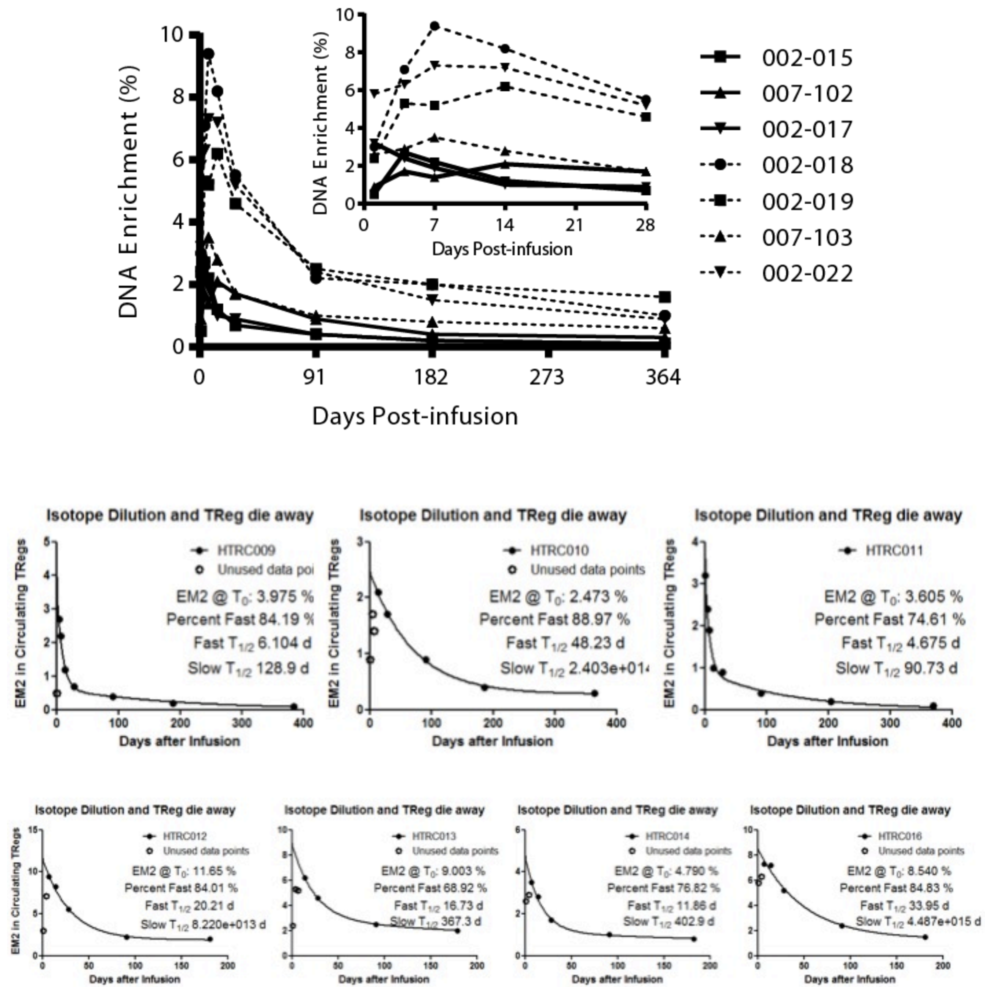
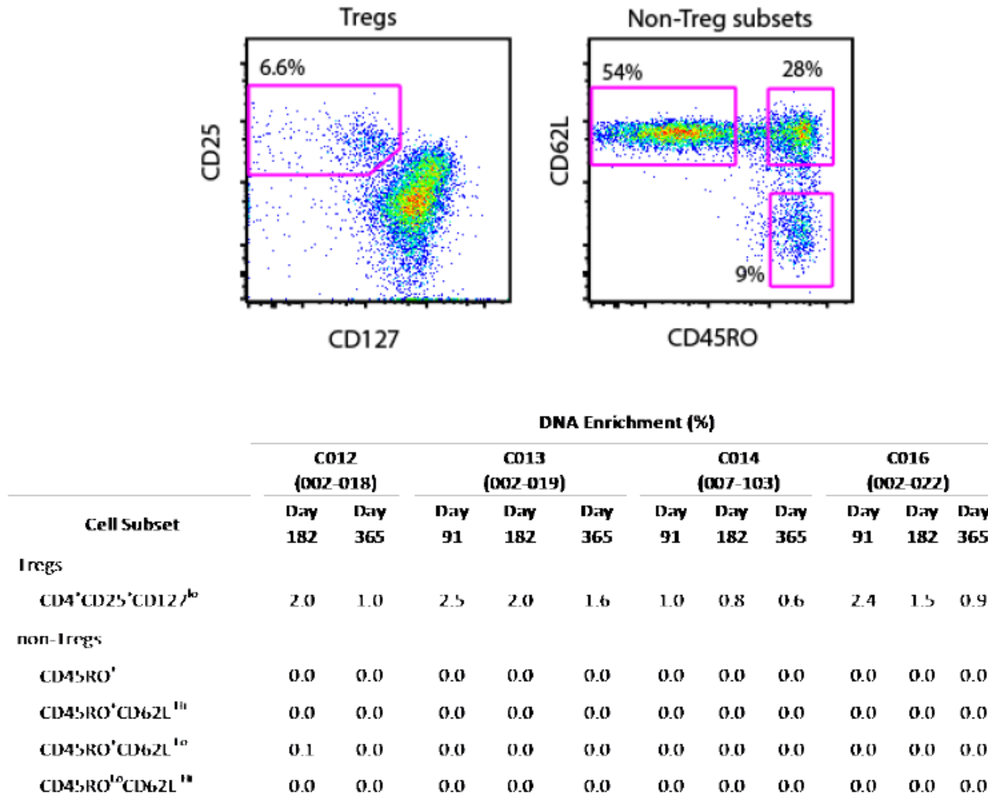


Figure 9, Treg Tracking by Stable Isotope Labeling

^{2}H label Tregs (target dose of 3.2 x10^{8} cells) from cohort three subjects (002-015, 007-102, and 002-017) and ^{2}H-labeled Tregs (target dose of 26 x10^{8} cells) from cohort four subjects (002-018, 002-019, 007-103, and 002-022) were infused as part of study treatment. All infused Tregs were approximately 60% enriched for the ^{2}H-label. (Top Panel) Peripheral blood was collected on days 1, 4, 7, 14, 28, 91, 182, and 364 days post infusion, and Tregs were isolated via FACS. ^{2}H isotopic enrichment of the purine deoxyribonucleosides in Tregs is plated on the y-axis. Background enrichment of unlabeled Tregs was ≤0.1% for each of the seven subjects. (Bottom Panel) Isotope dilution and Treg die away is shown for each subject.

To investigate the possibility that the cells may have changed their phenotype and the label was not captured in the sorting strategy for Tregs, we sorted the cells based on several non-Treg phenotypes, including activated memory cells, to see if deuterium label was found in other CD4+ T cell subsets. At no time up to 365 days was any deuterium observed in any other subset other than bona fide Tregs (Figure 10).



Cell Subset	DNA Enrichment (%) C012 (002-018) Day 182	C012 (002-018) Day 365	C013 (002-019) Day 91	C013 (002-019) Day 182	C013 (002-019) Day 365	C014 (007-103) Day 91	C014 (007-103) Day 182	C014 (007-103) Day 365	C016 (002-022) Day 91	C016 (002-022) Day 182	C016 (002-022) Day 365
Tregs											
$CD4^{+}CD25^{+}CD127^{lo}$	2.0	1.0	2.5	2.0	1.6	1.0	0.8	0.6	2.4	1.5	0.9
non-Tregs											
$CD45RO^{+}$	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
$CD45RO^{+}CD62L^{hi}$	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
$CD45RO^{+}CD62L^{lo}$	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
$CD45RO^{lo}CD62L^{hi}$	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0

Figure 10, Absence of evidence for label in non-Tregs

(Top Panel) Shown are representative FACS plots for subsets that were sorted for determination of ^{2}H-enrichment. Tregs were defined as CD4+CD25+CD127^{lo} while non-Tregs (non- CD25+CD127^{lo} CD4+ cells) were further subsetted into CD45RO+, CD45RO+CD62L^{lo}, CD45RO+CD62L^{hi}, and CD45RO-CD62L^{hi} fractions. (Bottom Panel) To address long-term stability of the infused expanded Tregs samples collected on days 91 (in 3 of 4 patients only), 182, and 365 were sorted into Treg and non-Treg subsets as shown in the panel above. Subsets were then analyzed by mass-spectrometry for ^{2}H-label, which was incorporated into the infused Tregs during the expansion. Values shown are % enrichment for ^{2}H and have an error of ±0.1.

1.3.2.1.5 Enhanced suppressive function of Polyclonal CD4+CD127lo/-CD25+ Tregs

In the study "A Phase 1 Safety Trial of CD4^{+}CD127$^{lo/-}$CD25^{+} Polyclonal Treg Adoptive Immunotherapy for the Treatment of Type 1 Diabetes" conducted under IND 14462 we examined the suppressive activity of the expanded Tregs *in vitro*. Initial studies were performed using fresh versus expanded Tregs from a series of healthy individuals. Decreasing numbers of Tregs were added to cultures containing peripheral blood

mononuclear cell (PBMC), and anti-CD3 and anti-CD28 mAb. As seen in Figure 11A, the expanded Tregs routinely suppressed the PBMC proliferation at significantly lower Tconv:Treg ratios than the non-expanded cells. Similarly, analysis of 3 expanded Treg preparations of the patients in the phase I trial demonstrated 4-8-fold greater suppressive activity than non-expanded Tregs from the same individual (Figure 11B).

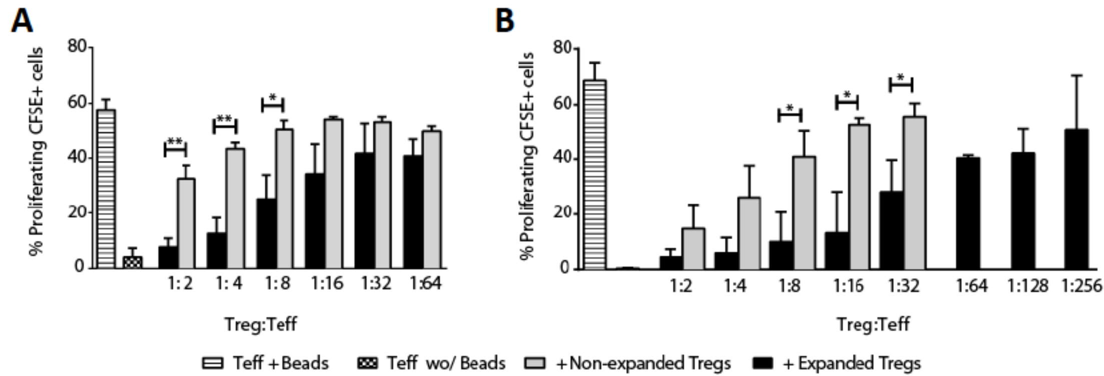


Figure 11, Expanded Treg function

*(A) In an in vitro culture, CFSE labeled Teff cells were cultured for 4 days in the presence of anti-CD3/CD28 antibody coated beads in the presence or absence of expanded/natural Tregs of same donor. T-cell proliferation in these cultures was analyzed by flow cytometry for CFSE dilution. Each condition set up in duplicate wells, was compared to cultures with Teff alone. Data are represented as mean + SEM for n=4 healthy individuals. (*P < 0.05 and **P < 0.01) (B) Suppression assays using CFSE-labeled CD4+CD127+CD25- cells sorted from standard PBMC as Teff cells cultured alone or activated with anti-CD3/anti-CD28 coated beads, and/ or co-incubated with Treg cells (from patients enrolled in this trial) show a consistent level of suppression. Comparison of mean of % proliferating CFSE+ cells with increasing ratio of Treg:Teff is shown for non- expanded Treg as compared to expanded Treg (n=3) (*P < 0.05).*

1.3.2.1.6 Metabolic Results

Although, the study was not designed to assess efficacy, we measured the stimulated C-peptide responses to a mixed meal over the 24-month period in the participants as a biomarker of safety. The area-under-the-curve (AUC) for 4-hour C-peptide measurements after a mixed-meal tolerance test (MMTT) is shown for all 14 subjects in Figure 12. C-peptide AUC appeared to be fairly constant over 2 years of follow-up for the six subjects in cohorts 1 and 2. In contrast, over the two years of follow-up, three of four subjects in cohort 3 had a decline in C-peptide of more than 60% and three of four subjects in cohort 4 also had a decline in C-peptide of more than 70%. The heterogeneity of diabetes progression as well as the dependence of progression on age and on duration of diabetes does not allow us to draw conclusive conclusions from this small number of subjects. An analysis of these data with respect to historical controls could not rule out the possibility that cohorts 1 and 2 had C-peptide results higher than expected, while cohorts 3 and 4 were not different than expected, given the natural history of diabetes. One subject in cohort 3 and 4 had sub-optimal glycemic control, which in and of itself could have contributed to more rapid beta cell loss as compared to those subjects who achieved idealized glycemic control at the prescribed American Diabetes Association targets. Insulin use was generally stable.

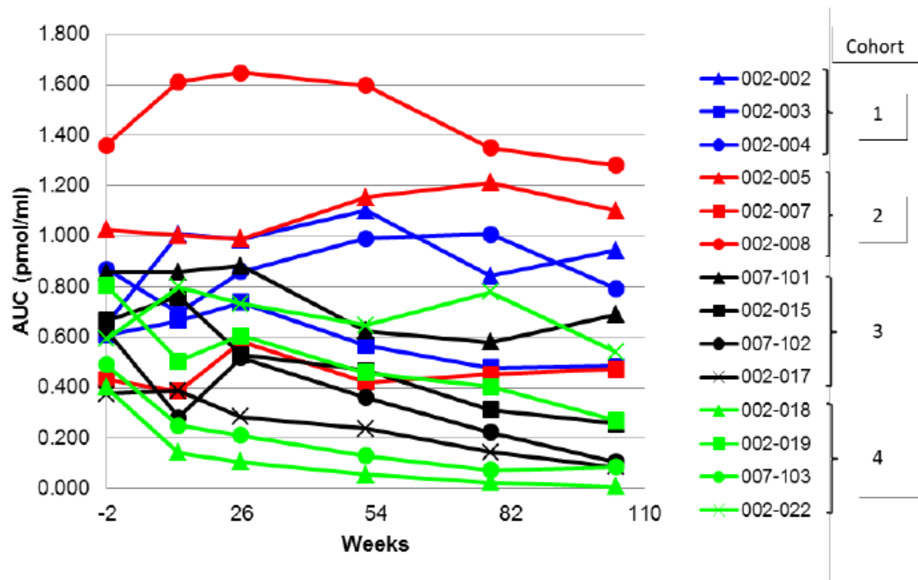


Figure 12, MMTT-stimulated 4 hour C-peptide AUC

C-peptide AUC is reported for fasting 4-hour mixed meal tolerance test without carbohydrate restriction for 3 days preceding test. The target glucose level at the start of the test was between 70 and 200 mg/dL. The baseline blood samples (-10 minutes and 0 minutes) were drawn, and then subjects drank Boost High Protein Nutritional Energy Drink® (Nestle Nutrition) at 6 kcal/kg (1 kcal/mL) to a maximum of 360 mL Blood was drawn at 15, 30, 60, 90, 120, 150, 180, 210, and 240 minutes following Boost dose. C-peptide AUC was calculated using the trapezoid rule. Shown are AUC results up to 2 years post infusion for cohort 1 (blue), cohort 2 (red), cohort 3 (black), and cohort 4 (green).

1.3.2.2 Prior Experience Using Polyclonal $CD4^{+}CD127^{lo/-}CD25^{+}$ Tregs in other disease settings

In addition to this preliminary experience with PolyTreg therapy in patients with TIDM, three other groups have performed trials in which Tregs were harvested from patients, expanded *ex vivo*, and then reinfused. The first is a small single-site study in cutaneous lupus being conducted at UCSF which has one active subject in the follow-up phase. To date, 2 of 3 cell products met release criteria for identity, purity, viability, and sterility. One product failed purity with unanticipated expression of CD8 on a small subset of CD4+FOXP3+ cells. This subset was pre-existing in this individual prior to expansion and the proportion was not markedly altered by the ex vivo expansion. This individual was initially lymphopenic at time of collection and expansion was sub-optimal at approximately 120-fold. The CD4lo/CD8hi final purity did not meet release criteria and the subject was discontinued prior to treatment.

As noted in Section 1.2, *Scientific Rationale*, patients undergoing stem cell transfer for leukemia have been treated with Tregs in an effort to prevent GvHD. One published trial reported no dose-limiting toxicities or increase in AEs in the year period following Treg infusion, when compared to historical controls. Incidences of severe acute GvHD were significantly reduced in patients who received Treg therapy, and for those developing GvHD, median time to onset of disease was prolonged. There was no increase in opportunistic infection or tumor relapse rates when compared to historical control patients undergoing the same regimen without Treg infusion [18]. Another study reported less GvHD, accelerated

immune reconstitution, reduced Cytomegalovirus (CMV) reactivation, and lower incidence of tumor relapse with robust graft vs. leukemia effect in patients who received polyclonal donor Treg therapy when compared to historical controls. These subjects received no adjunct immunosuppression for GvHD prevention [44]. In another trial, PolyTreg therapy was well-tolerated in children with recent-onset T1DM who had reduced insulin requirement relative to patients who did not receive PolyTregs [19]. These experiences provide a solid foundation for this trial.

1.4 Study Product Background

The study product under investigation in this trial consists of purified autologous PolyTregs that have been expanded *ex vivo* from the subject's own peripheral blood. The manufacturing facility, the cGMP process for isolation and expansion of the Tregs, and the final formulation and release testing are described in detail in Section 5, *Description of Study Product*.

1.5 Known and Potential Risks and Benefits of Study Product

1.5.1 Known and Potential Benefits of Study Product

The potential benefit is that treatment with autologous PolyTregs might provide a means to reduce the symptoms and long-term complications of pemphigus without requiring chronic immune suppression. This goal is important, because current approaches to the treatment of pemphigus are incompletely effective and often cause adverse effects that can be severe and, occasionally, fatal. If this treatment strategy is effective, it has the potential to restore self-tolerance and spare patients the risks of chronic immunosuppressive therapy.

1.5.2 Known and Potential Risks of Study Product/Treatment

Therapy with autologous PolyTregs carries the potential risk that the expanded PolyTregs might be contaminated with Teffs that could conceivably cause exacerbation rather than suppression of disease. This protocol describes a detailed set of preliminary experiments that provides reassurance that the transferred cells will be suppressive, not stimulatory, in effect (Section 1.3.1, *Pre-Clinical Studies*).

Safety, especially with regard to protection against infection, is a paramount concern in studies of novel approaches to immunotherapy. Although prior studies have been encouraging about the safety of infused PolyTregs, the data are still quite limited. This trial has been designed to proceed slowly and carefully through the dose-escalation process in order to limit the risk to study subjects.

1.5.2.1 Infection

As with any therapy that suppresses the immune system, there is a risk of developing infections. It should be noted that on a theoretical basis, this risk is minimal, since the total input of Tregs is far below the resident population.

1.5.2.2 T-Cell Suppression

Tregs are known to suppress naïve T cell responses to a variety of antigens. Less is known about ongoing immune responses especially to viruses and bacteria. It is not known whether Tregs will alter protective immunity.

1.5.2.3 Infusion Reaction

Side effects reported from previous human trials involving T cell infusions include transient fever, chills, and/or nausea. Appropriate pre-medications (see Section 5.3.1, *Preparation for Administration*) have mitigated the effects of these reactions. Infusion reactions have been limited. In the previous T1DM study, only four AEs in four subjects were reported, in the 24 hours following infusion of PolyTregs. Two were mild headache, one was mild nausea and one was mild abdominal pain.

With any T cell therapy there is a theoretical concern for cytokine release syndrome (CRS). However, to date, there have been no documented cases.

1.5.2.4 Lymphoproliferative Disease

Treg immunosuppression has been shown to enhance growth of already present tumors in some small animal model systems. Thus, complications such as lymphoproliferative disease are possible on a theoretical basis. Clinical experience in transplant recipients suggests that the risk of lymphoproliferative disease is highest in those who develop a primary Epstein-Barr virus (EBV) infection while immunosuppressed. Thus individuals without prior exposure to EBV are excluded from this study. In previously exposed individuals, EBV reactivation is associated with a degree of immunosuppression higher than that likely to be observed in this study since the total input of Tregs is far below the resident population. Nonetheless, careful attention will be paid to the potential for this complication.

1.5.2.5 Loss of Tumor Surveillance

T lymphocytes are one major component of tumor surveillance and it is possible that cells that inhibit T lymphocytes could impair this function. There has not been evidence of *de novo* development of tumors in preclinical models. No issues have been noted in previous clinical trial experience, which includes subjects that have been followed for five years, as of the time of this submission.

1.5.2.6 Reproductive risks

There may be an unexpected risk to an unborn or nursing child as described in Section 5.6.3, *Reproductive Risks*.

1.5.2.7 Worsening Disease

It is theoretically possible that some of the PolyTreg cells could convert to Teffs, which could increase pemphigus disease activity. No issues have been noted in previous clinical trial experience in other autoimmune indications, which includes subjects having been followed for five years, at of the time of this submission.

1.5.2.8 Procedure-related risks

In addition to the risk that might be associated with PolyTreg infusion, study subjects also will be exposed to the risks of multiple skin biopsies. In general, the risks of skin biopsy are similar to blood draw or any other procedure that punctures the skin. Discreet, hidden sites are chosen whenever possible, and infectious complications are very rare even in immunocompromised hosts.

Other potential risks of participation in this study are those associated with study-related procedures including blood draws and intravenous access.

1.6 Rationale for Study

1.6.1 Rationale for the Phase I Design

This phase I dose-escalation study design exposes only small numbers of subjects to the cell infusions, separated by defined intervals. The built-in delays in enrollment avoid exposing multiple subjects simultaneously as potential toxicities are assessed in a step-by-step fashion. The cohort size is chosen to allow detection of high frequency Serious Adverse Events (SAE) at a given dose. The proposed inclusion of two cohorts ensures that the total safety experience will include a large enough number of subjects exposed over the two doses in order to support the optimal design for a phase II trial.

1.6.2 Rationale for the Selection of Doses

Two dosing groups will be employed in this trial: 1 x 10^8 and 2.5 x 10^8 polyclonal Tregs. The lower dose will aid in detection of biomarkers and labeling of cells, and also allow us to compare Treg dosing to dosing regimens used in other Treg trials.

The dose of PolyTregs is selected based on a combination of practical considerations of manufacturing capacity, expected Treg numbers in pemphigus patients, the available safety data and a predicted efficacious dose of the PolyTreg product currently in clinical trials for the treatment of T1DM and cutaneous lupus/SLE.

In previous versions of this protocol, we chose to forego the low-dose cohort that had been included in the ALE08 Protocol (1.0 x 10^8) based on (i) increasing evidence in other trials that a higher dose would be safe, and (ii) the assumption that a higher dose might be more likely to demonstrate the desired biologic affect (successful trafficking to affected skin). However new data is now available from mechanistic studies in one SLE patient [54] enrolled in the ALE08 Protocol and in the first pemphigus patient enrolled in APG01 Protocol. The data indicates that a lower cell dose is sufficient to observe an impact on skin inflammation (Figure 13) [54].

At the same time, we have encountered challenges in the low yield of PolyTregs that appear to be pemphigus-specific. For these reasons, we believe that the feasibility of the study will be enhanced by reducing the target number of the PolyTreg to be infused without compromising our ability to demonstrate the desired biologic activity. The choice of PolyTregs dose in earlier cases of SLE, pemphigus, and other diseases was somewhat arbitrary, based in large part on what was technically feasible. We now have evidence that the use of a lower dose is probably sufficient for the proof-of-concept purpose of this study, and this change is very likely to enhance feasibility. An inclusion criterion for absolute Treg count at screening has also been added to increase the likelihood of meeting the target dose.

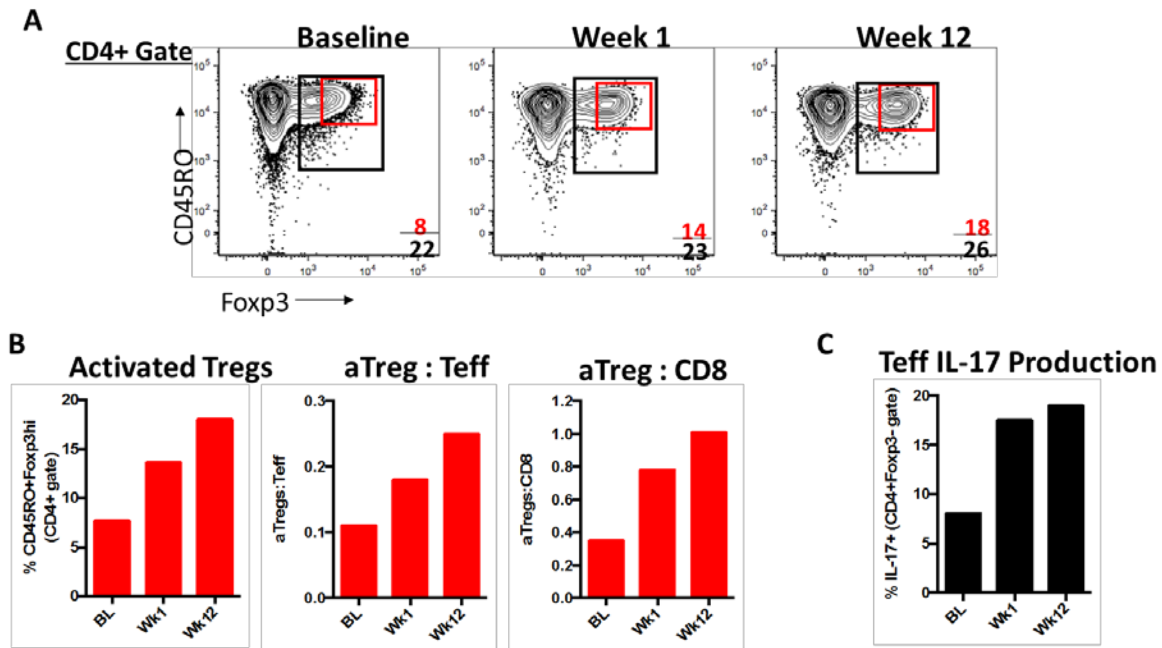


Figure 13, Activated Tregs Accumulated in Skin with Time After Adoptive Transfer for the first subject on APG01

Treg adoptive transfer resulted in a 2-fold increase in activated Tregs in skin from baseline to week 12 (A and B), and increased aTreg to Teff and aTreg to CD8 ratios (B). There was also increased IL-17 production from Teffs after Treg adoptive transfer (C). These are similar to the results observed in our SLE patient.

1.6.2.1 Dosing Estimate Based on Rodent Models

The effective dose of Tregs for controlling autoimmunity in humans is currently unknown. Data from animal models demonstrate that a high percentage of Tregs, 600% of that found in the steady state, is needed to prevent transplant rejection and autoimmunity in a non-manipulated setting. This is likely due to the very high frequency of autoreactive conventional T cells present in these settings. Thus, a significant number of Tregs is likely to be needed for efficacy. These considerations support the idea of maximizing Treg dosing to the extent permitted by manufacturing capacity and available safety data when Tregs are used in the setting of appropriate immune suppression.

1.6.2.2 Manufacturing Capacity

In the phase I Treg study in T1DM conducted under IND 14462, UCSF has shown that 2.6 x 10^9 expanded Tregs can be produced for infusion in the majority of patients. In recent trials we have focused on a maximum dose of 10 x 10^8 for feasibility reasons and in order to compare between disease cohorts.

1.6.2.3 Safety of T Cell Therapy

The standard dose of 10 x 10^9 cells per infusion of non-Treg autologous polyclonal T cells has been tested and found to be safe in hundreds of subjects to date [55].

The highest dose tested for safety in the phase I Treg study in T1DM conducted under IND 14462 was 2.6 x 10^9 (range 2.35-2.94 x 10^9). Adverse events in the trial have generally been mild or moderate in severity as described in Section 1.3.2.1, *Prior Experience Using Polyclonal CD4+CD127lo/-CD25+ Tregs in T1DM.*

Based on the information summarized above, it is estimated that hundreds of millions to billions of PolyTregs will be needed to control pemphigus. Because the safety profile of PolyTreg therapy in this patient population has not been established, APG01 will begin with a dose of 1.0 x 10^8 cells to determine its safety and impact on Treg trafficking to the skin.

In the above T1DM study, the minimum interval between subject doses was 6 days and serious adverse events reported were assessed to be 'unlikely' or 'unrelated' to study therapy. Based on this data, the APG01 protocol will require the previous subject to reach Week 2 prior to initiating the next subject's blood donation.

1.6.3 Rationale for the Selection of the Patient Population

This phase I trial is being conducted in subjects with pemphigus for two principal reasons. First, the selection of this population allows us to examine the impact of PolyTreg therapy not only in the peripheral blood, but also in the skin in patients with pemphigus. Mild to moderately active disease is being targeted in this phase I safety study, with a PDAI severity score of 3-10 being required for screening (with activity score no higher than 12 at time of PolyTreg infusion) [56, 57]. Second, cutaneous manifestations of pemphigus can be severe, refractory, and life-threatening, with significant insensible losses and impaired barrier function leaving patients susceptible to serious infections. Therefore, this patient population warrants examination of a novel therapy that would be of utility if the treatment proves to have a favorable risk:benefit ratio.

1.6.4 Rationale for the Mechanistic Studies to be Performed on Skin Biopsies

Skin biopsies will enable the determination of whether the infused PolyTregs migrate to active lesions in an affected organ, whether they persist and/or proliferate there, whether they maintain their function, and how they affect local mechanisms that contribute to immunopathology. Three (4 mm) punch samples are needed at each time point to allow: (1) tracking of deuterium $^2H_2$-labeled Tregs and Treg clonality *via* T Cell Receptor (TCR) sequencing; (2) whole transcriptome RNA sequencing to determine the effects PolyTreg infusion on inflammatory and immunoregulatory pathways in the target tissue; and (3) flow cytometric quantification of inflammatory cell subsets and immunologic biomarkers in skin, allowing comparison to blood. These mechanistic studies will be critical to the understanding of the biologic effects of PolyTreg therapy.

2 STUDY OBJECTIVES AND PURPOSE

The objective of this trial is to determine the safety profile of PolyTregs in subjects with pemphigus.

2.1 Primary Objective

- To evaluate significant events that occur when using PolyTregs and establish the dose recommended for further clinical investigation in subjects with pemphigus

A significant event will be defined as a National Cancer Institute – Common Terminology Criteria for Adverse Events (NCI-CTCAE) Grade 3 or higher AE which is related (possibly related or related) to study drug or any SAE which is related to study drug.

2.2 Secondary Objectives

- To characterize the safety of PolyTregs in subjects with pemphigus
- To provide preliminary data addressing the efficacy of PolyTregs
- To demonstrate the presence of and assess the persistence and function of transferred deuterium-labeled Tregs in peripheral blood and skin
- To assess disease specific and immunologic biomarkers in peripheral blood and skin
- To determine whether the PolyTregs proliferate in blood and/or skin

3 STUDY DESIGN

3.1 Description of Study Design

This phase I trial will be conducted as an open-label, dose-escalation, multicenter trial in adult patients with active pemphigus. We expect approximately four clinical sites to participate (UCSF and approximately 3 remote sites). The study will employ a sequential dose-escalation design with a maximum of twelve subjects receiving a single infusion of *ex vivo* selected and expanded autologous PolyTregs. The primary study endpoint is the number of significant events, defined as any related NCI-CTCAE Grade 3 or higher AE or any related SAE. Related is defined as being possibly related or related to the *ex vivo* expanded autologous PolyTregs, as determined by the safety review committee (SRC, consisting of the protocol chair, representatives from the DAIT-Statistical and Clinical Coordinating Center [DAIT-SACCC], and representatives of DAIT) that will review the laboratory data and clinical experience of all subjects in the cohort. Pemphigus relapses/flares will be assessed using the consensus definition of 3 or more new lesions a month that do not heal spontaneously within 1 week or by the extension of established lesions in a patient who has achieved disease control [58]; however, only flare components assessed as AEs of NCI-CTCAE Grade 3 or higher or SAEs will contribute to the evaluation of significant events.

Table 3.1 Dose cohorts for the Study

Cohort	**Subjects #**	**Target Cell Dose**	**Allowable Dose Range** (-30% to +20%)	**Volume (ml)**	**Concentration (cells/ml)**	**Allowable Concentration Range** (-30% to +20%)
1	4-6	1.0×10^8	0.7×10^8 to 1.2×10^8	100	1.0×10^6	0.7×10^6 to 1.2×10^6
2	4-6	2.5×10^8	1.75×10^8 to 3.0×10^8	100	2.5×10^6	1.75×10^6 to 3.0×10^6

Two dosing groups will be employed in this trial: 1.0×10^8 and 2.5×10^8 PolyTregs. Each cohort will enroll 4-6 subjects with pemphigus. Each subject in cohorts 1 and 2 will receive an infusion and must reach Week 2 prior to the subsequent subject's blood donation. A schematic representation of an individual's participation is shown in Figure 1. The study dose can be escalated after the DSMB and SRC evaluates the safety data collected when the fourth subject in cohort 1 completes Week 8. After reviewing the data from the first four subjects, the DSMB and SRC will recommend adding additional subject(s) to the first cohort, advancing enrollment in the second cohort, or stopping the trial. A schematic representation the study's cohort design is shown in Figure 2.

Since a significant event in this trial can occur from toxicity from the study drug or disease worsening (e.g., a pemphigus flare), the standard 3 + 3 design rules for dose escalation will not be employed. Therefore, if a Grade 3 or higher AE or any SAE occurs in a subject during his/her first year of study participation, enrollment will be suspended until the SRC can convene to review the event. If this occurs, the SRC may decide at that time to proceed with enrollment, adjust the dose as described above, continue the suspended enrollment until the Data and Safety Monitoring Board (DSMB) can review the data, or place the trial on hold. The SRC will review all Grade 3 or higher AEs or any SAEs that occur throughout the study. However, since the likelihood of events being related to the investigational treatment diminishes over time, Grade 3 or higher AEs or any SAEs occurring during a subject's second or third year of the study will not result in an immediate suspension of enrollment, donation, and treatment, unless the SRC determines that such action is indicated. Provided the SRC can review the event within 5 business days of awareness, events judged to be significant will drive protocol management in terms of proceeding, suspending enrollment/treatment pending ad hoc DSMB input, or placing the study on hold pending further evaluation.

DSMB input will be required for the evaluation of some specific events. Description of these events and additional directives are outlined in Section 5.5.4, *Safety Stopping Guidance*.

Accrual of all subjects is expected to take approximately 1.5 years.

3.1.1 Management Plan for Out of Range PolyTreg Doses

For subjects in cohort 1, any Treg product with less than 0.7×10^8 PolyTregs will not be infused and subject will be replaced. In cohort 2, products falling short of the minimal defined PolyTreg dose may be infused for up to maximum dose of cohort 1 in up to 2 subjects (not to exceed 6 total subjects in cohort 1). The SRC will review available information after the first occurrence of the dose falling short of target range. Occurrence of a second product not meeting the specified dose will result in a comprehensive SRC review of the manufacturing issues prior to the trial proceeding.

If a higher dose of PolyTregs is attained than specified for a given cohort, the maximum defined dose for that cohort will be infused. Unused product will be stored for future unspecified research commensurate with subject informed consent.

3.1.2 Stratification, Randomization, and Blinding

This is an open-label, phase I trial. Randomization will not be performed and site staff will not be masked to the subject's dosage.

3.1.2.1 Subject Completion and Early Termination

A subject is considered to have completed the study if he/she has completed the Week 156 visit.

In order to better understand safety through the peri- and proximal post-Treg infusion period, subjects who withdrew for non-safety issues prior to Week 12 will be replaced.

3.2 Description of Primary Endpoint

3.2.1 Primary Safety Endpoint

The primary study endpoint is the number of significant events in each cohort through Week 52, defined as any related NCI-CTCAE Grade 3 or higher AE or any related SAE.

3.3 Description of Secondary Endpoints

3.3.1 Secondary Safety Endpoints

The secondary safety endpoints include the following:

- Number of significant events in each cohort through Week 156
- All AEs through Week 156
- All NCI-CTCAE Grade 3-5 AEs through Week 52
- All NCI-CTCAE Grade 3-5 AEs through Week 156
- All SAEs through Week 156
- All infection related events through Week 156
- All infusion reactions, defined as any adverse reaction of NCI-CTCAE Grade 1 and higher within 24 hours of infusion

3.3.2 Secondary Efficacy Endpoints

The secondary efficacy endpoints include the following:

- Change in pemphigus disease area index (PDAI) score from baseline to Weeks 1, 2, 8, 12, 26, 39, 52, 78, 104, 130, and 156
- Change in Desmoglein 1 and 3 titers by ELISA from baseline to Weeks 1, 2, 8, 12, 26, 39, 52, 78, 104, 130, and 156
- Time to relapse (flare)
- Number of participants on prednisone dose ≤10 mg/day at Weeks 12, 26, 39, 52, 78, 104, 130, and 156

3.4 Description of Exploratory Endpoints

3.4.1 Exploratory Safety Endpoints

- All pemphigus relapses/flares, as defined by consensus definition of 3 or more new lesions a month that do not heal spontaneously within 1 week or by the extension of established lesions in a patient who has achieved disease control through Week 156
- Absolute and change from baseline in clinical chemistry, hematology, and urinalysis after treatment

3.4.2 Exploratory Efficacy Endpoints

The exploratory efficacy endpoints include the following:

- Change in Skindex-29 scores (symptoms domain, emotions domain, functioning domain, overall score) from baseline to Weeks 1, 2, 8, 12, 26, 39, 52, 78, 104, 130, and 156
- Change in patient global assessment (PGA) from baseline to Weeks 1, 2, 8, 12, 26, 39, 52, 78, 104, 130, and 156
- Change in physician global assessment (PhGA) from baseline to Weeks 1, 2, 8, 12, 26, 39, 52, 78, 104, 130, and 156
- Number of participants on prednisone dose 0 mg/day at Weeks 12, 26, 39, 52, 78, 104, 130, and 156
- Number of participants requiring an increase in prednisone dose relative to last recorded prednisone dose at any time prior to Weeks 12, 26, 39, 52, 78, 104, 130, and 156

3.4.3 Exploratory Mechanistic Endpoints

The exploratory mechanistic endpoints include the following:

- Determine the relative frequency of adoptively transferred Tregs in blood and skin by quantifying transferred deuterium-labeled Tregs in peripheral blood and skin from Week 1 to 12. In addition, TCR sequencing may be performed on sorted Tregs from either skin or blood.
- Perform whole transcriptome RNA sequencing to determine the effects of PolyTreg infusion on inflammatory and immunoregulatory pathways in the target tissue (skin)
- Quantify inflammatory cell subsets and immunologic biomarkers in blood and skin, including changes in lymphocyte subsets, cytokine profile, and autoantibody production
- Changes in disease specific and immunologic biomarkers in peripheral blood including lymphocyte subsets, cytokine profiles, polyclonal and antigen-specific Treg function (flow cytometry with MHC II-tetramer staining), and autoantibody production from baseline to Weeks 1, 2, 8, and 12

- Changes in disease specific and immunologic biomarkers in skin including lymphocyte subsets, polyclonal and antigen-specific Treg function (flow cytometry with MHC II-tetramer staining), and cytokine profiles from baseline to Weeks 1 and 12

4 SELECTION OF SUBJECTS

Subjects with pemphigus must grant written informed consent prior to the subject undergoing any study-related procedure, including screening tests and medication adjustments, when applicable.

4.1 Inclusion Criteria

Subjects who meet all of the following criteria are eligible for enrollment into the study:

1. Ability to provide informed consent
2. Age 18-75 years at Screening Visit
3. Diagnosis of PV or PF, defined by H&E and direct immunofluorescence staining of skin biopsy at any time prior to enrollment
4. Pemphigus treated with systemic corticosteroids within the 2 years prior to screening (historic or current) or treated with rituximab at least 12 months prior.
5. Presence of:
    a. anti-Dsg3 antibodies (>20.0 U/ml) at screening visit consistent with diagnosis of pemphigus vulgaris, or
    b. anti-Dsg1 antibodies (>20.0 U/ml) at screening visit consistent with diagnosis of pemphigus foliaceus
6. Active PV or PF as defined by PDAI overall activity score 3-10 at screening visit, and PDAI overall activity score 1-12 at baseline visit
7. Positive test for EBV antibody
8. Adequate venous access to support draw of 400 ml whole blood and infusion of investigational therapy
9. An absolute Treg count of ≥42 cells/µL within 6 weeks prior to whole blood collection at Week -2

4.2 Exclusion Criteria

Subjects who meet any of the following criteria are ineligible to participate in the study:

1. Initiation of systemic corticosteroid therapy, prednisone dose > 25 mg/d (or equivalent) or change in prednisone dose within 4 weeks prior to screening
2. Addition of a new medication, or change in the dose of any background medication used to treat any aspect of pemphigus within the timeframes listed below. Specifically:
    a. methotrexate, mycophenolate mofetil, mycophenolic acid, azathioprine, cyclosporine or dapsone within the 6 weeks prior to screening or in the time between screening and study drug infusion

   b. IVIG within 12 weeks prior to screening or in the time between screening and study drug infusion (subjects on IVIG must be on stable dose for at least 12 weeks prior to screening)
   c. treatment with cyclophosphamide within 12 weeks prior to screening or in the time between screening and study drug infusion

3. Doses of background medications at screening:
   a. methotrexate > 25 mg/week
   b. mycophenolate mofetil > 3000 mg/d
   c. mycophenolic acid > 1080 mg/bid
   d. azathioprine > 200 mg/d
   e. cyclosporine > 2 mg/kg/d
   f. dapsone >250 mg/d
   g. IVIG > 4mg/kg IV monthly
4. Use of rituximab within the 12 months prior to screening
5. Change in dosing frequency, concentration, or applied surface area of topical steroids and/or topical calcineurin inhibitors within 2 weeks prior to screening
6. Paraneoplastic pemphigus
7. Pemphigus erythematosus
8. Pemphigus vegetans
9. IgA pemphigus
10. Drug-induced pemphigus
11. Blood donation within 10 weeks prior to baseline visit (Day 0)
12. Hemoglobin < 10 g/dl
13. White blood cell (WBC) count < 3,000/ mm^3 (equivalent to < 3 x10^9/L)
14. Absolute lymphocyte count < 800/mm^3 (equivalent to < 0.8 x10^9/L)
15. Absolute neutrophil count < 1,500/mm^3 (equivalent to < 1.5 x10^9/L)
16. Platelets < 100,000/mm^3 (equivalent to < 100 x 10^9/L)
17. Liver function test (aspartate aminotransferase [AST], alanine aminotransferase [ALT], or alkaline phosphatase [ALK]) results that are ≥ 2 times the upper limit of normal (ULN)
18. Direct bilirubin > ULN
19. End stage renal disease (estimated glomerular filtration rate [eGFR] < 20 ml/min/1.73m^2 using the CKD-EPI equation [59])
20. At or within three months of screening:
   a. positive QuantiFERON®-TB Gold test or positive purified protein derivative tuberculin skin test (PPD) (>5mm induration, regardless of Bacille Calmette Guerin [BCG] vaccine administration) unless completion of treatment has been documented for active TB
   b. an indeterminate QuantiFERON®-TB Gold test unless followed by a subsequent negative PPD or negative QuantiFERON®-TB Gold test as well

as a consultation with and clearance by local infectious disease (ID) department

21. Recent or ongoing active bacterial, viral, fungal, or opportunistic infections requiring systemic anti-infective therapy
22. Evidence of current or prior infection with human immunodeficiency virus (HIV), hepatitis B (as assessed by HBsAg and anti-HBc Ab) or hepatitis C (as assessed by anti- HCV Ab)
23. Detectable circulating EBV or CMV genomes or active infection
24. Chronic infection that is currently being treated with suppressive anti-infective therapy, including but not limited to tuberculosis, pneumocystis, CMV, herpes zoster, and atypical mycobacteria, with the exception of historical orolabial or localized cutaneous herpes simplex infections treated with suppressive anti-viral therapy
25. Receipt of a live-attenuated vaccine within 12 months prior to screening
26. Concomitant malignancies or a history of malignancy, with the exception of completely treated basal cell carcinoma of the skin
27. Pregnancy
28. Lactating or breastfeeding
29. Unwilling or unable to use reliable method(s) of contraception (as described in Section 5.6.3, *Reproductive Risks*)
    a. For females of child-bearing potential, from four weeks prior to Day 0 through 1 year after Treg dosing
    b. For males, from the day of Treg infusion (baseline visit) to three months after Treg infusion
30. Use of an investigational therapeutic medication, or other biologic medications except rituximab, within the past 90 days, or 5 half-lives prior to screening, whichever is greater
31. Concomitant medical condition that places the subject at risk by participating in this study, including but not limited to:
    a. another severe, systemic autoimmune disease or condition (besides pemphigus) requiring systemic immunosuppressive therapy (e.g., rheumatoid arthritis, SLE, systemic sclerosis, primary Sjorgen's syndrome, primary vasculitis, psoriasis, multiple sclerosis, ankylosing spondylitis, and inflammatory bowel disease), or
    b. severe, progressive, or poorly controlled renal, hepatic, hematological, gastrointestinal, pulmonary, cardiac, or neurological disease, or
    c. history of significant infection or recurrent infection that, in the investigator's opinion, places the subject at risk by participating in this study, or
    d. any other concomitant medical condition that, in the investigator's opinion, places the subject at risk by participating in this study
32. Comorbidities requiring glucocorticoid therapy, including those which have required three or more courses of systemic glucocorticoids within the previous 12 months
33. Current or history within the past year of substance abuse
34. Inability to comply with study and follow-up procedures

4.2.1 Co-enrollment Guidelines

Subject may be in observational registries or cohorts as long as the combined blood draw totals do not exceed the limits of the local institutional review boards and objectives do not confound the current study.

5 DESCRIPTION OF STUDY PRODUCT

5.1.1 Product Description

The name of the drug product is: *Ex Vivo* Expanded Autologous $CD4^{+}CD127^{lo/-}CD25^{+}$ Polyclonal Regulatory T Cells (PolyTregs). PolyTregs are cells that have been collected from the donor by phlebotomy, purified by Ficoll density gradient, and subsequently selected for the target $CD4^{+}CD127^{lo/-}CD25^{+}$ T cell subset by FACS, using three monoclonal antibodies specific for the cell surface antigens CD4, CD127, and CD25. The isolated cells are then expanded using anti-CD3/anti-CD28-coated magnetic microbeads in cell culture medium containing deuterated glucose ([6,6-$^{2}H_2$]-Glucose) (see Section 6.5.1 for more information on deuterium labeling) for a total of 14 days before harvesting. Following expansion, anti-CD3/anti-CD28 beads are removed via a magnetic separation system, and cell cultures are combined, washed and adjusted to desired concentration.

The $CD4^{+}CD127^{lo/-}CD25^{+}$ PolyTreg product is a sterile cell suspension of fresh, non-cryopreserved cells in a mixture of 75% FDA approved infusion solution conforming to USP standards (49.02% (v/v) Plasma-Lyte A, 49.02% (v/v), Dextrose 5% with 0.45% NaCl, and 1.96% (v/v)) and 25% Human Serum Albumin (HSA). The final product is administered via IV over a 10-30 minute period by gravity.

The product will be manufactured at UCSF Human Islet and Cellular Transplantation Facility (HICTF) and GMP Facility (San Francisco, CA).

***Ex Vivo* Expansion of Human Autologous PolyTregs**

Study subjects will provide up to a target of 400 ml of whole blood via phlebotomy to obtain an adequate number of cells for product manufacture. Whole blood will be transported to the manufacturing facility for processing. Previous studies [60] have shown that this process can be conducted successfully using cells obtained locally or at other sites that ship to UCSF.

PBMC will be isolated by Ficoll gradient density centrifugation. The pooled lymphocytes will be stained with clinical-grade fluorescently conjugated antibodies and sorted for $CD4^{+}CD127^{lo/-}CD25^{+}$ T cells using a commercial cell sorter. Tregs will be expanded per standard operating procedures adapted from optimized methods previously described [23]. This method uses co-stimulation of the Tregs with anti-CD3 and anti-CD28 immobilized on magnetic beads. Enriched Tregs will be cultured in growth medium containing human serum and deuterated glucose and stimulated to proliferate over a 14-day period in the presence of IL-2 and above-mentioned beads. Cultures will be maintained in sterile culture vessels or bags in a humidified 37°C 5% CO_2 incubator throughout the culture period. Cells will be counted every 2 to 3 days and transferred to vessels of increasing size to maintain appropriate cell density as cells proliferate, and fresh medium and IL-2 will be added at each cell passage. Cells will be restimulated with additional anti-CD3/anti-CD28 beads at day 9 of the culture period. Cultures will be tested for presence of bacteria, fungus, mycoplasma, and

endotoxin at several interim time points during the expansion period. Following expansion, the beads will be removed via a magnetic separation and the cells will be concentrated, consolidated, and resuspended in sterile infusion solution at the required concentration.

The investigational product will be quarantine refrigerated and will be released with an Interim CoA. The final CoA will be provided once all sterility test results are available.

Studies will be run on the final product for infusion to identify the dominant Treg clones.

Results from bacterial and fungal testing of the final product will be available 14 days and 4 weeks post-infusion, respectively.

5.1.2 Packaging and Labeling of Study Product

The Tregs product will be manufactured for subjects at UCSF and the remote sites. It will be shipped to the remote clinical sites via next-day service using validated conditions and containers. Qualified staff at the remote sites will receive the PolyTregs, verify the recorded shipment temperatures were maintained within the validated range, and assess viability and recovery of the product according to UCSF established SOPs.

PolyTregs will be supplied fresh, in sterile 150 ml infusion bags. Label information will be consistent with applicable regulations. All information on the completed label will be verified as accurate by at least two individuals prior to transport to the infusion site.

5.1.3 Storage and Handling of Study Product

The Treg product is delivered to the bedside in a temperature controlled container (2-10°C) and is removed from the container just before being administered to the subject. The infusion of the product must occur before expiration time as indicated on the CoA.

Unused product will be disposed in accordance with established standard operating procedures or placed in archive if authorized by the subjects on the informed consent form.

5.2 Dosage Regimen

A single infusion of 1.0×10^8 or 2.5×10^8 PolyTregs will be administered.

5.3 Administration of Study Product

5.3.1 Preparation for Administration

On the day of scheduled infusion, blood chemistries, a complete blood count (CBC) with differential blood count, and a pregnancy test (for women of childbearing potential) will be obtained locally and reviewed prior to infusion of the PolyTregs. A pre-infusion checklist (see Section 15.6, *Pre-Infusion Required Checklist*) will be used to review relevant clinical and laboratory data prior to infusion. Cell infusion may be held based on the pre-infusion checklist at the discretion of the site investigator. A history of any recent illness or fever will be obtained. The subject should be both asymptomatic and the illness should be resolved before infusion. See Section 5.3.4 for additional criteria for withholding study treatment.

Subjects will be pre-medicated with acetaminophen 650 mg by mouth and diphenhydramine hydrochloride 25-50 mg by mouth or intravenous (IV), approximately 30 minutes prior to the infusion of Tregs. These medications may be repeated every six hours as needed. A course of non-steroidal anti-inflammatory (NSAID) medication may be prescribed if the patient exhibits a fever not relieved by acetaminophen. Patients will not receive systemic glucocorticoids higher than their usual dose such as hydrocortisone, prednisone, prednisolone (Solu-Medrol) or dexamethasone (Decadron).

Prior to administration, the identity of the subject and of the lot are verified by two individuals at the clinical site.

5.3.2 Administration

After double verification of cell therapy product and recipient identification, the cells will be infused via a peripheral IV line with an 18-gauge needle primed with saline per established standard operating procedures. All doses will be administered by peripheral IV line via gravity in approximately 20 to 30 minutes. Following administration of the PolyTregs, the product bag, tubing, and peripheral IV line will be flushed with normal saline to ensure complete dose is infused.

Vital signs (temperature, respiratory rate, pulse, and blood pressure) will be taken immediately prior, midway, and at completion of the infusion, and then every 15 minutes (+/- 3 minutes) for at least one hour and longer at this interval as indicated to confirm vital signs are stable. From then on, vital signs will be monitored approximately every hour until four hours post-infusion and approximately every four hours thereafter until discharge.

The IV catheter will be maintained after the infusion until discharge and the subject will remain in the clinical research unit for 24 hours. The subject will be contacted by telephone daily for the next 2 days to assess condition and AEs.

If a study subject experiences symptoms suggestive of an infusion reaction during administration, study treatment will be slowed or temporarily stopped. The subject will be monitored until symptoms resolve. If symptoms can be resolved with acetaminophen, diphenhydramine, or other medication (other than glucocorticoids) at the discretion of the investigator, the infusion may be restarted at the original rate. (Section 5.5.1 *Criteria for Interruption of Study Treatment*)

Emergency medical equipment (i.e., emergency crash code cart) will be made available for the infusion in case the subject has an allergic response, severe hypotensive crisis, or any other reaction to the infusion.

5.3.3 Action Plan for Post Release Positive Sterility Results

The results of aerobic bacterial culture, anaerobic bacterial culture, and fungal culture on the final product will not be available prior to product release. In the event of a post-release bacterial or fungal positive result, the finding will be reported to the clinical investigator, institutional review board (IRB), and FDA and a temporary suspension in enrollment will be in effect until a cause is identified and remedied, if required (see Section 5.5.4, *Safety Stopping Guidance*).

The clinical team (site PI[s]) will consult with local infectious disease specialists, and anti-microbial therapy will be considered for the subject as deemed appropriate by the clinical team. The cause of the sterility failure will be investigated and necessary corrective actions will be implemented. The sterility failure, results of investigation of the cause, and corrective actions will be reported to the FDA within 30 calendar days of the receipt of the positive culture test result.

5.3.4 Criteria for Withholding Study Treatment

Infusion of study product is to be withheld if the subject is experiencing any of the following on the day of infusion:

- Fever > 38.0°C
- Acute Diarrhea and/or Vomiting
- Other signs/symptoms of an intercurrent NCI-CTCAE Grade 2 or higher infection.
- Significant pemphigus relapse/flare (PDAI >12)
- Other elements precluding infusion as per the study pre-infusion checklist (see Section 15.6, *Pre-Infusion Required Checklist*)
- Other comorbid conditions which in the opinion of the investigator would pose risk to the subject with administration of the study product

5.4 Repeat Screening When Infusion is Not Given as Scheduled

If the participant completes the Week -2 visit (400ml blood donation) and does not receive the infusion on Day 0 as scheduled, the participant may be eligible to return to repeat screening no sooner than 8 weeks following the preceding blood donation. If the repeat Week -2 visit (blood donation) is scheduled:

- Less than or equal to 12 weeks after initial blood donation, only Hematology and whole blood quantitative PCR for EBV/CMV viral load must be repeated, with other screening tests repeated as clinically indicated
- Greater than 12 weeks after initial blood donation, all screening assessments must be repeated

5.5 Treatment Interruption and Discontinuation

Treatment will consist of a single infusion of *ex vivo* expanded PolyTregs. Once a PolyTreg infusion has concluded, the treatment phase of the protocol is complete. Thus, the question of subsequent withdrawal from treatment will not arise. Therefore, all subjects who undergo an infusion will continue per protocol unless they choose to withdraw (see Section 5.5.3, *Subject Withdrawal from the Study*).

5.5.1 Criteria for Interruption of Study Treatment

If a study subject experiences symptoms suggestive of an infusion reaction during administration, study treatment will be slowed or temporarily stopped. The subject will be monitored until symptoms resolve. If symptoms can be resolved with acetaminophen, diphenhydramine, or other medication (other than glucocorticoids) at the discretion of the investigator, the infusion may be restarted at the original rate, provided none of the criteria in Section 5.5.2 *Criteria for Permanent Discontinuation of Study Treatment* have occurred.

5.5.2 Criteria for Permanent Discontinuation of Study Treatment

Study treatment will be discontinued permanently for any individual subject under the following conditions:

1. At any time prior to or during the PolyTreg infusion at the request of the subject
2. If investigators or NIAID determine that the subject's health, safety, and/or well-being are threatened
3. If an infusion reaction symptom that caused a slow or temporary stop of the infusion cannot be resolved with acetaminophen or diphenhydramine
4. If administration of glucocorticoids to treat an infusion reaction is required
5. If a subject experiences any of the following:
    a. NCI-CTCAE Grade 3 or higher infusion-related reaction
    b. NCI-CTCAE Grade 3 or higher cytokine release syndrome
    c. Any other infusion related SAE

Subjects who discontinue protocol-specified treatment will be treated as medically indicated according to physician discretion. Refer to Section 5.5.3.1, *Procedures for Subject Withdrawal from the Study.*

5.5.2.1 Procedures for Discontinuation of Protocol-Specified Treatment Requirements

Whenever possible, subjects who received an abbreviated infusion that was terminated due to a possible infusion reaction should complete all remaining scheduled study visits including all exams, procedures, assessments, and tests. Furthermore, if discontinuation is due to safety concerns, subjects will be given appropriate care under medical supervision beyond the last scheduled study visit, if necessary, until the symptoms of any AE resolve or the subject's condition becomes stable. If the site PI determines that completion of these visits is not clinically appropriate for the subject or if the subject elects not to complete these visits, the subject will be withdrawn from the study per the guidelines in Section 5.5.3.1, *Procedures for Subject Withdrawal from the Study.*

5.5.3 Subject Withdrawal from the Study

When a subject is withdrawn from the study, protocol-specified treatment requirements are discontinued, and study-related visits, exams, procedures, assessments, tests, and data collection are terminated. Individual subjects will be withdrawn from the study under the following conditions:

1. The subject did not receive the planned infusion after two blood donations for PolyTreg manufacturing
2. The subject withdraws consent
3. The investigator or NIAID believes it is in the best interest of the subject
4. The subject is lost to follow-up

5.5.3.1 Procedures for Subject Withdrawal from the Study

Whenever possible, subjects who withdraw from the study will be asked to consent to an end-of-study evaluation, in which all scheduled exams, procedures, and laboratory tests

planned for Week 12 will be performed; unless the early withdrawal is after that time, in which case all scheduled exams, procedures, and laboratory tests scheduled for Week 52 will be performed; unless the early withdrawal is after that time, in which case all scheduled exams, procedures, and laboratory tests scheduled for Week 156 will be performed. Furthermore, if discontinuation is due to safety concerns, subjects will be given appropriate care under medical supervision beyond the last scheduled study visit, if necessary, until the symptoms of any AE resolve or the subject's condition becomes stable. After this end-of-study visit, the PI (or designated treating physician) may continue to follow the subject to manage clinical care, but no additional study-related data will be collected.

5.5.4 Safety Stopping Guidance

The SRC will suspend enrollment after a Grade 3 or higher AE or any SAE (as described in Section 3.1, *Description of Study Design)* in this trial in order to review the event, to review any additional events that occurred in the trial, and to make a decision on how to proceed. In the event of a temporary suspension in enrollment for a Grade 3 or higher AE or any SAE or a post release positive sterility result (see Section 5.3.3, *Action Plan for Post Release Positive Sterility Results*), no new subjects will be consented or started on therapy with PolyTregs; subjects who already received PolyTregs will continue to be followed. Subjects in the screening phase of the study may continue screening procedures (e.g., blood tests), but a skin biopsy to confirm Inclusion Criteria # 3 (see Section 4.1, *Inclusion Criteria*) should be deferred. Enrollment will not occur until the SRC review is complete.

A significant event in this trial can occur either due to toxicity from the study drug or disease worsening (e.g., a pemphigus relapse/flare) assessed as related to the investigational treatment.

The SRC will make this determination, seeking advice from the DSMB, if necessary. After SRC review, the study will: 1) proceed with enrollment as originally planned, 2) continue to suspend enrollment until the DSMB can review and advise on the event, or 3) be placed on hold pending further evaluation of the event, consideration of potential study modifications, and review by the DSMB, IRB and FDA.

For Grade 3 or higher AEs or any SAEs occurring in the first year of the study for each participant, DSMB input will be required for the evaluation of the following events unless the event was judged clearly unrelated to the investigational treatment:

- two Grade 3 or higher AEs or SAEs that occurs during the first 12 weeks of each participant in each cohort
- any Grade 4 or 5 AE
- two or more Grade 3 AEs of the same preferred term in a cohort
- any Grade 3 AE that is part of a pemphigus relapse/flare using the consensus definition of 3 or more new lesions a month that do not heal spontaneously within 1 week or by the extension of established lesions in a patient who has achieved disease control [58]

For Grade 3 or higher AEs or any SAEs occurring in the second or third years of the study for each participant, DSMB input will be required for the evaluation of the following events unless the event was judged clearly unrelated to the investigational treatment:

- any Grade 4 or 5 AE
- two or more Grade 3 AEs of the same preferred term in a cohort

In addition to the pre-scheduled data reviews and planned safety monitoring, the DSMB will review any event that potentially impacts safety at the request of the SRC or DAIT, NIAID. The DSMB will have the discretion to recommend actions regarding study conduct and continuation as a consequence of any planned or unplanned monitoring activity.

Otherwise, the DSMB will be informed in real time per the following criteria:

- During Year 1 of each subject's participation:
  - any decision of the SRC to add additional subjects based on confirmation of a significant event (as defined as any related Grade 3 or higher AEs or any SAEs)
  - any malignancy
- During Years 2 and 3 of each subject's participation:
  - any Grade 3 event that is part of a pemphigus relapse/flare using the consensus definition of 3 or more new lesions a month that do not heal spontaneously within 1 week or by the extension of established lesions in a patient who has achieved disease control [58]
  - any malignancy

5.6 Toxicity Management Plan for Study Product

See Section 5.5.2, *Criteria for Permanent Discontinuation of Study Treatment* for details on when to slow, temporarily discontinue, or permanently discontinue the study drug infusion for toxicities.

5.6.1 Infection

Based on the potential for Tregs to have immunosuppressive effects, subjects should be closely monitored for the development of signs and symptoms of infection, particularly opportunistic infections after treatment including reactivation of EBV and CMV.

5.6.1.1 Vaccination

Participants will be advised to obtain and will be offered tetanus, diphtheria, and pertussis (Tdap) and inactivated seasonal influenza vaccinations prior to treatment. Any such vaccination must be completed either prior to day -21, or after Week -2's whole blood donation and before day -7.

5.6.1.2 Herpes Simplex Virus

Infection with Herpes Simplex Virus (HSV), with predominance of mucocutaneous involvement, has been associated with pemphigus [61]. The protocol allows for participation of individuals receiving suppressive anti-viral treatment for orolabial or localized cutaneous herpes simplex infection (see Section 4.2, *Exclusion Criteria* #24). Individuals presenting

with oral erosions or ulcerations at screening will undergo viral culture to rule out active mucocutaneous HSV infection.

5.6.1.3 Epstein-Barr Virus

Assessment for the presence of EBV genomes will be carried out according to the schedule outlined in Table 6.1, *Schedule of Events*. Management will depend on the presence or absence of symptoms and on the viral load.

For those with no or NCI-CTCAE Grade 1 or 2 symptoms judged at least possibly related to EBV reactivation:

- If EBV viral load is undetectable, study proceeds as per Table 6.1, *Schedule of Events*
- If EBV viral load is detectable and < 2000 DNA copies per 10^6 PBMCs monitoring of EBV viral load may be increased as determined clinically necessary by the investigator
- If EBV viral load is ≥ 2000 DNA copies per 10^6 PBMCs monitoring of EBV viral load will be increased

For those with NCI-CTCAE Grade 3 or higher symptoms judged at least possibly related to EBV reactivation:

- If EBV viral load is undetectable, monitoring of EBV viral load will be increased as determined clinically necessary by the investigator
- If EBV viral load is detectable, monitoring of EBV viral load will be increased and appropriate treatment will be considered as determined clinically necessary by the investigator

5.6.1.4 Cytomegalovirus

Assessment for the presence of CMV genomes will be carried out according to the schedule outlined in Table 6.1, *Schedule of Events*. Management will depend on the presence or absence of symptoms and on the viral load.

For those who were seropositive at study entry:

- Subjects whose viral load increases 5-fold between scheduled assessments or is > 10,000 DNA IU/ml at any point during the study will be evaluated for CMV retinitis, pneumonia and gastroenteritis/colitis
- Participants with an active infection may be treated with gancyclovir as determined by the investigator. CMV viral load will be monitored by polymerase chain reaction (PCR) until it is < 10,000 DNA IU/ml

For those who were seronegative at study entry:

- Subjects whose viral load is > 2,000 DNA IU/ml will be evaluated for CMV retinitis as clinically indicated, as well as CMV pneumonia and gastroenteritis/colitis
- Participants with an active infection may be treated with gancyclovir as determined by the investigator. CMV viral load will be monitored by PCR until it is < 2,000 DNA IU/ml

For all subjects:

- If a participant develops any symptoms of the eye, lungs, or intestines consistent with CMV infection, additional blood will be drawn for appropriate laboratory analyses as determined by the investigator. Additionally, for those with visual symptoms, an ophthalmological exam will be performed.

5.6.2 Infusion Reaction

Section 5.3.1, *Preparation for Administration* describes the pre-medications required to help prevent infusion reactions and the medications that may be used to treat them.

Patients will not receive systemic glucocorticoids higher than their usual dose such as hydrocortisone, prednisone, prednisolone (Solu-Medrol) or dexamethasone (Decadron) at any time, except in the case of a severe or life-threatening emergency since this may have an adverse effect on T cells. If glucocorticoids are required for an acute infusion reaction, an initial dose of hydrocortisone 100 mg IV is suggested.

5.6.3 Reproductive Risks

There may be an unexpected risk to an unborn or nursing child. Pregnant and breastfeeding women will be excluded from participation in the study. Females must have a negative pregnancy test prior to enrolling in the study. Female subjects must agree not to participate in a conception process (e.g., active attempt to become pregnant or in vitro fertilization) and must agree to use a reliable and effective form of birth control (i.e., oral contraceptives, barrier methods, or abstinence) for one year after Treg dosing. Male subjects must agree to not participate in a conception process (e.g., active attempt to impregnate or sperm donation) and use birth control (i.e., oral contraceptives by female partners, barrier methods, or abstinence) for three months after Treg dosing. At every study visit the sexual activity of subjects of reproductive age will be re-assessed. If a subject who was previously sexually inactive becomes sexually active, s/he will be counseled about the need to use a reliable and effective form of birth control. Female subjects will also be required to undergo a urine pregnancy test prior to cell administration. A positive pregnancy test will result in holding of scheduled cell administration. Pregnancy tests will also be done at subsequent study visits for female subjects as described in Table 6.1, *Schedule of Events.*

Investigators of female subjects of childbearing potential on concurrent Mycophenolate Mofetil (MMF), and those subjects themselves, whether or not they plan to become pregnant, are strongly encouraged to participate in Mycophenolate Risk Evaluation and Mitigation Strategy (REMS), as described in Section 7.6.1, *Mycophenolate REMS Program.*

5.6.4 Malignancies

As the T lymphocytes are one major component of tumor surveillance it is theoretically possible that cells that inhibit T lymphocytes could impair this function. Therefore, the recommendations for individuals at higher risk for skin cancer apply to this research population and exposure to sunlight and UV light should be limited by wearing protective clothing and using a sunscreen with high protection factor. Refer to Section 5.7, *Concurrent Medication and Therapy.*

Subjects over the age of 50 should have age-appropriate cancer screenings at the investigator's discretion prior to enrollment and throughout the course of the study. For additional information, consult the American Cancer Society (ACS) reference below:

http://www.cancer.org/healthy/toolsandcalculators/reminders/screening-recommendations-by-age

5.6.5 Skin Biopsies

Three 4mm skin punch biopsies from affected skin will be collected under local anesthesia for proposed mechanistic studies. In addition, APG01 will minimize discomfort and scarring by numbing a single site and performing all three 4 mm punch biopsies in a contiguous fashion, with a single resultant incision, when possible. In cases where this is not technically feasible (e.g., lesion is too small), an alternative approach is to obtain a total of three punch biopsies from two or more separate sites; when possible, punches may still be placed in contiguous fashion to minimize the total number of individual, smaller incisions. In addition to the above biopsies for mechanistic studies, a single standard 4 mm punch biopsy will be obtained during screening for subjects without prior histopathologic confirmation of their pemphigus.

In all cases, the following prioritized list of considerations will inform the investigator's clinical judgment when selecting skin biopsy sites:

1. Wound environment. Investigators will avoid repeat biopsies directly through prior biopsy scars or biopsy of any areas that appear to be potentially secondarily infected.
2. Optimal cosmesis. Preference will be given to more cosmetically favorable areas (e.g., avoid central face when possible) with favorable skin mobility to facilitate the best possible closure.
3. Abundance and size of skin lesions. Preference will be given to more numerous and larger lesions so that biopsies may be obtained from the same body region.
4. Activity of lesions. Though the activity of lesions may change throughout study duration, preference will be given to active lesions at each time point.

Skin biopsy on Week 12 may be performed on unaffected skin if no lesional skin present.

- Please note topicals should be held from the planned biopsy location for the 7 days prior to each skin biopsy. See Section 5.7, *Concurrent Medications and Therapies* and 5.8, *Prohibited Medications* for specific information on use of topical agents prior to skin biopsies.

Skin biopsy sutures should be removed per local standards.

5.6.6 Digital Photography

Standardized total body photographs, regional photographs (e.g., torso, extremities, mouth) and close-up photographs of selected areas with active disease will be taken to monitor disease activity. The subject may decline photographs at any time during the study in instances where the subject is sensitive to being photographed. For additional instructions regarding Digital Photography, refer to the APG01 Manual of Procedures (MOP).

5.7 Concurrent Medications and Therapy

- If subjects are on prednisone at the Screening visit, subjects may continue to be treated with stable doses of prednisone not to exceed <25 mg/d until Week 12.
  - After Week 12, prednisone may be tapered at the discretion of the investigator, at a suggested rate of 5-10 mg decrease per month down to 10 mg/d, and then 1-5 mg decrease per month down to 0 mg/d.
  - Prednisone dose may be increased during the study for flares of pemphigus and for conditions that are not related to pemphigus based on the PI's clinical judgment. Increases in prednisone should occur only if absolutely necessary, and are discouraged for reasons other than pemphigus flares during the first 12 weeks of the trial. Stress doses of glucocorticoids may be used when clinically indicated.
- If subjects are on background immunosuppressive/immunomodulatory medications at the Screening visit, subjects should continue to be treated with stable doses of these agents until Week 12 including methotrexate ≤ 25 mg/week, mycophenolate mofetil ≤ 3000 mg/d, mycophenolic acid ≤ 1080 mg/bid, azathioprine ≤ 200 mg/d, cyclosporine ≤ 5 mg/kg/d, dapsone ≤ 250 mg/d and IVIG ≤ 4mg/kg monthly.
  - After Week 12, changes in the above background medications may be made per the investigator's judgment. Thus, one medication may be stopped and another medication started. The dose of any of the medications must not exceed those specified above at any point during the study period.
- If a subject is having increased pemphigus disease activity at any point during the study, they will be treated according to the best clinical judgment of the investigator.
- If subjects are on topical agents at the Screening visit, subjects must continue to be treated with stable amounts (stable percentage and approximate volume) of these agents, including steroids and calcineurin inhibitors, until Week 12. After Week 12, the amount of the topical medications may be altered per the investigator's judgment.
  - Of note, topical medications must be held from the planned biopsy location for one week prior to each scheduled skin biopsy. Topical medications must be held 7 days to skin biopsy on Day 0 and may be resumed after the skin biopsy at Week 1. Topical medications must again be held for 7 days prior to the final biopsy at Week 12, and may be resumed after this procedure.

In addition, iron supplementation and medications for bone health are encouraged as follows:

- Daily multivitamin containing iron for at least 90 days after the blood donation visit
- Medications for bone health may include any or all of the following: calcium carbonate or citrate (up to 1500 mg/d of elemental calcium), vitamin D (up to 2000 IU/day), and bisphosphonates

In addition, the use of broad spectrum (ultraviolet A [UVA] and ultraviolet B [UVB]) sunscreen through the entirety of the trial is encouraged.

5.8 Prohibited Medications

Prohibited medications include:

- throughout the duration of the trial:
  - immunosuppressive medications other than those specified in Section 5.7, *Concurrent Medications and Therapy*
  - live attenuated vaccines
- 7 days prior to each skin biopsy at the site of the biopsy:
  - topical steroids, topical calcineurin inhibitors

5.9 Procedures for Monitoring Subject Compliance

Subjects will not be self-administering any protocol-specified treatment. Therefore, their compliance will be monitored by their appearance for scheduled study visits, including acquisition of peripheral blood for expansion of Tregs, a subsequent single PolyTreg infusion, and follow-up visits for monitoring.

6 ASSESSMENT OF SAFETY AND EFFICACY

6.1 General Assessments

- Informed consent: written informed consent will be obtained before any study assessments or procedures are performed
- Demographics: date of birth, gender, self-identified race, self-identified ethnicity
- Medical History: Lifetime history of malignancy and family history of autoimmune disease. All other history should be from within 10 years prior to Screening Visit
- Comprehensive physical examination, at screening visit only
- Brief physical examination focused on participant's current complaints and clinical status
- Detailed Skin Examination
- Adverse Events. Participants will be assessed for adverse events at every visit
- Adverse Events Phone Assessment. Phone assessment for adverse events will be conducted at Day 2 and 3 and Weeks 3, 5 and 14
- Concomitant medications: Concomitant medications will be documented for 12 weeks prior to screening, and subsequently will be recorded at every visit
- Vital signs: height (at screening only), weight, temperature, heart rate, respiratory rate, sitting systolic blood pressure, sitting diastolic blood pressure will be assessed at every visit

6.2 Clinical Laboratory Assessments

- Chest x-ray: unless chest x-ray has been performed within 3 months of the Screening visit and documented test results are available
- Serum pregnancy test
- Urine pregnancy test: Stat studies at week -2 and baseline visits

- Hematology: CBC with differential and platelets
- Chemistry: BUN, creatinine, AST, ALT, total bilirubin, direct bilirubin, alkaline phosphatase, albumin, sodium, potassium, chloride, and bicarbonate
- Infectious disease testing: HIV serology, HBsAg, anti-HBc Ab, anti-HBs Ag, and anti-HCV Ab (and HCV RNA if anti-HCV Ab test is reactive), unless performed within 3 months of the Screening visit and documented test results are available
- EBV & CMV IgG, unless performed within 3 months of the Screening visit and documented test results are available
- EBV & CMV viral load. See Section 5.6.1.3, *Epstein-Barr Virus* and Section 5.6.1.4, *Cytomegalovirus* for management guidelines
- TB testing: either a PPD or QuantiFERON®-TB Gold In-Tube test, unless performed within 3 months of the Screening visit and documented test results are available, or unless subject is known to have a positive or indeterminate test and has documentation of appropriate therapy
- Urinalysis with microscopic analysis
- Desmoglein 1/3 autoantibodies by ELISA
- Treg absolute count in peripheral blood

6.3 Disease-Specific Assessments

- Skin biopsy for histopathology and direct immunofluorescence. Not required if results are documented prior to screening (see Section 4.1, *Inclusion Criteria* for details)
- Pemphigus Disease Area Index (PDAI): see Section 15.1, *PDAI*
- Skindex-29: see Section 15.2, *Skindex-29* [62, 63]
- PGA: see Section 15.3, *Patient's Global Assessment*
- PhGA: see Section 15.4, *Physician's Global Assessment*
- Digital photography: see Section 0, *Digital Photography* for details on types of photographs as well as adjustments for subject consent

6.4 Study Procedures

- Blood donation: approximately 400 ml will be colleced into a blood bag
- PolyTreg infusion: the Baseline and Day 1 visits are both a part of the 24-hour observation hospitalization for the PolyTreg infusion (see Section 5.3.2, *Administration* for details)
- Specimen collection for mechanistic studies
    - Skin biopsies: Anatomic location and disease activity will be recorded for each biopsy site

  - See Section 5.6.5, *Skin Biopsies,* 5.7, *Concurrent Medications and Therapies* and 5.8, *Prohibited Medications* for information on use of topical agents prior to skin biopsies
  - See Section 5.6.5, *Skin Biopsies* for the considerations to determine location of the biopsies as well as procedural steps to minimize discomfort and scarring
  - See Section 6.5, *Mechanistic Assessments* for information on the studies that will be conducted on these biopsies
- Blood: Sodium heparin (green top) tubes

6.5 Mechanistic Assessments

These mechanistic studies are designed to demonstrate the presence, persistence, and function of adoptively transferred Tregs in the blood and skin. Functionally analyzing both the adoptively transferred Tregs as well as endogenous T cell subsets in both the blood and skin enables comparison of the influence of adoptively transferred Tregs on the systemic immune system as well as on the local immune milieu in inflamed tissue. The following mechanistic studies will be performed: (i) mass spectrometry of transferred $^2H_2$-labeled Tregs in blood and skin and/or TCR sequencing to demonstrate the presence and assess the persistence of adoptively transferred Tregs; (ii) functional immunoflow cytometry on blood and skin to assess how transferred Tregs influence the cellular infiltrate and cytokine expression both systemically and in the target tissue; and (iii) whole transcriptome RNA-sequencing to assess gene expression changes in skin after Treg adoptive transfer.

6.5.1 Treg Tracking by Stable Isotope Labeling

APG01 relies on transferring PolyTregs that have been expanded hundreds-of-fold *in vitro*, which would be indistinguishable from endogenous Tregs by standard surface markers. However, because the cells are being expanded *ex vivo*, it provides a unique opportunity to label the cells prior to infusion to monitor engraftment and persistence of the administered PolyTregs. For over a decade, the Hellerstein group [64, 65] and others using their techniques, have applied stable isotope labeling with mass spectrometric analysis to measure the replication of murine and human cells *in vitro* and *in vivo*. Importantly, stable isotopes are non-radioactive and non-toxic, and they have been safely used as cellular, molecular, and metabolic markers in patients and healthy controls for more than six decades.

APG01 will label Tregs from pemphigus patients with deuterium by including the $^2H_2$ label in the culture medium during the entirety of the expansion phase *in vitro* (Day 0 to Day 14) prior to infusion in the subject. In this technique, the $^2H_2$ label is incorporated into the deoxyribose (dR) moiety in replicating DNA via the *de novo* synthesis pathway. Based on data from *in vitro* labeling studies, it is expected for 60% of purine deoxyribonucleosides in DNA strands to be labeled by deuterium at the time of infusion. After infusion, both PBMCs and single cell suspensions from skin biopsy specimens will be isolated and stored at time points as shown in Table 6.1, *Schedule of Events*. DNA prepared from these samples will be analyzed by gas chromatography-mass spectrometry in order to detect isotopic enrichment of the purine deoxyribonucleosides (deoxyadenosine [dA] or deoxyguanosine [dG]) [64, 66]. This method will identify infused Tregs in the systemic circulation and in the target tissue unequivocally. Based on estimates of the number of Tregs in the body (~1 x

10^{10}), 1.0 x 10^8 and 2.5 x 10^8 transferred autologous Tregs in cohorts 1 and 2, respectively, will represent between 2.5% and 10% of total Tregs. Preliminary studies in patients with T1DM have confirmed that this method is capable of detecting transferred Tregs in the blood (see Figure 9). Based on these calculations, deuterium labeling is predicted to be detectable for at least four divisions of the administered Tregs. This method of detection should be especially advantageous at early time points (up to 28 days) post-infusion, prior to significant division and/or loss of Tregs. Importantly, between 3 x 10^6 and 5 x 10^6 live cells will be isolated from a single 4 mm punch biopsy of affected skin. Approximately 3-5% of these cells are Tregs, enabling a sample between 9 x 10^4 and 1.5 x 10^5 Tregs, which is well within the sensitivity of this assay [67].

6.5.2 Whole Transcriptome RNA-Sequencing

In order to determine if and how adoptively transferred Tregs influence diseased tissue (i.e., skin), APG01 will perform whole transcriptome RNA-sequencing of skin biopsy samples taken from lesional skin before and after Treg adoptive transfer. This will allow for a comprehensive assessment of how adoptively transferred Tregs influence gene expression in skin, with a specific focus on pathways known to be important in the pathogenesis of pemphigus. As proof of concept, UCSF has recently performed RNA-sequencing on Tregs and Teff cells isolated from normal human skin. These populations were purified from relatively small samples of skin harvested from normal healthy volunteers (Figure 14A). Initial pathway analyses in Tregs compared to Teff cells have revealed IL-27-mediated signaling to be significantly increased and the IL-23 pathway to be significantly decreased in Tregs (Figure 14B). In a similar fashion, APG01 will comprehensively compare lesional skin pre-and post-Treg transfer to elucidate the major pathways that Tregs influence in skin.

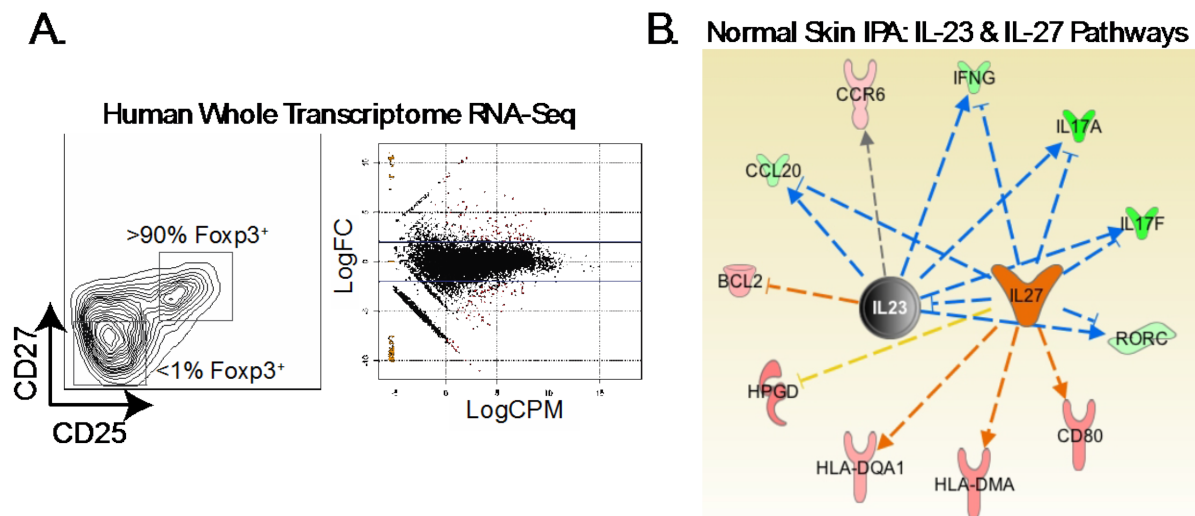


Figure 14, Whole transcriptome RNA-sequencing of Tregs and Teff cells isolated from normal human skin

(A) Tregs and mTeff cells were sorted from normal skin using expression of CD25 and CD27. RNA was isolated from purified cell fractions (from 3 different healthy volunteers) and subjected to whole transcriptome RNA-sequencing. Scatter plot shows grouped Treg vs. Teff cell comparisons. (B) Ingenuity® Pathway Analyses of the IL-23 and IL-27 pathways showing genes increased (arrows) and inhibited (line ends) identified by RNA-seq to have greater than 2-fold changes between mTregs and

mTeff cells with log p-values<0.05. Genes in the IL-27 pathway were significantly activated in mTregs (activation Z-score of +2.4; p-value $5.4x10^{-4}$) and genes in the IL-23 pathway were shown to be significantly inhibited in mTregs (inhibition Z-score of -2.4; p-value $3.0x10^{-7}$).

6.5.3 Functional Immunophenotyping of Skin and Blood

Cryopreserved PBMCs and single cell suspensions from fresh skin biopsies will be analyzed using multiparameter flow cytometry to assess changes in specific immune subsets following administration of expanded Tregs. The functionality of lymphocyte populations in blood and skin will be analyzed, specifically focusing on the cellular composition of the inflammatory infiltrate, T cell activation and intracellular cytokine expression profiles. T Helper Cell (T_H) subset differentiation (T_H1, T_H2, and T_H17) and cytokine expression from Teffs and Tregs will be compared between blood and skin. Analyses will focus on Treg activation markers and proteins known to be involved in Treg function, including but not limited to: CD25, CD127, CTLA-4, GARP, CD39, Ki67, and CD27.

6.5.4 Treg Suppression Assays

Frozen PBMCs will be thawed and Tregs will be isolated by flow-based sorting using CD4, CD25 and CD127 (and in some cases CD45RA and CD45RO) antibodies. Tregs will be assayed for their ability to suppress polyclonal T cell proliferation using either carboxyfluorescein diacetate succinimidyl ester (CFSE) or ^{3}[H]-thymidine in standard mixed suppression assays.

6.5.5 Autoantibody Analysis

Autoantibody titers, including Anti-Dsg1 and 3, will be assessed.

6.5.6 Lymphocyte Subset Analysis

Lymphocyte subsets include, but are not limited to (CD3 [T] cells, CD4 [helper] T cells, CD8 [cytotoxic] T cells, CD4+Foxp3+ Tregs, CD19 [B] cells, CD16 and CD56 Natural Killer [NK] cells).

6.5.7 Serum Cytokine Analysis

Collected serum will be stored for the measurement of cytokines. Serum will be assessed for pro-inflammatory cytokines (e.g., PDGF, IL-1ß, IL-6, IL-12, TNFα) and anti-inflammatory cytokines (e.g., TGF-ß, IL-10) by enzyme-linked immunosorbent assay (ELISA) and/or Luminex® bead assay to determine the effect of Treg treatment.

6.5.8 Treg Epigenetic Analysis

DNA purified from whole blood will be used to measure the frequency of Tregs in peripheral blood by means of epigenetic analysis. Analysis will be performed using the methylation-sensitive quantitative PCR (qPCR) method developed by Epiontis (Berlin, Germany) [68, 69]. This assay targets the demethylated state of the TSDR of the FOXP3 gene – a highly specific marker of Tregs. The proportion of $CD4^{+}CD25^{+}CD127^{-}$ cells as determined by flow cytometry strongly correlates with the proportion of demethylation of FOXP3 TSDR as

measured by qPCR. The proportion of demethylated FOXP3 will be normalized to proportion of demethylated CD3 DNA. The FOXP3 to CD3 ratio as determined by epigenetic analysis of methylation state strongly correlates with the ratio of Tregs to $CD3^+$ cells as measured by flow cytometry [70].

6.5.9 Antigen Specific Studies

Because the antigenic targets in pemphigus are well defined, mechanistic studies will include attempts at quantifying T cell responses to the pemphigus autoantigens, Dsg1 & Dsg3, before and after PolyTreg therapy. These will include, but are not limited to, quantification of Dsg1- and/or Dsg3-specific T cells in peripheral blood and skin using MHC class II tetramers. In addition, we will determine the magnitude of antigen-specific T cell responses in peripheral blood before and after PolyTreg therapy by performing autologous mixed lymphocyte reactions with increasing concentrations of exogenous Dsg1 and/or Dsg3 peptides. Together, these studies will provide critical information regarding the number of antigen-specific pathogenic T cells and the functionality of these cells before and after PolyTreg therapy.

6.6 Unscheduled Visits

If disease activity increases or other concerns arise between regularly scheduled visits, subjects should be instructed to contact study personnel to determine whether an “unscheduled” visit should occur. The following evaluations will be performed at each unscheduled visit:

- Adverse Event Assessment
- Concomitant medication assessment
- Physical Examination
- Other evaluations may be performed at the investigator’s discretion

If the unscheduled visit is due to an increase in Pemphigus disease activity, these additional evaluations should also be performed:

- PDAI
- Desmoglein 1/3 autoantibodies by ELISA
- Skindex-29 (QOL)
- Physician’s Global Assessment (PhGA)
- Patient’s Global Assessment (PGA)
- Hematology Panel
- Chemistry Panel
- Digital Photography (optional)
- Lymphocyte subsets (optional)
- Mechanistic Blood Draw (optional)

Table 6.1, Schedule of Events *(2 pages)*																					
Visit Name	Screening[E]	Wk -2	Base-line	Day 1	Day 2	Day 3	Wk 1	Wk 2	Wk 3	Wk 5	Wk 8	Wk 12	Wk 14	Wk 26	Wk 39	Wk 52	Wk 78	Wk 104	Wk 130	Wk 156	Un-sched-uled
Visit Window *(days)*	-49 to -42	-16 to -14	0	0	0	0	±3	±3	±3	±7	±7	±7	±7	±28	±28	±28	±28	±28	±28	±28	
Clinical Draw (ml)[G]	41	0	20.5	0	0	0	14	14	0	0	18	18	0	18	12	12	12	12	12	12	12
Study Product (ml)	0	400	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Mechanistic Draw (ml)	0	0	35	0	0	0	30	35	0	0	30	35	0	35	0	35	0	0	0	0	35
Visit Draw Total (ml)	41	400	55.5	0	0	0	44	49	0	0	48	53	0	53	12	47	12	12	12	12	47
General Assessments (Section 6.1)																					
Informed consent	X																				
Demographics	X																				
Medical History	X																				
Physical Examination	X	X	X				X	X			X	X		X	X	X	X	X	X	X	X
Vital Signs	X	X	X	X			X	X			X	X		X	X	X	X	X	X	X	X
Medications Assessment	X		X				X	X			X	X		X	X	X	X	X	X	X	X
AE Assessment		X	X	X			X	X			X	X		X	X	X	X	X	X	X	X
AE Assessment by Phone					X	X			X	X			X								
Laboratory Assessments (Section 6.2)																					
Chest X-ray	X																				
Serum pregnancy test	X																				
Stat urine pregnancy test[F]		X	X[D]																		
Urine pregnancy test											X	X									
Hematology	X		X[D]				X	X			X	X		X	X	X	X	X	X	X	X
Chemistry	X		X[D]								X	X		X	X	X	X	X	X	X	X
Infectious Disease testing	X[B]																				
EBV & CMV IgG	X[B]																				
EBV & CMV Viral Load	X[B]						X	X			X	X		X							
TB Testing (PPD or QuantiFERON®-TB Gold In-Tube Test)	X[B]																				
Urinalysis with microscopic analysis	X															X					
Treg absolute count in peripheral blood	X																				
Disease Specific Assessments (Section 6.3)																					
Skin Biopsy for histopathology[A]	X[A]																				
PDAI	X		X				X	X			X	X		X	X	X	X	X	X	X	X

Table 6.1, Schedule of Events *(2 pages)*																					
Visit Name	Screening^E	Wk -2	Base-line	Day 1	Day 2	Day 3	Wk 1	Wk 2	Wk 3	Wk 5	Wk 8	Wk 12	Wk 14	Wk 26	Wk 39	Wk 52	Wk 78	Wk 104	Wk 130	Wk 156	Un-sched-uled
Visit Window *(days)*	-49 to -42	-16 to -14	0	0	0	0	±3	±3	±3	±7	±7	±7	±7	±28	±28	±28	±28	±28	±28	±28	
Desmoglein 1/3 by ELISA	X		X				X	X			X	X		X	X	X	X	X	X	X	X
Skindex-29 (QoL)	X		X				X	X			X	X		X	X	X	X	X	X	X	X
Physician's Global Assessment (PhGA)	X		X				X	X			X	X		X	X	X	X	X	X	X	X
Patient's Global Assessment (PGA)	X		X				X	X			X	X		X	X	X	X	X	X	X	X
Digital photography	X		X				X	X			X	X		X	X	X	X	X	X	X	X^C
Study Procedures																					
Blood Donation		X																			
PolyTreg Infusion			X																		
Mechanistic Assessments																					
Skin Biopsies			X				X					X									X^C
Lymphocyte subsets			X					X				X		X		X					X^C
Mechanistic Studies			X				X	X			X	X		X		X					X^C

A. Skin biopsy for histopathology at screening is required only if historic information does not satisfy Inclusion Criterion #3 (see Section 4.1, Inclusion Criteria).
B. If a subject is rescreened within 3 months and there is no clinical indication to warrant a repeat test, previously negative EBV & CMV IgG, TB, and infectious disease tests do not need to be repeated.
C. At the Unscheduled Visit, the digital photography, skin biopsies, lymphocyte subsets, and mechanistic studies are optional.
D. On the day of the scheduled infusion (Baseline Visit), blood chemistries, a complete blood count CBC) with differential blood count, and a pregnancy test (for women of childbearing potential) will be obtained and reviewed locally prior to infusion of the PolyTregs.
E. Screening Labs should be drawn within the window that complies with overall blood volume limitations per NIH guidance of no more than 550 cc in an 8-week period.
F. Stat Pregnancy Test may be a urine or Point of Care (POC) test.
G. The Clinical Draw blood volumes are approximate volumes that may vary across sites based on collection materials available.

7 SAFETY MONITORING AND REPORTING

7.1 Overview

This section defines the types of safety data that will be collected under this protocol and outlines the procedures for appropriately collecting, grading, recording, and reporting that data. AEs that are classified as serious according to the definition of health authorities must be reported promptly (per Section 7.5, *Reporting of Adverse Events and Serious Adverse Events to the Sponsor: DAIT/NIAID*) and appropriately to the investigational new drug application (IND) sponsor (DAIT, NIAID), principal investigators in the trial, IRBs, and health authorities. Information in this section complies with *International Conference on Harmonization (ICH) Guideline E2A: Clinical Safety Data Management: Definitions and Standards for Expedited Reporting, ICH Guideline E-6: Guideline for Good Clinical Practice,* and applies the standards set forth in the NCI-CTCAE*, Version 4.0*: https://ctep.cancer.gov/protocolDevelopment/electronic_applications/ctc.htm#ctc_40

7.2 Definitions

7.2.1 Adverse Event (or Adverse Experience)

Any untoward or unfavorable medical occurrence in a human subject, including any abnormal sign, symptom, or disease, temporally associated with the subject's participation in the research, whether or not considered related to the subject's participation in the research (modified from the definition of AEs in the 1996 ICH E-6 Guidelines for Good Clinical Practice)." [From Office for Human Research Protections (OHRP) "Guidance on Reviewing and Reporting Unanticipated Problems Involving Risks to Subjects or Others and Adverse Events (1/15/07)" http://www.hhs.gov/ohrp/policy/advevntguid.html]

7.2.2 Adverse Reaction and Suspected Adverse Reaction

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

7.2.3 "Expected" versus "Unexpected" Suspected Adverse Reaction

A SAR is considered "expected" when it is listed in the investigator brochure, the general investigational plan, or the Protocol. A SAR is considered "unexpected" when its nature (specificity), severity, or rate of occurrence is not consistent with applicable product information as described in the safety information provided in the investigator brochure, the general investigational plan, or the protocol (21 CFR 312.32(a) and ICH E2A). A serious unexpected suspected adverse drug reaction is referred to as a SUSAR. For this study,

expectedness will be determined by product information provided in the investigator brochure (IB).

7.2.4 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) and ICH E2A):

1. Death
2. 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.
3. Inpatient hospitalization or prolongation of existing hospitalization
4. Persistent or significant incapacity or substantial disruption of the ability to conduct normal life functions
5. Congenital anomaly or birth defect
6. Important medical 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 subject and may require medical or surgical intervention to prevent one of the outcomes listed above.

Elective hospitalizations or hospital admissions for the purpose of conduct of protocol-mandated procedures (i.e. the 24-hour observation hospitalization for the drug infusion) are not to be reported as an SAE unless hospitalization is prolonged due to complications.

If an event meets any of the above SAE definitions, regardless of the relationship of the event to investigational product, the event must be reported to the sponsor as described in Section 7.5.2, *Reporting of Serious Adverse Events and Grade 3 and Higher Clinical Events.*

7.3 Collection and Recording of Adverse Events

7.3.1 Investigational Product

The investigational product in this protocol is *ex vivo* Expanded Autologous $CD4^{+}CD127^{lo/-}$ $CD25^{+}$ Polyclonal Regulatory T Cells (PolyTregs).

7.3.2 Methods of Collection

AEs (including SAEs) may be discovered through any of these methods:

- Observing the subject
- Questioning the subject in an objective manner
- Receiving an unsolicited complaint from the subject
- In addition, an abnormal value or result from a clinical or laboratory evaluation (including, but not limited to, a radiograph, an ultrasound, or an electrocardiogram) can also indicate an AE, as defined in Section 7.4, *Grading and Attribution of Adverse Events*. The evaluation that produced the result should be followed until that value or result returns to normal or can be explained and the subject's safety in no longer at risk.

7.3.3 Methods of Recording

7.3.3.1 Recording Adverse Events

Throughout the study, the investigator will record all clinical AEs and all laboratory AEs per the following criteria on the appropriate AE electronic case report form (eCRF) regardless of their severity or relation to study medication or study procedure.

- From time of signing of informed consent until start of investigational product infusion: all SAEs
- From start of investigational product infusion until 24 hours post infusion: all NCI-CTCAE Grade 1 and higher AEs
- From 24 hours post-infusion until Week 52: all NCI-CTCAE Grade 2 and higher AEs
- From Week 52 until Week 156: all SAEs and all NCI-CTCAE Grade 3 and higher AEs

Irrespective of study period, all clinical and laboratory events regardless of severity should be recorded in the medical research record for the duration of the trial.

The investigator will treat subjects experiencing AEs appropriately and observe them at suitable intervals until their symptoms resolve or their status stabilizes. Once recorded, an AE will be followed until it resolves with or without sequelae, or until the end of study participation, or after the subject prematurely withdraws (without withdrawing consent)/or is withdrawn from the study, whichever occurs first.

7.3.3.2 Recording Serious Adverse Events

Serious AEs will be recorded on the appropriate AE/SAE eCRF. All requested information on the AE eCRF and SAE eCRF should be provided, if available, for submission to the DAIT-SACCC, DAIT, NIAID, and health authorities as outlined in Section 7.5, *Reporting of Adverse Events and Serious Adverse Events to the Sponsor: DAIT/NIAID*.

Once recorded, an SAE will be followed until it resolves with or without sequelae, or until the end of study participation, whichever occurs first. End of study participation is the final study visit, either at time of study completion or when a subject prematurely withdraws or is withdrawn.

7.4 Grading and Attribution of Adverse Events

7.4.1 Grading Criteria

The study site will grade the severity of AEs experienced by the study subjects according to the criteria set forth in the NCI-CTCAE v4.0. This document (referred to herein as the NCI-CTCAE manual) provides a common language to describe levels of severity, to analyze and interpret data, and to articulate the clinical significance of all AEs. The NCI- CTCAE has been reviewed by the Protocol Chairs and has been deemed appropriate for the subject population to be studied in this protocol.

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

Grade 1 = mild AE

Grade 2 = moderate AE

Grade 3 = severe and undesirable AE

Grade 4 = life-threatening or disabling AE

Grade 5 = death

If NCI-CTCAE criteria are defined for grading an abnormal value or result from a clinical or laboratory evaluation (including, but not limited to, a radiograph, an ultrasound, or an electrocardiogram), then a treatment-emergent AE is defined as an increase in grade from Baseline (Day 0) or from the last post-baseline value that doesn't meet grading criteria. Changes in grade from screening to Baseline (Day 0) will also be recorded as outlined in Section 7.3.3.1, *Recording Adverse Events*. If a specific event or result from a given clinical or laboratory evaluation is not included in the NCI-CTCAE manual, then an abnormal result would be considered an AE if changes in therapy or monitoring are implemented.

AEs that are related to disease activity will be graded according to the plan outlined above. However, an increase in disease activity leading to an AE should also be reflected in standard measures of disease activity (PDAI) measured at regularly scheduled visits.

To facilitate identification of a safety signal associated with increased disease activity, the PDAI disease activity measures will be used throughout the study to monitor changes in disease activity over time. The DSMB will receive reports on these assessments for each cohort at regularly scheduled reviews.

7.4.2 Attribution Definitions

The relation, or attribution, of an AE to an investigational product will initially be determined by the site investigator. The site investigator will also record the initial determination of attribution on the appropriate AE eCRF. Final determination of attribution for safety reporting will be decided by DAIT, NIAID with input from the SRC. The relationship of an adverse event to study therapy regimen or procedures will be determined using the descriptors and definitions provided in Table 7.1 (below).

Table 7.1 Attribution of Adverse Events

Code	Descriptor	Relationship (to primary investigational product and/or other concurrent mandated study therapy or study procedure)
Unrelated Categories		
1	Not Related	The adverse event is clearly not related: there is insufficient evidence to suggest a causal relationship.
Related Categories		
2	Possibly Related	The adverse event has a reasonable possibility to be related; there is evidence to suggest a causal relationship.
3	Related	The adverse event is clearly related.

7.5 Reporting of Adverse Events and Serious Adverse Events to the Sponsor: DAIT/NIAID

7.5.1 Reporting of Adverse Events

This section describes the responsibilities of the site investigator to report AEs to the study sponsor (DAIT/NIAID) via the DAIT-SACCC using the AE eCRF. Timely reporting of adverse events as specified by study phase in Section 7.3.3.1, *Recording Adverse Events* is expected.

Unless otherwise noted below in Section 7.5.2, *Reporting of Serious Adverse Events and Grade 3 and Higher Clinical Events*, for serious adverse events and events of special interest, AEs should be recorded on the AE eCRF within five (5) business days of the site learning of the event(s). Whenever possible, a diagnosis should be provided, rather than compilation of signs/symptoms, with grade of the event determined by highest grade of the sign/symptom component.

The exception to this reporting timeframe is the reporting of serious adverse events and Grade 3 or higher clinical events which require reporting within 24 hours of discovery as described in Section 7.5.2 below.

7.5.2 Reporting of Serious Adverse Events and Grade 3 and Higher Clinical Events

This section describes the responsibilities of the site investigator to report serious adverse events and non-serious Grade ≥ 3 events to the sponsor via the electronic CRF (eCRF). Timely reporting of adverse events is required by 21 CFR and ICH E6 guidelines.

The adverse events outlined below must be reported by the site investigators to DAIT/NIAID via the DAIT-SACCC regardless of relationship or expectedness to study intervention **within 24 hours of discovery of the event:**

- All SAEs per 21 CFR 312.32 definitions (see Section 7.2.4, *Serious Adverse Event*)
- All other Clinical events with a NCI-CTCAE Grade 3 or greater severity

Note: clinical events include signs/symptoms, diagnoses, and laboratory abnormalities with clinical consequence (defined as the requirement for intervention, correction, increased monitoring, or further evaluation). Inclusive in this category is any NCI-CTCAE Grade 3 or greater clinical/laboratory component associated with a pemphigus relapse/flare per protocol Section 3.4.1 Exploratory Safety Endpoints.

The SAE eCRF will be used to capture all SAEs and non-serious Grade ≥ 3 clinical events for submission to the sponsor (DAIT/NIAID). All requested information on the SAE eCRF should be provided. Unavailable details of the event at the time of the initial report should not delay submission of known information. The initial report should include at a minimum: AE term, relationship to PolyTregs, and as applicable, reason why event is serious per the definitions in Section 7.2.4 *Serious Adverse Event*.

Supplementary CRF pages including medical history, concomitant medications, demographics, study drug administration, and death must be provided as applicable. As additional details become available, the SAE eCRF should be updated and submitted. With

each iteration of the form, the investigator (or designated sub-investigator) must sign the form electronically.

For additional information regarding SAE reporting, contact Rho Product Safety (DAIT-SACCC):

7.5.3 DAIT, NIAID Reporting to the Health Authority

After an AE requiring 24 hour reporting (per Section 7.5.2, *Reporting of Serious Adverse Events and Grade 3 and Higher Clinical Events*) is submitted by the site investigator and assessed by DAIT, NIAID, there are two options for DAIT, NIAID to report the AE to the appropriate health authorities:

- **Annual IND Report.** This option applies if the AE is classified as one of the following:
  - Serious, expected, SARs (see Section 7.2.2, *Adverse Reaction and Suspected Adverse Reaction,* and Section 7.2.3, *"Expected" versus "Unexpected" Suspected Adverse Reaction*)
  - Serious and not a SAR (see Section 7.2.2, *Adverse Reaction and Suspected Adverse Reaction*)

Note that all AEs (not just those requiring 24 hour reporting) will be reported in the Annual IND Report.

- **Expedited Safety Report.** This option applies if the AE is classified as one of the following:
  - Serious and unexpected suspected adverse reaction (SUSAR) (see Section 7.2.2, *Adverse Reaction and Suspected Adverse Reaction* and Section 7.2.3, *"Expected" versus "Unexpected" Suspected Adverse Reaction*)
    The sponsor must report any SAR that is both serious and unexpected. The sponsor must report an AE as a SAR only if there is evidence to suggest a causal relationship between the study drug and AE, such as:
    - A single occurrence of an event that is uncommon and known to be strongly associated with drug exposure
    - One or more occurrences of an event that is not commonly associated with drug exposure, but is otherwise uncommon in the population exposed to the drug
    - Aggregate analysis of specific events observed in a clinical trial (such as known consequences of the underlying disease or condition under investigation or other events that commonly occur in the study population

independent of drug therapy) that indicates those events occur more frequently in the drug treatment group than in a concurrent or historical control group

- Any findings from studies: The sponsor must report any findings from other epidemiological or clinical studies, pooled analysis of multiple studies, or animal or in vitro testing that suggest a significant risk in humans exposed to the drug that would result in a safety-related change in the protocol, informed consent, investigator brochure or other aspects of the overall conduct of the study.

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

7.5.4 Reporting of Adverse Events to IRBs

All investigators must report AEs and SAEs in a timely fashion to their respective IRBs in accordance with applicable regulations and local reporting guidelines.

All IND Safety Reports to the FDA will be distributed by the DAIT, NIAID or designee to all participating institutions for site IRB submission.

7.6 Pregnancy Reporting

Although pregnancy is not an SAE, information about any pregnancy should be reported promptly to the DAIT-SACCC on the same timeline as an SAE for tracking purposes (Section 7.5.2, *Reporting of Serious Adverse Events and Grade 3 and Higher Clinical Events*).

All pregnancies 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 in a study subject or a partner of a study subject. A pregnant subject should be instructed to stop taking study medication. The investigator should report to the DAIT-SACCC all pregnancies within one business day (as described in Section 7.5.2, *Reporting of Serious Adverse Events and Grade 3 and Higher Clinical Events*) using the Pregnancy eCRF. The investigator should counsel the subject and discuss the risks of continuing with the pregnancy and the possible effects on the fetus. Monitoring of the pregnant subject should continue until the conclusion of the pregnancy, and follow-up data detailing the outcome of the pregnancy should be submitted to the DAIT-SACCC by updating the Pregnancy eCRF. When possible, similar information should be obtained for a pregnancy occurring in a partner of a study subject.

Information requested about the delivery will include:

- Subject’s enrollment ID
- Gestational age at delivery
- Birth weight, length, and head circumference
- Gender

- Appearance, pulse, grimace, activity, and respiration (APGAR) score at one minute, five minutes, and 24 hours after birth, if available
- Any abnormalities

Should the pregnancy result in a congenital abnormality or birth defect, an SAE must be submitted to the DAIT-SACCC using the SAE reporting procedures described above.

7.6.1 Mycophenolate REMS Program

APG01 investigators of participating subjects taking concurrent MMF are strongly encouraged to register with the FDA's REMS program (www.mycophenolaterems.com). An investigator will be strongly encouraged to report to the REMS program any pregnancy occurring in an APG01 female subject while she is taking MMF or within the first six weeks following discontinuation of MMF treatment.

7.7 Reporting of Other Safety Information

An investigator should promptly notify the DAIT-SACCC when an "unanticipated problem involving risks to subjects or others" is identified, which is not otherwise reportable as an AE.

7.8 Review of Safety Information

7.8.1 Study Management Team Review

The study management team (SMT) will receive monthly reports from the DAIT-SACCC compiling new and accumulating safety information on, including but not limited to AEs, SAEs, and pregnancies recorded by the sites on appropriate eCRFs.

In addition, the SRC will review safety data collected within dosing cohorts to define significant events (see Section 3.1, *Description of Study Design*).

7.8.2 Medical Monitor Review

The Medical Monitor (MM), as part of the SMT and SRC, will review all information listed above in Section 7.8.1, *Study Management Team Review*. In addition, the MM will receive SAE and pregnancy reports for review and triage after the DAIT-SACCC is made aware of these events (see Sections 7.5.2, *Reporting of Serious Adverse Events and Grade 3 and Higher Clinical Events* and 7.6, *Pregnancy Reporting*).

7.8.3 DSMB Review

The DSMB will review the safety data collected when the fourth subject in cohort 1 completes Week 8. In addition, the DSMB will review accumulating safety data at least yearly during planned DSMB Data Review Meetings. Data for the planned safety reviews will include, at a minimum, a listing of all reported AEs and SAEs. To ensure subject safety between Data Review Meetings, the DSMB will be informed of all expedited safety reports in a timely manner.

In addition to the pre-scheduled data reviews and planned safety monitoring, the DSMB may be called upon for ad hoc reviews or emergency meetings (see Section 5.5.4, *Safety Stopping Guidance*). The DSMB will have the discretion to recommend actions regarding study conduct and continuation as a consequence of any planned or unplanned monitoring activity.

8 STATISTICAL CONSIDERATIONS AND ANALYTICAL PLAN

8.1 Sample Size and Power

No formal power analyses were conducted since the study objectives require no hypothesis testing. However, the ability to detect at least 1 significant event at different frequencies was considered. Table 8.1 illustrates the probability of observing at least one significant event assuming different example scenarios.

Table 8.1 Probability of Observing at least 1 Event in any Cohort under Different Scenarios

	True Probability of Event		
	0.1	0.2	0.3
Probability of observing at least 1 event in 4 subjects	0.34	0.59	0.76
Probability of observing at least 1 event in 6 subjects	0.47	0.74	0.88

The number of subjects to be enrolled will depend upon the observed safety profile and SRC review, which will determine the number of subjects per dose level, the number of dose escalations, and the number of cohorts.

8.2 Analysis Populations

8.2.1 Donation Population

The donation population will include all subjects who initiated the blood donation.

8.2.2 Safety Population

The safety population will include all subjects who received the PolyTregs infusion.

8.3 Description of the Analyses

Due to the exploratory nature of this study, no confirmatory inferential analyses are planned. Descriptive statistics (such as medians, quartiles, and ranges for continuous data and percentages for categorical data) will be used to summarize patient characteristics, safety, efficacy, and mechanistic parameters. These summaries will be presented overall and separately for the subjects in the different dosing groups.

8.3.1 Safety Analysis

All safety summaries will be performed using the Safety population.

AEs including changes in laboratory values will be graded according to the NCI-CTCAE Version 4.0 (http://ctep.cancer.gov/reporting/ctc.html). The frequency of AEs will be summarized by system organ class, preferred term, severity (grade), and relationship to PolyTregs as specified by the SRC and by the site. Relationship to PolyTregs will be categorized as either related (possibly, probably, or related) or unrelated (unlikely or not related). For the primary safety endpoint defined in Section 3.2.1, *Primary Safety Endpoint*, and each key safety endpoint defined in Section 3.3.1*, Secondary Safety Endpoints*, the number and percentage of subjects experiencing the event will be summarized.

Pemphigus flares defined in Section 3.4.1, *Exploratory Safety Endpoints,* will be summarized separately through Week 156 using appropriate descriptive statistics.

Laboratory results by parameter will be displayed in listings and graphically, where appropriate, to illustrate changes in laboratory results over time.

SAEs will also be summarized using the donation population to assess SAEs occurring from donation until PolyTreg infusion.

8.3.2 **Efficacy Analysis**

All efficacy endpoints will be summarized using appropriate descriptive statistics. For each change from baseline efficacy endpoint defined in Section 3.3.2, *Secondary Efficacy Endpoints,* and Section 3.4.2, *Exploratory Efficacy Endpoints,* change from baseline will be summarized at each visit outlined in the respective endpoint. Time to relapse (flare) will be measured from the day of PolyTreg infusion. For each endpoint related to prednisone dosing, the number of participants meeting the dosing criteria at Weeks 12, 26, 39, 52, 78, 104, 130, and 156 will be summarized. The efficacy summaries will be completed using the safety population.

8.3.3 **Mechanistic Analysis**

Appropriate descriptive statistics will be computed for the change in level of transferred deuterium-labeled Tregs in peripheral blood and skin from Week 1 at Week 12, changes in disease specific and immunologic biomarkers in peripheral blood and skin from baseline to Week 12, and for the relative frequency of adoptively transferred Tregs in blood and skin at each visit assessment. The mechanistic summaries will be completed using the safety population.

8.4 Interim Analysis

Results of interim analyses will be reported to the DSMB for planned Data Review Meetings. Reports prepared for these meetings will focus on study conduct and subject safety and may include information on enrollment, site activation status, protocol deviations, subject status and demographics and safety analyses. Similar reports, with emphasis on the safety analyses, will be provided to the SRC each time the study is paused due to a significant event and after the completion of each cohort.

8.4.1 Interim Analysis of Safety Data

The SRC will review data on significant events as described in Section 3.1, *Description of Study Design* and Section 5.5.4, *Safety Stopping Guidance*. Detailed listings of significant events, AEs, and safety-related laboratory values will be generated for the SRC review. The safety analyses will be completed overall and by dosing group using the safety population.

The DSMB will periodically review safety data as described in Section 5.5.4, *Safety Stopping Guidance* and Section 7.8.3, *DSMB Review.* The safety analyses for this trial will be descriptive rather than inferential. Detailed listings and summary tabulations of AEs and safety-related laboratory values will be generated. The safety analyses will be completed overall and by dosing group using the safety population.

8.5 Other Statistical Considerations

8.5.1 Covariates

Due to the samples size of this phase I trial, the impact of additional covariates will not be explored.

8.5.2 Multiple Comparisons and Multiplicity

Since this is a phase I study, all safety, efficacy, and mechanistic analyses are exploratory in nature. Therefore, no adjustment for multiple comparisons is needed.

8.5.3 Examination of Subgroups

Due to the sample size of this phase I trial, no additional subgroup analyses will be performed.

8.5.4 Missing Data

Standard procedures will be used to ensure that data are complete and accurate as possible. Due to the exploratory nature of this study, no imputation for missing data will be done; however per Section 3.1.2.1, *Subject Completion and Early Termination*, if an infused subject does not complete 12 weeks of follow-up, an additional subject will be recruited in order to fully understand safety through 12 weeks of treatment.

8.5.5 Changes to the Statistical Analysis Plan

The principal features of the design of this study and of the plan for statistical analysis of the data are outlined in this protocol. Additional details will be included in a Statistical Analysis Plan (SAP) before initiating analyses. Any changes to that plan will be documented in the Final Study Report and will be approved by the Protocol Chairs before being initiated.

9 ACCESS TO SOURCE DATA AND DOCUMENTS

Each participating site will maintain the highest degree of confidentiality permitted for the clinical and research information obtained from subjects participating 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, each site must permit authorized representatives of the IND sponsor, the DAIT-SACCC, and health authorities to examine (and when required by applicable law, to copy) clinical records for the purposes of quality assurance reviews, audits, and evaluation of the study safety and progress. Unless required by the laws permitting copying of records, only the coded identity associated with documents or other subject data may be copied (obscuring any personally identifying information). Authorized representatives as noted above are bound to maintain the strict confidentiality of medical and research information that may be linked to identified individuals. Participating sites will normally be notified in advance of auditing visits.

All subject records and study documentation will be kept after the protocol is completed. This will include all documentation of AEs, records of study drug receipt and dispensation, and all IRB correspondence. All study records will be kept for at least two years after the investigation is completed.

10 DATA COLLECTION, QUALITY CONTROL AND QUALITY ASSURANCE

The investigator is required to keep accurate records to ensure the conduct of the study is fully documented. The period of record retention should be consistent with the record retention policies of the sponsoring agency or applicable regulatory agencies. However, in certain instances, documents should be retained for a longer period if required by the applicable regulatory agency or by the National Institutes of Health.

The investigator will report all major protocol deviations to DAIT, NIAID and the DAIT-SACCC per the instructions in the APG01 Manual of Procedures. The DAIT-SACCC will forward reports of protocol deviations to the responsible DAIT, NIAID medical officer for review as specified in the ACE Manual of Procedures.

The DAIT-SACCC 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.

Data will be obtained from a variety of sources including, but not limited to laboratory notebooks, automated instrument output files, and clinical subject charts. Data from these source materials will be transmitted to the DAIT-SACCC via one of two mechanisms. Data collected electronically at central laboratories will be transferred electronically directly from the laboratory to the DAIT-SACCC using standard secure data transfer procedures. Data collected at the clinical sites will be transmitted to the DAIT-SACCC using an internet-based remote data entry system. Clinical site personnel use an internet browser to key data into eCRFs; each CRF page is submitted to the clinical database electronically as the page is completed. Univariate data validation tests are performed as the data are keyed. The clinical database is backed up nightly; backup tapes are saved in a secure, off-site location. At any time, authorized site personnel may log in to the remote data entry system, review and correct previously entered data, or key additional data. The data will be further validated per the study data validation plan via a series of computerized and manual edit checks, and all relevant data queries will be raised and resolved on an ongoing basis. Complete, clean data will be frozen to prevent further inadvertent modifications. All discrepancies will be reviewed and any resulting queries will be resolved with the investigators and amended in the database. All elements of data entry (i.e., time, date, verbatim text, and the person performing

the data entry) will be recorded in an electronic audit trail to allow all data changes in the database to be monitored and maintained in accordance with federal regulations.

The DAIT-SACCC will periodically visit the participating clinical sites and audit the source documents in order to validate the data in the DAIT-SACCC central database. Data will be provided using the subject's enrollment number; the DAIT-SACCC will not collect personally identifying information such as the subject's name or social security number. Subjects will provide demographic information such as race, ethnicity, and birth date.

Data collected by the DAIT-SACCC will be held in the strictest confidence, and are protected from access that could reveal personally identifying information about any subject in the trial.

11 ETHICAL CONSIDERATIONS AND COMPLIANCE WITH GOOD CLINICAL PRACTICE

The study will be conducted according to Good Clinical Practice (GCP) guidelines, U.S. 21 CFR Part 50 – Protection of Human Subjects, and Part 56 – Institutional Review Boards.

11.1 Compliance with Good Clinical Practices

This trial will be conducted in compliance with the protocol, current GCPs recommended by the ICH, and the applicable regulatory requirements for participating institutions. These include the tenets of the Declaration of Helsinki and review and approval by the appropriate ethics review committee or IRBs of participating organizations. The DAIT-SACCC will assure compliance through a program of quality assurance audits performed both at participating sites and within the DAIT-SACCC for data quality and adherence to protocol requirements. The DAIT-SACCC is operated by Rho Federal Systems Division, Inc. (RhoFED), Chapel Hill, North Carolina under a cooperative agreement from NIAID.

11.2 Institutional Review Board

Each participating institution must provide for the review and approval of this protocol and associated informed consent documents by an appropriate ethics review committee or IRB. Any amendments to the protocol or consent materials must be approved by the IRB and submitted to the FDA before they are placed into use. In both the United States and in other countries, only institutions holding a current Federal Wide Assurance (FWA) issued by the OHRP at the Department of Health and Human Services (DHHS) may participate.

The investigator will inform the IRB of serious or unexpected AEs that might occur during the study and are likely to affect the safety of the subjects, or the conduct of the study. The investigators will comply fully with all IRB requirements for both the reporting of AEs, protocol or consent form changes, as well as any new information pertaining to the use of the study medication that might affect the conduct of the study.

11.3 Informed Consent

The principles of informed consent in the current edition of the Declaration of Helsinki, as well as compliance with all IRB requirements, will be implemented in the study, before any protocol-specified procedures are carried out. A standard consent form for subject participation will be provided with the protocol to each institution. Any modifications to the

standard information in the template will require review and approval by DAIT, NIAID prior to IRB submission. Informed consent will be obtained in accordance with 21 CFR 50.52. Information may be given to subjects in oral, written, or video form by the investigator. All prospective subjects will be given ample time to read the consent form, and ask questions, before signing.

If subjects are to be enrolled who do not speak and read English, the consent and subject materials must be translated into the language appropriate for the enrolling subject. Translated documents must be certified to contain the complete descriptions provided in the English version of the document. If an interpreter is used to provide or assist in describing the consent materials to an enrolling subject, the interpreter must also sign the consent materials certifying their involvement with the consent process.

After completion, a copy of the signed consent form will be given to the subject. The original signed consent form will be kept on file in the subject's study chart, available for inspection by regulatory authorities, both federal and institutional.

11.4 Data and Safety Monitoring Board

The responsibility for reviewing the ethical conduct of the study and for monitoring reports of evidence of adverse or beneficial effect is assigned to the DAIT Autoimmunity DSMB. The DSMB is an independent group composed of biomedical ethic experts, physicians, and other scientists who are responsible for continuing review of study information. The DSMB makes recommendations to DAIT, NIAID on issues affecting the course and conduct of this clinical study.

11.5 Study Termination

In the event that the study is terminated, subjects who have received study treatment will be followed for the study's three years of safety follow-up.

12 FINANCING AND INSURANCE

Participating institutions must comply with their institution's policies on compensation, insurance, and indemnity. Institutions must have adequate liability insurance coverage to satisfy their local and national requirements for study participation.

13 PUBLICATION POLICY

The ACE policy on publication of study results will apply to this study. Authorized participants may find details regarding the policy statement on the ACE internet website Study investigators are encouraged to communicate and publish study results with prior notification of DAIT, NIAID. The following procedure is suggested:

1. Manuscripts, abstracts, posters and other material for public distribution will be submitted to DAIT, NIAID at least 30 days prior to submission for publication or public presentation.
2. DAIT, NIAID will review and comment on the proposed material within 30 days.
3. DAIT, NIAID may ask that confidential information be deleted or redacted in this case where a patent may be filed or where confidential information is involved. Publication

or presentation may be delayed up to 60 additional days in order to file a patent application.

14 REFERENCES

1. Payne, A.S., et al., *Desmosomes and disease: pemphigus and bullous impetigo.* Curr Opin Cell Biol, 2004. **16**(5): p. 536-43.
2. Martin, L.K., et al., *A systematic review of randomized controlled trials for pemphigus vulgaris and pemphigus foliaceus.* J Am Acad Dermatol, 2011. **64**(5): p. 903-8.
3. Ratnam, K.V., K.L. Phay, and C.K. Tan, *Pemphigus therapy with oral prednisolone regimens. A 5-year study.* Int J Dermatol, 1990. **29**(5): p. 363-7.
4. Mentink, L.F., et al., *Randomized controlled trial of adjuvant oral dexamethasone pulse therapy in pemphigus vulgaris: PEMPULS trial.* Arch Dermatol, 2006. **142**(5): p. 570-6.
5. Beissert, S., et al., *A comparison of oral methylprednisolone plus azathioprine or mycophenolate mofetil for the treatment of pemphigus.* Arch Dermatol, 2006. **142**(11): p. 1447-54.
6. Chams-Davatchi, C., et al., *Randomized controlled open-label trial of four treatment regimens for pemphigus vulgaris.* J Am Acad Dermatol, 2007. **57**(4): p. 622-8.
7. Rose, E., et al., *Intravenous dexamethasone-cyclophosphamide pulse therapy in comparison with oral methylprednisolone-azathioprine therapy in patients with pemphigus: results of a multicenter prospectively randomized study.* J Dtsch Dermatol Ges, 2005. **3**(3): p. 200-6.
8. Chrysomallis, F., et al., *Treatment of oral pemphigus vulgaris.* Int J Dermatol, 1994. **33**(11): p. 803-7.
9. Ioannides, D., F. Chrysomallis, and J.C. Bystryn, *Ineffectiveness of cyclosporine as an adjuvant to corticosteroids in the treatment of pemphigus.* Arch Dermatol, 2000. **136**(7): p. 868-72.
10. Anhalt, G.J. and L.A. Diaz, *In vivo studies of antibody dependent acantholysis.* Immunol Ser, 1989. **46**: p. 291-316.
11. Amagai, M., et al., *Use of autoantigen-knockout mice in developing an active autoimmune disease model for pemphigus.* J Clin Invest, 2000. **105**(5): p. 625-31.
12. Ahmed, A.R., et al., *Treatment of pemphigus vulgaris with rituximab and intravenous immune globulin.* N Engl J Med, 2006. **355**(17): p. 1772-9.
13. Veldman, C., et al., *Dichotomy of autoreactive Th1 and Th2 cell responses to desmoglein 3 in patients with pemphigus vulgaris (PV) and healthy carriers of PV-associated HLA class II alleles.* J Immunol, 2003. **170**(1): p. 635-42.
14. Yokoyama, T. and M. Amagai, *Immune dysregulation of pemphigus in humans and mice.* J Dermatol, 2010. **37**(3): p. 205-13.
15. Xu, R.C., et al., *The imbalance of Th17 and regulatory T cells in pemphigus patients.* Eur J Dermatol, 2013. **23**(6): p. 795-802.
16. Sugiyama, H., et al., *CD4+CD25high regulatory T cells are markedly decreased in blood of patients with pemphigus vulgaris.* Dermatology, 2007. **214**(3): p. 210-20.
17. Tang, Q. and J.A. Bluestone, *Regulatory T-cell physiology and application to treat autoimmunity.* Immunol Rev, 2006. **212**: p. 217-37.
18. Brunstein, C.G., et al., *Infusion of ex vivo expanded T regulatory cells in adults transplanted with umbilical cord blood: safety profile and detection kinetics.* Blood, 2011. **117**(3): p. 1061-70.

19. Marek-Trzonkowska, N., et al., *Clinical application of regulatory T cells in type 1 diabetes.* Pediatr Diabetes, 2013. **14**(5): p. 322-32.
20. Scalapino, K.J., et al., *Suppression of disease in New Zealand Black/New Zealand White lupus-prone mice by adoptive transfer of ex vivo expanded regulatory T cells.* J Immunol, 2006. **177**(3): p. 1451-9.
21. Scalapino, K.J. and D.I. Daikh, *Suppression of glomerulonephritis in NZB/NZW lupus prone mice by adoptive transfer of ex vivo expanded regulatory T cells.* PLoS One, 2009. **4**(6): p. e6031.
22. Tang, Q., et al., *In vitro-expanded antigen-specific regulatory T cells suppress autoimmune diabetes.* J Exp Med, 2004. **199**(11): p. 1455-65.
23. Putnam, A.L., et al., *Expansion of human regulatory T-cells from patients with type 1 diabetes.* Diabetes, 2009. **58**(3): p. 652-62.
24. Esensten, J.H., D. Wofsy, and J.A. Bluestone, *Regulatory T cells as therapeutic targets in rheumatoid arthritis.* Nat Rev Rheumatol, 2009. **5**(10): p. 560-5.
25. Brennan, T.V., et al., *Requirements for prolongation of allograft survival with regulatory T cell infusion in lymphosufficient hosts.* J Surg Res, 2011. **169**(1): p. e69-75.
26. Lever, W.F., *Pemphigus and pemphigoid.* Charles C. Thomas, 1965: p. 15–66.
27. Schmidt, E. and D. Zillikens, *Modern diagnosis of autoimmune blistering skin diseases.* Autoimmun Rev, 2010. **10**(2): p. 84-9.
28. Amagai, M., et al., *Pemphigus vulgaris antigen (desmoglein 3) is localized in the lower epidermis, the site of blister formation in patients.* J Invest Dermatol, 1996. **106**(2): p. 351-5.
29. Shirakata, Y., et al., *Lack of mucosal involvement in pemphigus foliaceus may be due to low expression of desmoglein 1.* J Invest Dermatol, 1998. **110**(1): p. 76-8.
30. Eyre, R.W. and J.R. Stanley, *Identification of pemphigus vulgaris antigen extracted from normal human epidermis and comparison with pemphigus foliaceus antigen.* J Clin Invest, 1988. **81**(3): p. 807-12.
31. Kowalczyk, A.P., et al., *Pemphigus sera recognize conformationally sensitive epitopes in the amino-terminal region of desmoglein-1.* J Invest Dermatol, 1995. **105**(2): p. 147-52.
32. Ding, X., et al., *Mucosal and mucocutaneous (generalized) pemphigus vulgaris show distinct autoantibody profiles.* J Invest Dermatol, 1997. **109**(4): p. 592-6.
33. Emery, D.J., et al., *Pemphigus foliaceus and pemphigus vulgaris autoantibodies react with the extracellular domain of desmoglein-1.* J Invest Dermatol, 1995. **104**(3): p. 323-8.
34. Tsunoda, K., et al., *Pathogenic autoantibody production requires loss of tolerance against desmoglein 3 in both T and B cells in experimental pemphigus vulgaris.* Eur J Immunol, 2002. **32**(3): p. 627-33.
35. Yokoyama, T., et al., *Antigen-independent development of Foxp3+ regulatory T cells suppressing autoantibody production in experimental pemphigus vulgaris.* Int Immunol, 2011. **23**(6): p. 365-73.
36. Veldman, C., et al., *Type I regulatory T cells specific for desmoglein 3 are more frequently detected in healthy individuals than in patients with pemphigus vulgaris.* J Immunol, 2004. **172**(10): p. 6468-75.
37. Leventhal, J.S.S., M.R., *Is it time to re-evaluate the treatment of pemphigus?* Journal of Drugs in Dermatology, 2012(11): p. 1200-1206.

38. Feldman, R.J. and A.R. Ahmed, *Relevance of rituximab therapy in pemphigus vulgaris: analysis of current data and the immunologic basis for its observed responses.* Expert Rev Clin Immunol, 2011. **7**(4): p. 529-41.
39. Colliou, N., et al., *Long-term remissions of severe pemphigus after rituximab therapy are associated with prolonged failure of desmoglein B cell response.* Sci Transl Med, 2013. **5**(175): p. 175ra30.
40. Michels, A.W. and P.A. Gottlieb, *Autoimmune polyglandular syndromes.* Nat Rev Endocrinol, 2010. **6**(5): p. 270-7.
41. Weigert, O., et al., *CD4+Foxp3+ regulatory T cells prolong drug-induced disease remission in (NZBxNZW) F1 lupus mice.* Arthritis Res Ther, 2013. **15**(1): p. R35.
42. Saadoun, D., et al., *Regulatory T-cell responses to low-dose interleukin-2 in HCV-induced vasculitis.* N Engl J Med, 2011. **365**(22): p. 2067-77.
43. Koreth, J., et al., *Interleukin-2 and regulatory T cells in graft-versus-host disease.* N Engl J Med, 2011. **365**(22): p. 2055-66.
44. Di Ianni, M., et al., *Tregs prevent GVHD and promote immune reconstitution in HLA-haploidentical transplantation.* Blood, 2011. **117**(14): p. 3921-8.
45. Qian, Y., et al., *Dissecting the anti-desmoglein autoreactive B cell repertoire in pemphigus vulgaris patients.* J Immunol, 2007. **178**(9): p. 5982-90.
46. Werner, G.T., *[Vaccination before foreign travel].* Krankenpflege (Frankf), 1987. **41**(4): p. 122-4.
47. Veldman, C.M., et al., *T cell recognition of desmoglein 3 peptides in patients with pemphigus vulgaris and healthy individuals.* J Immunol, 2004. **172**(6): p. 3883-92.
48. Tampoia, M., et al., *Diagnostic accuracy of enzyme-linked immunosorbent assays (ELISA) to detect anti-skin autoantibodies in autoimmune blistering skin diseases: a systematic review and meta-analysis.* Autoimmun Rev, 2012. **12**(2): p. 121-6.
49. Hori, S., *Lineage stability and phenotypic plasticity of Foxp3(+) regulatory T cells.* Immunol Rev, 2014. **259**(1): p. 159-72.
50. Baron, U., et al., *DNA demethylation in the human FOXP3 locus discriminates regulatory T cells from activated FOXP3(+) conventional T cells.* Eur J Immunol, 2007. **37**(9): p. 2378-89.
51. Sanchez Rodriguez, R., et al., *Memory regulatory T cells reside in human skin.* J Clin Invest, 2014. **124**(3): p. 1027-36.
52. Bluestone, J.A., et al., *Type 1 diabetes immunotherapy using polyclonal regulatory T cells.* Sci Transl Med, 2015. **7**(315): p. 315ra189.
53. Tang, Q. and K. Lee, *Regulatory T-cell therapy for transplantation: how many cells do we need?* Current opinion in organ transplantation, 2012. **17**(4): p. 349-54.
54. Dall'Era, M., et al., *Adoptive Regulatory T Cell Therapy in a Patient with Systemic Lupus Erythematosus.* Arthritis Rheumatol, 2018.
55. Bersenev, A. and B.L. Levine, *Convergence of gene and cell therapy.* Regen Med, 2012. **7**(6 Suppl): p. 50-6.
56. Boulard, C., et al., *Calculation of cut-off values based on the Autoimmune Bullous Skin Disorder Intensity Score (ABSIS) and Pemphigus Disease Area Index (PDAI) pemphigus scoring systems for defining moderate, significant and extensive types of pemphigus.* Br J Dermatol, 2016. **175**(1): p. 142-9.
57. Shimizu, T., et al., *Grading criteria for disease severity by pemphigus disease area index.* J Dermatol, 2014. **41**(11): p. 969-73.

58. Murrell, D.F., et al., *Consensus statement on definitions of disease, end points, and therapeutic response for pemphigus.* J Am Acad Dermatol, 2008. **58**(6): p. 1043-6.
59. Fund, A.K. *Blood Test: eGFR*. 2014 [cited 2014 September 18, 2014]; Equation]. Available from: http://www.kidneyfund.org/kidney-health/kidney-tests/kidney-test-egfr.html.
60. Bluestone, J.A., *Unpublished Data on File.* University of California, San Francisco, 2014.
61. Oliveira-Batista, D.P., et al., *Laboratory diagnosis of herpesvirus infections in patients with Pemphigus vulgaris lesions.* Intervirology, 2013. **56**(4): p. 231-6.
62. Chren, M.M., et al., *Improved discriminative and evaluative capability of a refined version of Skindex, a quality-of-life instrument for patients with skin diseases.* Arch Dermatol, 1997. **133**(11): p. 1433-40.
63. Paradisi, A., et al., *Quality-of-life assessment in patients with pemphigus using a minimum set of evaluation tools.* J Am Acad Dermatol, 2009. **60**(2): p. 261-9.
64. Macallan, D.C., et al., *Measurement of cell proliferation by labeling of DNA with stable isotope-labeled glucose: studies in vitro, in animals, and in humans.* Proc Natl Acad Sci U S A, 1998. **95**(2): p. 708-13.
65. Macallan, D.C., et al., *Measurement of proliferation and disappearance of rapid turnover cell populations in human studies using deuterium-labeled glucose.* Nat Protoc, 2009. **4**(9): p. 1313-27.
66. Busch, R., et al., *Measurement of cell proliferation by heavy water labeling.* Nat Protoc, 2007. **2**(12): p. 3045-57.
67. Wu, D., et al., *High-throughput sequencing detects minimal residual disease in acute T lymphoblastic leukemia.* Sci Transl Med, 2012. **4**(134): p. 134ra63.
68. Huehn, J., J.K. Polansky, and A. Hamann, *Epigenetic control of FOXP3 expression: the key to a stable regulatory T-cell lineage?* Nat Rev Immunol, 2009. **9**(2): p. 83-9.
69. Sehouli, J., et al., *Epigenetic quantification of tumor-infiltrating T-lymphocytes.* Epigenetics, 2011. **6**(2): p. 236-46.
70. Wiencke, J.K., et al., *Epigenetic biomarkers of T-cells in human glioma.* Epigenetics, 2012. **7**(12): p. 1391-402.
71. Daniel, B.S., et al., *Severity score indexes for blistering diseases.* Clin Dermatol, 2012. **30**(1): p. 108-13.
72. Rosenbach, M., et al., *Reliability and convergent validity of two outcome instruments for pemphigus.* J Invest Dermatol, 2009. **129**(10): p. 2404-10.

15 APPENDICES

- 15.1: PDAI
- 15.2: Skindex-29 (version 2)
- 15.3: Patient's Global Assessment
- 15.4: Physician's Global Assessment
- 15.5: Subject Self-Reported Demographics Optional Source Document
- 15.6: Pre-Infusion Required Checklist

15.1 PDAI

Pemphigus Disease Area Index (PDAI) [71]: The PDAI was developed by consensus by the International Pemphigus Definitions Committee in 2008 [58] PDAI consists of total sum of scores for Activity and Damage for skin and scalp, and Activity alone for mucous membrane. The maximum total activity score is 250 and maximum damage score is 13; the maximum total PDAI score is 263. The PDAI was found to be more reproducible than the ABSIS in a recent study of 15 patients with pemphigus [72]. PDAI also correlated more closely with Physician Global Assessment score [72]. It is considered to be a reliable tool for clinical trials as it is able to detect small differences in patients with mild or partially treated disease [71].

Pemphigus Disease Area Index (PDAI)

Skin	Activity		Damage
Anatomical Location	Erosion/Blisters or new erythema		Post-inflammatory hyperpigmentation or erythema from resolving lesion
	0 absent 1 1-3 lesions, up to one >2 cm in any diameter, none > 6 cm 2 2-3 lesions, at least two > 2 cm diameter, none > 6cm 3 >3 lesions, none > 6 cm diameter 5 >3 lesions, and/or at least one >6 cm 10 >3 lesions, and/or at least one lesion >16 cm diameter or entire area	Number lesions if ≤ 3	0 absent 1 present
Ears			
Nose			
Rest of the face			
Neck			
Chest			
Abdomen			
Back, buttocks			
Arms			
Hands			
Legs			
Feet			
Genitals			
Total skin	/120		/12

SAMPLE

Scalp

Scalp	Erosion/Blisters or new erythema	Post-inflammatory hyperpigmentation or erythema from resolving lesion
	0 absent 1 in one quadrant 2 two quadrants 3 three quadrants 4 affects whole skull 10 at least one lesion > 6 cm	0 absent 1 present
Total Scalp (0-10)	/10	/1

Mucous membrane

Anatomical Location	Erosion/Blisters	
	0 absent 1 1 lesion 2 2–3 lesions 5 >3 lesions or 2 lesions >2 cm 10 entire area	Number lesions if ≤ 3
Eyes		
Nose		
Buccal mucosa		
Hard palate		
Soft palate		
Upper gingiva		
Lower gingiva		
Tongue		
Floor of mouth		
Labial mucosa		
Posterior pharynx		
Anogenital		
Total Mucosa	/120	

Total Activity Score: ______ **Total Damage Score** ______

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15.2 Skindex-29 (version 2) [62]

The Skindex-29 is a skin-specific quality of life (QoL) tool consisting of 29 items that measure patient symptoms, emotions, and function [58].

APG01: PolyTregs for Pemphigus Required Source Document Manual of Procedures

SUBJECT SOURCE DOCUMENTATION
FOR THE
SKINDEX29 DERMATOLOGY SURVEY

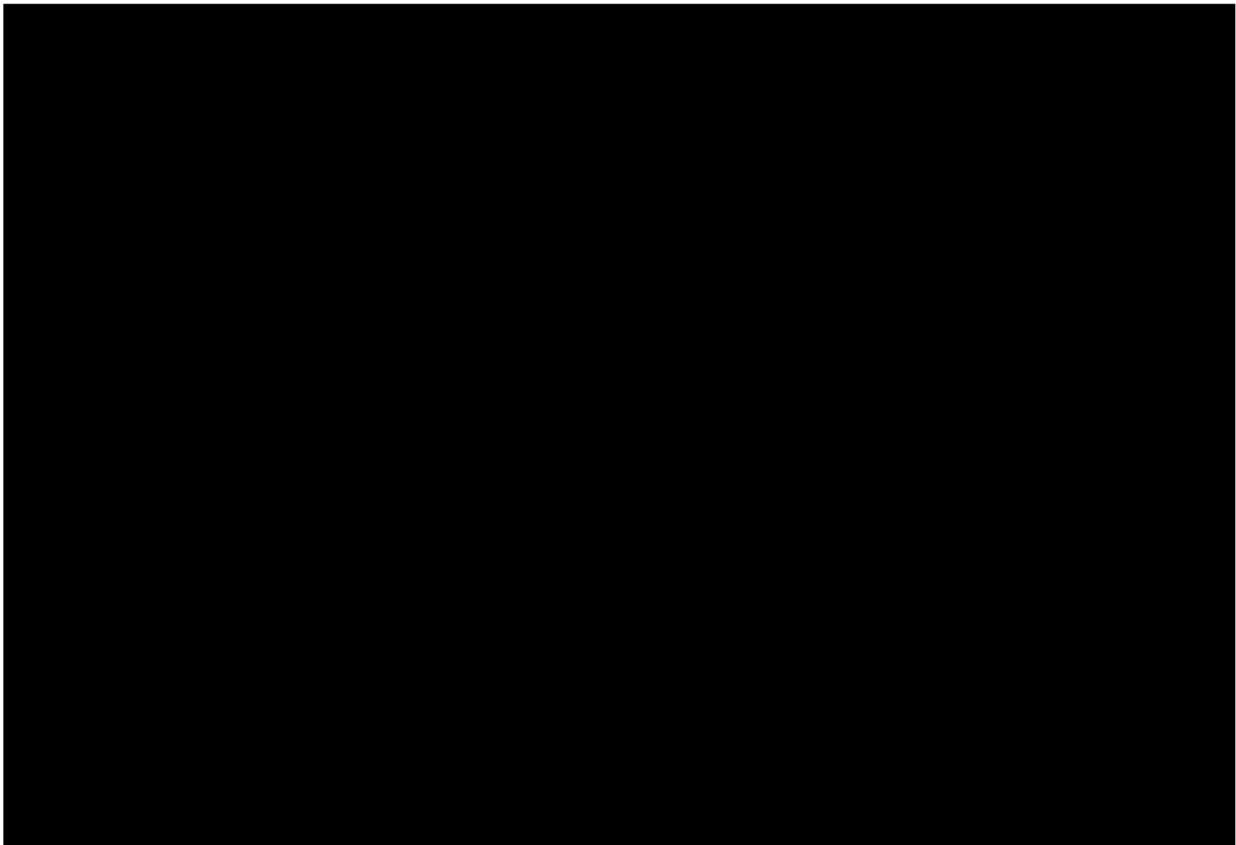
SAMPLE

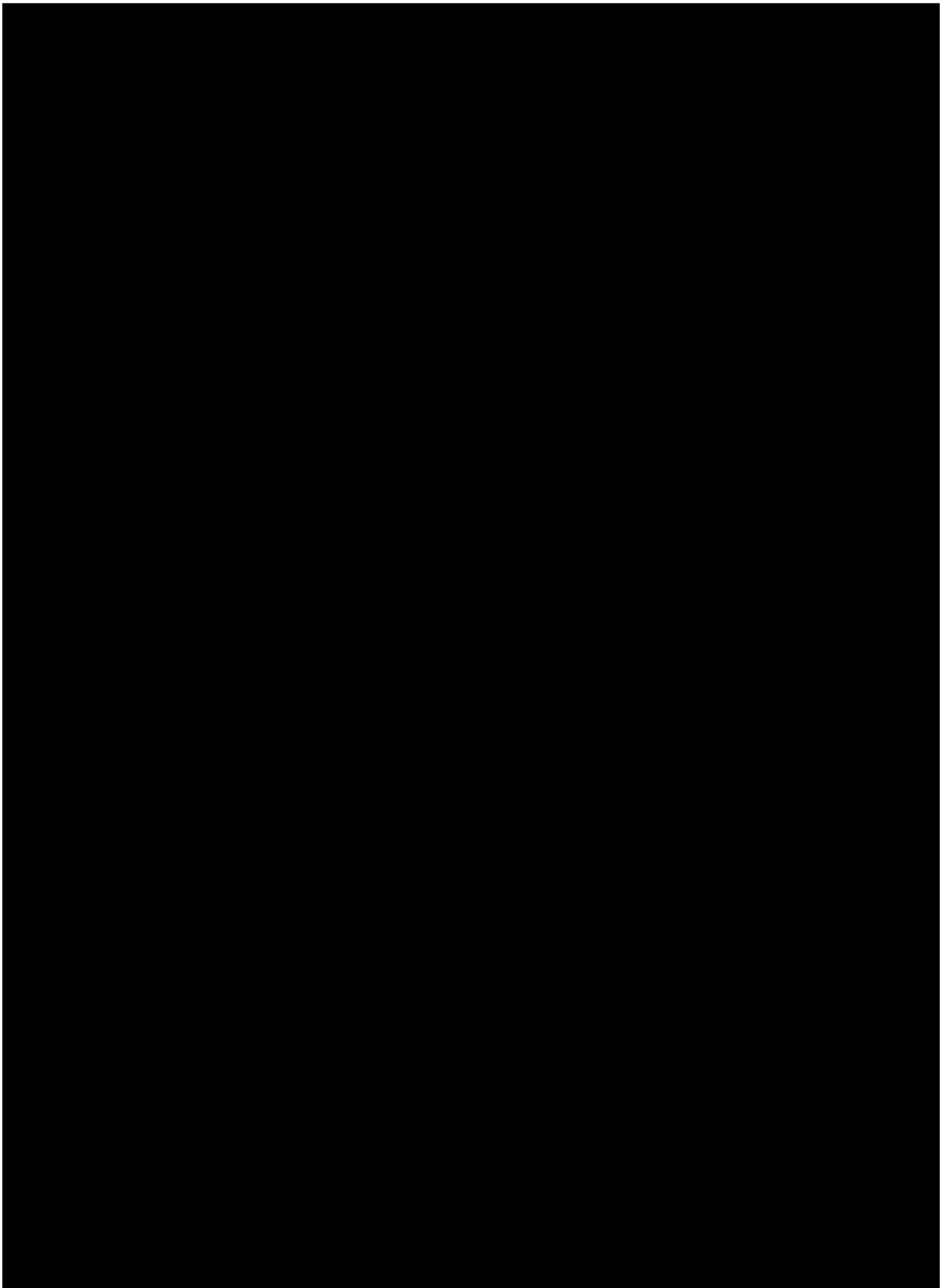
Subject ID #: ____________________

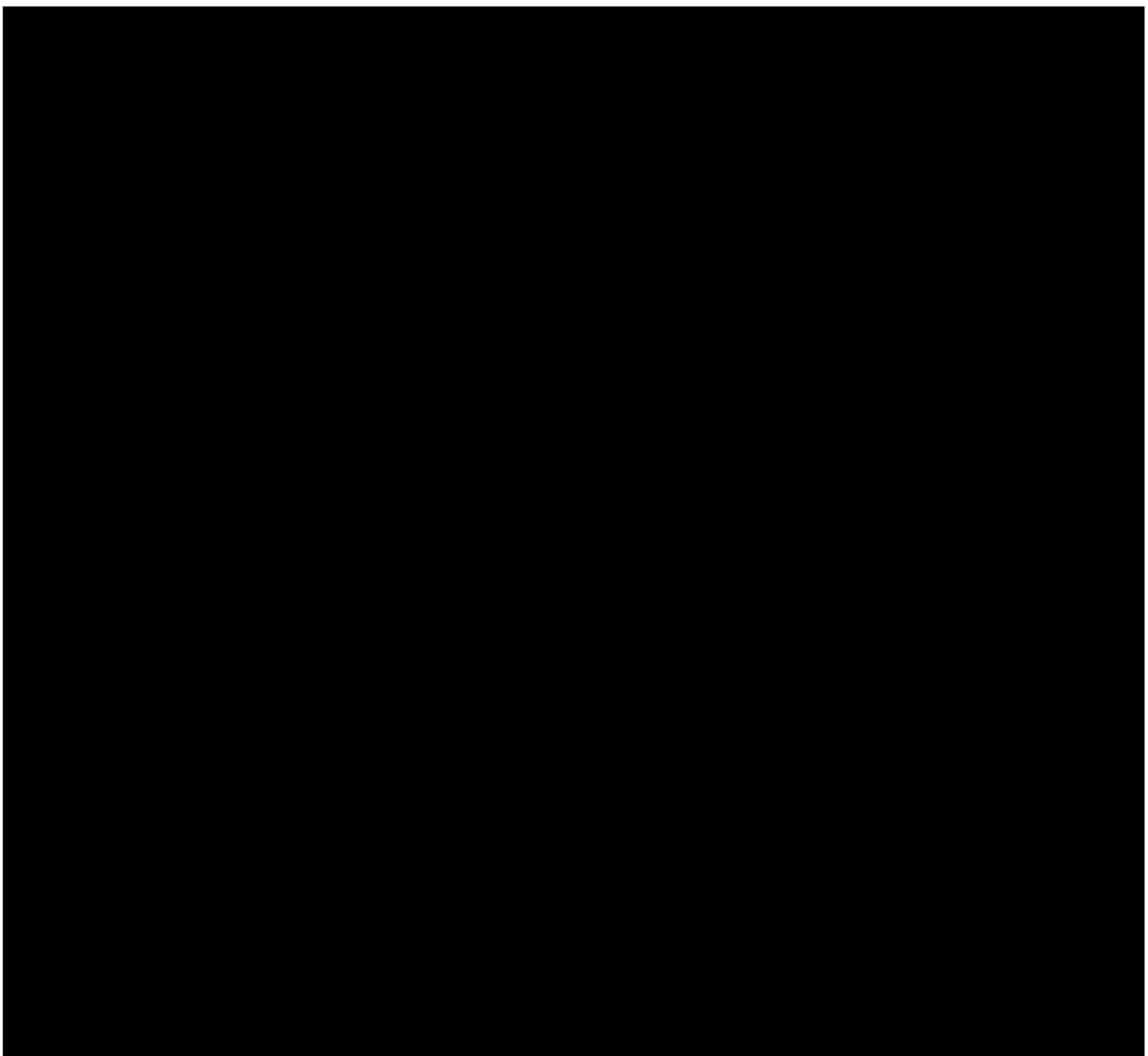
Subject Instructions:
Please complete the attached survey and return it to your APG01 Study Coordinator.
Please initial and date this form as indicated.

Subject Initials:__________________ Date survey completed:__________________

APG01: Skindex29 Required Source Document 06 September 2016







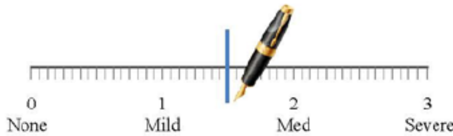
15.3 Patient's Global Assessment

APG01: PolyTregs for Pemphigus | Required Source Document | Manual of Procedures

PATIENT'S GLOBAL ASSESSMENT:
(3in)

Subject ID #: ____________________

EXAMPLE



SAMPLE

YOUR RESPONSE:

Please rate your current pemphigus disease activity on the scale below, with 0 being no disease activity and 3 being severe disease activity.

---------------------------------- **For Site Coordinator Use Only** ----------------------------------

Site Coordinator Directions: Using the markings provided, measure from the "0" to the vertical line placed by the subject. Enter the distance in inches as indicated below, then transfer the pertinent information onto the appropriate eCRF page, and place this document in the subject's research record.

Length of line
(from 0 to vertical assessment line) ______________________ in *(per markings provided)*

Initials of site personnel measuring the line: _________________ Date: ________________

APG01: Patient's Global Assessment Required Source Document 27 October 2016

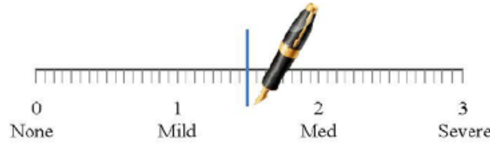
15.4 Physician's Global Assessment

APG01: PolyTregs for Pemphigus Required Source Document Manual of Procedures

PHYSICIAN'S GLOBAL ASSESSMENT:
(3in)

Subject ID #: ______________________

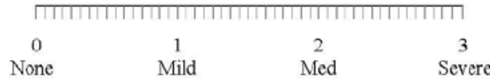
EXAMPLE



SAMPLE

YOUR RESPONSE:

Please rate the patient's current pemphigus disease activity on the scale below, with 0 being no disease activity and 3 being severe disease activity.



---------------------------------- **For Site Coordinator Use Only** ---------------------------------

Site Coordinator Directions: Using the markings provided, measure from the "0" to the vertical line placed by the physician. Enter the distance in inches as indicated below, then transfer the pertinent information onto the appropriate eCRF page, and place this document in the subject's research record.

Length of line
(from 0 to vertical assessment line) ______________________ in *(per markings provided)*

Initials of site personnel measuring the line: __________________ Date: _________________

APG01: Physician's Global Assessment Required Source Document 27 October 2016

15.5 Subject Self-Reported Demographics Optional Source Document

SAMPLE

SUBJECT SELF-REPORTED DEMOGRAPHICS OPTIONAL SOURCE DOCUMENT

Rave EDC Folder: Screening *Rave EDC Page: Demography*

Subject ID #: ___________________

Subject instructions: Please complete the survey by checking the box or boxes that most closely identify your race and ethnicity. Check multiple boxes if necessary. Initial and date this form as indicated and return it to your APG01 Study Coordinator.

Date of Birth: ________________________ **Gender:** ☐ Male ☐ Female
MM/DD/YYYY

Ethnicity: ☐ Hispanic or Latino ☐ Not Hispanic or Latino

Race:

White:
- ☐ White, not otherwise specified
- ☐ Eastern European
- ☐ European, not otherwise specified
- ☐ Mediterranean
- ☐ Middle Eastern
- ☐ North Coast Of Africa
- ☐ Western European
- ☐ White Caribbean
- ☐ White North American
- ☐ White South or Central American

Black or African American:
- ☐ Black, not otherwise specified
- ☐ African American
- ☐ African Black (both parents born in Africa)
- ☐ Caribbean Black
- ☐ South or Central American Black

Other:
- ☐ Unknown
- ☐ Other, specify:

Asian:
- ☐ Asian, not otherwise specified
- ☐ Asian Indian/South Asian
- ☐ Chinese
- ☐ Filipino
- ☐ Guamanian
- ☐ Korean
- ☐ Japanese
- ☐ Vietnamese
- ☐ Other Southeast Asian

Native Hawaiian or Other Pacific Islander:
- ☐ Hawaiian
- ☐ Native Pacific Islander, not otherwise specified
- ☐ Samoan

American Indian or Alaska Native:
- ☐ Native American, not otherwise specified
- ☐ American Indian, not otherwise specified
- ☐ Caribbean Indian
- ☐ Native Alaskan/Eskimo/Aleut
- ☐ South or Central American Indian

Subject Initials:____________________ Date survey completed:_________________

15.6 Pre-Infusion Required Checklist

APG01 PRE-INFUSION REQUIRED CHECKLIST

Complete this form in preparation for the PolyTreg Infusion for all subjects who have a Baseline (Day 0) visit.
Please note that all steps listed here must be completed before the start of the infusion.

Subject ID #: ____________________

Procedures/Steps *Answer to each item must be Yes or N/A as applicable to proceed with the PolyTreg infusion.*	Yes	No	N/A
1. Subject's medical history has been updated & does not meet any **Criteria for Withholding Study Treatment (Protocol Section 5.3.4)**.	❑	❑	
2. Subject's concomitant medication record has been updated & does not contain prohibited medications as per **Protocol Section 5.7**.	❑	❑	
3. Adverse Event assessment has been completed and does not meet any **Criteria for Withholding Study Treatment (Protocol Section 5.3.4)**.	❑	❑	
4. Pre-Infusion Physical Examination with Vital Signs has been conducted with no findings meeting **Criteria for Withholding Study Treatment (Protocol Section 5.3.4).**	❑	❑	
5. All baseline assessments as per **Protocol Table 6.1, Schedule of Events,** have been completed. *This includes PDAI, desmoglein 1/3, Skindex-29, Physician's Global Assessment, Patient's Global Assessment, Digital Photography of lesions, & urinalysis with microscopic analysis.*	❑	❑	
6. PDAI total score is between, and inclusive of, 1 to 12.	❑	❑	
Laboratory Assessments			
7. Subject's Day 0 urine pregnancy test is negative (if woman of childbearing potential).	❑	❑	❑
8. Subject's Day 0 Hematology Panel has been drawn, reviewed, and confirmed to not meet any **Criteria for Withholding Study Treatment (Protocol Section 5.3.4).** *Hematology Panel includes a CBC with differential and platelets.*	❑	❑	
9. Subject's Day 0 Chemistry Panel has been drawn, reviewed, and confirmed to not meet any **Criteria for Withholding Study Treatment (Protocol Section 5.3.4)**. *Chemistry Panel includes BUN, creatinine, AST, ALT, total bilirubin, direct bilirubin, ALK, albumin, sodium, potassium, chloride and bicarbonate.*	❑	❑	
Infusion Eligibility			
If any of the above criteria 1-9 have been checked as "No" then the subject is ineligible to receive the PolyTreg infusion. **I have reviewed the above elements and confirm the subject is eligible to proceed with the PolyTreg Infusion** ______________________ ____/____/____ ___:___ AM❑ PM❑ **Investigator Signature** **Date** **Time** **If No, specify exclusion below:**	❑	❑	
Comments:			
Continue Pre-Infusion Checklist on next page			

SAMPLE

APG01 PRE-INFUSION REQUIRED CHECKLIST

Complete this form in preparation for the PolyTreg Infusion for all subjects who have a Baseline (Day 0) visit.
Please note that all steps listed here must be completed before the start of the infusion.

Subject ID #: ____________________

Baseline Mechanistic Assessments:** To be obtained after eligibility has been confirmed above* ***Answer to each item must be Yes or N/A as applicable to proceed with the PolyTreg infusion.	Yes	No	N/A
10. Three skin biopsies were obtained and processed per **Protocol Section 5.5.5** and **MOP Section XX**.	❑	❑	
11. Lymphocyte subsets were drawn (*3mL in lavender top EDTA tube).*	❑	❑	
12. Mechanistic specimens were drawn *(20mL in two green top sodium heparin tubes).*	❑	❑	
Certificate of Analysis/Chain of Custody Documentation			
13. Upon receipt of the subject's Treg product for infusion from the HICTF, the Interim Certificate of Analysis and the product's Chain of Custody have been reviewed and signed.	❑	❑	
Comments:			

SAMPLE

Signature/Initials: ____ **Date:** ___/___/___